

MrBayes version 3.2 Manual: Tutorials and Model Summaries

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Preface

When we started working on MrBayes in 1999-2000, we knew that there was a need for a Bayesian MCMC package for phylogenetics, which was more flexible and easier to use than the software available then. Nevertheless, we were astonished by how quickly users took MrBayes to their hearts. The original release paper (Huelsenbeck and Ronquist, 2001), which appeared in *Bioinformatics* in 2001, quickly became a fast-track paper in *Computer Science*, as did the release note for version 3 (Ronquist and Huelsenbeck, 2003), published in *Bioinformatics* in 2003. To date, these two papers have accumulated more than 20,000 citations.

With the release of version 3.2 (Ronquist et al., 2012b), MrBayes has come of age. Through the years, we have added to, extended, and rewritten the program to cover most of the models used in standard statistical phylogenetic analyses today. We have also implemented a number of techniques to speed up calculations, improve convergence, and facilitate Bayesian model averaging and model choice. Version 3.2 has also undergone considerably more testing than previous versions of MrBayes. This is not to say that MrBayes 3.2 is bug free but it should be considerably more stable than previous versions. As in previous versions, we have done our best to document all of the available models and tools in the online help and in this manual.

Version 3.2 of MrBayes also marks the end of the road for us in terms of major development. Extending the original code has become increasingly difficult, and with version 3.2 we are at a point where we feel we need to explore new approaches to Bayesian phylogenetics. Perhaps most importantly, model specifications in MrBayes are strongly limited by the constraints of the Nexus language. In a separate project, RevBayes, we hope to provide a generic computing environment that al-

allows users to build complex phylogenetic models interactively, from small building blocks. However, such flexibility requires more of the user, and it may not be popular with everyone. For standard analyses, MrBayes will remain adequate for years to come and it is our intention to maintain the code base as long as the program remains heavily used and we have the resources to do so.

The MrBayes project would not have been possible without the help from many people. First and foremost, we would like to thank Maxim Teslenko, who has played an important role in fixing bugs and adding functionality to MrBayes during the last couple of years. Among other things, Maxim is responsible for a large part of the work involved in supporting BEAGLE, and in implementing the stepping-stone method for estimating marginal likelihoods. Maxim has also contributed several sections to this manual.

We are grateful for the financial support of the project provided by the Swedish Research Council, the National Institutes of Health, and the National Science Foundation. We are also deeply indebted to students, colleagues and numerous users of MrBayes, who have helped the project along in important ways. We are not going to try to enumerate them here, as any attempt to do so is likely to result in major omissions. However, we do want to express the overwhelming feeling of gratitude we feel for the generosity with which people have shared ideas, bug fixes and other valuable tips through the years. This feedback alone makes all the hours we have put into developing MrBayes worthwhile. Thank you, all of you!

Last but not least, we would like to thank our families for the unwavering support they have provided throughout the project. During intense programming periods, or when we have taught MrBayes workshops around the world, they have had to cope with absent-minded fathers, aloof visitors, and absent husbands. We realize that the childish enthusiasm we have shown when a new model resulted in some incomprehensible numbers scrolling by on the screen has been poor compensation. Thank you so much for all of your support and your sacrifices; we love you!

November, 2011

Fredrik Ronquist

John Huelsenbeck

Chapter 1

Introduction

MrBayes 3 is a program for Bayesian inference and model choice across a large space of phylogenetic and evolutionary models. The program has a command-line interface and should run on a variety of computer platforms, including large computer clusters and multicore machines. Depending on the settings, MrBayes analyses may demand a lot of your machine, both in terms of memory and processor speed. Many users therefore run more challenging analyses on dedicated computing machines or clusters. Several computing centers around the globe provide web access to such services. This said, many standard analyses run fine on common desktop machines.

This manual explains how to use the program. After introducing you to the program in this chapter, we first walk you through a simple analysis (chapter 2), which will get you started, and a more complex partitioned analysis, which uses more of the program's capabilities (chapter 3). This is followed by a set of shorter tutorials covering a range of common types of analyses (chapter 4).

We then cover the capabilities of the program in more detail (chapter 5), followed by some details on the evolutionary models that are implemented (chapter 6). The manual ends with a series of diagrams giving a graphical overview of the models and some proposal mechanisms implemented in the program (Appendix A). For more detailed information about commands and options in MrBayes, see the command reference that can either be downloaded from the program web site

or generated from the program itself (see section 1.4 below). All the information in the command reference is also available on-line when using the program.

The manual assumes that you are familiar with the basic concepts of Bayesian phylogenetics. If you are new to the subject, we recommend one of several recent reviews (Lewis, 2001; Holder and Lewis, 2003; Ronquist and Deans, 2010). The early papers introducing Bayesian phylogenetic methods (Li, 1996; Mau, 1996; Rannala and Yang, 1996; Mau and Newton, 1997; Larget and Simon, 1999; Mau et al., 1999; Newton et al., 1999) are also worthwhile reading. The basic MCMC techniques are described in Metropolis et al. (1953) and Hastings (1970), and the Metropolis-coupled MCMC used by MrBayes was introduced by Geyer (1991). Some recent general textbooks on Bayesian inference and MCMC methods include Gilks et al. (1996), Carlin and Louis (2000), Gelman et al. (2003), and Gamerman and Lopes (2006).

1.1 Conventions Used in this Manual

Throughout the document, we use `typewriter font` for things you see on screen or in a data file, and **bold typewriter font** for things you should type in. Alternative commands, options, file names, etc are also given in `typewriter font`.

1.2 Acquiring and Installing MrBayes

MrBayes 3 is distributed without charge by download from the MrBayes web site, <http://mrbayes.net>, or from the linked code repository on GitHub <https://github.com/NBISweden/MrBayes>. If someone has given you a copy of MrBayes 3, we strongly suggest that you download the most recent version from the official MrBayes site. The site also gives detailed information about installation on different platforms (Window, Macintosh, UNIX) and describes how you can report bugs or contribute to the project.

MrBayes 3 is a plain-vanilla program that uses a command line interface and

therefore behaves virtually the same on all platforms — Macintosh, Windows and Unix. Perhaps the easiest way of installing the program is to compile it from source code in a Unix environment, as described in the **README** and **INSTALL** documents in the MrBayes repository. The program may also be available as binaries that run on Windows or Macintosh computers.

MrBayes comes with reasonably fast code for computing likelihoods, partly implemented using so-called SIMD (single instruction, multiple data) technologies like SSE, AVX and FMA. However, MrBayes will run faster with the specialized BEAGLE library (Ayres et al., 2012), which makes even better use of the available computing resources. For instance, BEAGLE allows you to take advantage of the processing power of your graphics card, which the standard MrBayes code does not. Installing the BEAGLE library and linking it to MrBayes can be challenging; please follow the instructions in the **INSTALL** file.

MrBayes is available both in a serial version and in a parallel version that uses MPI (message passing interface) instructions to distribute computations across several processors or processor cores. The serial version does not support multithreading, which means that you will not be able to utilize more than one core on a multi-core machine for a single MrBayes analysis. If you want to utilize all cores, you need to run the MPI version of MrBayes. The MPI version must be compiled from source and will only run under Unix/Linux. Most Windows machines allow you to install a parallel Linux partition, and all Macintosh computers come with a Unix system under the hood, which will support MPI. Refer to the **README** and **INSTALL** documents for further instructions on how to compile and run the MPI version.

The MrBayes package comes with example data files. These are intended to show various types of analyses you can perform with the program, and you can use them as templates for your own analyses. In the tutorials given in chapters 2 to 4, you can learn more about how to set up various types of analyses based on these example data files.

1.3 Getting Started

Start MrBayes by double-clicking the application icon (or typing `./mb` or simply `mb` depending on your system) and you will see the information below:

```
MrBayes v3.2.7

(Bayesian Analysis of Phylogeny)

Distributed under the GNU General Public License

Type "help" or "help <command>" for information
on the commands that are available.

Type "about" for authorship and general
information about the program.

MrBayes >
```

Note the `MrBayes >` prompt at the bottom, which tells you that MrBayes is ready for your commands.

Changing the Size of the MrBayes Window

Some MrBayes commands will output a lot of information and write fairly long lines, so you may want to change the size of the MrBayes window to make it easier to read the output. On Macintosh and Unix machines, you should be able to increase the window size simply by dragging the margins. On a Windows machine, you cannot increase the size of the window beyond the preset value by simply dragging the margins but you can change both the size of the screen buffer and the console window by right-clicking on the title bar of the MrBayes window and then selecting “Properties” in the menu that appears. Make sure the “Layout” tab is selected in the window that appears, and then set the “Screen Buffer Size” and “Window Size” to the desired values.

1.4 Getting Help

At the **MrBayes** > prompt, type **help** to see a list of the commands available in MrBayes. Most commands allow you to set values (options) for different parameters. If you type **help <command>**, where <command> is any of the listed commands, you will see the help information for that command as well as a description of the available options. For most commands, you will also see a list of the current settings at the end. Try, for instance, **help lset** or **help mcmc**. The **lset** settings table at the end should look like this:

Parameter	Options	Current Setting
Nucmodel	4by4/Doublet/Codon/Protein	4by4
Nst	1/2/6/Mixed	1
Code	Universal/Vertmt/Invermt/Yeast/Mycoplasma/ Ciliate/Echinoderm/Euplotid/Metmt	Universal
Ploidy	Haploid/Diploid/Zlinked	Diploid
Rates	Equal/Gamma/LNorm/Propinv/ Invgamma/Adgamma/Kmixture	Equal
Ngammacat	<number>	4
Nlnormcat	<number>	4
Nmixtcat	<number>	4
Nbetacat	<number>	5
Omegavar	Equal/Ny98/M3	Equal
Covarion	No/Yes	No
Coding	All/Variable/Informative/Nosingletons Noabsencesites/Nopresencesites/ Nosingletonabsence/Nosingletonpresence	All
Parsmodel	No/Yes	No

Note that MrBayes 3 supports abbreviations of commands and options, so in many cases it is sufficient to type the first few letters of a command or option instead of the full name.

A complete list of commands and options can be generated as an ASCII text file by giving the command **manual** to MrBayes. See the output of the command **help manual** for more information.

1.5 Reporting and Fixing Bugs

If you find a bug in MrBayes, we are grateful if you tell us about it using the bug reporting functions of GitHub, as explained on the MrBayes web site (www.mrbayes.net). Advanced users may be interested in fixing bugs themselves in the source code. Refer to section 5 of this manual for information on how to contribute bug fixes, improved algorithms or expanded functionality to other users of MrBayes.

1.6 License and Warranty

MrBayes is free software; you can redistribute it and/or modify it under the terms of the GNU General Public License as published by the Free Software Foundation; either version 3 of the License, or (at your option) any later version.

The program is distributed in the hope that it will be useful, but WITHOUT ANY WARRANTY; without even the implied warranty of MERCHANTABILITY or FITNESS FOR A PARTICULAR PURPOSE. See the GNU General Public License for more details (<http://www.gnu.org/copyleft/gpl.html>).

1.7 Citing the Program

If you wish to cite the program, you can simply refer to the most recent release note (Ronquist et al., 2012a). That is,

Ronquist, F., M. Teslenko, P. van der Mark, D. Ayres, A. Darling, S. Höhna, B. Larget, L. Liu, M. A. Suchard, and J. P. Huelsenbeck. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61:539–542.

This manual should be cited as an online publication, for example:

Ronquist, F., J. P. Huelsenbeck, M. Teslenko, and J. A. A Nylander (2019) MrBayes version 3.2 manual: tutorials and model summaries. Distributed with the software from <https://github.com/NBISweden/MrBayes>

For more tips on citations, you can run the `citations` command in the program, which will give you a number of other relevant citations for the program and its models and algorithms.

Chapter 2

Tutorial: A Simple Analysis

This section walks you through a simple MrBayes example analysis to get you started. It is based on the `primates.nex` data file and will guide you through a basic Bayesian MCMC analysis of phylogeny, explaining the most important features of the program. There are two versions of the tutorial. You will first find a Quick-Start version for impatient users who want to get an analysis started immediately. The rest of the section contains a much more detailed description of the same analysis.

2.1 Quick Start Version

There are four steps to a typical Bayesian phylogenetic analysis using MrBayes:

1. Read the Nexus data file
2. Set the evolutionary model
3. Run the analysis
4. Summarize the samples

In more detail, each of these steps is performed as described in the following paragraphs:

1. At the MrBayes > prompt, type **execute primates.nex**. This will bring the data into the program. When you only give the data file name (**primates.nex**), MrBayes assumes that the file is in the current directory. If this is not the case, you have to use the full or relative path to your data file, for example **execute ../examples/primates.nex**. If you are running your own data file for this tutorial, beware that it may contain some MrBayes commands that can change the behavior of the program; delete those commands or put them in square brackets to follow this tutorial.
2. At the MrBayes > prompt, type **lset nst=6 rates=invgamma**. This sets the evolutionary model to the GTR substitution model with gamma-distributed rate variation across sites and a proportion of invariable sites (“GTR+I+ Γ ”). If your data are not DNA or RNA, if you want to invoke a different model, or if you want to use non-default priors, refer to the rest of this manual and the Appendix for more help.
 - 3.1. At the MrBayes > prompt, type **mcmc ngen=20000 samplefreq=100 printfreq=100 diagnfreq=1000**. This will start the Markov chain Monte Carlo simulation. It will also ensure that you get at least 200 samples from the posterior probability distribution, and that diagnostics are calculated every 1,000 generations. For larger data sets you probably want to run the analysis longer and sample less frequently. The default sample and print frequency is 500, the default diagnostic frequency is 5,000, and the default run length is 1,000,000. You can find the predicted remaining time to completion of the analysis in the last column printed to screen.
 - 3.2. If the standard deviation of split frequencies is below 0.01 after 20,000 generations, stop the run by answering **no** when the program asks **Continue the analysis? (yes/no)**. Otherwise, keep adding generations until the value falls below 0.01. If you are interested mainly in the well-supported parts of the tree, a standard deviation below 0.05 may be adequate.
 - 4.1. Type **sump** to summarize the parameter values using the same burn-in as the diagnostics in the **mcmc** command. The program will output a table with summaries of the samples of the substitution model parameters, including the mean, mode, and 95 % credibility interval (region of Highest Posterior Density,

HPD) of each parameter. Make sure that the potential scale reduction factor (PSRF) is reasonably close to 1.0 for all parameters; if not, you need to run the analysis longer. The same applies for the effective sample size (ESS). If the ESSs are somewhere below 100, you may want to run longer.

4.2. Summarize the trees using the same burn-in as the `mcmc` command by typing `sumt`. The program will output a cladogram with the posterior probabilities for each split and a phylogram with mean branch lengths. Both trees will also be printed to a file that can be read by FigTree (Rambaut and Drummond, 2012) and other tree-drawing programs, such as TreeView (Page, 1996) and Mesquite (Maddison and Maddison, 2006).

It does not have to be more complicated than this; however, as you get more proficient you will probably want to know more about what is happening behind the scenes. The rest of this section explains each of the steps in more detail and introduces you to all the implicit assumptions you are making and the machinery that MrBayes uses in order to perform your analysis.

2.2 Thorough Version

2.2.1 Getting Data into MrBayes

To get data into MrBayes, you need a so-called Nexus file that contains aligned nucleotide or amino acid sequences, morphological (“standard”) data, restriction site (binary) data, or any mix of these four data types. The Nexus data file is often generated by another program, such as Mesquite (Maddison and Maddison, 2006). Note, however, that MrBayes version 3 does not support the full Nexus standard, so you may have to do a little editing of the file for MrBayes to process it properly. In particular, MrBayes uses a fixed set of symbols for each data type and does not support user-defined symbols. The supported symbols are {A, C, G, T, R, Y, M, K, S, W, H, B, V, D, N} for DNA data, {A, C, G, U, R, Y, M, K, S, W, H, B, V, D, N} for RNA data, {A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y, V, X} for protein data, {0, 1} for restriction (binary) data, and {0, 1, 2, 3, 4, 5, 6, 5, 7, 8, 9} for standard (morphology) data.

In addition to the standard one-letter ambiguity symbols for DNA and RNA listed above, ambiguity can also be expressed using the Nexus parenthesis notation. For instance, if there is uncertainty whether a taxon have state 2 or 3 for a specific character, it can be coded as (23) (or (2,3)). The Nexus format also allows coding taxa as polymorphic — where a taxon truly have both character states 2 and 3. One would then use braces instead of paranthesis: {23}.

MrBayes, however, does not make any distinction between polymorphic states or uncertainty in states, but treat them the same way (as uncertainty), so it does not matter whether you use parentheses or braces.

If you have other symbols in your matrix than the ones supported by MrBayes, you need to replace them before processing the data block in MrBayes. You also need to remove the **Equate** and **Symbols** statements in the **Format** line if they are included. Unlike the Nexus standard, MrBayes supports data blocks that contain mixed data types as described in the tutorial in chapter 3.

To put the data into MrBayes type **execute <filename>** at the **MrBayes >** prompt, where **<filename>** is the name of the input file. To process our example file, type **execute primates.nex** or simply **exe primates.nex** to save some typing (MrBayes allows you to use the shortest unambiguous version of a command). Note that the input file must be located in the same folder (directory) where you started the MrBayes application (or else you will have to give the path to the file) and the name of the input file should not have blank spaces, or it will have to be quoted. If everything proceeds normally, MrBayes will acknowledge that it has read the data in the **DATA** block of the Nexus file by outputting some information about the file read in.

2.2.2 Specifying a Model

All of the commands are entered at the **MrBayes >** prompt. At a minimum two commands, **lset** and **prset**, are required to specify the evolutionary model that will be used in the analysis. Usually, it is also a good idea to check the model settings prior to the analysis using the **showmodel** command. In general, **lset** is used to define the structure of the model and **prset** is used to define the prior probability distributions on the parameters of the model. In the following, we

will specify a GTR+I+ Γ model (a General Time Reversible model with a proportion of invariable sites and a gamma-shaped distribution of rates across sites) for the evolution of the mitochondrial sequences and we will check all of the relevant priors. We assume that you are familiar with the common stochastic models of molecular evolution.

In general, a good start is to type **help lset**. Ignore the help information for now and concentrate on the table at the bottom of the output, which specifies the current settings. It should look like this:

Model settings for partition 1:

Parameter	Options	Current Setting
Nucmodel	4by4/Doublet/Codon/Protein	4by4
Nst	1/2/6/Mixed	1
Code	Universal/Vertmt/Invermt/Yeast/Mycoplasma/ Ciliate/Echinoderm/Euplotid/Metmt	Universal
Ploidy	Haploid/Diploid/Zlinked	Diploid
Rates	Equal/Gamma/LNorm/Propinv/ Invgamma/Adgamma/Kmixture	Equal
Ngammacat	<number>	4
Nlnormcat	<number>	4
Nmixtcat	<number>	4
Nbetacat	<number>	5
Omegavar	Equal/Ny98/M3	Equal
Covarion	No/Yes	No
Coding	All/Variable/Informative/Nosingletons Noabsencesites/Nopresencesites/ Nosingletonabsence/Nosingletonpresence	All
Parsmodel	No/Yes	No

First, note that the table is headed by **Model settings for partition 1**. By default, MrBayes divides the data into one partition for each type of data you have in your DATA block. If you have only one type of data, all data will be in a single partition by default. How to change the partitioning of the data will be explained in the tutorial in chapter 3.

The **Nucmodel** setting allows you to specify the general type of DNA model. The **Doublet** option is for the analysis of paired stem regions of ribosomal DNA and the **Codon** option is for analyzing the DNA sequence in terms of its codons. We

will analyze the data using a standard nucleotide substitution model, in which case the default `4by4` option is appropriate, so we will leave `Nucmodel` at its default setting.

The general structure of the substitution model is determined by the `Nst` setting. By default, all substitutions have the same rate (`Nst=1`), corresponding to the F81 model (or the JC model if the stationary state frequencies are forced to be equal using the `prset` command, see below). We want the GTR model (`Nst=6`) instead of the F81 model so we type `lset nst=6`. MrBayes should acknowledge that it has changed the model settings.

The `Code` setting is only relevant if the `Nucmodel` is set to `Codon`. The `Ploidy` setting is also irrelevant for us. However, we need to change the `Rates` setting from the default `Equal` (no rate variation across sites) to `Invgamma` (gamma-shaped rate variation with a proportion of invariable sites). Do this by typing `lset rates=invgamma`. Again, MrBayes will acknowledge that it has changed the settings. We could have changed both `lset` settings at once if we had typed `lset nst=6 rates=invgamma` in a single line.

We will leave the `Ngammacat` setting (the number of discrete categories used to approximate the gamma distribution) at the default of 4. In most cases, four rate categories are sufficient. It is possible to increase the accuracy of the likelihood calculations by increasing the number of rate categories. However, the time it will take to complete the analysis will increase in direct proportion to the number of rate categories you use, and the effects on the results will be negligible in most cases.

Of the remaining settings, it is only `Covarion` and `Parsmodel` that are relevant for single nucleotide models. We will use neither the parsimony model nor the covariotide model for our data, so we will leave these settings at their default values. If you type `help lset` now to verify that the model is correctly set, the table should look like this:

Model settings for partition 1:

Parameter	Options	Current Setting

Nucmodel	4by4/Doublet/Codon/Protein	4by4

Nst	1/2/6/Mixed	6
Code	Universal/Vertmt/Invermt/Yeast/Mycoplasma/ Ciliate/Echinoderm/Euplotid/Metmt	Universal
Ploidy	Haploid/Diploid/Zlinked	Diploid
Rates	Equal/Gamma/LNorm/Propinv/ Invgamma/Adgamma/Kmixture	Invgamma
Ngammacat	<number>	4
Nlnormcat	<number>	4
Nmixtcat	<number>	4
Nbetacat	<number>	5
Omegavar	Equal/Ny98/M3	Equal
Covarion	No/Yes	No
Coding	All/Variable/Informative/Nosingletons Noabsencesites/Nopresencesites/ Nosingletonabsence/Nosingletonpresence	All
Parsmodel	No/Yes	No

2.2.3 Setting the Priors

We now need to set the priors for our model. There are six types of parameters in the model: the topology, the branch lengths, the four stationary frequencies of the nucleotides, the six different nucleotide substitution rates, the proportion of invariable sites, and the shape parameter of the gamma distribution of rate variation. The default priors in MrBayes work well for most analyses, and we will not change any of them for now. By typing **help prset** you can obtain a list of the default settings for the parameters in your model. The table at the end of the help information reads:

Model settings for partition 1:		
Parameter	Options	Current Setting
Tratiopr	Beta/Fixed	Beta(1.0,1.0)
Revmatpr	Dirichlet/Fixed	Dirichlet(1.0,1.0,1.0,1.0,1.0,1.0)
Aamodelpr	Fixed/Mixed	Fixed(Poisson)
Aarevmatpr	Dirichlet/Fixed	Dirichlet(1.0,1.0,...)
Omegapr	Dirichlet/Fixed	Dirichlet(1.0,1.0)
Ny98omega1pr	Beta/Fixed	Beta(1.0,1.0)
Ny98omega3pr	Uniform/Exponential/Fixed	Exponential(1.0)
M3omegapr	Exponential/Fixed	Exponential
Codoncatfreqs	Dirichlet/Fixed	Dirichlet(1.0,1.0,1.0)
Statefreqpr	Dirichlet/Fixed	Dirichlet(1.0,1.0,1.0,1.0)
Shapepr	Uniform/Exponential/Fixed	Exponential(1.0)
Ratecorrpr	Uniform/Fixed	Uniform(-1.0,1.0)
Pinvarpr	Uniform/Fixed	Uniform(0.0,1.0)
Covswitchpr	Uniform/Exponential/Fixed	Uniform(0.0,100.0)

Symdirihyperpr	Uniform/Exponential/Fixed	Fixed(Infinity)
Topologypr	Uniform/Constraints/Fixed/ Speciestree	Uniform
Brlenspr	Unconstrained/Clock/Fixed	Unconstrained:GammaDir(1.0,0.100,1.0,1.0)
Treeagepr	Gamma/Uniform/Fixed/ Truncatednormal/Lognormal/ Offsetlognormal/Offsetgamma/ Offsetexponential	Gamma(1.00,1.00)
Speciationpr	Uniform/Exponential/Fixed	Exponential(10.0)
Extinctionpr	Beta/Fixed	Beta(1.0,1.0)
Fossilizationpr	Beta/Fixed	Beta(1.0,1.0)
SampleStrat	Random/Diversity/Cluster/ FossilTip	Random
Sampleprob	<number>	1.00000000
Popsizepr	Lognormal/Gamma/Uniform/ Normal/Fixed	Gamma(1.0,10.0)
Popvarpr	Equal/Variable	Equal
Nodeagepr	Unconstrained/Calibrated	Unconstrained
Clockratepr	Fixed/Normal/Lognormal/ Exponential/Gamma	Fixed(1.00)
Clockvarpr	Strict/Cpp/TK02/Igr/Mixed	Strict
Cpratepr	Fixed/Exponential	Exponential(0.10)
Cppmultdevpr	Fixed	Fixed(0.40)
TK02varpr	Fixed/Exponential/Uniform	Exponential(1.00)
Igrvarpr	Fixed/Exponential/Uniform	Exponential(10.00)
Ratepr	Fixed/Variable=Dirichlet	Fixed
Generatepr	Fixed/Variable=Dirichlet	Fixed

We need to focus on **Revmatpr** (for the six substitution rates of the GTR rate matrix), **Statefreqpr** (for the stationary nucleotide frequencies of the GTR rate matrix), **Shapepr** (for the shape parameter of the gamma distribution of rate variation), **Pinvarpr** (for the proportion of invariable sites), **Topologypr** (for the topology), and **Brlenspr** (for the branch lengths).

The default prior probability density is a flat Dirichlet (all values are 1.0) for both **Revmatpr** and **Statefreqpr**. This is appropriate if we want estimate these parameters from the data assuming no prior knowledge about their values. It is possible to fix the rates and nucleotide frequencies but this is generally not recommended. However, it is occasionally necessary to fix the nucleotide frequencies to be equal, for instance in specifying the JC and SYM models. This would be achieved by using `prset statefreqpr=fixed(equal)`.

If we wanted to specify a prior that put more emphasis on equal nucleotide frequencies than the default flat Dirichlet prior, we could for instance use `prset statefreqpr=Dirichlet(10,10,10,10)` or, for even more emphasis on equal frequencies, `prset statefreqpr=Dirichlet(100,100,100,100)`. The sum of

the numbers in the Dirichlet distribution determines how focused the distribution is, and the balance between the numbers determines the expected proportion of each nucleotide (in the order A, C, G, and T). Usually, there is a connection between the parameters in the Dirichlet distribution and the observations. For example, you can think of a Dirichlet(150,100,90,140) distribution as one arising from observing (roughly) 150 A's, 100 C's, 90 G's and 140 T's in some set of reference sequences. If the reference sequences are independent but clearly relevant to the analysis of your sequences, it might be reasonable to use those numbers as a prior in your analysis.

In our analysis, we will be cautious and leave the prior on state frequencies at its default setting. If you have changed the setting according to the suggestions above, you need to change it back by typing **prset statefreqpr=Dirichlet(1,1,1,1)** or **prs st=Dir(1,1,1,1)** if you want to save some typing. Similarly, we will leave the prior on the substitution rates at the default flat Dirichlet(1,1,1,1) distribution.

The **Shapepr** parameter determines the prior for the α (shape) parameter of the gamma distribution of rate variation. We will leave it at its default setting, an Exponential distribution with mean 1.0. This will put highest probability on low α values (see section 6.6.1).

The prior for the proportion of invariable sites is set with **Pinvarpr**. The default setting is a uniform distribution between 0 and 1, an appropriate setting if we don't want to assume any prior knowledge about the proportion of invariable sites.

For topology, the default **Uniform** setting for the **Topologypr** parameter puts equal probability on all distinct, fully resolved topologies. The alternative is to introduce some constraints on the tree topology, but we will not attempt that in this analysis.

The **Brlenspr** parameter can either be set to unconstrained, clock-constrained, or fixed. If **Fixed** is chosen then branch lengths will not be updated by the MCMC in the program but will be fixed to the current tree (requires a user-provided starting tree). For trees without a molecular clock ("unconstrained") there are currently five different branch length priors that can

be applied. The default is a compound Dirichlet prior, which essentially puts a prior on the total length on the tree, and then assigns prior lengths on individual branches by dividing the tree length using a Dirichlet prior. More details can be found in `build` in help documentation (by issuing **help prset**).

2.2.4 Checking the Model

To check the model before we start the analysis, type **showmodel**. This will give an overview of the model settings. In our case, the output will be as follows:

Model settings:

```
Data not partitioned --
  Datatype   = DNA
  Nucmodel   = 4by4
  Nst        = 6
              Substitution rates, expressed as proportions
              of the rate sum, have a Dirichlet prior
              (1.00,1.00,1.00,1.00,1.00,1.00)
  Covarion   = No
  # States    = 4
              State frequencies have a Dirichlet prior
              (1.00,1.00,1.00,1.00)
  Rates      = Invgamma
              The distribution is approximated using 4 categories.
              Shape parameter is exponentially
              distributed with parameter (1.00).
              Proportion of invariable sites is uniformly dist-
              ributed on the interval (0.00,1.00).
```

Active parameters:

```
Parameters
-----
Revmat           1
Statefreq        2
Shape            3
Pinvar           4
Ratemultiplier   5
Topology         6
Brlens           7
-----

1 -- Parameter = Revmat
```

```

      Type      = Rates of reversible rate matrix
      Prior     = Dirichlet(1.00,1.00,1.00,1.00,1.00,1.00)

2 -- Parameter = Pi
      Type      = Stationary state frequencies
      Prior     = Dirichlet

3 -- Parameter = Alpha
      Type      = Shape of scaled gamma distribution of site rates
      Prior     = Exponential(1.00)

4 -- Parameter = Pinvar
      Type      = Proportion of invariable sites
      Prior     = Uniform(0.00,1.00)

5 -- Parameter = Ratemultiplier
      Type      = Partition-specific rate multiplier
      Prior     = Fixed(1.0)

6 -- Parameter = Tau
      Type      = Topology
      Prior     = All topologies equally probable a priori
      Subparam. = V

7 -- Parameter = V
      Type      = Branch lengths
      Prior     = Unconstrained:GammaDir(1.0,0.1000,1.0,1.0)

```

Note that we have seven types of parameters in our model. All of these parameters, except the rate multiplier, will be estimated during the analysis (to fix them to some predetermined values, use the **prset** command and specify a fixed prior). To see more information about each parameter, including its starting value, use the **showparams** command. The **startvals** command allows one to set the starting values, separately for each chain if desired.

2.2.5 Setting up the Analysis

The analysis is started by issuing the **mcmc** command. However, before doing this, we recommend that you review the run settings by typing **help mcmc**. In our case, we will get the following table at the bottom of the output:

Parameter	Options	Current Setting
-----------	---------	-----------------

Ngen	<number>	1000000
Nruns	<number>	2
Nchains	<number>	4
Temp	<number>	0.100000
Reweight	<number>,<number>	0.00 v 0.00 ^
Swapfreq	<number>	1
Nswaps	<number>	1
Samplefreq	<number>	500
Printfreq	<number>	1000
Printall	Yes/No	Yes
Printmax	<number>	8
Mcmcdiagn	Yes/No	Yes
Diagnfreq	<number>	5000
Diagnstat	Avgstddev/Maxstddev	Avgstddev
Minpartfreq	<number>	0.10
Allchains	Yes/No	No
Allcomps	Yes/No	No
Relburnin	Yes/No	Yes
Burnin	<number>	0
Burninfrac	<number>	0.25
Stoprule	Yes/No	No
Stopval	<number>	0.05
Savetrees	Yes/No	No
Checkpoint	Yes/No	Yes
Checkfreq	<number>	2000
Filename	<name>	primates.nex.<p/t>
Startparams	Current/Reset	Current
Starttree	Current/Random/ Parsimony	Current
Nperts	<number>	0
Data	Yes/No	Yes
Ordertaxa	Yes/No	No
Append	Yes/No	No
Autotune	Yes/No	Yes
Tunefreq	<number>	100

The **Ngen** setting is the number of generations for which the analysis will be run. It is useful to run a small number of generations first to make sure the analysis is correctly set up and to get an idea of how long it will take to complete a longer analysis. We will start with 20,000 generations but you may want to start with an even smaller number for a larger data set. To change the **Ngen** setting without starting the analysis we use the **mcmcp** command, which is equivalent to **mcmc** except that it does not start the analysis. Type **mcmcp ngen=20000** to set the

number of generations to 20,000. You can type **help mcmc** to confirm that the setting was changed appropriately.

By default, MrBayes will run two simultaneous, completely independent analyses starting from different random trees (**Nruns=2**). Running more than one analysis simultaneously allows MrBayes to calculate convergence diagnostics on the fly, which is helpful in determining when you have a good sample from the posterior probability distribution. The idea is to start each run from a different, randomly chosen tree. In the early phases of the run, the two runs will sample very different trees but when they have reached convergence (when they produce a good sample from the posterior probability distribution), the two tree samples should be very similar.

To make sure that MrBayes compares tree samples from the different runs, check that **Mcmcdiagn** is set to yes and that **Diagnfreq** is set to some reasonable value. The default value of 5,000 is more appropriate for a larger analysis, so change the setting so that we compute diagnostics every 1,000th generation instead by typing **mcmcp diagnfreq=1000**.

MrBayes will now calculate various run diagnostics every **Diagnfreq** generation and print them to a file with the name **<Filename>.mcmc**. The most important diagnostic, a measure of the similarity of the tree samples in the different runs, will also be printed to screen every **Diagnfreq** generation. Every time the diagnostics are calculated, either a fixed number of samples (**burnin**) or a percentage of samples (**burninfrac**) from the beginning of the chain is discarded. The **relburnin** setting determines whether a fixed burnin (**relburnin=no**) or a burnin percentage (**relburnin=yes**) is used. By default, MrBayes will discard the first 25 % samples from the cold chain (**relburnin=yes** and **burninfrac=0.25**).

By default, MrBayes uses Metropolis coupling to improve the MCMC sampling of the target distribution. The **Swapfreq**, **Nswaps**, **Nchains**, and **Temp** settings together control the Metropolis coupling behavior. When **Nchains** is set to 1, no heating is used. When **Nchains** is set to a value n larger than 1, then $n - 1$ heated chains are used. By default, **Nchains** is set to 4, meaning that MrBayes will use 3 heated chains and one “cold” chain. In our experience, heating is essential for some data sets but it is not needed for others. Adding more than

three heated chains may be helpful in analyzing large and difficult data sets. The time complexity of the analysis is directly proportional to the number of chains used (unless MrBayes runs out of physical RAM memory, in which case the analysis will suddenly become much slower), but the cold and heated chains can be distributed among processors in a cluster of computers and among cores in multicore processors using the MPI version of the program, greatly speeding up the calculations. See also section 5.3.8 for more information on Metropolis coupling.

The **Samplefreq** setting determines how often the chain is sampled; the default is every 500 generations. This works well for moderate-sized analyses but our analysis is so small and is likely to converge so rapidly that it makes sense to sample more often. Let us sample the chain every 100th generation instead by typing **mcmc samplefreq=100**. For really large data sets that take a long time to converge, you may even want to sample less frequently than the default, or you will end up with very large files containing tree and parameter samples.

When the chain is sampled, the current values of the model parameters are printed to file. The substitution model parameters are printed to a **.p** file (in our case, there will be one file for each independent analysis, and they will be called **primates.nex.run1.p** and **primates.nex.run2.p**). The **.p** files are tab delimited text files that can be imported into most statistics and graphing programs. The topology and branch lengths are printed to a **.t** file (in our case, there will be two files called **primates.nex.run1.t** and **primates.nex.run2.t**). The **.t** files are Nexus tree files that can be imported into programs like PAUP* (Swofford, 1998), Mesquite, and FigTree. The root of the **.p** and **.t** file names can be altered using the **Filename** setting.

The **Printfreq** parameter controls the frequency with which brief info about the analysis is printed to screen. The default value is 1,000. Let us change it to match the sample frequency by typing **mcmc printfreq=100**.

When you set up your model and analysis (the number of runs and heated chains), MrBayes creates starting values for the model parameters. A different random tree with predefined branch lengths is generated for each chain and most substitution model parameters are set to predefined values. For instance,

stationary state frequencies start out being equal and unrooted trees have all branch lengths set to 0.1. The starting values can be changed by using the **Startparams** command. For instance, user-defined trees can be read into MrBayes by executing a Nexus file with a **trees** block. The available user trees can then be assigned to different chains using the **Startparams** command. After a completed analysis, MrBayes keeps the parameter values of the last generation and will use those as the starting values for the next analysis unless the values are reset using **mcmc starttree=random startparams=reset**.

Since version 3.2, MrBayes prints all parameter values of all chains (cold and heated) to a checkpoint file every **Checkfreq** generations, by default every 2,000 generations. The checkpoint file has the suffix **.ckp**. If you run an analysis and it is stopped prematurely, you can restart it from the last checkpoint by using **mcmc append=yes**. MrBayes will start the new analysis from the checkpoint; it will even read in all the old trees and include them in the convergence diagnostics. At the end of the new run, you will have parameter and tree files that are indistinguishable from those you would have obtained from an uninterrupted analysis.

2.2.6 Running the Analysis

Finally, we are ready to start the analysis. Type **mcmc**. MrBayes will first print information about the model and then list the proposal mechanisms that will be used in sampling from the posterior distribution. In our case, the proposals are the following:

The MCMC sampler will use the following moves:

With prob.	Chain will use move
0.93 %	Dirichlet(Revmat)
0.93 %	Slider(Revmat)
0.93 %	Dirichlet(Pi)
0.93 %	Slider(Pi)
1.85 %	Multiplier(Alpha)
1.85 %	Slider(Pinvar)
9.26 %	ExtSPR(Tau,V)
9.26 %	ExtTBR(Tau,V)
9.26 %	NNI(Tau,V)
9.26 %	ParsSPR(Tau,V)

```

37.04 %   Multiplier(V)
12.96 %   Nodeslider(V)
5.56 %    TLMultiplier(V)

```

The exact set of proposals and their relative probabilities may differ depending on the exact version of the program that you are using. Note that MrBayes will spend most of its effort changing the topology (Tau) and branch length (V) parameters. In our experience, topology and branch lengths are the most difficult parameters to integrate over and we therefore let MrBayes spend a large proportion of its time proposing new values for those parameters. The proposal probabilities and tuning parameters can be changed with the `Propset` command, but be warned that inappropriate changes of these settings may destroy any hopes of achieving convergence.

After the initial log likelihoods, MrBayes will print the state of the chains every 100th generation, like this:

```

Chain results (20000 generations requested):

  0 -- [-8849.255] (-8762.724) (-8987.103) (-8764.951) * [-8802.317] (-8424.093) (-8889.702) (-8566.483)
100 -- (-6964.051) [-6564.318] (-6585.769) (-7091.917) * (-6978.196) (-6959.121) [-6832.584] (-6918.296) -- 0:00:00
200 -- (-6331.866) (-6429.968) [-6064.254] (-6553.628) * (-6373.717) (-6481.652) [-6370.470] (-6395.154) -- 0:00:00
300 -- (-6244.179) (-6321.599) [-6025.263] (-6348.894) * (-6300.468) (-6276.345) [-6164.928] (-6257.516) -- 0:00:00
400 -- (-6206.423) (-6220.442) [-5983.629] (-6294.857) * (-6161.822) (-6151.696) (-6121.981) [-6110.477] -- 0:00:00
500 -- (-6186.992) (-6143.353) [-5976.339] (-6255.509) * (-6093.662) (-6136.777) [-6071.812] (-6099.497) -- 0:00:00
600 -- (-6176.634) (-6139.131) [-5960.670] (-6214.204) * (-6091.991) (-6129.092) [-6049.816] (-6075.710) -- 0:00:00
700 -- (-6112.019) (-6103.034) [-5944.847] (-6145.807) * (-6026.301) (-6075.044) [-5986.287] (-6069.480) -- 0:00:27
800 -- (-6082.030) (-6049.371) [-5952.450] (-6076.208) * (-6029.975) (-6031.923) [-5984.259] (-6052.163) -- 0:00:24
900 -- (-6071.824) (-6040.252) [-5931.429] (-6040.448) * (-6011.106) (-6022.905) [-5976.582] (-6051.125) -- 0:00:21
1000 -- (-6018.607) (-5969.229) [-5921.752] (-6031.270) * (-6006.262) (-6021.765) [-5942.360] (-6039.305) -- 0:00:19

Average standard deviation of split frequencies: 0.028570

1100 -- (-6002.039) (-5931.818) [-5903.026] (-6030.727) * (-6012.134) (-5993.487) [-5908.674] (-6040.577) -- 0:00:17
...
19100 -- (-5723.134) (-5728.224) [-5724.674] (-5722.268) * (-5729.337) (-5732.352) [-5723.627] (-5728.838) -- 0:00:00
19200 -- (-5724.510) (-5726.167) [-5727.321] (-5726.341) * (-5732.863) (-5732.101) [-5724.573] (-5727.017) -- 0:00:00
19300 -- (-5723.553) (-5729.459) [-5725.622] (-5728.011) * (-5726.456) (-5734.877) [-5725.750] (-5723.521) -- 0:00:00
19400 -- (-5726.202) (-5729.561) [-5726.133] (-5737.466) * (-5726.154) (-5735.261) (-5725.677) (-5725.421) -- 0:00:00
19500 -- (-5720.607) (-5730.406) (-5729.461) (-5725.360) * (-5721.939) (-5736.619) (-5717.324) (-5726.372) -- 0:00:00
19600 -- (-5717.783) (-5732.331) (-5725.260) [-5727.780] * [-5727.295] (-5742.071) (-5722.216) (-5727.507) -- 0:00:00
19700 -- [-5717.220] (-5726.029) (-5723.541) (-5727.178) * (-5729.795) (-5737.784) [-5728.311] (-5724.124) -- 0:00:00
19800 -- [-5723.804] (-5719.361) (-5721.537) (-5731.023) * (-5727.843) (-5737.280) (-5727.455) [-5723.834] -- 0:00:00
19900 -- (-5725.641) (-5726.235) [-5725.040] (-5723.821) * (-5728.999) (-5734.996) [-5727.100] (-5730.544) -- 0:00:00
20000 -- (-5731.566) (-5723.933) [-5726.992] (-5727.836) * (-5728.071) (-5734.746) (-5722.002) [-5728.053] -- 0:00:00

Average standard deviation of split frequencies: 0.000520

Continue with analysis? (yes/no):

```

If you have the terminal window wide enough, each generation of the chain will print on a single line.

The first column lists the generation number. The following four columns with negative numbers each correspond to one chain in the first run. Each column represents one physical location in computer memory, and the chains shift

positions in the columns as the run proceeds (it is actually only the temperature that is shifted). The numbers are the log likelihood values of the chains. The chain that is currently the cold chain has its value surrounded by square brackets, whereas the heated chains have their values surrounded by parentheses. When two chains successfully change states, they trade column positions (places in computer memory). If the Metropolis coupling works well, the cold chain should move around among the columns; this means that the cold chain successfully swaps states with the heated chains. If the cold chain gets stuck in one of the columns, then the heated chains are not successfully contributing states to the cold chain, and the Metropolis coupling is inefficient. The analysis may then have to be run longer. You can also try to reduce the temperature difference between chains, which may increase the efficiency of the Metropolis coupling.

The star column separates the two different runs. The last column gives the time left to completion of the specified number of generations. This analysis approximately takes 1 second per 1,000 generations. Because different moves are used in each generation, the exact time varies somewhat for each set of 100 generations, and the predicted time to completion will be unstable in the beginning of the run. After a while, the predictions will become more accurate and the estimated remaining time will decrease more evenly between generations.

2.2.7 When to Stop the Analysis

At the end of the run, MrBayes asks whether or not you want to continue with the analysis. Before answering that question, examine the average standard deviation of split frequencies. As the two runs converge onto the stationary distribution, we expect the average standard deviation of split frequencies to approach zero, reflecting the fact that the two tree samples become increasingly similar. In our case, the average standard deviation is down to 0.0 already after 1,000 generations and then stays at very low values throughout the run. Your values can differ slightly because of stochastic effects but should show a similar trend.

In larger and more difficult analyses, you will typically see the standard deviation of split frequencies come down much more slowly towards 0.0; the standard

deviation can even increase temporarily, especially in the early part of the run. A rough guide is that an average standard deviation below 0.01 is very good indication of convergence, while values between 0.01 and 0.05 may be adequate depending on the purpose of your analysis. The `sumt` command (see below) allows you to examine the error (standard deviation) associated with each clade in the tree. Typically, most of the error is associated with clades that are not very well supported (posterior probabilities well below 0.95), and getting accurate estimates of those probabilities may not be an important depending on the purpose of the analysis.

Given the extremely low value of the average standard deviation at the end of the run, there appears to be no need to continue the analysis beyond 20,000 generations so when MrBayes asks `Continue with analysis? (yes/no):` stop the analysis by typing `no`.

Although we recommend using a convergence diagnostic, such as the standard deviation of split frequencies, there are also simpler but less powerful methods of determining when to stop the analysis. The simplest technique is to examine the log likelihood values (or, more exactly, the log probability of the data given the parameter values) of the cold chain, that is, the values printed to screen within square brackets. In the beginning of the run, the values typically increase rapidly (the absolute values decrease, since these are negative numbers). In our case, the values increase from below -8400 to around -5725 in the first few thousand generations. This is the “burn-in” phase and the corresponding samples are typically discarded. Once the likelihood of the cold chain stops to increase and starts to randomly fluctuate within a more or less stable range, the run may have reached stationarity, that is, it may be producing a good sample from the posterior probability distribution. At stationarity, we also expect different, independent runs to sample similar likelihood values. Trends in likelihood values can be deceiving though; you’re more likely to detect problems with convergence by comparing split frequencies than by looking at likelihood trends.

When you stop the analysis, MrBayes will print several types of information useful in optimizing the analysis. This is primarily of interest if you have difficulties in obtaining convergence, which is unlikely to happen with this analysis. We give a few tips on how to improve convergence at the end of the

following section.

2.2.8 Summarizing Samples of Model Parameters

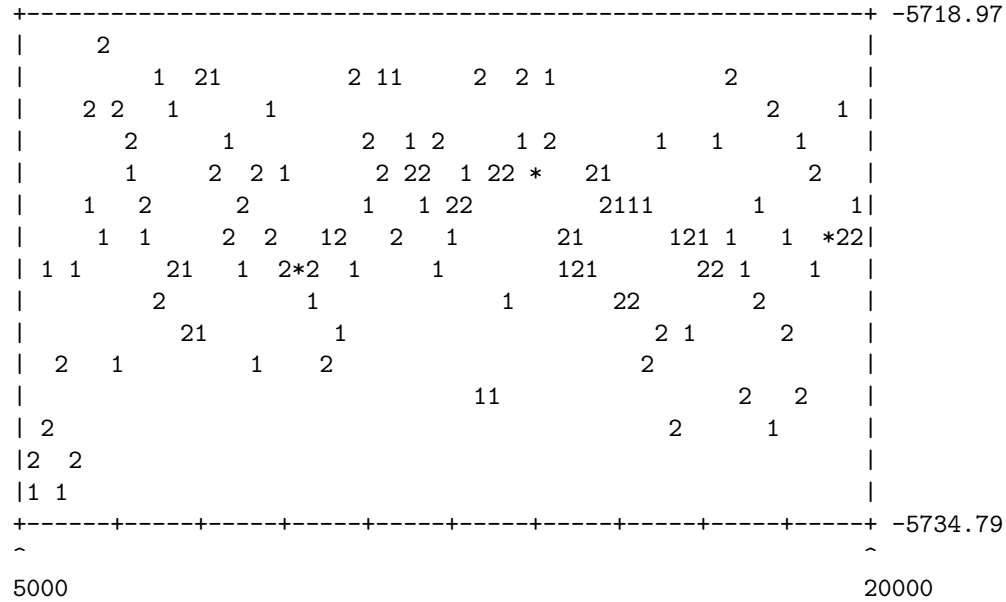
During the run, samples of the substitution model parameters have been written to the `.p` files every `samplefreq` generation. These files are tab-delimited text files that look something like this:

```
[ID: 0184438524]
Gen    LnL      LnPr      TL      r(A<->C)  ... pi(T)      alpha      pinvar
0      -8.849255e+03 4.262056e+01 4.200000e-01 1.666667e-01 ... 2.500000e-01 1.000000e+00 0.000000e+00
100    -6.564318e+03 1.426025e+01 1.722886e+00 1.499856e-01 ... 2.500000e-01 1.000000e+00 8.643869e-02
...
19900  -5.725040e+03 4.019204e+00 2.936465e+00 4.674104e-02 ... 2.517984e-01 4.555687e-01 8.178856e-02
20000  -5.726992e+03 2.358156e+00 3.184675e+00 4.674104e-02 ... 2.517984e-01 4.689211e-01 4.150474e-02
```

The first number, in square brackets, is a randomly generated ID number that lets you identify the analysis from which the samples come. The next line contains the column headers, and is followed by the sampled values. From left to right, the columns contain: (1) the generation number (**Gen**); (2) the log likelihood of the cold chain (**LnL**); (3) the log likelihood of the prior (**LnPr**); (4) the total tree length (the sum of all branch lengths, **TL**); (5) the six GTR rate parameters (**r(A<->C)**, **r(A<->G)** etc); (6) the four stationary nucleotide frequencies (**pi(A)**, **pi(C)** etc); (7) the shape parameter of the gamma distribution of rate variation (**alpha**); and (8) the proportion of invariable sites (**pinvar**). If you use a different model for your data set, the `.p` files will of course be different.

MrBayes provides the `sump` command to summarize the sampled parameter values. By default, the `sump` command uses the same burn-in as the convergence diagnostics in the `mcmc` command. This should be appropriate if we use these diagnostics to determine when we have an appropriate sample from the posterior. Thus, we can summarize the information in the `.p` file by simply typing `sump`. By default, `sump` will summarize the information in the `.p` file or files generated most recently, but the filename can be changed if necessary. The `relburnin=yes` option specifies that we want to give the burn-in in terms of a fraction (relative burn-in) rather than as an absolute value. The `burninfrac` option specifies the desired burn-in fraction.

The `sump` command will first generate a plot of the generation versus the log probability of the data (the log likelihood values). If we are at stationarity, this plot should look like “white noise”, that is, there should be no tendency of increase or decrease over time. The plot should look something like this:



If you see an obvious trend in your plot, either increasing or decreasing, you very likely need to run the analysis longer to get an adequate sample from the posterior probability distribution. Furthermore, if you see a clear separation of the two chains (all the 1:s vs. all the 2:s), that is also an indication that the two runs did not converge on the same target distribution, and that you might require longer runs.

At the bottom of the `sump` output, there is a table summarizing the samples of the parameter values:

Parameter	Mean	Variance	95% HPD Interval		Median	min ESS*	avg ESS	PSRF+
			Lower	Upper				
TL	3.157259	0.110374	2.498745	3.787651	3.133277	14.34	54.22	0.999
r(A<->C)	0.042789	0.000086	0.024422	0.056206	0.042196	16.58	38.21	0.999
r(A<->G)	0.467506	0.002449	0.368281	0.554633	0.469500	11.91	15.60	0.998
r(A<->T)	0.035821	0.000061	0.023179	0.051031	0.036272	15.00	18.17	1.007
r(C<->G)	0.032281	0.000197	0.007671	0.058215	0.029646	19.70	38.75	1.019
r(C<->T)	0.399001	0.001674	0.325954	0.474863	0.399301	17.63	24.72	1.007
r(G<->T)	0.022602	0.000289	0.000512	0.054123	0.017937	8.58	30.61	1.074

pi(A)	0.356156	0.000166	0.331900	0.378428	0.356370	56.29	70.70	1.009
pi(C)	0.321006	0.000156	0.300738	0.344609	0.321326	15.28	48.65	1.006
pi(G)	0.080853	0.000047	0.067370	0.093071	0.080178	19.82	30.34	1.004
pi(T)	0.241985	0.000119	0.223346	0.265659	0.241647	21.20	52.94	1.002
alpha	0.533518	0.024674	0.329657	0.817921	0.488774	61.71	94.24	1.030
pinvar	0.111808	0.005969	0.001041	0.252672	0.103843	71.95	95.00	1.018

For each parameter, the table lists the mean and variance of the sampled values, the lower and upper boundaries of the 95 % credibility interval, and the median of the sampled values. The parameters are the same as those listed in the .p files: the total tree length (TL), the six reversible substitution rates ($r(A \leftrightarrow C)$, $r(A \leftrightarrow G)$, etc), the four stationary state frequencies ($pi(A)$, $pi(C)$, etc), the shape of the gamma distribution of rate variation across sites ($alpha$), and the proportion of invariable sites ($pinvar$). Note that the six rate parameters of the GTR model are given as proportions of the rate sum (the Dirichlet parameterization). This parameterization has some advantages in the Bayesian context; in particular, it allows convenient formulation of priors. If you want to scale the rates relative to the G–T rate, just divide all rate proportions by the G–T rate proportion.

The last three columns in the table contains information about the mixing and convergence of the MCMC. The ESS (Effective Sample Size; Ripley (1987)) is the (estimated) effective number of independent draws from the posterior distribution that the MCMC managed to sample. A small ESS (< 100) would indicate poor mixing behaviour, suggesting that you need to run longer simulations. The Potential Scale Reduction Factor (PSRF; Gelman and Rubin (1992)), is based on comparing the estimated between-chain variance with the within-chain variance for a parameter. If we have a good sample from the posterior probability distribution, these values should be close to 1.0. A reasonable goal might be to aim for values between 1.0 and 1.2 but it can be difficult to achieve this for all parameters in the model in larger and more complicated analyses.

2.2.9 Summarizing Tree Samples

Trees and branch lengths are printed to the .t files. These files are Nexus-formatted tree files with a structure like this:

```

#NEXUS
[ID: 0184438524]
[Param: tree]
begin trees;
  translate
    1 Tarsius_syrichta,
    2 Lemur_catta,
    3 Homo_sapiens,
    4 Pan,
    5 Gorilla,
    6 Pongo,
    7 Hylobates,
    8 Macaca_fuscata,
    9 M_mulatta,
    10 M_fascicularis,
    11 M_sylvanus,
    12 Saimiri_sciureus;
  tree gen.0 = [&U] (((10:2.000000e-02,((((12:2.000000e-02,...
  ...
  tree gen.20000 = [&U] (((7:1.869397e-01,(6:1.712180e-01,...
end;

```

To summarize the tree and branch length information, while using the same burnin as above when summarizing the other model parameters, type **sumt**.

The **sumt** command will output, among other things, summary statistics for the taxon bipartitions, a tree with clade credibility (posterior probability) values, and a phylogram (if branch lengths have been saved). The output first gives a key to each partition in the tree sample using dots for the taxa that are on one side of the partition and stars for the taxa on the other side. For instance, the 14th partition (ID 14) in the output below represents the clade *Homo* (taxon 3) and *Pan* (taxon 4), since there are stars in the third and fourth positions and a dot in all other positions.

List of taxa in bipartitions:

```

1 -- Tarsius_syrichta
2 -- Lemur_catta
3 -- Homo_sapiens
4 -- Pan
5 -- Gorilla
6 -- Pongo
7 -- Hylobates
8 -- Macaca_fuscata
9 -- M_mulatta

```

```

10 -- M_fascicularis
11 -- M_sylvanus
12 -- Saimiri_sciureus

```

Key to taxon bipartitions (saved to file "primates.nex.parts"):

```

ID -- Partition
-----
1 -- .*****
2 -- .*.....
3 -- ..*.....
4 -- ...*.....
5 -- ....*.....
6 -- .....*.....
7 -- .....*.....
8 -- .....*.....
9 -- .....*....
10 -- .....*..
11 -- .....*.
12 -- .....*
13 -- .....****.
14 -- ..**.....
15 -- .*****
16 -- .....**...
17 -- .*****.
18 -- ..*****.
19 -- ..*****.
20 -- ..***.....
21 -- .....***..
-----

```

Then it gives a table over the informative bipartitions (the ones with more than one taxon included), specifying the number of times the partition was sampled (#obs), the probability of the partition (Probab.), the standard deviation of the partition frequency (Sd(s)) across runs, the min and max of the standard deviation across runs (Min(s) and Max(s)) and finally the number of runs in which the partition was encountered. In our analysis, there is overwhelming support for a single tree, so all partitions in this tree have a posterior probability of 1.0 (or very close to 1.0).

Summary statistics for informative taxon bipartitions
(saved to file "primates.nex.tstat"):

ID	#obs	Probab.	Sd(s)+	Min(s)	Max(s)	Nruns

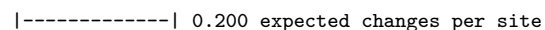
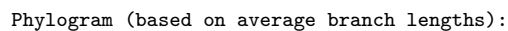
13	302	1.000000	0.000000	1.000000	1.000000	2
14	302	1.000000	0.000000	1.000000	1.000000	2
15	302	1.000000	0.000000	1.000000	1.000000	2
16	302	1.000000	0.000000	1.000000	1.000000	2
17	302	1.000000	0.000000	1.000000	1.000000	2
18	302	1.000000	0.000000	1.000000	1.000000	2
19	302	1.000000	0.000000	1.000000	1.000000	2
20	302	1.000000	0.000000	1.000000	1.000000	2
21	301	0.996689	0.004683	0.993377	1.000000	2

We then get a table summarizing branch and node parameters, in our case the branch lengths. The indices in this table refer to the key to partitions. For instance, `length[14]` is the length of the branch corresponding to partition ID 14. As we noted above, this is the branch grouping humans and chimps. The meaning of most of the values in this table is obvious. The last two columns give a convergence diagnostic, the Potential Scale Reduction Factor (PSRF), and the number of runs in which the partition was encountered. The PSRF diagnostic is the same used for the regular parameter samples, and it should approach 1.0 as runs converge.

Summary statistics for branch and node parameters
(saved to file "primates.nex.vstat"):

Parameter	Mean	Variance	95% HPD Interval		Median	PSRF+	Nruns
			Lower	Upper			
length[1]	0.531436	0.008411	0.378396	0.744535	0.530547	1.006	2
length[2]	0.372177	0.005349	0.239386	0.522698	0.367659	0.998	2
length[3]	0.051257	0.000138	0.028920	0.073525	0.050418	1.000	2
length[4]	0.064483	0.000190	0.041188	0.094009	0.064052	0.997	2
length[5]	0.060566	0.000223	0.032939	0.087111	0.059372	0.997	2
length[6]	0.151097	0.000855	0.102240	0.215567	0.147996	1.008	2
length[7]	0.181224	0.001226	0.118437	0.244907	0.175819	1.007	2
length[8]	0.019283	0.000038	0.007797	0.031080	0.018823	1.007	2
length[9]	0.022357	0.000045	0.010939	0.034789	0.021591	0.997	2
length[10]	0.057082	0.000131	0.038134	0.079448	0.055902	1.002	2
length[11]	0.076942	0.000490	0.037907	0.122557	0.075693	0.998	2
length[12]	0.465255	0.007233	0.280258	0.608086	0.465252	0.997	2
length[13]	0.297782	0.005323	0.163584	0.431633	0.291874	0.999	2
length[14]	0.031277	0.000159	0.010224	0.054248	0.029928	0.997	2
length[15]	0.132457	0.002687	0.034449	0.226634	0.126070	0.999	2
length[16]	0.134438	0.001637	0.067021	0.221458	0.131206	1.002	2
length[17]	0.275791	0.003801	0.175685	0.405595	0.273442	1.000	2
length[18]	0.060090	0.000501	0.020345	0.106085	0.059751	0.997	2
length[19]	0.036908	0.000128	0.016550	0.057338	0.035712	1.002	2
length[20]	0.088113	0.000594	0.038292	0.129339	0.085182	0.998	2
length[21]	0.047375	0.000386	0.012946	0.087369	0.047096	1.000	2

Clade credibility values:



In the background, the **sumt** command creates five additional files. The first is a **.parts** file, which contains the key to taxon bipartitions. The second and third are the **.tstat** and **.vstat** files, which contain the summaries of partition statistics and branch length statistics, respectively.

The next is the **.con.tre** file, which includes the consensus trees. By default, the consensus tree with all the relevant clade support values and branch length information is printed in a format suitable for FigTree. Once the **.con.tre** file is opened in FigTree, you can display statistics such as the posterior probability and its associated standard deviation on each clade in the tree.

Alternatively, you can specify **conformat=simple** when doing **sumt** to get the consensus tree in a simpler format that can be opened in most tree drawing programs. The simple format contains two different trees, one with both branch lengths and support values, and one with only branch lengths. Be sure to select the appropriate one if you wish to display both branch lengths and support values.

The last file generated by the **sumt** command is the **.trprobs** file, which contains the trees that were found during the MCMC search, sorted by posterior probability. This allows you to examine the trees contained in various credible sets of trees. For instance, the 95 % credible set contains the most probable trees up to an accumulated posterior probability of 95 %. In our case, this file will only contain one tree, but data sets with more topological uncertainty can produce long lists of trees in the **.trprobs** files.

Chapter 3

Tutorial: A Partitioned Analysis

MrBayes handles a wide variety of data types and models, as well as any mix of these models. In this example we will look at how to set up a simple analysis of a combined data set, consisting of data from four genes and morphology for 30 taxa of gall wasps and outgroups. A similar approach can be used, e.g., to set up a partitioned analysis of molecular data coming from different genes. The data set for this tutorial is found in the file `cynmix.nex`.

3.1 Getting Mixed Data into MrBayes

First, open up the Nexus data file in a text editor. The `DATA` block of the Nexus file should look familiar but there are some differences compared to the `primates.nex` file in the format statement:

```
Format datatype=mixed(Standard:1-166,DNA:167-3246)
      interleave=yes gap=- missing=?;
```

First, the `datatype` is given as `mixed` followed by a specification of the content of the matrix: it contains standard (morphology) characters in columns 1–166 and DNA characters in the remaining columns. The `mixed` datatype is an extension to the Nexus standard, which originated in MrBayes 3. It may not be compatible with other phylogenetics programs.

Second, the matrix is specified to be interleaved. It is often convenient to specify mixed data in interleaved format, with each block consisting of a natural subset of the matrix, such as the morphological data or one of the gene regions.

3.2 Dividing the Data into Partitions

By default, MrBayes partitions the data according to data type. There are only two data types in the matrix, so the default model will include only a morphology (standard) and a DNA partition. To divide the DNA partition into gene regions or other appropriate subsets, it is convenient to first specify character sets. In principle, this can be done from the command line but it is easier to do it in a MrBayes block in the data file. At the end of the `cynmix.nex`, there is such a block (starting with `begin mrbayes;`). Any text between square brackets (`[]`) are comments and ignored by MrBayes. Note also that each command ends with a semicolon.

The relevant lines for setting up partitions starts with the command `charset`. That command simply associates a name with a set of characters. For instance, the character set `COI` is defined to include characters 167 to 1244. The next step is to define a partition of the data according to genes and morphology. This is accomplished with the line:

```
partition favored = 5: morphology, COI, EF1a, LWRh, 28S;
```

The elements of the `partition` command are: (1) the name of the partitioning scheme (`favored`); (2) an equal sign (`=`); (3) the number of character divisions in the scheme (`5`); (4) a colon (`:`); and (5) a list of the characters in each division, separated by commas. The list of characters can simply be an enumeration of the character numbers (the above line is equivalent to `partition favored = 5: 1-166, 167-1244, 1245-1611, 1612-2092, 2093-3246;`) but it is often more convenient to use predefined character sets as given in the `mrbayes` block. The `end;` statement closes the MrBayes block.

When we read this block into MrBayes, we will have defined variables that can be used for setting up a partitioned analysis with the first character division

being morphology, the second division being the COI gene, etc. Exit your text editor, launch MrBayes and type **execute cynmix.nex** to read in your data and the variables. The final step is to tell MrBayes that we want to work with this partitioning of the data instead of the default partitioning. We do this using the **set** command:

```
set partition = favored;
```

3.3 Specifying a Partitioned Model

Before starting to specify the partitioned model, it is useful to examine the default model. Type **showmodel** and you should get this table as part of the output:

Active parameters:

Parameters	Partition(s)				
	1	2	3	4	5
-----	-----	-----	-----	-----	-----
Statefreq	1	2	2	2	2
Ratemultiplier	3	3	3	3	3
Topology	4	4	4	4	4
Brlens	5	5	5	5	5
-----	-----	-----	-----	-----	-----

There is a lot of other useful information in the output of **showmodel** but this table is the key to the partitioned model. We can see that there are five partitions in the model and five active (free) parameters. There are two stationary state frequency parameters, one for the morphological data (parameter 1) and one for the DNA data (parameter 2). Then there is also a ratemultiplier (3), a topology parameter (4) and a set of branch length parameters (5). All three are the same for all partitions.

Now, assume we want a separate GTR+ Γ +I model for each gene partition. All the parameters should be estimated separately for the individual genes. Assume further that we want the overall evolutionary rate to be (potentially) different across partitions, and that we want to assume gamma-shaped rate variation for the morphological data. We can obtain this model by using **lset** and **prset** with

the **applyto** mechanism, which allows us to apply the settings to specific partitions. For instance, to apply a GTR+ Γ +I model to the molecular partitions, we type **lset applyto=(2,3,4,5) nst=6 rates=invgamma**. This will produce the following table when **showmodel** is invoked:

Active parameters:

Parameters	Partition(s)				
	1	2	3	4	5
-----	-----	-----	-----	-----	-----
Revmat	.	1	1	1	1
Statefreq	2	3	3	3	3
Shape	.	4	4	4	4
Pinvar	.	5	5	5	5
Ratemultiplier	6	6	6	6	6
Topology	7	7	7	7	7
Brlens	8	8	8	8	8
-----	-----	-----	-----	-----	-----

As you can see, all molecular partitions now evolve under the correct model but all parameters (**statefreq**, **revmat**, **shape**, **pinvar**) are shared across partitions. To unlink them such that each partition has its own set of parameters, type: **unlink statefreq=(all) revmat=(all) shape=(all) pinvar=(all)**. Gamma-shaped rate variation for the morphological data is enforced by typing **lset applyto=(1) rates=gamma**. The trickiest part is to allow the overall rate to be different across partitions. This is achieved using the **ratepr** parameter of the **prset** command. By default, **ratepr** is set to **fixed**, meaning that all partitions have the same overall rate. By changing this to **variable**, the rates are allowed to vary under a flat Dirichlet prior. To allow all our partitions to evolve under different rates, type **prset applyto=(all) ratepr=variable**.

The model is now essentially complete but there is one final thing to consider. Typically morphological data matrices do not include all types of characters. Specifically, morphological data matrices do not usually include any constant (invariable) characters. Sometimes, autapomorphies are not included either, and the matrix is restricted to parsimony-informative characters. For MrBayes to calculate the probability of the data correctly, we need to inform it of this ascertainment (coding) bias. By default, MrBayes assumes that standard data sets include all variable characters but no constant characters. If necessary, one

can change this setting using `lset coding`. We will leave the `coding` setting at the default, though, which is `variable` for standard (morphology) data. Now, `showmodel` should produce this table:

Active parameters:

Parameters	Partition(s)				
	1	2	3	4	5
-----	-----				
Revmat	.	1	2	3	4
Statefreq	5	6	7	8	9
Shape	10	11	12	13	14
Pinvar	.	15	16	17	18
Ratemultiplier	19	19	19	19	19
Topology	20	20	20	20	20
Brlens	21	21	21	21	21
-----	-----				

3.4 Running the Analysis

When the model has been completely specified, we can proceed with the analysis essentially as described above in the tutorial for the `primates.nex` data set.

However, in the case of the `cynmix.nex` dataset, the analysis will have to be run longer before it converges.

When looking at the parameter samples from a partitioned analysis, it is useful to know that the names of the parameters are followed by the character division (partition) number in curly braces. For instance, `pi(A){3}` is the stationary frequency of nucleotide A in character division 3, which is the EF1a division in the above analysis.

In this section we have used a separate Nexus file for the MrBayes block. Although one can add this command block to the data file itself, there are several advantages to keeping the commands and the data blocks separate. For example, one can create a set of different analyses with different parameters in separate command files and submit all those files to a job scheduling system on a computer cluster. It is important to remember, though, that MrBayes uses the name of the file containing the character matrix as the default for all output files.

Thus, if you run all your analyses in the same directory, results from different analyses will overwrite each other.

To change this behavior, include the command **mcmcp filename=<filename>;** in each of your run files, just before issuing the **mcmc** command, using a different file name for each run file. For instance, if you wish to name the output files from one analysis using the root **analysis1**, you use the line **mcmcp filename=analysis1;.** The files will then be named **analysis1.run1.t**, **analysis1.run2.t**, etc. An alternative approach is to run each analysis in a separate directory, in which case the naming of the output files will not be an issue.

Chapter 4

More Tutorials

In this chapter, we provide a number of brief tutorials that cover a wide range of common models and analyses in MrBayes. We use a slightly different format for these tutorials. First, we assume that you are familiar with the basic working of the program, so we skip uninteresting details when they should be obvious. Also, in addition to the conventions used previously, we will often simply give the command line, including the **MrBayes** > prompt. What you need to type in is simply the text after the prompt.

You may wish to start each tutorial by switching off warnings and asking MrBayes to quietly finish each analysis after the requested number of generations, without asking you if you want to extend the analysis. You can accomplish this with the command line:

```
MrBayes > set autoclose=yes nowarn=yes
```

The tutorials are largely independent of each other, so it should be possible to skip directly to a tutorial that is of particular interest. However, some tutorials form a natural sequence of successively more advanced analyses of the same data set, so following all of the tutorials in order should give some added insight.

The tutorials by no means cover the entire range of models or analytical options supported by MrBayes. In chapters 5 and 6 we describe all component models and algorithms that are available, so refer to these for complete coverage. A

graphical overview of the models implemented in MrBayes 3.2 is provided in Appendix A.

4.1 An Amino Acid Analysis

In this tutorial, we will explore a couple of different approaches to amino acid analyses. First, we will ask MrBayes to integrate over a predetermined set of fixed rate matrices, and then we will repeat the analysis using a fixed rate matrix. To start the tutorial, first read in the amino acid data:

```
MrBayes > execute avian_ovomucoids.nex
```

To ask MrBayes to sample across fixed amino acid rate matrices, we use the `prset` command:

```
MrBayes > prset aamodelpr=mixed
```

Now we run the analysis as usual. The analysis is much slower than a normal nucleotide analysis, so let us try first and see if we can get convergence without using Metropolis coupling (heated chains). We do this by asking for a single chain per analysis using `nchains=1` in the `mcmc` command. Although convergence might be slightly slower if measured in terms of the number of generations, the analysis will run much faster without the overhead of the heated chains. We are still using the default of two parallel runs, so we can monitor topological convergence on the fly. For the dataset in this tutorial, our previous results indicate that about 300,000 generations are sufficient for decent topological convergence. Run 300,000 generations without heating using the command:

```
MrBayes > mcmc nchains=1 ngen=300000
```

Now we can summarize the parameter and tree samples as usual using the `sumt` and `sump` commands. The output of the `sump` command will include a table giving the posterior probabilities of the amino acid models:

```
Model probabilities above 0.050
Estimates saved to file "avian_ovomucoids.nex.mstat".
```

Posterior	Standard	Min.	Max.
-----------	----------	------	------

Model	Probability	Deviation	Probability	Probability
aamodel[Jones]	1.000	0.000	1.000	1.000

This particular dataset overwhelmingly supports the Jones model, so this may be the only model sampled after the burn-in phase. This means that the move mechanism trying to change the amino acid model will rarely or ever have its proposals accepted after the burn-in phase. However, the sample from the posterior may nevertheless be accurate. To determine whether we have an appropriate sample from the posterior, we simply compare the model probabilities across independent runs. The range of probabilities, from minimum to maximum, across independent analyses is given in the table. In our case, we have two independent analyses, both of which are putting all of the probability on the Jones model (both minimum and maximum probabilities are 1.000). This is good evidence that we have an adequate sample from the posterior, since each run started with a randomly selected amino acid rate matrix.

If desired, it is possible to run the same data set under another amino acid model, for instance the Dayhoff model, by fixing the rate matrix. Do this by running the analysis with the following commands:

```
MrBayes > prset aamodelpr=fixed(dayhoff)
MrBayes > mcmc nchains=1 ngen=300000
```

Note that the likelihoods printed to screen during the run are much worse (about 100 log likelihood units lower) than those obtained when we sampled over rate matrices according to their posterior probabilities. As you will discover, the analysis will not be much faster per generation if we fix the rate matrix, which may be somewhat counter-intuitive. The reason is that only a single rate matrix is used in any one generation of the chain, even if we change the rate matrix between generations. The effect of sampling across rate matrices on the speed of convergence is data-dependent. Convergence may be slower or faster when you mix over rate matrices, depending on the effects of the rate matrices on the complexity of the posterior.

We end this tutorial by pointing out that protein-coding nucleotide sequences can also be analyzed using amino acid models in MrBayes. MrBayes simply uses

the specified genetic code to translate the codons to amino acids, generating relevant ambiguous amino acid state sets when appropriate, before running the analysis. For instance, we would run a mixed amino acid model analysis on the protein-coding viral sequences in the `replicase.nex` data set using:

```
MrBayes > execute replicase.nex
MrBayes > lset nucmodel=protein
MrBayes > prset aamodelpr=mixed
MrBayes > mcmc nchains=1 ngen=300000
```

4.2 Identifying Positively Selected Sites

The purpose of this analysis is to identify positively selected sites in a protein-coding viral sequence. We will use the data in the file `replicase.nex`.

First, we read in the data:

```
MrBayes > execute replicase.nex
```

Then we specify that we want to use a codon model with omega variation across sites, and that we want MrBayes to report positive selection probabilities and site omega values (dN/dS ratios).

```
MrBayes > lset nucmodel=codon omegavar=ny98
MrBayes > report possel=yes siteomega=yes
```

This is going to be a slow analysis, so we will run relatively short analyses and will therefore reset the sampling frequency and the frequency with which we print to screen to once every 20 generations, so that we can monitor the run adequately and get more samples than we would otherwise. In the same vein, we will increase the frequency with which we calculate convergence diagnostics to once every 500 generations. Even though it is possible to speed up the analysis by running it without Metropolis coupling, our results indicate that the mixing across model parameters benefits considerably from the heated chains in this case, so we will not attempt that. Our results indicate that it is possible to get decent if not perfect convergence within about 10,000 generations, so let us try that:

```
MrBayes > mcmc printfreq=20 samplefreq=20 diagnfreq=500
MrBayes > mcmc ngen=10000
```

Now we examine the parameter samples by using the **sump** command. The output will be dominated by a long table summarizing the estimates of the posterior distributions of the model parameters, including the probabilities of sites being in the positively selected class, and the weighted site omega values (dN/dS ratios):

Parameter	Mean	Variance	95% HPD Interval		Median	min ESS*	avg ESS	PSRF+
			Lower	Upper				
TL	6.063468	0.869372	4.156027	7.813660	6.025831	5.08	12.36	1.014
omega(-)	0.069961	0.000184	0.047403	0.095480	0.068025	6.48	7.61	1.048
omega(N)	1.000000	0.000000	1.000000	1.000000	1.000000	NA	NA	NA
omega(+)	1.657224	1.111911	1.000940	4.105477	1.112150	34.57	84.80	0.999
pi(-)	0.827655	0.001925	0.764926	0.906755	0.828821	10.20	11.70	0.999
pi(N)	0.125519	0.002765	0.009573	0.206275	0.128064	16.95	33.05	1.166
pi(+)	0.046826	0.003448	0.000100	0.172577	0.015650	20.67	43.00	1.119
pi(AAA)	0.014750	0.000007	0.011227	0.019912	0.014817	10.95	12.02	1.010
pi(AAC)	0.014741	0.000009	0.009607	0.019714	0.015200	7.43	37.31	1.488
pi(AAG)	0.021098	0.000010	0.015839	0.026900	0.020483	11.36	17.71	1.089
...								
pr+(1,2,3)	0.012812	0.000861	0.000000	0.059747	0.000743	40.76	171.75	1.080
pr+(4,5,6)	0.000332	0.000000	0.000000	0.001532	0.000036	43.44	195.80	1.092
pr+(7,8,9)	0.000068	0.000000	0.000000	0.000316	0.000003	83.01	223.65	1.054
...								
omega(1,2,3)	0.103900	0.002723	0.054415	0.176367	0.089053	16.88	20.23	1.014
omega(4,5,6)	0.071107	0.000195	0.048813	0.097142	0.069085	6.47	7.37	1.041
omega(7,8,9)	0.070189	0.000188	0.048528	0.096028	0.068360	6.46	7.48	1.046
...								

First, note that the effective sample size (ESS) is still somewhat low for many parameters. The total ESS across the two runs is the double of the average ESS (avg ESS). The total in this analysis is typically below the recommended minimum of 100 to 200, so we need to run the analysis longer to get a publication-quality sample from the posterior. The potential scale reduction factor (PSRF) also indicates that there is some heterogeneity between the two independent runs. Nevertheless, the analysis is clearly approaching convergence. We should not have to run it much longer to get an accurate sample from the posterior, and we might expect the sample obtained so far to be reasonably close to the correct values.

Now focus on the summaries of the parameter samples. From the top, the parameters are total tree length (TL), the omega values (dN/dS ratios) for the negatively selected, neutral, and positively selected sites (**omega(-)**, **omega(N)**, and **omega(+)**), the frequencies of the site categories (**pi(-)**, **pi(N)**, and **pi(+)**), and the stationary state frequencies of the codons (**pi(AAA)**, **pi(AAC)**, etc).

The table ends with the probabilities of individual sites being in the positively selected class, with `pr+(1,2,3)` being the probability of the codon corresponding to nucleotide sites 1–3 being positively selected, `pr+(4,5,6)` being the probability for the codon corresponding to sites 4–6 being positively selected, etc.

The positive selection probabilities are followed by the site omega values, which represent a weighted average for each site across all categories of omega values, with `omega(1,2,3)` being the value for the codon corresponding to nucleotide sites 1–3, `omega(4,5,6)` the value for the codon corresponding to sites 4–6, etc.

Looking at the values, we note that about 85 % (`pi(-)`) of the sites are under strong constraining selection (`omega(-)=0.0699`). Only a small fraction of sites, around 5 %, are under positive selection (`pi(+)`).

Examination of the positive selection probabilities and codon site omega values reveals which sites are most likely to be experiencing positive selection (a high value for `pr+(n,n,n)`, and `omega(n,n,n) > 1.0`).

4.3 Sampling Across the GTR Model Space

A standard approach to Bayesian phylogenetics is to first select an appropriate substitution model using a model testing approach, such as those implemented in `jModelTest` (Posada, 2008) or `MrModeltest` (Nylander, 2004). An alternative, more elegant approach, is to sample across the substitution model space in the Bayesian MCMC analysis itself (Huelsenbeck et al., 2004), removing the need for a priori model testing. The purpose of this tutorial is to demonstrate how to set up an analysis that integrates out the uncertainty about the correct substitution model by sampling across the entire general time reversible (GTR) model space. We will use the data in the file `primates.nex`.

First, we read in the data:

```
MrBayes > execute primates.nex
```

Then we specify that we want to use a gamma model of rate variation across sites, and that we want to sample across the GTR model space:

```
MrBayes > lset nst=mixed rates=gamma
```

We can now run the analysis as usual. The **sump** command will give relevant summaries of the parameter samples, including the probabilities of the sampled substitution models. We assess convergence by making sure that these probabilities are the same across independent runs. This is a sample table from an analysis using two independent runs on the tutorial data set:

```
Model probabilities above 0.050
Estimates saved to file "primates.nex.mstat".
```

Model	Posterior Probability	Standard Deviation	Min. Probability	Max. Probability
gtrsubmodel[121123]	0.196	0.015	0.186	0.207
gtrsubmodel[121121]	0.099	0.007	0.094	0.104
gtrsubmodel[121321]	0.066	0.017	0.053	0.078
gtrsubmodel[121324]	0.063	0.013	0.054	0.072
gtrsubmodel[121323]	0.062	0.008	0.057	0.068
gtrsubmodel[123323]	0.055	0.004	0.053	0.058

The models are labeled using a so-called restricted growth function. The six different substitution rates are given in the order r_{AC} , r_{AG} , r_{AT} , r_{CG} , r_{CT} , r_{GT} . The first rate is labeled “1”. The next rate is labeled with the next higher, unused integer if it is different, and otherwise with the same integer as the partition it belongs to. For instance, `gtrsubmodel[111111]` refers to the Jukes–Cantor or F81 model, `gtrsubmodel[123456]` to the GTR model, and `gtrsubmodel[121121]` to the HKY model, and so on.

For this particular data set, we observe that there is considerable uncertainty concerning the correct substitution model. The model with the highest posterior probability is `gtrsubmodel[121123]`, which differs from the HKY model only in that the G–T substitution rate is different from the other transversion rates, presumably lower. The HKY model has the second best posterior probability of all the models reaching above the reporting threshold of 0.050. All other estimates of model parameters derived from this analysis, the topology estimate for instance, will be based on an average across the sampled substitution models, each one weighted according to its posterior probability.

To assess convergence when you sample across the GTR substitution model space, focus on the variation across runs in estimated model probabilities. The table gives the standard deviation as well as the range of estimated values across runs. Here, we see some heterogeneity across runs in model probabilities, but most models receive similar probabilities in the two independent runs.

Please note, the following sections of the manual is not revised for v.3.2.7!

4.4 Testing a Topological Hypothesis

In this tutorial, we will use Bayes factor comparisons to test a topological hypothesis, namely that humans are more closely related to chimps than to other primates. Specifically, we will contrast the hypothesis that humans and chimps form a monophyletic group (M_1), with the hypothesis that humans and chimps do not form a monophyletic group (M_2). In order to do this, we need to compute the ratio of the marginal likelihoods of the two models, M_1 and M_2 . This ratio is known as the Bayes factor. We will use the data in the file `primates.nex` for the analysis.

First, read in the data using:

```
MrBayes > execute primates.nex
```

Now let us specify a GTR + I + Γ model:

```
> lset nst=6 rates=invgamma
```

Before we can test the hypothesis, we need to specify a hard constraint and a negative constraint. The hard constraint will allow us to force a partition to always be present in the sampled trees. The negative constraint will allow us to sample across all trees that do not contain the specified partition. Specify the constraints using the following commands:

```
MrBayes > constraint humanchimp = Homo_sapiens Pan
```

```
MrBayes > constraint nohumanchimp negative = Homo_sapiens Pan
```


MrBayes provides two methods for estimating marginal model likelihoods. The first is based on the harmonic mean of the likelihood values of the MCMC samples. It is simple to compute but it is a pretty rough estimate of the model likelihood. To obtain a more accurate model likelihood, MrBayes provides the stepping-stone method. First, let us use the harmonic mean estimate of the model likelihoods of the two models we want to compare. First we enforce the positive constraint, run an mcmc analysis with 100,000 generations, and use sump to get the harmonic mean estimate:

```
MrBayes > prset topologypr=constraints(humanchimp)
MrBayes > mcmc ngen=100000
MrBayes > sump
```

In the output from the `sum` command, focus on the table summarizing the likelihoods of the MCMC samples:

```
Estimated marginal likelihoods for runs sampled in files
"primates.nex.run1.p" and "primates.nex.run2.p":
(Use the harmonic mean for Bayes factor comparisons of models)
```

```
(Values are saved to the file primates.nex.lstat)
```

Run	Arithmetic mean	Harmonic mean
1	-5720.59	-5729.69
2	-5720.75	-5735.25
TOTAL	-5720.67	-5734.56

It is the harmonic mean that we will use as the initial estimate of the model likelihood. The estimate of the logarithm of the model likelihood is -5734.56 , with a little bit of heterogeneity between the runs.

Now we enforce the negative constraint, and repeat the procedure using the commands:

```
MrBayes > prset topologypr=constraints(nohumanchimp)
MrBayes > mcmc ngen=100000
MrBayes > sump
```

Our run produced the following estimates of the log of the model likelihoods:

Run	Arithmetic mean	Harmonic mean
1	-5727.08	-5738.92
2	-5725.67	-5737.63
TOTAL	-5726.15	-5738.47

The harmonic mean estimate for this model is -5738.47 in log units, which is about 4 log units worse than the previous model. A log difference in the range of 3–5 is typically considered strong evidence in favor of the better model, while a log difference above 5 is considered very strong evidence (Kass and Raftery, 1995). Thus, the harmonic mean estimator indicates that we have strong evidence in favor of human and chimp being each others closest relatives, with Gorilla and other primates being more distant.

It may also be interesting to look at the best estimate of the phylogeny under the assumption that humans and chimps are not each others sister groups. Do this by typing **sumt**. As you will see, this tree groups chimps and gorillas together, with humans being just outside, as one might have expected.

Let us now repeat the comparison using the more accurate stepping-stone sampling approach. Instead of using the **mcmc** command followed by the **sump** command, we simply use the **ss** command instead, which will produce the estimated model likelihood directly. The stepping-stone analysis moves from the posterior to the prior through a number of steps in which the sampled distribution is a mixture of varying proportions of the two.

Ideally, one would like to see evidence of convergence among independent runs in each of the steps of the stepping-stone sampling algorithm. As the algorithm moves close to the prior, however, we expect this to be difficult. This will be especially true for the topology parameter, as the number of trees with similar probability will become huge for any reasonably-sized set of taxa.

To help you monitor convergence during stepping-stone sampling, MrBayes will print the average standard deviation of split frequencies across runs if you use at least two independent, parallel runs. Each step in the algorithm is treated as an independent MCMC sampling problem. The only burn-in phase used by the

algorithm occurs before the first step of the algorithm. Once the step sampling starts, each step simply serves as the burn-in for the next one. The length of the initial burn-in can be specified either in terms of the number of samples to be discarded (positive numbers) or in terms of step lengths (negative numbers). By default, the initial burn-in phase is as long as one of the subsequent steps in the algorithm, that is, `burninss=-1`.

To obtain an adequate sample from most of the steps in the algorithm, we will use 50 steps (the default) with 5,000 generations each, for a total of 250,000 generations. If we add the initial burn in of 5,000 generations (one step), the total run length will be 255,000. To monitor convergence twice during each step, we set the diagnostics frequency to once every 2,500 generations. Stepping-stone analysis under the two models using these settings will be generated by the following commands:

```
MrBayes > prset topologypr=constraints(humanchimp)
MrBayes > ss ngen=250000 diagnfreq=2500
MrBayes > prset topologypr=constraints(nohumanchimp)
MrBayes > ss
```

The output from the `ss` command will include the following table, which is given here for the first model:

Marginal likelihood (in natural log units) estimated using stepping-stone sampling based on 50 steps with 5000 generations (10 samples) within each step.

Run	Marginal likelihood (ln)
1	-5788.13
2	-5785.85
Mean:	-5786.45

In our run, the second model produced the following version of the same table:

Marginal likelihood (in natural log units) estimated using stepping-stone sampling based on 50 steps with 5000 generations (10 samples) within each step.

Run	Marginal likelihood (ln)
1	-5797.09
2	-5798.19
Mean:	-5797.50

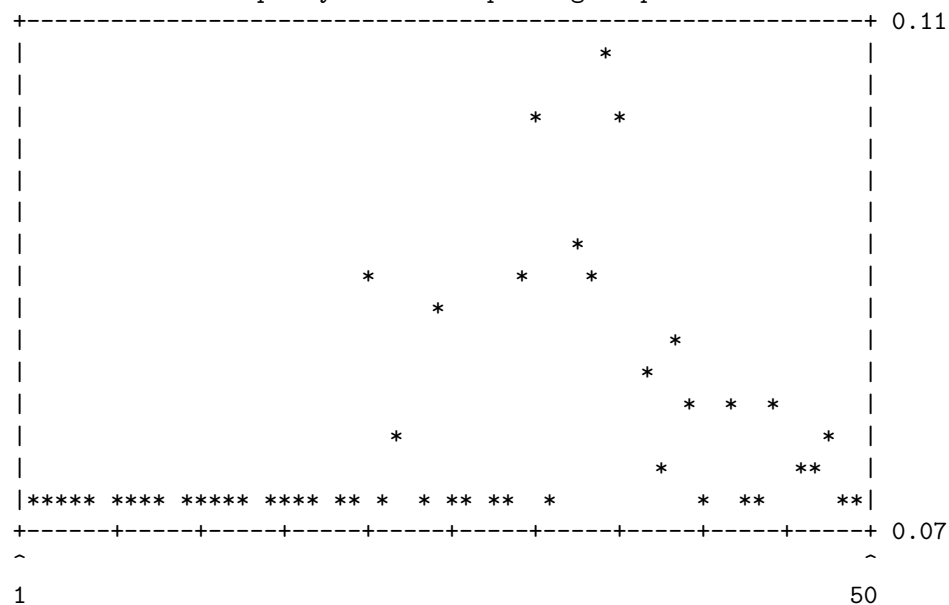
The model likelihoods are thus -5786.45 for the first model and -5798.19 for the

second (in natural log units). We first note that both likelihoods are considerably smaller than the corresponding values based on the harmonic mean estimator. This is an expected effect due to the reliance of the harmonic mean estimator on rare samples of very low likelihood. Short runs are unlikely to include such samples, resulting in the harmonic mean estimator usually being biased upwards in practice.

We also note that the more accurate model likelihoods suggest a larger difference between the two models, about 11 log likelihood units. Thus, we conclude that the better model (humans and chimps do form a monophyletic group) is very strongly supported by the Bayes factor.

Finally, let us examine one of the convergence diagnostics plots from one of our stepping-stone analyses. It will probably look something like this:

Plot of average standard deviation of split frequencies across steps.
Points at -1.0 (y-axis) indicates that there were no splits
above minimum frequency for corresponding step.



Here, we see the expected pattern of low average standard deviation of split frequencies in the early steps of the algorithm (to the left in the diagram), and difficulties of sampling across topology space when we move closer to the prior (to the right in the diagram). By increasing the length of each step, it should be possible to improve convergence also in the steps close to the prior, and therefore

increase the precision of the estimated model likelihoods. However, the similarity between the two independent estimates of the model likelihood we obtained in this analysis suggests that the accuracy is already reasonably good.

4.5 Testing the Strict Molecular Clock

In this tutorial, we will use a Bayes factor comparison to test the strict clock model against the non-clock model for an example data set, `primates.nex`. The data set is unusual in that a standard non-clock analysis suggests that the evolution of these sequences may be fairly well explained by a strict clock. The analysis is quite similar to the previous one, so you might find it useful to go through that tutorial first. The basic idea is that we want to compare the marginal likelihoods of the two models (non-clock and strict clock) against each other. To do that we need to estimate the marginal likelihoods either using the rough harmonic mean method or the more accurate stepping-stone method. We will use both in this tutorial and compare the results.

As usual, we first read in the data:

```
> execute primates.nex
```

Then we specify a GTR + I + Γ model:

```
MrBayes > lset nst=6 rates=invgamma
```

To estimate marginal likelihoods of the two models using harmonic means of the likelihoods of the MCMC samples, we simply run an ordinary MCMC analysis and obtain the appropriate values using the standard `sump` command.

Specifically, we first run the standard non-clock model for 100,000 generations and summarize the obtained samples using `sump`:

```
MrBayes > mcmc ngen=100000  
MrBayes > sump
```

In the `sump` output, we focus on the table summarizing the means of the likelihoods of the MCMC samples obtained after the burn-in:

Run	Arithmetic mean	Harmonic mean
1	-5720.67	-5735.68
2	-5719.97	-5730.58
TOTAL	-5720.26	-5734.99

The harmonic mean suggests that the marginal likelihood of the standard non-clock model is -5734 log units. Notice, however, some heterogeneity between the runs.

Now we repeat the procedure under the strict clock model. To enforce branch lengths to conform to a strict clock, we choose one of the clock priors for the branch lengths. Specifically, we are going to use the uniform prior on clock trees. The other commands are the same as those used previously:

```
> prset brlenspr=clock:uniform
> mcmc ngen=100000
> sump
```

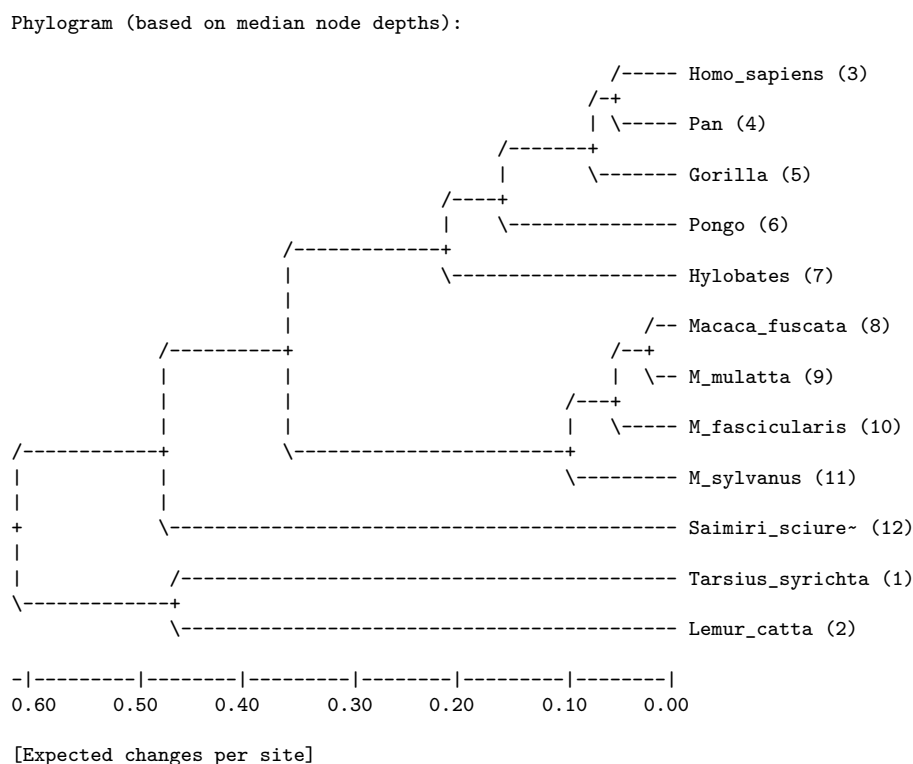
Again, we focus on the table summarizing the means of the likelihoods of the MCMC samples:

Run	Arithmetic mean	Harmonic mean
1	-5719.69	-5726.23
2	-5719.44	-5733.01
TOTAL	-5719.55	-5732.32

The harmonic mean is lower for the strict-clock mode than for the non-clock model, the log values being -5732 and -5734 , respectively. This is as we suspected, but the difference is small and there is overlap between the estimates obtained in individual runs. In this case, we undoubtedly need the increased accuracy of the stepping-stone sampling method.

Before moving on to the stepping-stone analysis, first compute a summary of the tree samples using the **sumt** command. Now focus on the phylogram, the second

tree, in the output from this command. It should look something like this:



We briefly want to point out two things. First, all tips in the tree have the same distance to the root in the consensus phylogram, the summary tree that is based on branch lengths. This is because the default assumption in a strict-clock model is that the tips are of the same age. This assumption can be altered as shown below in the tutorial on dating. Second, the clock tree is rooted, unlike a tree resulting from a non-clock analysis. An unrooted tree is drawn by convention such that it is rooted on the outgroup taxon. This means that it will have a basal trichotomy, or a basal polytomy of higher degree if outgroup relationships are unresolved. The specified outgroup taxon (by default the first taxon in the matrix) will be the first member lineage of the basal trichotomy. A strict-clock analysis, however, produces a rooted tree, which should have a dichotomy at the root unless there is uncertainty concerning the resolution near the base of the tree. Such a tree does not have to be artificially rooted for drawing purposes using an outgroup. Thus, *the outgroup setting is irrelevant in analyses of clock tree models*. The outgroup setting is only used when drawing unrooted trees.

Now let us continue with a more accurate assessment of the marginal model likelihoods using the stepping-stone method. It estimates the model likelihood by sampling a series of distributions that represent different mixtures of the posterior distribution and the prior distribution (Xie et al., 2011). The stepping-stone algorithm is invoked with the `ss` command, and it uses the same computational machinery as the `mcmc` command for each of the steps. Let us run the algorithm for 50 steps (the default) of 5,000 generations each, which makes a total of 250,000 generations. We will adjust the default frequency of calculating convergence diagnostics to once every 2,500 generations to get two reports from each step in the algorithm. By default, the algorithm uses an initial burn-in corresponding to one step, which corresponds to setting the `burninss` option to `-1`. The total length of the analysis will therefore be 255,000 generations.

As before, the run needs to be repeated for each of the two models. The following set of commands will run stepping-stone sampling on the non-clock model. Note that we first reset the prior on branch lengths to the default, an independent exponential prior with the rate parameter 10.0. An alternative way of resetting the model to the default is to execute the data set again. If you choose that alternative, do not forget to specify the GTR + I + Γ model before running the stepping-stone method.

```
> prset brlenspr=unconstrained:exp(10.0)
> ss ngen=255000 diagnfreq=2500
```

The estimated marginal likelihoods are found in a table printed by the `ss` command after the analysis is completed. Here are the estimates from our run:

Run	Marginal likelihood (ln)
1	-5791.38
2	-5792.04
Mean:	-5791.66

We now run the same analysis on the strict clock model:

```
> prset brlenspr=clock:uniform
> ss ngen=255000 diagnfreq=2500
```


We got the following estimates of the marginal model likelihoods:

Run	Marginal likelihood (ln)

1	-5774.94
2	-5774.75

Mean:	-5774.84

First we note that there is good agreement between the two independent analyses for both models, indicating that we now have accurate estimates of the marginal model likelihoods. If we compare these estimates with the harmonic mean estimates, it is striking that they are both much lower. Although the harmonic mean estimator is unbiased if you let the number of samples go to infinity, it relies heavily on rarely sampled states with low likelihood. Short runs tend not to include such samples, so the harmonic mean estimate is often considerably inflated in practice.

We also see that the strict-clock model is now almost 17 log likelihood units better than the non-clock model. A difference exceeding 5 log likelihood units is usually considered very strong evidence in favor of the better model (Kass and Raftery, 1995). If you are familiar with likelihood ratio tests, it may seem counter-intuitive that the strict clock model can be so strongly preferred. After all, the strict clock is just a special case of the non-clock model, so the likelihood *must* necessarily be higher, or at least as high, for the latter. How can the strict-clock model still be preferred in the Bayes factor test?

The answer has to do with the number of parameters. For a data set with n taxa, a non-clock model has roughly $2n$ independent branch length parameters, whereas a strict clock model only has around n independent node times. The other model parameters, the ones associated with the topology and the substitution model, are so few in comparison to the branch lengths or node times that they can be neglected. Thus, a strict clock model is much more parsimonious than a non-clock model in the number of parameters, and in Bayesian model choice, there is a built-in correction for the number of parameters.

One way of explaining the correction is by noting that comparing marginal

model likelihoods is the same thing as comparing the average likelihood across the joint prior distribution over all model parameters. By including more parameters, we are almost always going to increase the peak likelihoods, but whether the average likelihood increases is a complex function of the distribution of likelihood values with respect to the joint prior distribution. If the simpler model already captures values close to the peak likelihoods of the more complex model, then the latter is likely to have a lower average likelihood when the values are spread out across its larger parameter space. This is what happens here, resulting in a strong preference for the strict clock model.

For a brief discussion on convergence diagnostics and the plot printed by the `ss` command, see the previous tutorial.

4.6 Using a Relaxed Clock Model

In this analysis, we will set up a relaxed clock model for the same data set, `primates.nex`, used in the previous two tutorials. A relaxed clock model is intermediate between a strict clock model and a non-clock model. It has one or a few more parameters than a strict clock model, but much fewer than a non-clock model. Unlike a non-clock model, it produces a rooted tree, but the information about the position of the root is not as strong as in a non-clock analysis. Because the information about the position of the root might be weak, it is often beneficial to add a rooting constraint to a relaxed clock analysis. It can help a relaxed clock analysis correctly infer rate variation close to the base of the tree, variation that might otherwise cause rooting artifacts. It can also help speed up convergence, since it may be difficult for an MCMC analysis to mix adequately across multiple alternative root positions in a relaxed clock tree; this is going to be the case for this data set. To speed up convergence, and to show you how to set up a rooting constraint, we will choose the rooting option for this tutorial. Specifically, we will accept the common view that lemurs form the sister group of other primates.

As usual, we first read in the data:

```
> execute primates.nex
```

Then we specify a GTR + I + Γ model:

```
MrBayes > lset nst=6 rates=invgamma
```

To specify a rooting constraint, we first need to know the order of the taxa in the data matrix, or their names. We can do this by looking into the data file or by printing the matrix using the **showmatrix** command. In either case, we will discover that the only lemur in the matrix, *Lemur catta*, is taxon number 2. To specify a hard constraint that includes all taxa but taxon 2, we can use the following command:

```
MrBayes > constraint ingroup = 1 3-.
```

The constraint command first gives the name of the constraint, and then the constraint is specified after the equal sign using a list of taxa, where the taxa can be specified using either their index in the matrix (1, 2, etc) or their name. A period is synonymous with the last taxon in the matrix, and a range is indicated using a dash. By default, the constraint is assumed to be positive or hard, that is, we wish to enforce the specified split or clade to always be present in the sampled trees.

To enforce the constraint, we also need to set the topology prior using the following command:

```
MrBayes > prset topologypr=constraints(ingroup)
```

Inside the parenthesis, it is possible to give one or more previously defined constraints, separated by a comma. We are now ready to specify the relaxed clock model, which is done in two steps. First, we select an underlying strict clock model. Let us use the simple uniform clock model, which would be specified with the command:

```
MrBayes > prset brelenspr=clock:uniform
```

Other options include the coalescent and the birth – death models. In the next step, we select a model for the variation of the clock rate across lineages.

MrBayes offers three relaxed clock models: the Thorne–Kishino 2002 (TK02), the compound poisson process (CPP), and the independent gamma rates (IGR) models. Let us use the independent gamma rates model, which is a continuous

uncorrelated model of rate variation across lineages. We invoke this model using:

```
MrBayes > prset clockvarpr=igr
```

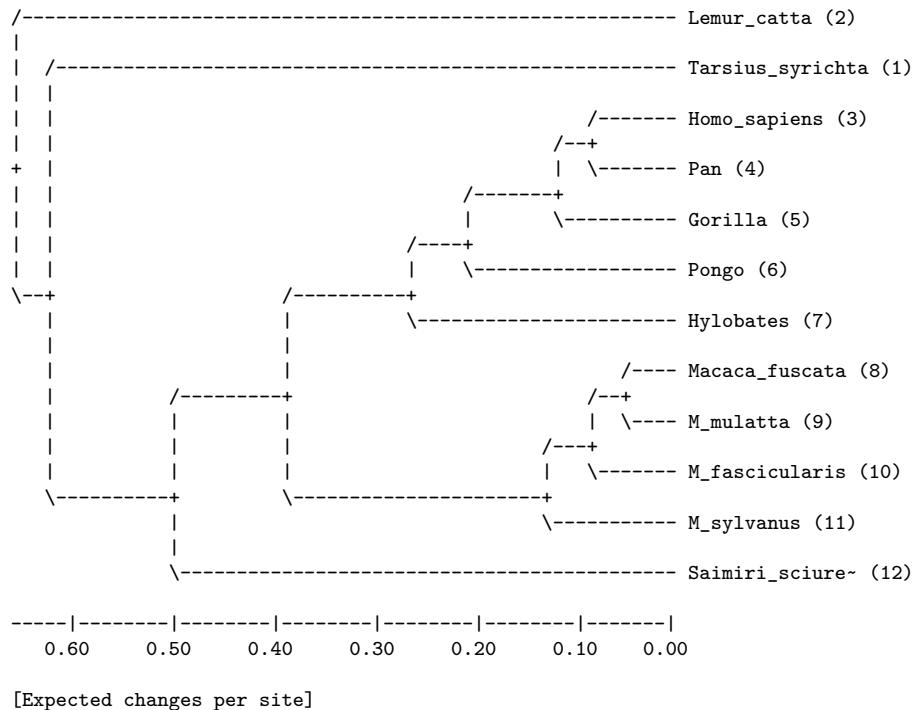
Each relaxed clock model has one or more additional parameters. In the case of the IGR model, it is the rate at which the variance of the effective branch length increases over time, **Igrvarpr**. Because the strict clock model fits this data set so well (see previous tutorial), we do not expect the variance to increase much over time. The default prior for this parameter, an exponential distribution with rate 10.0, should be more than generous enough for our analysis.

To check the model settings, it is wise to first run the **showmodel** command. Once you are convinced that the model settings are OK, run the analysis for 100,000 generation using:

```
MrBayes > mcmc ngen=100000
```

We can now verify using the **sumt** command that the tree is rooted correctly and that it looks reasonable. We focus on the phylogram, the second tree to be displayed:

Phylogram (based on median node depths):



Note that the strict clock tree that we obtained in the previous analysis was rooted differently, with *Lemur* and *Tarsius* together forming the sister group of the remaining primates. The reason that the strict clock analysis prefers to put the root there can be understood by going back to the non-clock tree (see chapter 2). By placing the root in this position, we minimize the difference between the non-clock and strict clock branch lengths.

If the rooting assumption we are using here is correct, then there must have been some changes in the evolutionary rate close to the root. Specifically, we should see a deceleration in the *Lemur* and *Tarsius* lineages, or an acceleration in their sister lineages. We can look for this pattern in the summary statistics for the branch and node parameters, output by the `sumt` command before the trees. The table is preceded by a key to the taxon bipartitions, which is critical to interpreting where the different parameters belong:

List of taxa in bipartitions:

```

1 -- Tarsius_syrichta
2 -- Lemur_catta
3 -- Homo_sapiens
4 -- Pan
5 -- Gorilla
6 -- Pongo
7 -- Hylobates
8 -- Macaca_fuscata
9 -- M_mulatta
10 -- M_fascicularis
11 -- M_sylvanus
12 -- Saimiri_sciureus
```

Key to taxon bipartitions (saved to file "primates.nex.parts"):

```

ID -- Partition
-----
0 -- *****
1 -- *.
2 -- .*
3 -- ..*
4 -- ...*
```

```

5 -- ....*.....
6 -- .....*.....
7 -- .....*.....
8 -- .....*.....
9 -- .....*....
10 -- .....*...
11 -- .....*.
12 -- .....*
13 -- ..**.....
14 -- *.*****
15 -- ..*****
16 -- .....**...
17 -- .....****.
18 -- ..*****.
19 -- ..***.....
20 -- ..****.....
21 -- ..*****.
22 -- .....***..
-----

```

Now let us find the ID of the partitions corresponding to the *Lemur* and *Tarsius* lineages, and their sister lineages. We first identify the taxon ID of *Lemur* and find that it is 2. Now we find the partition ID that has a single star in the second position; it is the bipartition that separates *Lemur catta* from all other taxa. Not surprisingly, the bipartition ID is 2. The sister lineage of *Lemur catta* includes all taxa except *Lemur catta*. To find the corresponding bipartition ID, we need to find the ID that has a partition descriptor with a single dot in the second position; all other positions should be filled with stars. This bipartition has ID 14. Similarly, we find that the bipartition IDs of *Tarsius syrichta* and its sister lineage are 1 and 15, respectively.

Now we examine the posterior distribution of IGR branch rates for these taxon bipartitions. If the clock is accelerated, the branch rate should be above 1.0; if the rate is decelerated, the branch rate should be below 1.0. This is the relevant rows of the table:

Parameter	Mean	Variance	95% HPD Interval		Median	PSRF+	Nruns
			Lower	Upper			
Igrbranchlens_rate[1]	0.916002	0.037357	0.545476	1.196117	0.947514	1.002	2

Igrbranchlens_rate[2]	0.688266	0.033214	0.345016	0.946393	0.703381	0.997	2
Igrbranchlens_rate[14]	0.776625	1.237010	0.000000	2.947193	0.424638	1.001	2
Igrbranchlens_rate[15]	2.635028	8.175572	0.634393	6.162516	1.809020	0.997	2

We see that there is considerable uncertainty about the branch rates but some of our predictions seem to be supported. In particular, the evolutionary rate is decelerated in the lineage leading to *Lemur catta*, with bipartition ID 2; the entire 95% HPD interval is below 1.0. Although the evidence is less compelling, there is also an indication that the rate is decelerated in the lineage leading to *Tarsius syrichta*, bipartition ID 1, and considerably accelerated in its sister lineage with bipartition ID 15. However, there is no evidence that the rate is accelerated in the sister lineage of *Lemur catta* with bipartition ID 14. On the contrary, both the mean and median indicate that this rate is more likely to be below than above 1.0.

To help you visualize parameters such as branch rates inferred by a relaxed clock model, the `sumt` command produces a `.con.tre` file that contains summaries of all branch and node parameter samples in a format that can be displayed by the program FigTree. This allows you, for instance, to color branches according to inferred evolutionary rates.

If you performed the Bayes factor comparison between the strict clock and non-clock models for this data set in the previous tutorial, you may wonder how the relaxed clock model stacks up. To make the comparison fair, let us compare the strict clock and relaxed clock models using the same rooting constraint that we enforced above. To get an accurate comparison, we run the stepping-stone sampling method. We refer to the previous two tutorials for details, but briefly give the commands here for the two stepping-stone analyses that are required for this comparison:

```
MrBayes > ss ngen=250000 diagnfreq=2500
MrBayes > prset clockvarpr=strict
MrBayes > ss
```

The results are summarized in the tables output by the `ss` command. The results we got are summarized below, with the relaxed clock model first, followed by the strict clock model:

```

Run   Marginal likelihood (ln)
-----
    1   -5785.05
    2   -5785.76
-----
Mean:  -5785.34

...

Run   Marginal likelihood (ln)
-----
    1   -5780.04
    2   -5781.53
-----
Mean:  -5780.53

```

Note that the models are close, but it is still the strict clock model that carries the day. The difference is almost five log units, which is considered strong evidence in favor of the better model Kass and Raftery (1995). Our runs indicate that the result is going to be similar if we were to repeat the comparison without enforcing a rooting constraint. We end by pointing out that this is a rather unusual data set; most other data sets will strongly favor relaxed over strict clock models.

4.7 Node Dating and Total-Evidence Dating

In this tutorial, we will use three different approaches to dating. First, we will show how to calibrate a tree based on information about the clock rate (rate dating), then we will date the tree based on node calibrations (node dating), and finally we will use total-evidence dating (or tip dating), in which the tree is dated based on simultaneous analysis of fossil and recent taxa. Rate dating and node dating will be demonstrated on a small data set, **primates.nex**, which has been analyzed in several previous tutorials, while a much larger data set, **hymfossil.nex** will be used to demonstrate total-evidence dating and node dating in a more typical setting.

We begin by reading in the smaller data set using the command

```
MrBayes > execute primates.nex
```


Next, we set the substitution model to GTR + I + Γ :

```
MrBayes > lset nst=6 rates=invgamma
```

We know from previous tutorials (see sections 4.5 and 4.6) that a strict clock model provides a good fit to the data set. Specifically, we choose a strict clock model with uniform branch lengths using the command:

```
MrBayes > prset brlenspr=clock:uniform
```

By default, the clock rate is going to be fixed to 1.0, which means that the age of the nodes in the tree will be measured in terms of the number of expected substitutions per site. If we want a dated tree, it is sufficient to change this assumption. For instance, let us assume that we know that the rate is approximately 0.01 ± 0.005 substitutions per site per million years. To use this assumption, we could for instance use a normal distribution as the prior for the clock rate, using 0.02 as the mean and 0.005 as the standard deviation. Since we give these values using millions of years as the unit, the resulting tree will be calibrated in millions of years.

Setting the prior on the clock rate is thus achieved with

```
MrBayes > prset clockratepr=normal(0.01,0.005)
```

There is one more thing to consider before we can run the analysis. By default, the clock rate is fixed to 1.0. This means that the tree age (or height, if you wish) measured in substitutions per site is the same as tree age measured in time units. As soon as these two measures are decoupled, it is important to consider whether the clock tree prior is defined in terms of substitution units or time units. MrBayes uses clock tree priors that are defined on parameters (speciation rate, extinction rate etc) measured in time rather than substitution units.

The uniform clock tree prior has only one prior parameter, tree age. The default prior is an exponential distribution with rate parameter 1.0, which usually works well as long as the clock rate is fixed to 1.0. When we change the clock prior, we usually need to reconsider this prior. For instance, we might want to modify the prior so that it has the same effect on branch lengths measured in substitution units. In our case, we want to change the default prior, with expected mean 1.0,

identify the bipartition IDs that we are interested in. We do that by referring to the bipartition table:

List of taxa in bipartitions:

```

1 -- Tarsius_syrichtha
2 -- Lemur_catta
3 -- Homo_sapiens
4 -- Pan
5 -- Gorilla
6 -- Pongo
7 -- Hylobates
8 -- Macaca_fuscata
9 -- M_mulatta
10 -- M_fascicularis
11 -- M_sylvanus
12 -- Saimiri_sciureus

```

Key to taxon bipartitions (saved to file "primates.nex.parts"):

```

ID -- Partition
-----
0 -- *****
1 -- *.....
2 -- .*.....
3 -- ..*.....
4 -- ...*.....
5 -- ....*.....
6 -- .....*.....
7 -- .....*.....
8 -- .....*.....
9 -- .....*...
10 -- .....*..
11 -- .....*.
12 -- .....*
13 -- ..***.....
14 -- .*****
15 -- .****.....
16 -- .....**...
17 -- .*****.
18 -- .****.....
19 -- .....****.
20 -- ..**.....
21 -- .....***..
22 -- **.....
-----

```

For instance, assume that we are interested in the age of the oldest split in the

tree, and the split between humans and chimps. The oldest split in the tree corresponds to the partition description with all stars, that is, partition ID 0. Humans (*Homo sapiens*) have taxon ID 3 and chimps (*Pan*) the taxon ID 4. The partition description corresponding to the human–chimp split thus should have stars in positions 3 and 4, and dots in all other positions. This is partition ID 20. In your analysis, the partition IDs may be different.

Now we go into the table of node and branch parameter estimates, and find the corresponding age estimates. Here are the relevant rows in this table in our analysis:

Parameter	Mean	Variance	95% HPD Interval		Median	PSRF+	Nruns
			Lower	Upper			
age[0]	59.590844	588.972831	26.805076	114.390416	52.435516	0.999	2
age[20]	5.361829	4.555993	2.300513	10.178956	4.955494	1.000	2

For instance, we see that the posterior distribution of the age of the human–chimp split has a mean of 5.4 Ma, a median of 5.0 Ma, and a 95% HPD ranging from 2.3 to 10.2 Ma. By default, the `sumt` command will print all the summaries of node and branch parameters to the tree in the file with the ending `.con.tre` in a format that can be understood by FigTree. When you open the file in FigTree, you will have access to a range of options for visualizing the parameter summaries of your interest.

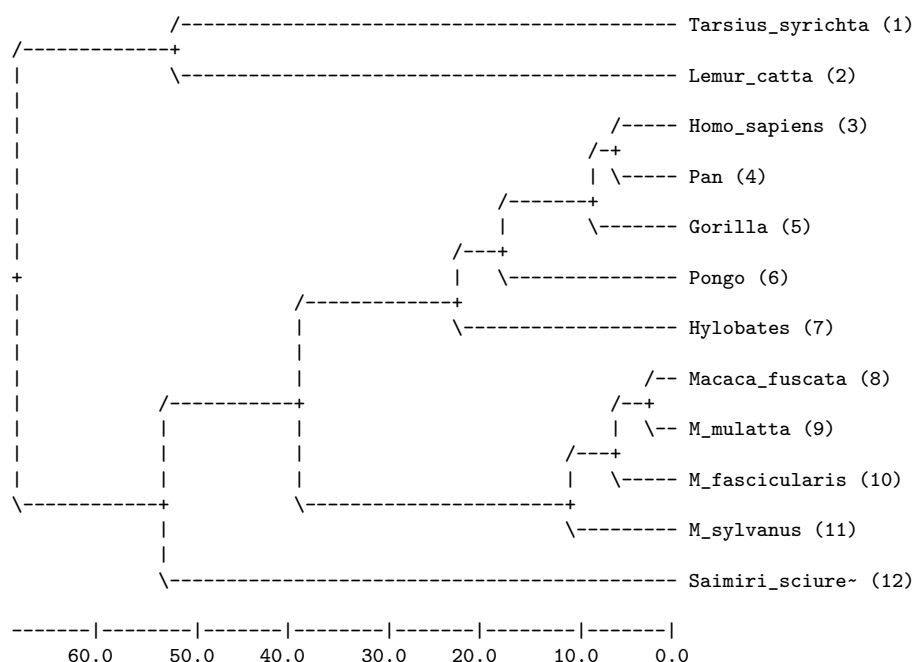
We now turn our attention to dating of the tree using node calibrations (node dating). Assume, for instance, that we have independent evidence suggesting that the human–chimp split occurred at least 5 Ma and not before 7 Ma. We might then choose to date the tree using a uniform prior from 5 to 7 Ma on the human–chimp split.

Dating the tree using a calibration node does not free us from setting appropriate priors on tree age and clock rate. We would like those priors to be set such that the main dating information comes from the calibrated node. Given how diffuse the divergence time estimates were under the priors chosen previously, it is clear that dating the human–chimp node as suggested will add information to the analysis. Thus, let us stick with the previous priors for tree age and clock rate,

and just add the human–chimp calibration. This is done in four steps. First, we define a constraint for the node of interest. Second, we associate the node with a calibration assumption. Third, we enforce the constrained nodes. Fourth, we enforce node ages to obey calibrations. Specifically, the MrBayes commands are:

```
MrBayes > constraint humanchimp = Homo_sapiens Pan
MrBayes > calibrate humanchimp=uniform(5,7)
MrBayes > prset topologypr=constraints(humanchimp)
MrBayes > prset nodeagepr=calibrated
```

After running the analysis and summarizing the tree samples, we can examine the resulting phylogram. It should look something like this:



If we look at the detailed summary of the age estimate of the human–chimp split, we now see that the distribution has a mean and a median of 6.0, and the HPD interval ranges from 5.0 to 6.9 (your results may be slightly different). Thus, the posterior is virtually the same as the prior for the calibration node, meaning that the tree age and clock rate priors have little influence on the dating of the tree. Also note that the tree is now estimated to be slightly older than previously, as one might have expected given that the previous median age estimate for the human–chimp split was around 5 Ma.

Regardless of how one approaches dating, it is important to keep in mind that the posterior distribution will always depend to a considerable extent on the prior. Therefore, it is important to consider the priors on the clock tree parameters (such as the tree age for the uniform prior), the node ages and the clock rate carefully. It is good practice to examine the influence of these priors on the dating results.

Now we turn our attention to a larger data set, and more complex dating scenarios. The data set, `hymfossil.nex`, is from a study of the early radiation of the Hymenoptera (Ronquist et al., 2012a), and includes 68 recent taxa and 45 fossil taxa. The data matrix comprises about 5 kb data from seven molecular markers for recent taxa, and about 350 morphological characters coded for both recent and fossil taxa.

At the end of the data file, in the MrBayes block, you will find two dating analyses specified. The first is based on the standard node dating approach, in which the fossil record is used to derive calibration points that can help date the tree of extant taxa. The second uses total-evidence dating, in which the fossils are analyzed together with the extant taxa. The advantage of the total-evidence approach is that it integrates out the uncertainty in the placement of the fossils in the tree, while simultaneously extracting the dating information they provide. Thus, it avoids the problem of translating the fossil record into node calibrations, and it does not suffer from the possible artifacts resulting from locking the clades corresponding to calibration nodes in the phylogenetic analysis.

Specifically, both analyses specified in the `hymfossil.nex` data file use two standard calibration nodes for the outgroups, one corresponding to the root of the tree and the other to the ancestor of Holometabola. In addition, the node dating analysis relies on seven hymenopteran calibration points derived from the 45 hymenopteran fossils. The fossils themselves, however, are not included in the analysis. In the total-evidence analysis, no node calibrations are used for the Hymenoptera. Instead, dating is based on simultaneous analysis of the fossil and recent taxa, taking the age of the fossils into account.

To run any of these two analyses, simply uncomment one of the two analysis blocks by removing the pair of square brackets enclosing it. Then you execute

the data file from MrBayes and use the standard `mcmc` command to run the analysis. We will not comment the results here but will simply walk you through the MrBayes block in the data file to help explain all the steps needed in setting up the node dating and total-evidence dating analyses.

The block starts out by defining a number of character sets. Then there is a set of lines that define the model for the morphological partition. It first defines a set of morphological characters to be ordered (`morphOrdered`), and then a set of morphological characters to be excluded (`morphExcluded`). Then these characters are ordered and excluded with the following two lines:

```
ctype ordered : morphOrdered;  
exclude morphExcluded;
```

The next set of lines define the partitioning of the data:

```
partition without_CO1_3 = 7: MV MS, 12S 16S, 18S, 28S,  
      CO1_12 CO1_3, Ef1aF1_12 Ef1aF2_12, Ef1aF1_3 Ef1aF2_3;  
exclude CO1_3;  
set partition=without_CO1_3;
```

Note that the morphological data (sets `MV` and `MS`) are lumped into one partition, as are the `12S` and `16S` sequences (because these are very short sequences). The protein-coding `CO1`, `Ef1aF1`, and `Ef1aF2` sequences are each divided into two character sets: one for the first and second codon positions and one for the third codon positions. Then the first and second codon positions of `Ef1aF1` and `Ef1aF2`, both nuclear protein-coding genes, are lumped into one partition, while the third codon positions of these sequences are modeled in a separate partition. Finally, only the the first and second codon positions of `CO1` are included in the analysis, in a separate partition; the third codon positions of `CO1` are excluded. MrBayes does not accept a partition definition that does not include all characters, so we need to include the third codon position sites of `CO1` in one partition, it does not matter which, before excluding them from the analysis. Here, they are included in the same partition as the first and second codon position sites of `CO1` before being excluded. Finally, we tell MrBayes that the partition we just defined is the one we want to use with the `set` command.

The next lines define the substitution models for the data partitions based on a priori model testing. You should already be familiar with the structure of these commands; if not, refer to the tutorial in chapter 3. The following block first defines an outgroup, to be used in drawing trees from unrooted non-clock analyses. This line is irrelevant for the dating analyses, since they produce rooted trees. The next line defines a set of taxa including the fossils, called **fossils**:

```
taxset fossils= Triassoxyela Asioxyela ...
```

This will be convenient when we want to exclude the fossils from the node dating analysis. Finally, all the constraints corresponding to the calibration nodes are defined:

```
constraint root = 1-.;
constraint hymenoptera = 10-69;
constraint holometabola = 3-69;
constraint holometabola_withFossils = 3-.;
...
```

Note that the file uses two versions of the holometabola constraint, one with the fossils for the total-evidence analysis and one without for the node dating analysis. Strictly speaking, this is not necessary because MrBayes will remove excluded taxa from the constraint before using it, so the second version of the constraint would have been adequate for both analyses.

We now come to the specification of the two dating analyses. The first is the total-evidence analysis, which starts out by specifying the date of all fossil taxa:

```
calibrate Triassoxyela=Fixed(235)
          Asioxyela=Fixed(235)
...
```

Here, we assume that the uncertainty concerning the age of each fossil is negligible compared to other sources of uncertainty, so we simply fix the age of each fossil instead of associating it with a prior probability distribution.

The next three lines set up an IGR relaxed clock model. You should recognize the commands from the tutorial in section 4.6.


```
prset brlenspr=clock:uniform;  
prset clockvarpr=igr;  
prset igrvarpr=exp(37.12);
```

An appropriate prior for the `igrvarpr` parameter, the variance increase parameter of the IGR model, was found by simulations. Specifically, strict-clock and non-clock branch lengths were inferred on the same topology, and then simulations under the IGR model were used to try to match the variation observed between them (Ronquist et al., 2012a). Stepping-stone sampling indicated that the IGR model matched these data better than the other two relaxed clock models provided by MrBayes.

The final five lines define a prior on the clock rate and sets the two outgroup calibration points using the same commands discussed previously in this tutorial:

```
prset clockratepr=lognorm(-7.08069,2.458582);  
calibrate root=offsetexp(315,0.01234568);  
calibrate holometabola_withFossils=offsetexp(302,0.0106383);  
prset topologypr=constraints(root,holometabola_withFossils);  
prset nodeagepr=calibrated;
```

Note that a tree age prior is not used here; instead, we define a root calibration point. MrBayes will use the root calibration as your tree age prior if you provide one; otherwise, it is the `treeagepr` setting that will be used to define the uniform prior on clock trees. The specific parameter values used in defining the priors on the calibration points and the clock rate are derived from the fossil record (Ronquist et al., 2012a).

This completes the definition of the total-evidence analysis. Let us now turn our attention to the node-dating analysis. It starts out by deleting the fossils from the analysis, using the previously defined taxon set called `fossils`:

```
delete fossils
```

It then continues by defining the relaxed clock model in the same way as the total-evidence analysis. This is followed by a specification of the clock rate prior and then the specification of the two outgroup and seven ingroup calibration points. The commands are essentially the same used in the total-evidence analysis. Note that all nine constraints have to be enforced when specifying the

topology prior:

```
prset topologypr=constraints(root,holometabola,hymenoptera,...,Vespina);
```

This completes the description of the analysis specifications. As you will notice, the file ends by setting up some `mcmc` parameters using the `mcmcp` command but it leaves it up to you to actually start the analysis.

4.8 Inferring Ancestral States

The purpose of the analysis discussed in this tutorial is to infer the ancestral state probabilities for a given node in a phylogeny, while simultaneously accommodating uncertainty in all other model parameters, including the topology of other parts of the tree. We will use the `cynmix.nex` dataset for the tutorial.

First, we read in the data using

```
MrBayes > execute cynmix.nex
```

This is the same data set we used for the partitioned analysis in chapter 3. It is a mixed data set consisting of morphological and molecular data. Let us assume we are interested in inferring the likely ancestral states of one of the morphological characters, say the first one in the matrix, for a particular ancestor in the tree, the ancestor of all gall wasps (family Cynipidae). The family includes taxa 1–29 of the 32 taxa included in the matrix.

To make sure we can infer the states of the morphological character of interest without inferring the states of all other morphological characters, we will treat it in a separate data partition. Otherwise, we will use the same partitioned model as in chapter 3. To set up the model, we need to type in the following lines:

```
MrBayes > partition ancstates = 6: 1, 2-166, COI, EF1a, LWRh, 28S
MrBayes > set partition=ancstates
MrBayes > lset applyto=(2) rates=gamma
MrBayes > lset applyto=(3,4,5,6) rates=invgamma nst=6
MrBayes > unlink statefreq=(all) revmat=(all) pinvar=(all) shape=(all)
MrBayes > prset ratepr=variable
```

Now we need to enforce a topological constraint corresponding to the family

Cynipidae, and then ask MrBayes to report ancestral states for that node and the relevant partition. We force the node corresponding to the Cynipidae to always be present in the sampled trees by first defining and then enforcing the constraint:

```
MrBayes > constraint cynipidae=1-29
MrBayes > prset topologypr=constraints(cynipidae)
```

To ask MrBayes to report ancestral states for the first partition, which contains only the first morphological character, we use:

```
MrBayes > report applyto=(1) ancstates=yes
```

We now run the analysis, say for 30,000 generations, which is barely enough to get adequate convergence. After the analysis is completed, we summarize the parameter samples, including those of the ancestral state probabilities, using the `sump` command:

```
MrBayes > mcmc ngen=30000
MrBayes > sump
```

The output from `sump` will include the state probabilities of the first character for the node corresponding to the most recent common ancestor of the Cynipidae. Here are the relevant lines in the parameter table from our analysis (they are found at the bottom of the table), based on a run of 100,000 generations:

Parameter	Mean	Variance		min ESS*	avg ESS	PSRF+
p(0){1@cynipidae}	0.582241	0.028763	...	28.07	30.99	1.004
p(1){1@cynipidae}	0.417759	0.028763	...	28.07	30.99	1.004

The first line gives the probability of state 0 for character 1 at the constrained node labeled “cynipidae”. The second line gives the probability of state 1 for the same character and the same node. The results indicate that the probability of the ancestral state being 0 at this node is only slightly higher than the probability of the state being 1.

4.9 The Multi-Species Coalescent

In this tutorial, we will set up an analysis using the multi-species coalescent. Unlike standard models, which are based on sequence concatenation, this model does not assume that gene trees are identical to the species tree. Instead, we model the evolution of individual gene trees within species trees using the coalescent model, extended to multiple species (Liu and Pearl, 2007; Edwards et al., 2007). The multi-species coalescent will take into account that gene trees and species trees may be different because of lineage sorting (deep coalescence). However, it does not model introgression or hybridization.

For this tutorial, we will use the `finch.nex` data set, which contains data from 30 loci for a set of four individuals belonging to four different species. As usual, we first read in the data:

```
MrBayes > execute finch.nex
```

The data file sets up a partition that divides the data into the 30 separate genes. The next step in specifying the multi-species coalescent is to define what species to which the different sequenced individuals belong. This is done using the `speciespartition` command. By default, it is assumed that each individual belongs to a separate species, which is the case we are dealing with, but let us nevertheless be explicit:

```
MrBayes > speciespartition species = SpeciesA: 1, SpeciesB: 2,  
                                SpeciesC: 3, SpeciesD: 4  
MrBayes > set speciespartition=species
```

The first line (broken here into two to fit the page, but you need to enter it as a single line in the console window) simply names each of the species and then lists the sequences that belong to that species. The list of sequences is a standard taxon list, as used by the `Taxset` command. This means that you can use either the index or the name of a sequence (“taxon”). Ranges are specified using a dash, and a period can be used as a synonym of the last sequence in the matrix. Here, each species that we name is represented by a single sequence, specified by its row index in the data matrix.

We now need to unlink the topology parameter of the 30 gene partitions, which is done using the `unlink` command:

```
MrBayes > unlink topology=(all)
```

To set the multi-species coalescent as the prior for each of the gene trees, we need to say both that the branch lengths should come from a coalescent process within a species tree and that the topology should be constrained by the species tree.

This is achieved using the lines:

```
MrBayes > prset topologypr=speciestree
MrBayes > prset brelenspr=clock:speciestree
```

Note that these settings need to go together to invoke the multi-species coalescent. Once the multi-species coalescent is invoked, the ordinary rate multiplier is split into a within-gene and an among-gene rate multiplier. The within-gene rate multiplier is referred to simply as the rate multiplier, while the among-gene rate multiplier is called the generate multiplier. Examine the model parameters by using the `showmodel` command to verify this.

We now turn our attention to the substitution model. Assume that we would like to use an HKY model with site rates modeled using a discrete gamma. This would be accomplished using the line:

```
MrBayes > lset nst=2 rates=gamma
```

Finally, we need to consider the prior on the population size. By default, it will be assumed that the population size is the same for all lineages in the tree. Let us change that to make the population size variable across lineages. For the prior on the population size, let us use a lognormal distribution with the mean on the log scale being equal to a population size of 100, and the standard deviation on the log scale being equal to 10. Since the natural log of 100 is about 4.6, and the natural log of 10 is about 2.3, this would be achieved using the lines:

```
MrBayes > prset popvarpr=variable
MrBayes > prset popsizepr=lognormal(4.6,2.3)
```

We can now sample from the model to estimate the posterior probability distribution. This is a large model with many parameters, so we need to run a

fair number of generations to get adequate convergence. To speed up the analysis, let us try to run it without heating (Metropolis coupling). We do that by setting the number of chains to one, `nchains=1`. Under these settings, our run seemed to produce an adequate sample within 1 M generations. To run an analysis of this length without Metropolis coupling, use:

```
MrBayes > mcmc ngen=1000000 nchains=1
```

By default, MrBayes runs two analysis in parallel, and computes convergence diagnostics on all gene trees as well as on the species tree. Ideally, we would like to see topological convergence on all trees. This is a sample output from our analysis:

```
Average standard deviation of split frequencies for topology 1: 0.007974
Average standard deviation of split frequencies for topology 2: 0.016684
Average standard deviation of split frequencies for topology 3: 0.015212
...
Average standard deviation of split frequencies for topology 31: 0.043427
```

In this list, the first 30 topologies correspond to the gene trees, and the last one to the species tree. To summarize the tree samples, simply use the **sumt** command, which will process all of the gene trees and then the species tree. Focus on the last tree, which is the species tree. It should look something like this:

```
Clade credibility values:

/----- SpeciesA (1)
|
|----- SpeciesB (2)
+
|----- SpeciesC (3)
|
\----- SpeciesD (4)

Phylogram (based on median node depths):

/----- SpeciesA (1)
|
|----- SpeciesB (2)
+
|----- SpeciesC (3)
|
\----- SpeciesD (4)

-----|-----|-----|-----|
          3.00e-04    2.00e-04    1.00e-04    0.00e-04
```

[Expected changes per site]

These summaries of the tree samples show that the species tree topology is unresolved. Note also that the species tree is very shallow, so the sequences are quite similar to each other. In the partition tables printed above the trees, it is possible to find the posterior probability for each of the individual clades found in the tree samples.

Chapter 5

Working with MrBayes

In this chapter, we cover the current capabilities of MrBayes, and provide some guidance on how you work most effectively with the program. We also discuss how to compile the program from source, and how to modify and extend it. Finally, we answer some frequently asked questions, and list the major differences between versions 3.1 and 3.2. The chapter ends with some pointers to additional information about the program.

The text is intended as a complement to the tutorials and the online information available by using the `help` command. We strongly recommend that you run through the relevant tutorials before looking for additional information in this chapter. Often, we will simply describe the relevant commands, so that you can obtain the appropriate online help information in the program itself. The online help information is always likely to be the most reliable, detailed, and up-to-date source of information on how to use MrBayes.

5.1 Reading in Data

The basic mechanism for bringing data into MrBayes is to execute a Nexus data file using the `execute` command. MrBayes supports much of the Nexus standard (Maddison, 1997) but not all of it. The most frequent problems in reading Nexus data files into MrBayes are caused by the lack of support for the `equale` macro

and the **symbols** list in the **format** command. These mechanisms are used to define non-standard symbols for use in a data matrix specification, but MrBayes does not support such non-standard symbols. Instead, MrBayes assumes a fixed set of standard symbols for the supported data types. These standard symbols include widely recognized ambiguity codes.

Specifically, the symbols understood by MrBayes are {A, C, G, T, R, Y, M, K, S, W, H, B, V, D, N} for DNA data, {A, C, G, U, R, Y, M, K, S, W, H, B, V, D, N} for RNA data, {A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y, V, X} for protein data, {0, 1} for restriction (binary) data, and {0, 1, 2, 3, 4, 5, 6, 5, 7, 8, 9} for standard (morphology) data. In addition to these symbols, MrBayes supports the use of separate symbols for gaps, missing characters, and “match” characters; the latter correspond to characters that match the state in the first taxon in the matrix. The special symbols are defined using the **gap**, **missing**, and **matchchar** parameters of the **format** command. For instance, **missing=?** specifies that a questionmark is used to denote missing characters.

Typically, the restriction to standard symbols is not a problem. However, some programs that write data to Nexus files will use **equate** macros and **symbols** lists even when they are not needed. If this is the case, you can simply delete the **equate** and **symbols** part of your **format** statement. Otherwise, you will have to do some editing of your data matrix to translate the symbols to the standard symbols used by MrBayes.

MrBayes supports all mechanisms defined in the Nexus standard for specifying partial ambiguities or polymorphisms. The states involved in the ambiguity can be specified with or without a separating comma, and they can be bracketed by curly braces or parentheses. For instance, using (A,G) or {AG} for a nucleotide character would both signify an ambiguity as to whether the correct state is A or G. This would be equivalent to using the standard ambiguity code R. Like other statistical phylogenetics programs, MrBayes treats partial ambiguity and polymorphism the same. All the character states in the specified set are assigned a conditional likelihood of 1.0; states that are not in the set have 0.0 likelihood.

Since version 3.2, MrBayes supports Nexus files that divide up the character matrix into a **taxa** block and a **characters** block instead of using a single **data**

block. If you have formatting errors in your matrix, MrBayes should report the line and position of the error, which should help you find the problem. After you have successfully read a data matrix into MrBayes, you can examine it using the `showmatrix` command.

5.2 Reading in Trees

Unlike previous versions of MrBayes, version 3.2 uses standard Nexus tree specifications to read in user-defined trees. Here is an example of a simple Nexus file containing a tree block with a single tree in it:

```
#NEXUS

begin trees;
  tree mytree = [&R] ((SpeciesA:0.15,SpeciesB:0.20):0.10,SpeciesC:0.25);
end;
```

Typically, a Nexus `trees` block will start with a `translate` statement, which allows taxon names to be replaced by indices in the tree specifications. If a `translate` statement is not present, the taxon names need to be spelled out in each tree statement.

Note that the Nexus tree statement includes a name for each tree; in this case, the tree is named `mytree`. It is this name that is used in MrBayes to refer to the tree and its branch lengths. The command `showusertrees` will print to screen a simple representation of the user-defined trees that are currently held in memory. If two user-defined trees have the same name, the second tree, when read in, will replace the first. All user-defined trees will be cleared from memory when a new data matrix is read in.

The Nexus tree format allows you to specify both trees with branch lengths and topologies — trees without branch lengths. Both rooted and unrooted trees are handled, and the trees need not be fully resolved. Typically, a special comment is used to explicitly declare whether a tree is rooted or unrooted. The special comments start with a bracket followed immediately by an ampersand. For instance, the special comment `[&R]` is used to declare that a tree is rooted.

When MrBayes prints trees to file, it uses such special comments to specify branch and node parameters that belong to the tree, for instance, branch rate parameters in a relaxed clock model. If you are interested in manipulating these parameters, we suggest that you set up the model you are interested in and then run a short MCMC analysis to the first checkpoint. The correct specification of all tree parameters can then be found by examining the checkpoint file, which will include a `trees` block with all relevant parameter values specified.

5.3 Dealing with Models

In this section, we discuss how to set up a model in MrBayes, how to choose a model, and how to detect and solve problems associated with model selection. As we will discover, one can either choose a model before the analysis using a priori testing procedures, or use Bayesian model choice criteria to select among models analyzed by MrBayes. Under certain conditions, MrBayes can automatically sample across a large model space. We also discuss how to detect and address problems with the selected models, and share some of our experiences with models for various types of data.

5.3.1 Setting up a Model

Since version 3.2, MrBayes sets up a default model as soon as you have read in a data matrix. The default model will have one partition for each data type, and will apply a simple substitution model to each of them. You modify the model settings primarily by using the `lset` and `prset` commands. Appendix A contains graphical summaries of the available models for different data types, and the appropriate `lset` and `prset` parameters needed to invoke them. Chapter 6 gives more detail about each model and how you set it up in MrBayes.

In principle, the `lset` command is used to set the structure of the model, while the `prset` command is used to set the prior parameters. However, the distinction is somewhat arbitrary, so check the options of both commands if you have difficulties finding a model option where you expect it to be.

Several auxiliary commands are used to support the specification of models. The **constraints** command allows you to specify constraints on the topology. Importantly, it allows you to fix and name interior nodes in the tree. The **calibrate** command is used to associate tips or fixed interior nodes with prior probability distributions on age. The **pairs** command defines pairs (doublets) for models on RNA stem sequences.

To set up a partitioned model, you first define the partition using the **partition** and **set** commands, possibly using previously named character sets specified with the **charset** command. Once that has been accomplished, you use the **applyto** option of **lset** and **prset** to apply model settings to particular parts of the data set. To link or unlink parameters across partitions, you use the **link** and **unlink** commands. For more instructions on how to set up a partitioned model, see the tutorial in chapter 3 and the model specification in the **cynmix.nex** example file. More details are provided in section 6.9.

You often want to specify models using different subsets of the characters or the taxa in a data matrix. The easiest way of doing this is to first specify appropriate character and taxon sets using the **charset** and **taxaset** commands. Both commands use a similar syntax: the set is given a name, which is followed by a list of the included characters or taxa. The list can be based on character and taxon indices, or on taxon names in the case of **taxaset**. A range of taxa or characters is specified by giving the first member, a dash, and then the last member in the range. A dot (period) can be used to represent the last character or taxon. Finally, a backslash can be used to specify a step criterion after a range. For instance, **1-**.

3 would refer to every third character from character 1 to the end. This mechanism is commonly used to group sites in protein-coding sequences based on their position in the codon.

Character and taxon sets are usually best defined in a MrBayes block in the data file. Here we give some examples of character and taxon sets defined in such a block. In principle, the same commands could be issued from the command line, without the ending semicolon. For these examples, we assume that the gene sequence starts with the first character being a site in the first codon position. Comments are given in square brackets; they are ignored by MrBayes when the

data file is processed.

```
charset COI = 1-756; [Identify a gene using a range]
charset COI_2nd = 2-756; \ 3 [Identify second codon position sites]
taxaset fossils = 10-30; [Create set with taxon 10 through 30]
```

Once character and taxon sets have been defined, they can be used to identify subsets for inclusion in a particular model. Characters are excluded using the **exclude** command, and included using the **include** command. For taxa, the corresponding commands are **delete** and **restore**. Note that the **delete** command does not delete the taxa from memory, it only results in the taxa being excluded temporarily until the next **restore** command is issued.

For a simple example, assume that we have a data set consisting of a mix of fossil and recent taxa, and would like to run an analysis on only recent taxa. We can do this by defining a taxon set called **fossils** and then exclude it from the analysis by using the following pattern (given here as lines in a data file, with an ending semicolon):

```
taxaset fossils = 10-30;
delete fossils;
```

For a second example, consider a mix of three genes: the mitochondrial gene COI and the nuclear genes Ef1a and CAD. First, let us set up a partition by gene:

```
charset COI = 1-756;
charset Ef1a = 757-1785;
charset CAD = 1786-.;
partition genes = 3:COI,Ef1a,CAD;
set partition=genes;
```

Now assume we want to run an analysis with only the nuclear genes. Intuitively, one might expect that this could be accomplished by simply using **exclude COI**. However, MrBayes does not allow parts in a data partition to be empty, so this would clash with the partition called **genes**. We first need to set up an appropriate partition that will not have any empty parts after COI has been excluded. The following pattern is one possibility:

```
partition nuclear_genes = 2:Ef1a COI,CAD;
set partition=nuclear_genes;
exclude COI;
```

This will give the appropriate two-gene partition with COI excluded. We end by noting that it is often better to partition sites in protein-coding sequences based on the codon position they occupy rather than on the gene to which they belong.

5.3.2 Selecting a model for analysis

MrBayes implements a vast number of different models. How does one select an appropriate model for analysis? There is a number of sophisticated techniques that can help you with this decision, but to some degree it still relies on your familiarity with the problem and your background knowledge of evolutionary processes. In particular, it is the choice of partitioning scheme and the type of substitution model (nucleotide, doublet, amino acid or codon) that necessarily depend to a large extent on your input.

Let us first discuss the selection of substitution model type. For non-coding sequence data, the choice is straightforward, as a standard four-by-four nucleotide model is the only sensible option. Ribosomal sequences are more difficult. If it is possible to reliably separate out the stem regions, it would be advantageous to use a doublet model for those and a standard four-by-four model for the loops. The reason is that the doublet model captures the dependencies between the substitutions at the paired stem sites. If the stem sites are modeled with the standard four-by-four model, the changes in stem sites will be weighted too heavily and this might cause problems if there are homoplasies in these characters. Of course, a doublet model is computationally more complex than a four-by-four model, so if this means you cannot get convergence in a reasonable amount of time, you may still be better off with the four-by-four model. If it is difficult or impossible to separate out the stem sites, then a standard four-by-four nucleotide model is the only option. Alignment is often a significant issue when analyzing ribosomal sequences. There are Bayesian approaches that accommodate alignment uncertainty, but none of them is implemented currently in MrBayes.

For protein-coding sequences, there are numerous options. Is it best to analyze such sequences using a nucleotide, amino acid or codon model? In principle, a codon model could also capture evolutionary processes at both the amino acid

and nucleotide levels, making it difficult to beat. The codon models currently implemented in MrBayes should do a decent job at modeling nucleotide substitution processes, but amino acid evolution is only represented by a single rate, omega, the ratio of nonsynonymous to synonymous changes. While the most sophisticated codon models allow omega to vary across sites, different amino acid substitutions always have the same rate. This is unlike the amino acid models, which can potentially model each of the 190 pairs of amino acids as having a separate substitution rate. The amino acid models, however, completely ignore nucleotide substitution processes. None of the models implemented in MrBayes consider the dependency among sites caused by the constraints on the 3D structure of the resulting protein.

On top of the model adequacy considerations, there is also computational complexity. Amino acid models are roughly 25 times slower than nucleotide models, and codon models are roughly 10 times slower than amino-acid models. If you are able to use BEAGLE to compute likelihoods on the GPU, you may observe less severe performance penalties, but amino-acid and codon models are still likely to be a lot slower than nucleotide models. This slowdown may compromise your ability to obtain an adequate sample of the posterior probability distribution, offsetting any gains in model adequacy.

There is much to suggest that, currently, four-by-four nucleotide models typically give you the best bang for the buck. However, when analyzing more difficult phylogenetic problems, especially problems involving deep divergences, amino acid models may be the best choice. Codon models, as currently implemented in MrBayes, are chiefly recommended for those interested in inferring dN/dS ratios and in finding positively selected sites. However, future studies may well show that, for difficult phylogenetic problems, more complex codon models may offer an interesting alternative to amino acid or nucleotide models.

When it comes to partitioning schemes, a standard approach is to group molecular data by gene. However, in most cases, you will be better off partitioning the sites based on other criteria. For nucleotide models, for instance, we often partition by origin (nuclear or mitochondrial) and codon position rather than by gene. A good scheme might be to group first and second codon position sites in one part and third codon position sites in another. One of several reasons

for preferring such a scheme is that sites in a protein-coding sequence often show a distinct bimodal pattern of rate variation across sites, with third codon position sites being significantly faster than first and second codon position sites. Such a bimodal distribution can often not be modeled adequately with the gamma or invariable sites plus gamma models of rate variation across sites. In fact, we have seen several instances where this misfit causes long branch attraction phenomena.

With amino acid models, it seems plausible that dividing up the sites by the selective environment of the amino acid residue will be better than dividing up the sites simply by gene. For instance, hydrophilic sites in one group and hydrophobic sites in another. Unfortunately, such a partitioning scheme requires detailed knowledge of protein structure or careful analysis of the aligned character matrix. MrBayes currently does not implement models that automatically finds these partitions and models for you.

If you have decided on using a four-by-four nucleotide model, then a very popular approach for selecting a specific substitution model is to use so-called model testing, as implemented in jModelTest (Posada, 2008) or MrModelTest (Nylander, 2004). The basic idea is to find a rough estimate of the tree, and then use one of several information criteria to find the best rate matrix and model of rate variation across sites. Often, such a procedure will suggest not only a specific model but also a set of specific parameter estimates.

Especially in older maximum likelihood algorithms for phylogenetic inference, there was a big performance penalty for estimating parameter values, so it was often a good idea to initially fix parameters to the rough estimates produced by the model-selection procedure. However, fixing values is against the Bayesian philosophy of integrating out uncertainty where possible. Furthermore, in the Bayesian approach, the computational penalty associated with estimating parameters rather than fixing them prior to analysis is typically insignificant. For these reasons, one would generally advise against fixing parameter values in Bayesian inference. Parameter estimates from a priori model testing also tend to be quite imprecise, so if one insists on fixing parameter estimates, it would probably be better to use more accurate estimates.

Another potential problem that may arise is that jModelTest suggests a specific

rate matrix that is not implemented in MrBayes. However, the Bayesian approach is robust to slight overparameterization, so we suggest that you simply substitute the preferred model with the next more complex model available in MrBayes.

Since version 3.2, MrBayes can automatically sample across the entire general time-reversible (GTR) model space (see section 5.3.5 below), which basically renders a priori model testing superfluous. An additional advantage of this approach is that your results will be based on a weighted average of the best substitution models instead of being based exclusively on a single model. The only potential drawback is that you may experience problems with mixing over the substitution model space. In our limited experience, however, this has not been a problem. If it is, you will detect it easily by examining the **sump** output from the analysis sampling across the GTR model space, as described in the tutorial in section 4.3. A similar option of sampling across rate matrices is available for fixed amino-acid rate matrices, as described below.

If you are interested in fixing parameters in MrBayes to specific values, you can do that using **prset <parameter_prior_name>=fixed (<value or commaseparated values>)**. For instance, if you want to fix the shape parameter of the gamma model to 0.12, use **prset shapepr=fixed(0.05)**. The graphical model summaries in Appendix A give the relevant **prset** options for particular types of parameters.

In general, the default prior distributions used by MrBayes are so-called flat, uninformative, or vague distributions. This is an appropriate choice if you want your posterior distribution to primarily be based on the data at hand rather than on your background knowledge. However, uninformative priors demand more of the data than more informative alternatives. Simply put, you can use a more complex model if you have informative priors than if you are using vague priors. This will be reflected both in the likelihood of obtaining symptoms of over-parameterization (see the following section) and in the Bayesian model choice criteria. A more informative prior will reduce the problems of over-parameterization and will tilt Bayesian model choice in favor of more complex models. If you want to use more informative priors in your analyses, it is easy to change the default ones using the **prset** command.

Remember that the prior distributions are part of a Bayesian model. Thus, if you want to compare the results of MrBayes with those of other Bayesian programs, remember to check that the priors are the same.

In many cases, one ends up with a small set of candidate models that cannot be separated meaningfully based on a priori considerations. If so, then it usually makes sense to analyze all models using the full Bayesian MCMC machinery. The stepping-stone algorithm (see below) can then be used to select among them based on Bayes factors and Bayesian model choice criteria.

5.3.3 Over-parameterization

Even though Bayesian inference is robust to modest over-parameterization, you can definitely run into problems if you introduce parameters willy-nilly in your model. What types of models should be avoided and what are the symptoms of over-parameterization?

A common cause of over-parameterization is the thought-less application of too complex rate matrices or models of rate variation across sites to data sets with little variation across the tree. For instance, a group of sites that hardly vary over the tree is unlikely to give much information about the six relative substitution rates of the GTR model. Therefore, the posterior is likely to largely reflect the prior. Similarly, if roughly half of the sites in your sequence are invariant across taxa, then it may be difficult to fit a gamma distribution to the few remaining variable sites. Similarly, if you partition your data sets into many small parts there may be too little information to accurately infer the relative evolutionary rate of each part.

A common symptom of over-parameterization is that you obtain posterior distributions that seem extreme in some sense. For instance, trees may have excessively long branches, some substitution rates may be extremely large or small, or partition rate multipliers may be extremely small or large. These phenomena are caused by the vague default priors used in MrBayes. When the posterior is dominated by the vague default priors, it will include values that are typically more extreme than you expect. One way of understanding this is that

you really have much more background knowledge than represented by the vague default priors.

A phenomenon related to over-parameterization is “burn out”. The likelihood monitored during the MCMC sampling typically increases in the early phase of a run (burn in) and then stabilizes close to the peak values. However, with over-parameterized models, one sometimes finds that the likelihood first increases but then *decreases* significantly before stabilizing. The cause of this behavior is the default starting values used in MrBayes. They often tend to be closer to the maximum likelihood estimate (MLE) of the parameter than a random sample from the default vague prior. For instance, the starting value of all relative rate parameters is set to 1.0 by default.

In most cases, you expect the bulk of the probability mass in the posterior probability distribution to be in the region close to the MLE. However, if your model is over-parameterized, there may not be enough information to focus the posterior around the MLE. Then it is possible that there is another region in parameter space with only moderate height (lower likelihood values) but considerably larger probability mass than the MLE region. It is like comparing the mass of a tower to the mass of a huge office complex. Even if the tower is considerably higher, its mass is going to be only a fraction of the mass of the office complex. Under these conditions, the likelihood will initially climb towards the peak of the MLE region, since the sampling starts close to that region of parameter space. After a while, it will find the larger probability mass, and the likelihood will decrease.

A particularly common situation is that the MLE region, which includes trees with short branch lengths, has considerably lower probability mass than a much larger region of tree space at longer branch lengths. The reason is that a standard phylogenetic model, particularly a non-clock model, is entirely dominated by the many branch length parameters. For instance, a non-clock GTR+I+ Γ model for 100 taxa has 10 free substitution model parameters but roughly 200 branch length parameters. As a branch becomes longer, its exact value has little influence on likelihood and we can end up with the huge office complex situation.

Regardless of the cause, there are two ways of correcting an over-parameterized

model: either you decrease the model complexity by reducing the number of model parameters, or you incorporate more background information into your prior distributions.

5.3.4 Bayesian Model Choice

Once a small set of models have been chosen for analysis, they can be compared using Bayesian model choice criteria. This is typically done through the calculation of Bayes factors, which can be interpreted as indicators of the strength of the evidence in favor of the best of two models.

Bayes factor is given by

$$BF_{12} = \frac{Pr(Data|Hypothesis_1)}{Pr(Data|Hypothesis_2)}$$

where *Data* is the data thought to have been generated by any of the candidate hypotheses *Hypothesis_k* ($k = 1, 2$). The expression $Pr(Data|Hypothesis_k)$ is called the marginal likelihood. The Bayes factors values are typically interpreted according to recommendations presented by Kass and Raftery (Kass and Raftery, 1995).

Unlike a hierarchical likelihood ratio test, the models compared with Bayes factors need not be hierarchically nested. A Bayes factor is calculated simply as the ratio of the marginal likelihoods of the two models being compared. The logarithm of the Bayes factor is the difference in the logarithms of the marginal model likelihoods.

The marginal likelihood of a model is difficult to estimate accurately but a very rough estimate may be obtained from the harmonic mean of the likelihood values of the MCMC samples (Newton and Raftery, 1994). MrBayes calculates this estimator when you summarize your samples with the command `sump`. In the output from the `sump` command, you will find the following table (it might look a little different depending on how many simultaneous runs you have performed; this table is for two runs):

Estimated marginal likelihoods for runs sampled in files
 "replicase.nex.run1.p", "replicase.nex.run2.p", etc:
 (Use the harmonic mean for Bayes factor comparisons of models)

Run	Arithmetic mean	Harmonic mean
1	-5883.41	-5892.10
2	-5883.82	-5892.81
TOTAL	-5883.60	-5892.52

For instance, assume we want to compare a GTR model with an HKY model. Then simply run two separate analyses, one under each model, and estimate the logarithm of the marginal likelihoods for the two models (using only samples from the stationary phase of the runs). Then simply take the difference between the logarithms of the harmonic means and find the corresponding interpretation in the table of Kass and Raftery (1995) (to use this table, you actually have to calculate twice the difference in the logarithm of the model likelihoods). The same approach can be used to compare any pair of models you are interested in. For instance, one model might have a group constrained to be monophyletic while the other is unconstrained, or one model can have gamma-shaped rate variation while the other assumes equal rates across sites. As stated above, models need not be hierarchically nested. An interesting property of the Bayes factor comparisons is that it can favor either the more complex model or the simpler model, so they need not be corrected for the number of parameters in the models being compared. Additional discussion of Bayesian model testing, with several examples, is found in Nylander et al. (2004).

5.3.5 Model Jumping

Bayesian MCMC model jumping provides a convenient alternative to model selection prior to the analysis. In model jumping, the MCMC sampler explores different models and weights the results according to the posterior probability of each model. In MrBayes, the model-jumping approach is available for two sets of models. One is for all models where a four-by-four nucleotide model is a

component, i.e., doublet and codon models in addition to the ordinary nucleotide models. Rather than selecting a substitution model before the analysis, the user can now sample across all 203 possible time-reversible rate matrices according to their posterior probability (Huelsenbeck et al., 2004). This can be accomplished simply by using the option `mixed` for the `Nst` parameter in the `lset` command. For example:

```
MrBayes > lset applyto=(all) nst=mixed
```

Currently, the program only applies model-jumping among subclasses in the GTR matrix, so there is not possible to sample across, for example, a GTR model with or without any assumption of rate variation across sites (e.g. GTR vs. GTR+ Γ vs. GTR+I+ Γ).

The second scenario where the program can apply model jumping is is the estimation of fixed-rate amino-acid substitution models. In this situation, the Markov chain will sample over models according to its probability. Currently, ten different rate matrices will be sampled (see section 6.2.2). To achieve the integration over rate matrices, one need to use the `mixed` option for `Aamodelpr` parameter in the `prset` command. For example:

```
MrBayes > prset applyto=(all) aamodelpr=mixed
```

5.3.6 Tuning Proposals

Manual Tuning

If you are using automatic optimization of proposal tuning parameters (auto-tuning), and your runs are reasonably long so that MrBayes has sufficient time to find the best settings, you should not have to adjust proposal tuning parameters manually. Auto-tuning is turned on by default and will take care of this for you.

However, auto-tuning does not change the frequencies with which different proposals are tried. By default, MrBayes uses topology proposals quite frequently, while other parameters are changed more rarely. If it is easy to get topological convergence on your data set, you may want to decrease the

frequency of topology proposals to get an adequate sample of other model parameters more quickly.

There is also a number of proposal mechanisms that are turned off by default, in particular topology proposals. If you have difficulties getting topological convergence, it might sometimes help to select a different mix of topology moves than the one used by default. For instance, the random SPR move tends to do well on some data sets but it is switched off by default because, in general, it is less efficient than the default moves.

You can add and remove proposal mechanisms, or change the frequency with which they are used, by adjusting their relative proposal probabilities using the `propset` command. Use `showmoves allavailable=yes` first to see a list of all the available moves.

In general, however, if your chains are not converging then it may be more productive to add more heated chains, especially if you have access to a large computer cluster, than it is to try to fine-tune proposal tuning parameters or proposal frequencies.

5.3.7 Monitoring Convergence

One of the most powerful ways of assessing MCMC convergence, is to compare samples obtained from independent MCMC analyses. It is typically the tree samples that are most divergent in phylogenetic analyses, and we therefore introduced the average standard deviation of split frequencies (ASDSF; (Lakner et al., 2008)) in MrBayes to allow quantitative assessment of the similarity among such samples.

ASDSF is calculated by comparing split or clade frequencies across multiple independent MCMC runs that ideally should be started from different randomly chosen starting trees (Lakner et al., 2008). ASDSF should approach 0.0 as runs converge to the same distribution. The frequencies of rare splits or clades are difficult to estimate accurately and these groupings are usually of marginal interest. Therefore, it may be advantageous to exclude them from the diagnostic. MrBayes allows the user to set a cutoff frequency (using the `Minpartfreq` option

for the `sumt` command); all splits or clades occurring minimally at that frequency (default value 0.10) in at least one of the runs will be incorporated in the ASDSF.

To allow users to monitor MCMC progress, MrBayes can run several analyses in parallel (by ensuring that the `nruns` parameter for the `mcmc` command is > 1) and report the average (ASDSF) or maximum standard deviation of split frequencies at regular intervals. For example (command given on several lines for readability):

```
MrBayes > mcmcp nruns=2 mcmcdiagn=yes diagnfreq=5000
MrBayes > mcmcp diagnstat=avgstddev minpartfreq=0.10
MrBayes > mcmcp relburnin=yes burninfrac=0.25 savetrees=no
```

All options given for the `mcmcp` command above are the default settings for MrBayes 3.2 (effectively the same as typing `mcmc`), but we want to highlight the relevant parts. Setting `nruns=2` will ensure that we can compare independent runs (two in this case), each started from a random starting tree. By using `mcmcdiagn=yes`, MrBayes will print a MCMC-diagnostics file, ending in `.mcmc`, with the convergence diagnostics calculated every `diagnfreq` generation. Here we have chosen the standard deviation of split frequencies to be based on the average (`diagnstat=avgstddev`), but there is also a choice of using the maximum (`diagnstat=maxstddev`). The `minpartfreq=0.10` will discard any partitions with a frequency < 0.10 from being included in the calculation of the diagnostic statistic. The `savetrees` option becomes relevant if you are using a relative burnin for run-time convergence diagnostics (as given by `relburnin=yes burninfrac=0.25`). Tree samples need to be deleted from split frequency counters as the cut-off point for the burnin moves during the run. If `savetrees` is set to `no`, tree samples to be discarded are read back in from file. If `savetrees` is set to `yes`, the tree samples to be removed will be stored in the internal memory instead. This can use up a lot of memory in large analyses.

If one wishes to rely on the ASDSF as the crucial statistic for deciding when to stop a run, then MrBayes provides a convenient “automatic stopping rule”. For example (note that other default values are omitted):

```
MrBayes > mcmc stoprule=yes stopval=0.01
```

In the example above, we start a MCMC run and allow MrBayes to check the

ASDSF (every `diagnfreq` generation, see above), and if the ASDSF value is below 0.01, the run will automatically stop. In order for the stopping rule to work, more than one run needs to be started.

More detailed diagnostics can be obtained using the `sump` and `sumt` commands after a run has completed. They include ASDSF across runs for each of the sampled clades in addition to the potential scale reduction factor (PSRF; (Gelman and Rubin, 1992)) for branch lengths, node times, and substitution model parameters. PSRF compares the variance within and between runs and should approach 1.0 as runs converge. MrBayes 3.2 also reports the effective sample size (ESS; (Ripley, 1987)), the number of effectively independent draws from the posterior distribution, widely used for single-run convergence diagnostics.

5.3.8 Metropolis Coupling

MrBayes uses Metropolis coupling to improve the MCMC sampling of the target distribution. The `Swapfreq`, `Nswaps`, `Nchains`, and `Temp` settings together control the Metropolis coupling behavior. When `Nchains` is set to 1, no heating is used. When `Nchains` is set to a value n larger than 1, then $n - 1$ heated chains are used. By default, `Nchains` is set to 4, meaning that MrBayes will use 3 heated chains and one “cold” chain. In our experience, heating is essential for some data sets but it is not needed for others. Adding more than three heated chains may be helpful in analyzing large and difficult data sets. The time complexity of the analysis is directly proportional to the number of chains used, but the cold and heated chains can be distributed among processors in a cluster of computers and among cores in multicore processors using the MPI version of the program, greatly speeding up the calculations.

MrBayes uses an incremental heating scheme, in which chain i is heated by raising its posterior probability to the power $1/(1 + i\lambda)$, where λ is the heating coefficient controlled by the `Temp` parameter. Every `Swapfreq` generation, two chains are picked at random and an attempt is made to swap their states. For many analyses, the default settings should work nicely. If you are running many more than three heated chains, however, you may want to increase the number of

swaps (`Nswaps`) that are tried each time the chain stops for swapping. If the frequency of swapping between chains that are adjacent in temperature is low, you may want to decrease the `Temp` parameter.

As illustrated by several of the tutorials above, it is sometimes possible to get a reasonable sample from the posterior without using Metropolis coupling, which will save you a lot of computational effort. The risk is that you will have to start over if you have difficulties getting convergence. Turn off heating by setting the `mcmc` option `nchains` to 1 and switch it on by setting `nchains` to a value larger than 1.

If you do use heated chains, try to make sure that the acceptance rates of swaps between adjacent chains are in the approximate range of 10 to 70 %. The acceptance rates are printed to the `.mcmc` file and to screen at the end of the run. The latter output contains a table that looks like this (you find the critical values in a different format in the `.mcmc` file):

	1	2	3	4

1		0.53	0.26	0.12
2	3347		0.55	0.31
3	3295	3337		0.53
4	3332	3396	3293	

It is the values just above the diagonal, $\{0.53, 0.55, 0.53\}$ in this case, that you should focus on. If the acceptance rates are low, say below 10 %, you can try decreasing the temperature constant (`mcmc temp=<value>`); if the acceptance rates are high, say above 70 %, you can try increasing it. Acceptance rates outside the optimal range do not invalidate the results from your analysis, but they do suggest that you could make your analysis more efficient.

We often observe considerable improvements in convergence and mixing for difficult problems when you use Metropolis coupling. If your chains are not converging, it may worthwhile to explore the possibility of adding more heated chains, especially if you have access to a cluster running the MPI version of MrBayes. The MPI version scales almost linearly with the number of chains, which means that you can add chains without making the analysis slower, provided that the number of chains per core stays the same. For more details on

the MPI version, see the next section and chapter 5.

If you decide to experiment with an increased number of chains, you may wish to simultaneously decrease the heating coefficient, so that the temperature difference between the cold chain and the hottest chain stays the same. See chapter 5.3.8 for more details on the heating coefficient.

5.3.9 Improving Convergence

There are several things you can do to improve the efficiency of your analysis. The simplest is to just increase the length of the run. However, the computational cost of doing so may be prohibitive. A better way is then to try improving the mixing behavior of the chain. First, examine the acceptance rates of the proposal mechanisms used in your analysis (output at the end of the run). The Metropolis proposals used by MrBayes work best when their acceptance rate is neither too low nor too high. A rough guide is to try to get them within the range of 10 % to 70 %. Rates outside this range are not necessarily a big problem but they typically mean that the analysis is inefficient. If the rate is too high, you can make the proposal bolder by changing tuning parameters (see Appendix) using the props command. Be warned, however, that changing tuning parameters of proposals and proposal probabilities may destroy any hope of getting convergence. For instance, you need at least one move changing each parameter in your model.

The next step is to examine the heating parameters if you are using Metropolis-coupled MCMC. If acceptance rates for the swaps between adjacent chains (the values close to the diagonal in the swap statistics matrix) are low, then it might be a good idea to decrease the temperature to make the cold and heated chains more similar to each other so that they can change states more easily. If acceptance rates for the swaps is high, then you may try to increase the temperature in order to flatten space for heated chain facilitating for them escape from local minima. Finally you may consider to increase number of parallel chain to improve the efficiency of the Metropolis coupling.

A good way of improving convergence is to start the analysis from a good tree

instead of starting it from a randomly chosen tree. To do this, you first define a good tree, with or without branch lengths, and put it in the tree block of a Nexus file. Say you name the tree `usertree`. Then start the chains from this tree using `startvals tau=usertree` just before you issue your `mcmc` command. Note that changing the number of chains or runs after you have set the starting tree will cause the starting trees to be changed. Therefore, set the number of runs and chains with `mcmcp` before you set the starting tree and run your analysis with the `mcmc` command.

A disadvantage with starting the analysis from a good tree is that it is more difficult to detect problems with convergence using independent runs. A compromise is to start each chain from a slightly perturbed version of a good tree. MrBayes can introduce random perturbations of a starting tree; this is requested using `mcmcp nperts=<integer_value>`.

5.3.10 Checkpointing

If you are running a long analysis and want to extend it, or if there is a risk that a long analysis will be inadvertently interrupted by hardware failure or other factors that are out of your control, checkpointing will come handy. The option `checkpoint` for the `mcmc` command will create a check-pointing file containing all the current parameter values of all chains. This is done on a regular interval as set by the `checkfreq` option (units are MCMC generations). The check-point file is written in the current directory with file suffix `‘.ckp’`.

To start from an existing `‘.ckp’` file, you need to use the `append=yes` option when starting a new (continued) MCMC analysis using the `mcmc` command. Also make sure that the output file names used in the previous run are the same as those in the current run.

5.4 Stepping-stone Sampling

5.4.1 Getting started using stepping-stone sampling

Since version 3.2 MrBayes allows you to use stepping-stone sampling (Xie et al., 2011) to estimating the marginal likelihood of the currently specified model. Stepping-stone sampling (SS) is considerably more accurate than the harmonic mean of the likelihoods from a standard MCMC run on the model (calculated by the `Sump` command) and it was shown to be more efficient than thermodynamic integration (Xie et al., 2011).

It is very easy to use stepping-stone sampling to estimate marginal likelihood. All you should do is to set up your model as usual, but instead of issuing command `mcmc` you issue command `ss` with the same arguments. In other words you may literally substitute `mcmc` with `ss` in your dataset file or the command sequence fed to MrBayes and that would produce estimate of marginal likelihood of your model. The only thing that you would need to adjust in order to get accurate estimate is to increase number of generations. You would probably need to increase number of generation by a factor of 10 compared with your ordinary `mcmc`. In most of the cases it does not have to be more complicated than that. For example, if you want to estimate marginal likelihood of a model which is setup in file `primates.nex` then you may issue the commands:

```
MrBayes > exe primates.nex
MrBayes > ss ngen=1000000 samplefreq=1000
```

That will start MCMC-like run. Once completed a table containing estimate of marginal likelihood for each run is printed along with the mean value:

```
Marginal likelihood (in natural log units) estimated using stepping-stone sampling based on
50 steps with 196000 generations (196 samples) within each step.
```

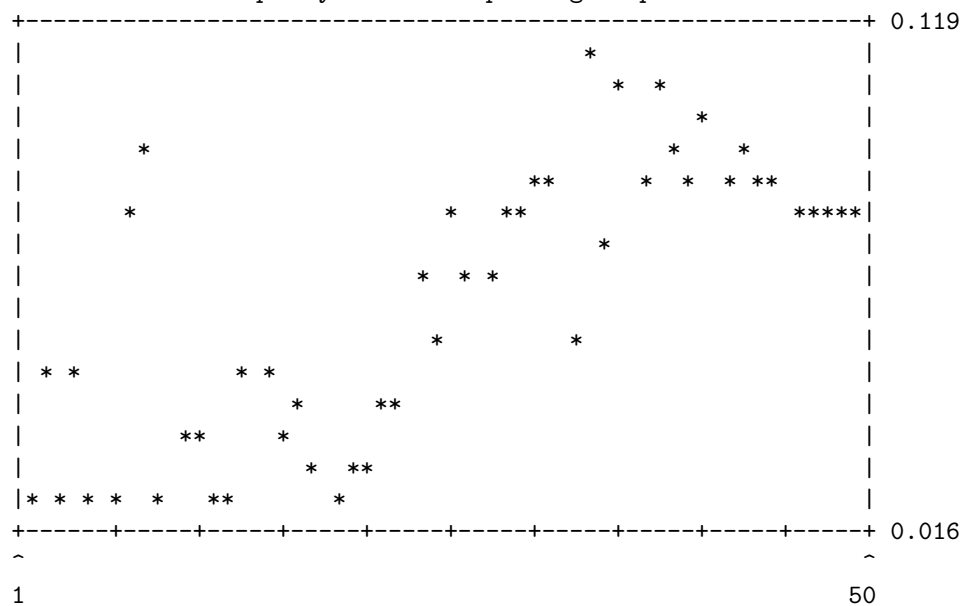
Run	Marginal likelihood (ln)
1	-6346.22
2	-6348.62
Mean:	-6346.82

Note that unlike with ordinary MCMC run you can not stop the SS-run and

process results for sampled generations or extend your run beyond originally specified length. Thus, it is extremely important to chose right length of the SS-run at the beginning, you will not be able to change it later on. That sad, it does not mean that if your SS-run did not finish it is complitly wasted. As we with any other MCMC run, if you enable checkpointing you can “resurrect” you run from the last checkpoint.

Now you know how to get the result, but how much we can trust the numbers? As usually you need to have at least two independent runs in order to get a feeling of result quality. The first thing to check would be to look at the difference of estimates among runs. Another thing that you may look at is the graph plotted below the table with marginal likelihood estimates:

Plot of average standard deviation of split frequencies across steps.
Points at -1.0 (y-axis) indicate that there were no splits
above minimum frequency for corresponding step.



The dots on this graph represent deviation of split frequencies among different runs as SS-run progresses. Ideally the values of deviation of split frequencies in the beginning of SS-run (x-axis around 1) should be as good as at the end of your ordinary MCMC analyzes on this dataset. It is quite normal that towards right hand side of the graph the values start to increase. If you have datasets with many taxons then towards the end of the run you may even get values

indicating that there were no splits above minimum frequency.

5.4.2 How stepping-stone sampling works

Stepping-stone sampling uses importance sampling to estimate each ratio in a series of discrete steps bridging the posterior and prior distributions. The importance distributions that are used are called power posterior distributions, and are defined as $Prior * Likelihood^\beta$. By varying β from 1 to 0, we get a series of distributions that connect the posterior ($\beta = 1$) to the prior ($\beta = 0$).

The power posterior distributions are sampled using MCMC. First, we start a standard MCMC chain on the posterior distribution, and let it run until we have reached the criterion specified by the `Burninss` option. After this, we step through the power posterior distributions until we reach the prior distribution. In each of the `Nsteps` steps, we sample from a new power posterior distribution with a distinct β value. The β values correspond to `Nsteps` evenly spaced quantiles in a `Beta(Alpha,1.0)` distribution. For the first sampling step, the β value is equal to the last quantile, i.e., it is close to 1.0. For each successive step, the β value takes on the value of the next quantile, in decreasing order, until it reaches the value of 0.0.

The `ss` procedure uses the same machinery as the standard `mcmc` algorithm, and shares most of its parameters with the `mcmc` and `mcmcpr` commands. All `mcmc` parameters, except those related to burnin, have the same meaning and usage in the `ss` command as they have in the `mcmc` command. However, the `mcmc` burnin parameters are ignored. Instead, the `ss` command uses its own burnin parameter, `Burninss`. The `ss` command also has its own parameters for specifying the number of steps and the shape of the Beta distribution from which the β values are computed.

Note that the `Ngen` parameter of `mcmc` is used to set the maximum number of generations processed, including both the burnin and the following steps in the stepping-stone sampling phase. For instance, assume that `Burninss` is set to `-1`, `Nsteps` to 49, `Ngen` to 1000000 and `Samplefreq` to 1000. We will then get 1,000 samples in total (1,000,000 / 1,000). These will fall into 50 bins, one of which

represents the burnin and is discarded. Each step in the algorithm will thus be represented by 20 samples.

The marginal likelihood estimated by SS is the multiplication of the ratios obtained from each step. Information about value of the ratio estimated at each step and run could be found in a file with .ss extension created during SS analyses. Since each ratio is stored in log units, the marginal likelihood in log units is obtained simply by summing of the log ratios across all steps. Examining the values of ratio estimates allows you to assess their quality and judge convergence of different runs for specific steps. If you observe huge differences among estimates for the same step then the runs did not converge for the step. If you observe poor convergence for later steps then in your next SS analyses you may consider reducing **Alpha** to increase skewness of the β values. If you have diagnostic turned the file contains information about standard deviation of split frequencies for each step. So, instead of looking at the graph with split frequencies plotted at the end of SS analyses, you may examine the .ss file to analyze precise values. If you have dataset with many taxa (> 20) you should not worry if standard deviation of split frequencies is poor or even can not be evaluated for later steps.

Ancestral State Reconstruction

MrBayes allows you to infer ancestral states at ancestral nodes using the full hierarchical Bayesian approach (integrating out uncertainty concerning topology and other model parameters). The basic approach is described by Huelsenbeck and Bollback (2001) as well as in a recent review (Ronquist, 2004). You first need to constrain the node you want to infer ancestral states for using a **constraint** definition and the **topologypr=constraints(...)** command as described above for constrained topology models. Then ancestral state reconstruction is requested using **report ancstates=yes**.

The probability of each state will be printed to the .p file(s) under the heading **p(<state_code>)character_number**. For instance, the probability of an A at site 215 in a nucleotide data set would be found under the heading **p(A){215}**. If you constrain several nodes in your data set, the node number will be given as

well. If you had constrained two nodes, the probabilities of the above character would be distinguished as $p(A)_{1}$ and $p(A)_{2}$. However, if you are interested in inferring ancestral states at two or more different nodes, we recommend running separate analyses, each constraining a single node. The reason is that when you focus on one node, you probably want to integrate over uncertainty in the rest of the tree, including the potential uncertainty concerning the presence of the other node(s).

Often, there is interest in mapping only one or a few characters onto trees inferred using largely other types of data. For instance, a behavioral or ecological trait may be mapped onto trees based on molecular data. To do this type of analysis in MrBayes, you would set up a mixed data set including both the character to be mapped and the data used to infer the phylogeny, with the character to be mapped in a separate data partition. How to do this is explained in the tutorial given in section 3 of this manual as well as in the description of partitioned models above. Typically, you also want to assume that the evolutionary rate for the mapped character is proportional to that of the other data (rather than identical). This is achieved by setting up a partitioned rate model using **prset ratepr=variable**. Then you need to set up a constraint for the node of interest, as described above. Finally, you request that ancestral states are inferred for the partition with the mapped character (there is no need to wade through ancestral state probabilities for the other partition(s)). For instance, if the character to be mapped is in partition 2, request ancestral state sampling using **prset applyto=(2) ancstates=yes**. Now only the ancestral states for the character of interest will be printed to the **.p** file(s). The sampled values can be summarized as usual with the **sump** command.

5.4.3 Convergence Diagnostics

The convergence diagnostics provided by MrBayes are quite powerful (see section 5.3.7) but they certainly do not exhaust the possibilities. Several programs will read MrBayes output files and will provide you with a number of additional ways in which you can examine the output from your analysis. Two of the most popular tools are Tracer (Rambaut et al., 2018) for the parameter (**.p**) files, and

AWTY (Nylander et al., 2007) for the tree (`.t`, `.con.tre`) files. Among other things, they provide nice graphical representations of the output from MCMC analyses. Another set of tools is provided by the CODA (Plummer et al., 2006) and RWTY (Warren et al., 2017) packages for R (R Core Team, 2018).

5.5 Working Effectively With MrBayes

5.5.1 Running MrBayes in Batch Mode

When you become more familiar with MrBayes, you will undoubtedly want to run it in batch mode instead of typing all commands at the prompt. This is done by adding a **MRBAYES** block to a Nexus file, either the same file containing the **DATA** block or a separate Nexus file. The **MRBAYES** block simply contains the commands as you would have given them from the command line, with the difference that each command line is ended with a semi-colon. For instance, a **MRBAYES** block that performs three single-run analyses of the data set in `primates.nex` under the GTR+ γ model and stores each result in a separate file is given below:

```
begin mrbayes;
  set autoclose=yes nowarn=yes;
  execute primates.nex;
  lset nst=6 rates=gamma;
  mcmc nruns=1 ngen=10000 samplefreq=10 file=primates.nex1;
  mcmc file=primates.nex2;
  mcmc file=primates.nex3;
end;
```

Since this file contains the `execute` command, it must be in a file separate from the `primates.nex` file. You start the analysis simply by typing **execute <filename>**, where filename is the name of the file containing the **MRBAYES** block. The `set` command is needed to change the behavior of MrBayes such that it is appropriate for batch mode. When `autoclose=yes`, MrBayes will finish the MCMC analysis without asking you whether you want to add more generations. When `nowarn=yes`, MrBayes will overwrite existing files without warning you, so make sure that your batch file does not inadvertently cause the deletion of

previous result files that should be saved for future reference.

The UNIX version of MrBayes can execute batch files in the background from the command prompt. Just type **mb <file> > log.txt &** at the UNIX prompt, where **<file>** is the name of your Nexus batch file, to have MrBayes run in the background, logging its output to the file **log.txt**. If you want MrBayes to process more than one file, just list the files one after the other with space between them, before the output redirection sign (**>**). When MrBayes is run in this way, it will quit automatically when it has processed all files; it will also terminate with an error signal if it encounters an error.

Alternatively, the UNIX version of MrBayes can also be run in batch mode using input redirection. For that you need a text file containing the commands exactly as you would have typed them from the command line. For instance, assume that your data set is in **primates.nex** and that you want to perform the same analyses specified above. Then type **mb < batch.txt > log.txt &** (note that both angle brackets are essential and must be included in the command line) with the **batch.txt** file containing this text:

```
set autoclose=yes nowarn=yes
execute primates.nex
lset nst=6 rates=gamma
mcmc ngen=10000 savebrlens=yes file=primates.nex1
mcmc file=primates.nex2
mcmc file=primates.nex3
quit
```

The **quit** command forces MrBayes to terminate. With previous versions of MrBayes we have had problems with infinite loops when the quit command is not included at the end of the file. This problem should have been solved since version 3.1.0.

5.5.2 Turbo-charging MrBayes with BEAGLE and MPI

Please note that the instructions in these sections are not up to date (Feb 2019). Please refer to the GitHub pages <https://github.com/NBISweden/MrBayes> for further details.

To speed up MrBayes analyses, you can try using the BEAGLE library or the MPI version of MrBayes. Let us start by examining the options provided by BEAGLE. This is a library that contains code for efficient likelihood calculations on both CPUs and GPUs. The CPU (central processing unit) is the core component of all computers, while a GPU (graphics processing unit) belongs on the video card that comes with most modern computers. The GPU specializes in performing small tasks on large sets of numbers very rapidly. Its power is typically used in processing video data for screen output, but recently it has become much easier to use GPUs for ordinary calculations. BEAGLE takes advantage of this for likelihood calculations.

By default, the Mac and Windows versions of MrBayes will come with the BEAGLE library installed. However, it is turned off, so you have to specifically change the settings to use BEAGLE. Regardless of your machine, you should be able to use BEAGLE on your CPU. However, it is the GPU code that will give you the dramatic speedups, and it currently requires that you have an NVIDIA video card and the appropriate CUDA drivers.

By default, MrBayes will use its built-in single-precision SSE likelihood calculators, which run on the CPU. They are quite fast on most machines, and should be similar in performance to the CPU routines in the BEAGLE library. The `set` command is used to switch to the BEAGLE likelihood calculators, and to set various BEAGLE-related options. Before looking into that, however, it may be a good idea to examine the available BEAGLE CPU and GPU resources. You can find out by using the following command:

```
MrBayes > showbeagle
```

If BEAGLE finds an available GPU resource, it will be listed here. This is the listing from a machine with an available GPU detected by BEAGLE:

```
Available resources reported by beagle library:
Resource 0:
Name: CPU
Flags: PROCESSOR_CPU PRECISION_DOUBLE PRECISION_SINGLE COMPUTATION_SYNC
      EIGEN_REAL EIGEN_COMPLEX SCALING_MANUAL SCALING_AUTO
      SCALING_ALWAYS SCALING_DYNAMIC SCALERS_RAW SCALERS_LOG
      VECTOR_NONE VECTOR_SSE THREADING_NONE
```

```

Resource 1:
Name: GeForce 9400M
Desc: Global memory (MB): 254 | Clock speed (Ghz): 1.10 | Number of cores: 16
Flags: PROCESSOR_GPU PRECISION_SINGLE COMPUTATION_SYNCH EIGEN_REAL
      EIGEN_COMPLEX SCALING_MANUAL SCALING_AUTO SCALING_ALWAYS
      SCALING_DYNAMIC SCALERS_RAW SCALERS_LOG VECTOR_NONE
      THREADING_NONE

```

It is the second entry that corresponds to the GPU. If you do not have one or more GPUs listed here, you will have to run the BEAGLE code on the processor.

By focusing on the table printed at the end of the output from the **help set** command, you can quickly find the current BEAGLE settings. Here is the table with only the relevant BEAGLE settings included:

Parameter	Options	Current Setting
Usebeagle	Yes/No	No
Beagledevice	CPU/GPU	CPU
Beagleprecision	Single/Double	Double
Beaglescaling	Always/Dynamic	Always
Beaglesse	Yes/No	No
Beagleopenmp	Yes/No	No

If you run BEAGLE on the CPU, then your best option (when these lines are written) is likely to be the double-precision SSE code with dynamic scaling. This would be requested with the following lines:

```

MrBayes > set usebeagle=yes beagledevice=cpu beagleprecision=double
MrBayes > set beaglescaling=dynamic beaglesse=yes

```

The default MrBayes likelihood calculator uses single-precision SSE code, which should theoretically be the fastest option on the CPU. Currently, BEAGLE only supports double-precision SSE code, which is slower. However, the code we use to call BEAGLE supports smart dynamic scaling, unlike the code calling the default calculator, which may well compensate for the slowdown caused by the increased precision. Therefore, you need to test both options before knowing which one will be faster on your machine.

To use the GPU, you simply switch from CPU to GPU (if you have an available GPU). With the GPU, the fastest option should be the single-precision code with

dynamic scaling. The SSE option is not applicable in the GPU case. The fastest GPU option should therefore be:

```
MrBayes > set usebeagle=yes beagledevice=gpu
MrBayes > set beagleprecision=single beaglescaling=dynamic
```

The GPU code can be a lot faster than the CPU code, particularly for amino acid and codon models. However, the length of the sequences also influences the speed-up. In general, the longer the sequences are, the better GPU performance you can expect. If the sequences are short, the overhead involved in shuffling data to and from the GPU may well overshadow any performance gain you get in the computation step. Try the various calculator options out in short runs before you decide on the best option for longer runs.

If you run multiple simultaneous runs or use Metropolis coupling, which is standard practice with MrBayes, then you can speed up your analyses considerably by using the MPI version of MrBayes. MPI stands for Message Passing Interface, which is a method for farming out computations to a set of parallel processors. To use MPI successfully, you need to have a machine with several processors or processor cores, and you need to be able to run an MPI implementation, such as Open MPI www.open-mpi.org. You can install Open MPI on virtually any machine running Unix or Linux, including all modern Macs. If you have a Windows machine, you can convert it to a dual-boot machine that will also run Linux in a separate partition, if you wish to run MPI on it. Computer clusters will almost always support MPI.

The MPI version of MrBayes is different from the serial version put on your machine by the standard Mac and Windows installers. Therefore, you need to compile the MPI version of MrBayes from source code. It is not difficult, if you are willing to run a few commands from a console window. Refer to chapter 5 for detailed instructions.

The MPI version of MrBayes farms out each chain to a different core or processor on your computer or computer cluster. To use MPI efficiently, you should make sure that the number of chains in your analysis is a multiple of the number of cores or processors on your system. For instance, assume you are running four runs with four chains each, giving a total of 16 chains. Compared to the serial

version of MrBayes, which uses just a single processor or core for this analysis, the MPI version can run the analysis almost twice as fast if it has access to two cores, almost four times as fast if it has access to four cores, and almost 16 times as fast if it has access to 16 cores. This is the maximum speedup; you will not be able to run the analysis on more cores than there are chains in the analysis.

The MPI version of MrBayes can use the BEAGLE library. However, modern computers almost always have more cores than GPUs. This means that BEAGLE may not help much in an MPI setting unless you are running on a specially designed cluster.

5.6 Working with the Source Code

Please note that the instructions in these sections are not up to date (Feb 2019). Please refer to the GitHub pages <https://github.com/NBISweden/MrBayes> for further details.

Compiling MrBayes from source code can be done with several different compilers targeting all the common operating systems: Macintosh, Windows, and Unix. The easiest way to build MrBayes on a Unix-like system is to use the GNU tool-chain. You can also compile MrBayes with Xcode (on a Macintosh) and the Microsoft Visual Studio suite (on a Windows machine).

Some performance gain can be achieved by using compiler optimization flags that are not set by default. Keep in mind, however, that not all such options are safe. A number of optimization methods might give a slowdown instead of a speedup, and some options relax numerical stringency, leading to a program that might produce incorrect results. If you encounter problems with MrBayes, please make sure they are not due to unusual compiler optimization settings before submitting a bug report.

5.6.1 Compiling and Running the MPI Version of MrBayes

Metropolis coupling or heating is well suited for parallelization. MrBayes 3 takes advantage of this and uses MPI to distribute heated and cold chains among available processors (Altekar et al., 2004). For instance, the default of two runs with four chains each makes for a total of eight chains, which the MPI version will farm out to different processors. The speedup is almost linear with the number of processors. Assume, for instance, that you want to run an analysis with four runs and four chains for a total of 16 chains. Compared to the serial version, this analysis will run almost twice as fast on two processors, almost four times as fast on four processors, and almost sixteen times as fast on sixteen processors. MrBayes will refuse to use more processors than the total number of chains, as there is no additional speedup beyond this point.

The MPI version will run both on clusters and on single machines with multicore CPUs, provided that the system supports MPI. The dominant current implementation of MPI is Open MPI, which runs on most Unix or Unix-like systems, including Macintoshes. For more information on Open MPI, see openmpi.org. Once you have Open MPI installed, compiling and running the parallel version of MrBayes will essentially be the same as if you were running on a computer cluster, with each core behaving as a separate processor.

Before running the MPI version of MrBayes, you will have to compile it on a system with an mpi compiler in addition to `autoconf` and `make`. Find out whether your system has an mpi compiler by typing `which mpicc`. If the system responds by printing a path, you have an MPI compiler, otherwise you do not. In addition, your system needs to have the relevant libraries in the correct locations, which is typically the case if you have an MPI compiler installed.

Once you have ensured that the system environment supports MPI compilation, you need to run the configure script with the `-enable-mpi=yes` option. Otherwise, the compilation process is quite similar to the one used to produce the serial version. A typical make session would look as follows:

```
~/mrbayes-3.2 $ ./configure --enable-mpi=yes
...
```

```

checking for mpicc... yes
...
~/mrbayes-3.2 $ make
mpicc -O3 -ffast-math -Wall -DNDEBUG -DUSECONFIG_H -c -o bayes.o bayes.c
mpicc -O3 -ffast-math -Wall -DNDEBUG -DUSECONFIG_H -c -o command.o command.c
mpicc -O3 -ffast-math -Wall -DNDEBUG -DUSECONFIG_H -c -o mbmath.o mbmath.c
mpicc -O3 -ffast-math -Wall -DNDEBUG -DUSECONFIG_H -c -o mcmc.o mcmc.c
mpicc -O3 -ffast-math -Wall -DNDEBUG -DUSECONFIG_H -c -o model.o model.c
mpicc -O3 -ffast-math -Wall -DNDEBUG -DUSECONFIG_H -c -o plot.o plot.c
mpicc -O3 -ffast-math -Wall -DNDEBUG -DUSECONFIG_H -c -o sump.o sump.c
mpicc -O3 -ffast-math -Wall -DNDEBUG -DUSECONFIG_H -c -o sumt.o sumt.c
mpicc -O3 -ffast-math -Wall -DNDEBUG -DUSECONFIG_H -c -o tree.o tree.c
mpicc -O3 -ffast-math -Wall -DNDEBUG -DUSECONFIG_H -c -o utils.o utils.c
mpicc -O3 -ffast-math -Wall -DNDEBUG -DUSECONFIG_H -c -o mbbeagle.o mbbeagle.c
mpicc -O3 -ffast-math -Wall -DNDEBUG -DUSECONFIG_H -c -o best.o best.c
mpicc -O3 -ffast-math -Wall -DNDEBUG -DUSECONFIG_H -lreadline -lm -o mb bayes.o
command.o mbmath.o mcmc.o model.o plot.o sump.o sumt.o tree.o utils.o mbbeagle.o
best.o -o mb

```

This produces an MPI-enabled version of MrBayes called **mb**. Make sure that the mpicc compiler is invoked instead of a normal C compiler; this is indicated by each of the compile lines starting with mpicc. It is perfectly normal if the build process takes some time; optimization of the source code is computationally demanding, especially for the largest source files such as mcmc.c.

Note that the default name of the executable is the same as for the serial version, **mb**. This means that the parallel version will overwrite any previously existing serial version kept in the same directory. If you would like to have both the serial and parallel versions installed on the same system, you probably want to name the differently and put both in your path. We often rename the parallel executable **pmb** to separate it from the serial version.

Although the MPI version is a lot faster than the serial version, it has one drawback: it does not support the readline library. This means that you do not have access to your command history, or command completion with the tab key. Therefore, we often prefer to run short experimental analyses and interactive post-hoc analyses using the serial version, even though we typically use the MPI version for most MCMC analyses.

How you run the MPI version depends on your system. Some implementations of MPI require you to invoke a daemon or virtual machine to set up the MPI execution environment. Once the MPI environment is ready, a parallel MrBayes job is typically started with a line such as

```
$ mpirun -np 4 mb batch.nex > log.txt &
```

This line calls the MPI version of MrBayes (just called **mb** here) and asks it to run the file **batch.nex** using four processors (**-np 4**), saving the screen output to the file **log.txt**. Specifically, the angle bracket is the output redirection flag, and the ampersand at the end ensures that the job is posted quietly so that you get the prompt back immediately. If you keep both a serial and a parallel version of MrBayes on your system, make sure you are using the parallel version with your **mpirun** command. When executing the command, you should get a process ID number back. It is handy to keep this number for future reference. For instance, you might want to kill your job if you find you made a mistake when starting it. You can do this using **kill -9 <PID>**, where **<PID>** is the process ID. Just make sure you kill the right process or some really bad things can happen.

To examine how the analysis is doing, you can simply look at the last few lines in the screen dump file. Say that you dump the screen output to a file called **log.txt**. We can look at the end of that file by using **tail log.txt**. If you would like the output to be dynamically updated, you can use **tail -f log.txt** instead, in which case each new line printed to the file **log.txt** will be echoed to screen. To stop the display of new lines, press Control-C (this will only stop the echoing of new lines, it will not affect the analysis). Another way of examining how the analysis is doing is to look into the output files from the analysis, such as the **.mcmc** file.

We strongly recommend that you use checkpointing with the MPI version of MrBayes. This allows you to restart the analysis from the last checkpoint, should your analysis crash or be terminated prematurely. By default, MrBayes outputs a checkpoint file every 100,000 generations. See section 5.3.10 for more details on checkpointing and how to restart your analysis from a checkpoint file.

There are two typical cases when parallel runs fail due to different MPI implementations getting mixed:

1. You install one MPI implementation on your system and after that you install another. In such cases, it could happen that, in your path, the compiler command **mpicc** and the command that runs MPI jobs, **mpirun**, refer to different MPI implementations. You may use **which mpicc** and

which mpirun to check which exact files are being called by these commands. Often **mpicc** and **mpirun** are symbolic links, so you have to follow them to see the actual files that get called. If the commands refer to different implementations, you will have to change one of them so that both refer to the same implementation.

2. Typically on a cluster you will have a variety of MPI implementations that could be loaded to your environment using the **module add** command. Check that the same module is added both during compilation and before the analysis. If they are different, your analyses will fail.

5.6.2 Compiling the Development Version of MrBayes

If you want to use recently added features that have not made it to an official release yet, you may want to compile the most recent development version of MrBayes. First a word of warning, however. The development version may not be as stable as the latest official release, so use it at your own risk.

If you decide you want to go ahead, the first step is to download (clone) the code repository of MrBayes from GitHub. On a Unix system with **git** installed, this is accomplished using the command line:

```
$ git clone --depth=1 https://github.com/NBISweden/MrBayes.git
```

In the copy of the MrBayes repository on the local system, you will find the compile instructions in the file **INSTALL**.

5.6.3 Modifying or Extending MrBayes

If you are interested in investigating or working with the source code, you first need to download the latest development version from the MrBayes repository on GitHub <https://github.com/NBISweden/MrBayes>. The download process was briefly described above in section 5.6.2.

If you are interested in contributing code with bug fixes, the best way is to create a “git pull request” using the functionality on GitHub. See

<https://help.github.com/articles/about-pull-requests/> for more information on pull requests.

5.7 Frequently Asked Questions

How are gaps and missing characters treated?

MrBayes uses the same method as most maximum likelihood programs: it treats gaps and missing characters as missing data. Thus, gaps and missing characters will not contribute any phylogenetic information. There is no way in which you can treat gaps as a fifth state in MrBayes (but see below for information on how you can use gap information in your analysis).

How do I use gap information in my analysis?

Often, insertion and deletion events contain phylogenetically useful information. Although MrBayes 3 is not able to do statistical multiple sequence alignment, treating the insertion-deletion process under a realistic stochastic model, there is nevertheless a way of using some of the information in the indel events in your MrBayes analysis: Code the indel events as binary characters (presence/absence of the gap) and include them as a separate binary (restriction) data partition in your analysis. See more information on this possibility in the section on the binary model in this manual.

How many data partitions can I have in MrBayes?

Since version 3.2, MrBayes does not limit the maximum number of partitions. You can use as many partitions as you can fit in the available memory. However, it may be difficult to reach convergence if your model includes an excessive number of parameters.

Does MrBayes run faster on a dual-processor or dual-core machine?

Yes, if you are using MPI versions of MrBayes or along with BEAGLE library you enable multithreading while compiling MrBayes. Note however, that you should not enable multithreading with beagle if you are using GPU card for

computation. When you enable multithreading make sure that you actually gain any performance before starting long analyses.

The Windows and Mac serial versions of MrBayes 3.2 distributed with installer packages are not multithreaded. So they will not take advantage of more than one processor on a single machine. However, you should be able to run two copies of MrBayes without noticeable decrease in performance on a dual-processor machine (provided you have enough RAM for both analyses).

If you run long and demanding analyses on a Mac, Unix, or Linux machine, you should consider using the MPI version of MrBayes. For instructions on compiling and running the MPI version, see section 5.6.1.

How much memory is required?

You can calculate the amount of memory needed to store the conditional likelihoods for an analysis roughly as $2 * (\# \text{ taxa}) * (\# \text{ chains in total} + 1) * (\# \text{ states in the Q matrix}) * (\# \text{ gamma categories}) * 4 \text{ bytes}$ (for the single-precision float version of the code; double the memory requirement for the double-precision code). Here ($\# \text{ chains in total}$) is the number of chains allocated for a given MrBayes process. In case of serial version of MrBayes all ($\# \text{ chains per run}$) * ($\# \text{ runs}$) chains are allocated to the only MrBayes process. In the case of an MPI run, multiple MrBayes processes are created and each of them is responsible for only a portion of the total number of chains. For example if we have 2 runs with 4 chains in each of the runs and we invoke an MPI run with 4 processes, then each process receives only 2 chains. The program will need slightly more memory for various book-keeping purposes but the bulk of the memory required for an analysis is typically occupied by the conditional likelihoods.

How do I fix the tree during an analysis?

From version 3.2, MrBayes allows you to fix the tree topology using the **topologypr** parameter of the **prset** command. Before doing that, you need to read in the topology you want to enforce. This is done by putting the topology in a regular trees block in a NEXUS file. The tree in the NEXUS file is then read into memory using the **execute** command of MrBayes. You can read in up to 100 trees. Each tree is referred to by the name it has been given in the NEXUS

file. Assume for instance that you named your tree topology “myTree”. Then you fix the topology parameter of your phylogenetic model to the topology of that tree with the MrBayes command `prset topologypr=fixed(myTree)`.

Note that the procedure above only fixes the tree topology, not the branch lengths. If you want to fix the branch lengths too, you need to use the `brlenspr` parameter of the `prset` command. First you fix the topology as described above, then you fix the branch lengths using `prset brlenspr=fixed(myTree)`. If it is a clock tree, you should use `prset brlenspr=clock:fixed(myTree)`.

Why does MrBayes not use my starting values?

A number of commands or actions will result in MrBayes resetting some or all of the starting values. For instance, starting values will be reset if you change the partitioning scheme, if you change the number of runs or chains, or if you change any model settings. MrBayes will warn you that it resets the starting values in these cases, but it is easy to miss that warning. If you want to set the starting values yourself, make sure the `startvals` command is the last command issued before you start the analysis. In addition, make sure you do not change the number of runs or chains, or reset the starting values in your `mcmc` command. If you need to change the number of runs or chains, then do this in a separate `mcmcpr` command before you set the starting values.

I set the seed and swapseed to the same number. Why are the analyses not repeatable?

Trying to exactly replicate an analysis is not normally something you would want to do, unless you are trying to replicate a bug before submitting a bug report. If you really do want to make sure that you can run analyses that are identical down to the last decimal, you need to be aware that, since version 3.2, MrBayes generates a default model, including starting values, already when it reads in a character matrix. Setting the seed and swapseed will not change those starting values. Thus, unless you make sure you reset all starting values after you have set the seeds, different runs will have different starting values and will produce slightly different results. The easiest way of making sure analyses are repeatable is to set the seed and swapseed before reading in the character matrix.

5.8 More Information

For more information on how to use MrBayes, turn to the MrBayes web site (www.mrbayes.net).

Chapter 6

Evolutionary Models in MrBayes

MrBayes implements a wide variety of evolutionary models for nucleotide, amino acid, restriction site (binary), and standard discrete data. In addition, there are several different ways of modeling the process generating phylogenetic trees. An overview of all the models is given in diagrammatic form in the Appendix. Here, we provide a brief description of each model with some comments on their implementation in MrBayes and advice on how you can apply them successfully to your data.

6.1 Nucleotide Models

MrBayes implements a large number of DNA substitution models. These models are of three different types. The “**4by4**” models are the usual simple models of nucleotide evolution. The “**Doublet**” model is intended for stem regions of ribosomal DNA, where nucleotides evolve in pairs. Finally, the “**Codon**” models group the nucleotides in triplets and model evolution based on these. The type of nucleotide model is set in MrBayes with **lset nucmodel**; for instance, if you want to use the doublet model, the command is **lset nucmodel=doublet**. The default setting is **4by4**.

In addition to these model types, version 3.2 of MrBayes also supports translation of protein-coding nucleotide data to amino acids before analysis,

effectively treating the nucleotide data as if it were amino acid data. To request this, use the command **lset nucmodel=aa**. The translation uses the current setting of the genetic code. The default code is the universal genetic code; several others can be selected using **lset code**; for instance, if you want to use the metazoan mitochondrial code, specify **lset code=metmt**.

The translation takes ambiguities into account. For instance, assume that the observed codon is **A?A**, that is, the second codon site is completely ambiguous. Then each of the possible unambiguous codons is translated to an amino acid separately, and all unique amino acids are then included in the final state set. Once the nucleotide sequences have been translated to amino acids, the analysis proceeds as if you had started with amino acid data. See section 6.2 for details on the available amino acid models.

6.1.1 Simple Nucleotide Models

There has been more work based on the simple four by four nucleotide models than on any other type of evolutionary model for molecular data. MrBayes 3 implements three main types of models; you select among those by setting the number of substitution types using **lset nst** to 1, 2, or 6. The widely used General Time Reversible (GTR) model has six substitution types (**lset nst=6**), one for each pair of nucleotides. The (unscaled) instantaneous rate matrix for the GTR model is (note that we order the rows and columns alphabetically - A, C, G, T - unlike some other authors)

$$Q = \begin{bmatrix} & [A] & [C] & [G] & [T] \\ [A] & - & \pi_C r_{AC} & \pi_G r_{AG} & \pi_T r_{AT} \\ [C] & \pi_A r_{AC} & - & \pi_G r_{CG} & \pi_T r_{CT} \\ [G] & \pi_A r_{AG} & \pi_C r_{CG} & - & \pi_T r_{GT} \\ [T] & \pi_A r_{AT} & \pi_C r_{CT} & \pi_G r_{GT} & - \end{bmatrix}$$

The GTR model (Tavare, 1986) has four stationary state frequencies ($\pi_A, \pi_C, \pi_G, \pi_T$) and six rate parameters ($r_{AC}, r_{AG}, r_{AT}, r_{CG}, r_{CT}, r_{GT}$). In total, there are eight free parameters, since one of the stationary state frequencies and

one of the substitution rates are determined by the others. By default, MrBayes uses a “flat” Dirichlet distribution (with all distribution parameters set to 1) as the prior for both the stationary state frequencies and the substitution rates. This is a reasonable uninformative prior that should work well for most analyses.

During the analysis, both the stationary state frequencies and substitution rates are estimated. If you want to fix the stationary state frequencies or substitution rates, you can do that by using the **prset** command. For instance, assume that we want to fix the stationary state frequencies to be equal, converting the GTR model to the so-called SYM model. This is achieved by **prset statefreqpr=fixed(0.25,0.25,0.25, 0.25)** or, more conveniently, **prset statefreqpr=fixed(equal)**. The substitution rate matrix now becomes

$$Q = \begin{bmatrix} & [A] & [C] & [G] & [T] \\ [A] & - & 0.25r_{AC} & 0.25r_{AG} & 0.25r_{AT} \\ [C] & 0.25r_{AC} & - & 0.25r_{CG} & 0.25r_{CT} \\ [G] & 0.25r_{AG} & 0.25r_{CG} & - & 0.25r_{GT} \\ [T] & 0.25r_{AT} & 0.25r_{CT} & 0.25r_{GT} & - \end{bmatrix}$$

and the stationary state frequencies are no longer estimated during the analysis.

Similarly, it is possible to fix the substitution rates of the GTR model using **prset revmatpr=fixed**. Assume, for instance, that we want to fix the substitution rates to be ($r_{AC} = 0.11$, $r_{AG} = 0.22$, $r_{AT} = 0.12$, $r_{CG} = 0.14$, $r_{CT} = 0.35$, $r_{GT} = 0.06$). Then the correct statement would be **prset revmatpr=fixed(0.11,0.22,0.12,0.14,0.35, 0.06)**. Note the order of the rates. The substitution rates can be given either as percentages of the rate sum, as here, or they can be scaled to the r_{GT} rate. The former representation is the simplex (Dirichlet) parameterization used internally by MrBayes. By default, MrBayes reports substitution rates in the simplex format but you can request conversion of sampled rates to the scaled format using the **report** command if you prefer this representation. One disadvantage with the scaled format is that the posterior distribution tends to be strongly skewed such that the arithmetic mean of the sampled values is considerably higher than the mode and the median. Therefore, consider using the median instead of the mean when reporting a posterior distribution sampled in the scaled format.

Before using the GTR model for some of your data, we recommend that you make sure there are at least a few possible substitutions of each type. For instance, if there is not a single GT substitution in your data, it will be difficult to estimate the GT substitution rate. In such cases, you should consider either the HKY model (`nst=2`) or the F81 model (`nst=1`) instead of the GTR model. The HKY model (Hasegawa, Kishino and Yano, 1985) has different rates for transitions (r_{ti}) and transversions (r_{tv}):

$$Q = \begin{bmatrix} & [A] & [C] & [G] & [T] \\ [A] & - & \pi_C r_{tv} & \pi_G r_{ti} & \pi_T r_{tv} \\ [C] & \pi_A r_{tv} & - & \pi_G r_{tv} & \pi_T r_{ti} \\ [G] & \pi_A r_{ti} & \pi_C r_{tv} & - & \pi_T r_{tv} \\ [T] & \pi_A r_{tv} & \pi_C r_{ti} & \pi_G r_{tv} & - \end{bmatrix}$$

The HKY model is often parameterized in terms of the ratio between the transition and transversion rates, $\kappa = r_{ti}/r_{tv}$, and this is the default format used by MrBayes when reporting samples from the posterior distribution. Internally, however, MrBayes uses the simplex (Dirichlet) parameterization in which the transition and transversion rates are expressed as percentages of the (unscaled) rate sum. If you prefer, you can have MrBayes report the sampled values using the Dirichlet format instead of the ratio format by using the command **report tratio=dirichlet**. The caveats described above for the GTscaled substitution rates also apply to the transition / transversion rate ratio. In the .p files, the ratio will be referred to as kappa and the transition and transversion rate proportions will be referred to as `ti` and `tv`. The setting of the **report tratio** option will determine whether you will see a single **kappa** column or the two `ti` and `tv` columns.

As with the GTR model, you can fix both the stationary state frequencies and the transition / transversion rate ratio of the HKY model. If you fix the stationary state frequencies to be equal, you will get the K2P model (Kimura, 1980).

Finally, MrBayes implements the F81 model (Felsenstein, 1981), which assumes that all substitution rates are equal but stationary state frequencies are not, that

is

$$Q = \begin{bmatrix} & [A] & [C] & [G] & [T] \\ [A] & - & \pi_C & \pi_G & \pi_T \\ [C] & \pi_A & - & \pi_G & \pi_T \\ [G] & \pi_A & \pi_C & - & \pi_T \\ [T] & \pi_A & \pi_C & \pi_G & - \end{bmatrix}$$

If the stationary state frequencies are fixed to be equal using **prset statefreqpr=fixed(equal)**, you will get the simplest of all nucleotide substitution models, the JC model (Jukes and Cantor, 1969).

A large number of other constrained versions of the GTR model are often used in Maximum Likelihood inference. Why do we not allow more substitution model types in MrBayes? One of the most important advantages of the Bayesian approach is that it allows you to integrate out uncertainty concerning the model parameters you have little information about. Thus, Bayesian inference is relatively robust to slight over-parameterization of your model. In addition, the MCMC sampling procedure is typically efficient in dealing with complex multi-parametric models. For these reasons, it is less important in the Bayesian context to find the simplest possible model that can reasonably represent your data. Therefore, if your intended model is not available in the program, we suggest that you simply select the next more complex model implemented in MrBayes.

How do I select the best substitution model for my analysis? A standard approach is to use a model testing procedure, such as those implemented in ModelTest (Posada, 1998), jModelTest (Posada, 2008), or MrModelTest (Nylander, 2004). However, a priori model testing comes with a number of serious drawbacks. It typically restricts the testing to a rough topology estimate, and at the end of the day you have to put all your money down on a single substitution model.

An alternative approach, which is more truly Bayesian, is to integrate out the uncertainty concerning the correct structure of the substitution model. This is accomplished by having the MCMC procedure sample across substitution models (Huelsenbeck et al., 2004), a method made available in version 3.2 of MrBayes. This will result in all parameter estimates being averaged across substitution

models, with each substitution model being weighted according to its posterior probability.

Specifically, growth function.

To sample across substitution models, simply use the command `lset nst=mixed` before running the analysis. At the end Refer to tutorial.

6.1.2 The Doublet Model

The doublet model of MrBayes is intended for stem regions of ribosomal sequences, where nucleotides pair with each other to form doublets. The nucleotide pairing results in strong correlation of substitutions across sites - when there is a substitution at one site it is typically accompanied by a compensatory substitution at the paired site. If the correlation between paired sites is not accounted for, parametric statistical methods will overestimate the confidence we should have in the best tree(s). Incidentally, the same is true for parsimony and the non-parametric bootstrap.

There are various ways to model the evolution of nucleotide doublets. One method is to focus on the common doublets, A-T and C-G in particular. MrBayes uses a more complex model, originally formulated by Schoniger and von Haeseler (1994), where all doublets are taken into account. The central idea in this model is that one common doublet is converted into another through a two-step process. In the first step, one of the nucleotides is substituted with another according to a standard four by four model of nucleotide change. In the second step, the matching nucleotide is changed according to the same standard four by four model. Thus, in this model there is no momentary change from one doublet to another doublet; this always occurs through an intermediate, rare doublet.

Assume that we are using a GTR model for the single nucleotide substitutions, that i and j are two different doublets, that d_{ij} is the minimum number of nucleotides that must be changed when going between i and j , and that mn is the pair of nucleotides that change when going between i and j when $d_{ij} = 1$. Then the elements q_{ij} of the instantaneous rate matrix Q of the doublet model

can be expressed as follows

$$q_{ij} = \begin{cases} 0 & \text{if } d_{ij} > 1 \\ \pi_j r_{min} & \text{if } d_{ij} = 1 \end{cases}$$

for the case when i differs from j ; the diagonal elements ($i = j$) are determined, as usual, to balance the rows in the instantaneous rate matrix to sum to zero. This gives the instantaneous rate matrix (only 7 rows and columns out of 16 shown):

$$Q = \begin{bmatrix} & [AA] & [AC] & [AG] & [AT] & [CA] & [CC] & \cdots & [TT] \\ [AA] & - & \pi_{AC}r_{AC} & \pi_{AG}r_{AG} & \pi_{AT}r_{AT} & \pi_{CA}r_{AC} & 0 & \cdots & 0 \\ [AC] & \pi_{AA}r_{AC} & - & \pi_{AG}r_{CG} & \pi_{AT}r_{CT} & 0 & \pi_{CC}r_{AC} & \cdots & 0 \\ [AG] & \pi_{AA}r_{AG} & \pi_{AC}r_{CG} & - & \pi_{AT}r_{GT} & 0 & 0 & \cdots & 0 \\ [AT] & \pi_{AA}r_{AT} & \pi_{AC}r_{CT} & \pi_{AG}r_{GT} & - & 0 & 0 & \cdots & \pi_{TT}r_{AT} \\ [CA] & \pi_{AA}r_{AC} & 0 & 0 & 0 & - & \pi_{CC}r_{AC} & \cdots & 0 \\ [CC] & 0 & \pi_{AC}r_{AC} & 0 & 0 & \pi_{CA}r_{AC} & - & \cdots & 0 \\ \cdots & \cdots & \cdots & \cdots & \cdots & \cdots & \cdots & \cdots & \cdots \\ [TT] & 0 & 0 & 0 & \pi_{AT}r_{AT} & 0 & 0 & \cdots & - \end{bmatrix}$$

Instead of using the GTR model, we could of course have used the HKY or F81 model, resulting in obvious modifications of the r_{mn} values. Use `lset nst` to change among those options; for instance, use `lset nst=6` to choose the GTR model. The default model is the F81 model (`lset nst=1`).

Before the doublet model can be used, it is necessary to specify all the nucleotide pairs in the alignment. This is done using the `pairs` command, most conveniently in a MrBayes block in a data file. For instance, `pairs 1:10, 2:9;` would pair nucleotide 1 with 10 and 2 with 9. See the `kim.nex` data file for an example of an analysis using the doublet model.

6.1.3 Codon Models

The codon models implemented in MrBayes are based on the first formulations of such models (Goldman and Yang (1994); Muse and Gaut (1994)). The approach is similar to that used in the doublet model. A codon can change to another only through steps of one nucleotide change at a time. These steps are modeled using

a standard four by four nucleotide model. There is one additional complication though: some nucleotide changes are synonymous while others lead to changes in amino acids and thus may be subject to selection (a factor modifying the substitution rate). Assume that i and j are different codons, that $a(i)$ is the amino acid coded for by codon i , that d_{ij} is the minimum number of nucleotide changes involved in changing between them, and that ω is the ratio of the non-synonymous to the synonymous substitution rate. The off-diagonal elements of the instantaneous rate matrix can now be defined as

$$q_{ij} = \begin{cases} 0 & \text{if } d_{ij} > 1 \\ \pi_j r_{min} & \text{if } d_{ij}=1 \text{ and } a(i) = a(j) \\ \pi_j r_{min} \omega & \text{if } d_{ij}=1 \text{ and } a(i) \neq a(j) \end{cases}$$

with the diagonal elements being defined to balance the rows of the instantaneous rate matrix, as usual. The single-nucleotide substitution rates can be modeled using the GTR, the F81, or the JC model, as before. Use `lset nst` to change among those options; for instance, use `lset nst=6` to choose the GTR model. The default model is the F81 model (`lset nst=1`).

Invoking the codon model is easy; just make sure that the aligned DNA or RNA sequences start with a nucleotide in codon position 1 and that they end with a nucleotide in codon position 3. Also, make sure that the sequences do not contain any stop codons. To figure out whether a codon is a stop codon and whether a particular single-nucleotide change involves an amino acid change, MrBayes uses one of several genetic codes. By default, MrBayes uses the universal code but you can select other codes by using the `lset code` command. Note that the codon models are computationally demanding. Whereas the computations for the simple four by four models need to deal with only 16 Q-matrix and transition probability elements (4x4), the codon model computations need to process more than 3,600 Q-matrix and transition probability elements resulting in these runs being roughly 200 times slower. The codon models also require much more memory than the four by four models, about 16 times as much.

The simplest codon model, described above, assumes that all amino acid sites are subject to the same level of selection (the same ω factor). However, MrBayes also implements models accommodating variation in selection across sites. This

allows you to detect positively selected amino-acid sites using a full hierarchical Bayes analysis (that is, an analysis that does not fix tree topology, branch lengths or any substitution model parameters but instead integrates out uncertainty in all these factors).

The ω variation models work much like the model accommodating rate variation across sites according to a gamma distribution. The likelihood of each site is calculated under several different ω values and then the values are summed to give the site likelihood. A difference is that the stationary frequency of each omega category is estimated, instead of being fixed as in the case of the gamma distribution. There are two variants implemented in MrBayes, and they are invoked using the **lset omegavar** option. In the Ny98 model (Nielsen and Yang, 1998), there are three classes with potentially different ω values: $0 < \omega_1 < 1$, $\omega_2 = 1$, and $\omega_3 > 1$. The M3 model also has three classes of ω values but these values are less constrained in that they only have to be ordered $\omega_1 < \omega_2 < \omega_3$. If, for instance, you would like to invoke the M3 model, use the command **lset omegavar=M3**.

When you use a model with variation in selection pressure across sites, you probably want to infer the positively selected sites. If you select **report possel=yes** before you start your analysis, MrBayes will calculate the probability of each site being in a positively selected omega class. For the M3 model, for instance, the likelihood of the site is calculated under each of the three categories, taking the category frequencies into account, and then the likelihoods are summed to yield the total likelihood of the site. Finally, the proportion of this sum originating from categories that are positively selected (those that have an ω value larger than 1); this is the posterior probability of the site being positively selected.

Once the probabilities of each site being positively selected are printed to file, they can be summarized using the standard **sump** command. When interpreting the output from the Ny98 model, it is helpful to know that **pi(-)**, **pi(N)** and **pi(+)** are the frequencies of the negatively selected, neutral and positively selected categories, respectively, and **omega(-)**, **omega(N)** and **omega(+)** are the corresponding ω values. The M3 model parameters are instead labeled **pi(1)**, **pi(2)** and **pi(3)** for the category frequencies and **omega(1)**, **omega(2)** and

`omega(3)` for the ω values. The probability of a codon being positively selected is labeled by the site numbers in the original alignment. Thus `pr+(16,17,18)` is the probability of the codon corresponding to the original nucleotide alignment sites 16, 17, and 18 being in a positively selected omega category.

6.2 Amino-acid Models

MrBayes implements a large number of amino-acid models. They fall in two distinct categories: the fixed-rate models and the variable-rate models. The former have both the stationary state frequencies and the substitution rates fixed, whereas one or both of these are estimated in the latter.

6.2.1 Fixed Rate Models

The Poisson model (Bishop and Friday, 1987) is the simplest of the fixed rate models. It assumes equal stationary state frequencies and equal substitution rates; thus, it is analogous to the JC model for nucleotide characters. The rest of the fixed-rate models have unequal but fixed stationary state frequencies and substitution rates reflecting estimates of protein evolution based on some large training set of proteins. These models include the Dayhoff model (Dayhoff et al., 1978), the Mtrev model (Adachi and Hasegawa, 1996), the Mtmam model (Cao et al. (1998); Yang et al. (1998)), the WAG model (Whelan and Goldman, 2001), the Rtrev model (Dimmic et al., 2002), the Cprev model (Adachi et al., 2000), the Vt model (Muller and Vingron, 2000) and the Blosum62 model (Henikoff and Henikoff, 1992). Each model is appropriate for a particular type of proteins. For instance, if you are analyzing mammal mitochondrial proteins, you might want to use the Mtmam model. Invoke that model by typing **`prset aamodelpr=fixed(mtmam)`**.

6.2.2 Estimating the Fixed Rate Model

MrBayes allows a convenient way of estimating the fixed-rate model for your amino acid data instead of specifying it prior to the analysis. If you choose this option, MrBayes will allow the MCMC sampler to explore all of the fixed-rate models listed above, including the Poisson model, by regularly proposing new models. When the MCMC procedure has converged, each model will contribute to your results in proportion to its posterior probability. For instance, if you are analyzing mammal mitochondrial proteins, it is likely that the Mtmam model will contribute most to the posterior distribution but it is possible that some other models, for instance the Mtrev model, will contribute significantly too. A nice feature of the MCMC model estimation is that the extra computational cost is negligible.

To allow so-called model jumping between fixed-rate amino acid models, simply set the prior for the amino acid model to mixed, **prset aamodelpr=mixed**, prior to analysis. During the run, MrBayes will print the index of the current model to the .p file(s) in the **aamodel** column. The indices of the models are as follows: 0 = Poisson, 1 = Jones, 2 = Dayhoff, 3 = Mtrev, 4 = Mtmam, 5 = Wag, 6 = Rtrev, 7 = Cprev, 8 = Vt, 9 = Blosum. When you use the **sump** command, you will get a table with the names of the amino acid models and their posterior probabilities.

6.2.3 Variable Rate Models

There are two variable-rate models implemented in MrBayes for amino acid data. The *Equalin model* is a “glorified” F81 model in that it allows the stationary state frequencies of all amino acids to be different but assumes the same

substitution rate. Thus, the instantaneous rate matrix for this model is

$$Q = \begin{bmatrix} & [A] & [R] & [N] & [D] & [C] & [Q] & \cdots & [V] \\ [A] & - & \pi_R & \pi_N & \pi_D & \pi_C & \pi_Q & \cdots & \pi_V \\ [R] & \pi_A & - & \pi_N & \pi_D & \pi_C & \pi_Q & \cdots & \pi_V \\ [N] & \pi_A & \pi_R & - & \pi_D & \pi_C & \pi_Q & \cdots & \pi_V \\ [D] & \pi_A & \pi_R & \pi_N & - & \pi_C & \pi_Q & \cdots & \pi_V \\ [C] & \pi_A & \pi_R & \pi_N & \pi_D & - & \pi_Q & \cdots & \pi_V \\ [Q] & \pi_A & \pi_R & \pi_N & \pi_D & \pi_C & - & \cdots & \pi_V \\ \cdots & \cdots & \cdots & \cdots & \cdots & \cdots & \cdots & \cdots & \cdots \\ [V] & \pi_A & \pi_R & \pi_N & \pi_D & \pi_C & \pi_Q & \cdots & - \end{bmatrix}$$

and it has 19 free parameters (20 stationary state frequencies minus one because the frequencies sum to 1) that will be estimated from data.

The other variable-rate model is the “glorified” GTR model, which allows all stationary state frequencies and substitution rates to vary. Thus, the instantaneous rate matrix for this model is

$$Q = \begin{bmatrix} & [A] & [R] & [N] & [D] & [C] & [Q] & \cdots & [V] \\ [A] & - & \pi_R r_{AR} & \pi_N r_{AN} & \pi_D r_{AD} & \pi_C r_{AC} & \pi_Q r_{AQ} & \cdots & \pi_V r_{AV} \\ [R] & \pi_A r_{AR} & - & \pi_N r_{RN} & \pi_D r_{RD} & \pi_C r_{RC} & \pi_Q r_{RQ} & \cdots & \pi_V r_{RV} \\ [N] & \pi_A r_{AN} & \pi_R r_{RN} & - & \pi_D r_{ND} & \pi_C r_{NC} & \pi_Q r_{NQ} & \cdots & \pi_V r_{NV} \\ [D] & \pi_A r_{AD} & \pi_R r_{RD} & \pi_N r_{ND} & - & \pi_C r_{DC} & \pi_Q r_{DQ} & \cdots & \pi_V r_{DV} \\ [C] & \pi_A r_{AC} & \pi_R r_{RC} & \pi_N r_{NC} & \pi_D r_{DC} & - & \pi_Q r_{CQ} & \cdots & \pi_V r_{CV} \\ [Q] & \pi_A r_{AQ} & \pi_R r_{RQ} & \pi_N r_{NQ} & \pi_D r_{DQ} & \pi_C r_{CQ} & - & \cdots & \pi_V r_{QV} \\ \cdots & \cdots & \cdots & \cdots & \cdots & \cdots & \cdots & \cdots & \cdots \\ [V] & \pi_A r_{AV} & \pi_R r_{RV} & \pi_N r_{NV} & \pi_D r_{DV} & \pi_C r_{CV} & \pi_Q r_{QV} & \cdots & - \end{bmatrix}$$

and the model has 19 free stationary state frequency parameters and 189 free substitution rate parameters. The Bayesian MCMC approach is good at handling uncertainty in multiparameter models, so the GTR model may be used successfully with moderate-size data sets, but the model is so parameter-rich that you need a fairly sizable data set to be able to estimate all parameters with reasonable precision.

The *GTR model* can be used to express a user-derived fixed rate model other than those already implemented in MrBayes. Simply use the **prset** command to fix the stationary state frequencies and substitution rates of the GTR model to the desired values. You need to set two options, **prset aarevmatpr=fixed(<190 comma-separated values>)** and **prset statefreqpr=fixed(<20 comma-separated values>)**. Once the values are fixed prior to analysis, the MCMC procedure will not change them and they will remain the same throughout the analysis.

6.3 Restriction Site (Binary) Model

MrBayes implements a simple F81-like model for restriction sites and other binary data. The instantaneous rate matrix for this model is simply

$$Q = \begin{bmatrix} & [0] & [1] \\ [0] & - & \pi_1 \\ [1] & \pi_0 & - \end{bmatrix}$$

Any asymmetry in the rate of 0 to 1 and 1 to 0 transitions is expressed in terms of the stationary state frequencies. Thus, if the stationary frequencies are $\pi_0 = 0.25$ and $\pi_1 = 0.75$, then the rate of 0 to 1 transitions is 3 times as high as the rate of transitions in the other direction ($\pi_1/\pi_0 = 3$).

A problem with some binary data sets, notably restriction sites, is that there is an ascertainment (coding) bias such that certain characters will always be missing from the observed data. It is impossible, for instance, to detect restriction sites that are absent in all of the studied taxa. MrBayes corrects for this bias by calculating likelihoods conditional on the unobservable characters being absent (Felsenstein, 1992). The ascertainment (coding) bias is selected using **lset coding**. There are five options: (1) there is no bias, all types of characters could, in principle, be observed (**lset coding=all**); (2) characters that are absent (state 0) in all taxa cannot be observed (**lset coding=noabsencesites**); (3) characters that are present (state 1) in all taxa cannot be observed (**lset coding=nopresencesites**); (4) characters that are constant (either state 0 or 1)

in all taxa cannot be observed (`lset coding=variable`); and (5) only characters that are parsimony-informative have been scored (`lset coding=informative`). For restriction sites it is typically true that all-absence sites cannot be observed, so the correct coding bias option is `noabsencesites`.

The binary model is useful for a number of character types other than restriction sites. For instance, the model can be used for gap characters. The presence and absence of gaps must be coded consistently for all characters; let us assume here that absence of a gap is coded as 0 and presence as 1. Since the detection of gaps is typically contingent on observing some sequence length variation, neither all-absence nor all-presence characters can be observed. Thus, the correct ascertainment bias for gap characters is `variable`. The parameters π_0 and π_1 would represent the rate at which insertions and deletions occur, respectively (assuming that state 0 denotes absence of a gap).

The binary model can also be used for ecological, morphological, or other binary characters of arbitrary origin. However, if the binary model is applied to more than one character, then there is an implicit assumption that the state labels are not arbitrary for those characters. That is, the 0 state in one character must somehow be comparable to the 0 state in the others. For instance, 0 could mean absence (or presence) of a particular type of feature, such as a wing vein, a restriction site, or a gap in a DNA sequence. It is not appropriate to apply the default binary model to a set of characters where the state labels are arbitrary, as is true of most morphological characters. Thus, we can possibly estimate the rate of loss versus gain of wing veins over a set of consistently coded wing venation characters, but we cannot compare the rate of loss of antennal articles to the rate at which a yellow patch evolves into a green patch. If state labels are truly arbitrary, then the stationary state frequencies of the binary model must be fixed to be equal, such that the estimation of model parameters becomes independent of the labeling of character states. An alternative is to consider the standard model, which provides more sophisticated ways of dealing with arbitrary state labels.

When is the correction for ascertainment bias important? This is strongly dependent on the size of the tree (the sum of the branch lengths on the tree). The larger the tree, the less important the correction for ascertainment bias

becomes. In our experience, when there are more than 20-30 taxa, even the most severe bias (only informative characters observed) is associated with an insignificant correction of the likelihood values.

6.4 Standard Discrete (Morphology) Model

The model used by MrBayes for “standard” discrete data is based on the ideas originally presented by Lewis (2001). Essentially, the model is analogous to a JC model except that it has a variable number of states, from 2 to 10. For instance, a three-state standard character would be evolving according to the instantaneous rate matrix

$$Q = \begin{bmatrix} & [0] & [1] & [2] \\ [0] & - & 1 & 1 \\ [1] & 1 & - & 1 \\ [2] & 1 & 1 & - \end{bmatrix}$$

Because all rates are the same, we can maintain the essential property of standard characters, namely that state labels are arbitrary. Thus, the standard model assures that you will get the same results regardless of the way in which you label the states.

This applies to the standard, program default, assumption of unordered character states. In ordered characters, on the other hand, evolution between some states is assumed to go through intermediate states. MrBayes implements a stochastic model for such characters. For a three-state character assumed to be ordered (by convention in the sequence 0-1-2), the instantaneous rate matrix is

$$Q = \begin{bmatrix} & [0] & [1] & [2] \\ [0] & - & 1 & 0 \\ [1] & 1 & - & 1 \\ [2] & 0 & 1 & - \end{bmatrix}$$

Note that the instantaneous rate of going between the two end states is 0, that

is, a transition from 0 to 2 or from 2 to 0 has to go through state 1. By default, MrBayes treats all standard characters as unordered. To change this, use the **ctype** command. For instance, if you want to treat characters 3 and 4 as ordered you need to include the statement **ctype ordered: 3 4;** in your MrBayes block (or enter it using the command line, if you prefer).

The number of states of each standard character is determined by MrBayes simply by looking at the state codes in your matrix. Thus, a three-state model will be used for a three-state character and a six-state model for a six-state character. MrBayes does not check that all state codes are used, it simply finds the largest state code in the matrix for each character. Thus, make sure that you use the state codes 0, 1, and 2 for a three-state character and state codes 0, 1, 2, 3, 4, and 5 for a six-state character.

Because state labels are arbitrary in the standard model, we cannot estimate unequal stationary state frequencies or substitution rates (recall that the stationary state frequencies are an important factor in determining the latter). However, it is still possible to allow the state frequencies (rates) to vary over sites according to some appropriate distribution. MrBayes uses a symmetric Dirichlet distribution for this purpose. For binary data, the analogy of the Dirichlet distribution is called the beta distribution; MrBayes uses Dirichlet and beta interchangeably for the distribution depending on context. The approach is similar to the one used to allow rate variation across sites according to a gamma distribution: we calculate the likelihood of a site assuming different discrete categories of asymmetry and then we sum the values to obtain the site likelihood.

The symmetric Dirichlet distribution has one parameter that determines its shape, just like the alpha parameter determines the shape of the gamma distribution. The larger the parameter of the symmetric Dirichlet, the less transition rate (stationary frequency) asymmetry there is across sites. By default, the parameter is fixed to infinity (**prset symdirihyperpr=fixed(infinity)**); this corresponds to the standard assumption of no transition rate asymmetry across sites: the rate of going from 0 to 1 is equal to the rate of going from 1 to 0 for all characters. The prior is called a hyperprior because it concerns a distribution that controls the distributions of other model parameters (stationary state frequencies in this case). If you want to allow transition rate (stationary

frequency) asymmetry in standard data, then simply select another (hyper)prior. For instance, you can fix the parameter to 1.0, which would result in a uniform prior on the proportions of the state frequencies.

In practice, MrBayes uses a discrete approximation of the Dirichlet distribution for binary characters; five categories are used by default (change this with `lset nbetacat`). For instance, assume that we fix the hyperprior to 1.0 and then evaluate the likelihood of one binary character using five discrete beta categories. MrBayes would then calculate the likelihood of the character assuming that the stationary state frequencies of the two states were 0.1 : 0.9, 0.3 : 0.7, 0.5 : 0.5, 0.7 : 0.3 and 0.9 : 0.1. The five category likelihoods would then be multiplied by 0.20 (there is a probability of 0.20 of being in each of the categories) and then summed up to give the total likelihood of the character. For multistate characters, MrBayes does not use the discrete approximation; instead, it uses the MCMC procedure to explore different stationary state frequency proportions.

6.5 Parsimony Model

MrBayes implements an incredibly parameter-rich model, first described by Tuffley and Steel (1997). It orders trees in terms of their maximum likelihood in the same way as the parsimony method would order them in terms of their parsimony score; hence we call it the parsimony model. The model is also referred to as the No-Common-Mechanism model because it treats each branch length for each character as a separate, completely independent parameter. In principle, a Bayesian MCMC analysis using the parsimony model should integrate out the branch lengths but MrBayes 3 uses a simpler approach, in which the branch lengths are fixed to their maximum likelihood values (infinity if there is a change on the branch and zero otherwise). This type of approach, where some parameters are fixed prior to the Bayesian analysis according to some non-Bayesian estimate, is typically referred to as an empirical Bayes method. Future versions of MrBayes may implement the true (hierarchical) Bayesian approach to the parsimony model but we expect the results to be very similar under both approaches.

The parsimony model is much less parsimonious with respect to parameters than any other model implemented in MrBayes. Consider, for instance, an analysis of 1,000 characters and 100 taxa. The parsimony model would have about 200,000 free parameters (200 branches times 1000 characters). A more typical GTR+ γ +I model would have only little more than 200 parameters, about 1,000 times fewer parameters. In this sense, the standard stochastic models are much more parsimonious than the parsimony model. Several problems are associated with the excessive number of parameters. Statistical inconsistency is perhaps the best known of these but, more fundamentally, a model like the parsimony model does not offer much in terms of generalities that can be inferred about the evolutionary process.

The Goldman (Goldman, 1993) model is another example of a parameter-rich stochastic model that orders trees in the same way as the parsimony method. In this model, the branch lengths are the same but the ancestral states are estimated for all characters and all nodes in the tree. For an analysis of 100 taxa and 1,000 characters, this results in approximately 100,000 free parameters. The Goldman model is actually very similar to the No- Common-Mechanism model; it makes little difference if the character-specific and tree-section- specific parameters are introduced at the nodes or at the branches. The Goldman model is not implemented in MrBayes.

We would like to emphasize that we do not recommend the use of the parsimony model. We have included it in MrBayes only to allow users to explore its properties and contrast it with the other models implemented in the program. The parsimony model is not the default model used for standard (morphological) data in MrBayes. The default standard data model is described above and is similar to the models used for nucleotide and amino acid data. By default, MrBayes does not use the parsimony model at all; you have to invoke it using **lset parsmodel=yes**.

6.6 Rate Variation Across Sites

By default, MrBayes assumes that all characters evolve at the same rate (measured in expected changes per site over the tree). There are different ways in which you can allow rate variation across sites. The simplest method is to assume that rates vary over sites according to a gamma distribution (Yang, 1993). The gamma model can be combined with spatial autocorrelation between the rates at adjacent sites, the autocorrelated gamma model. A completely different approach to rate variation across sites is to allow a proportion of sites to be invariable. This model can be combined with the gamma model but not with the autocorrelated gamma model. In addition, MrBayes also implements new ways of modelling rate-variation (log normal, and k -mixtures, see below). Finally, it is possible to divide characters into groups evolving at different rates, the partitioned or site specific rate model.

6.6.1 Gamma-distributed Rate Model

The commonly used model of gamma-shaped rate variation across sites is invoked using **lset rates=gamma**. The shape of the gamma distribution is determined by the so-called α (alpha) parameter. When this parameter is small (below 1), the distribution takes on an L-shaped form with a few sites evolving rapidly while most sites are conserved. Conversely, when α is above 1 the distribution becomes similar to a normal distribution with less and less variation in rates across sites as α becomes larger.

In practice, the gamma distribution is approximated using a small number of discrete rate categories (Yang, 1994). By default, four rate categories are used; you can change this setting by using **lset ngammacat**. For instance, if you want to use eight discrete rate categories the appropriate command is **lset ngammacat=8**. The computational complexity is proportional to the number of categories used. An analysis with four discrete gamma categories is four times as slow as an analysis with no rate variation across sites and twice as fast as one with eight categories.

The shape parameter (α) of the gamma distribution is similar to the shape parameter of the Dirichlet distribution of stationary state frequencies used for standard data (see above) in that it controls the distribution of another model parameter (the site rates). Therefore, the prior probability distribution used for the shape parameter can be referred to as a hyperprior. The default prior used in MrBayes is an exponential distribution with the rate set to 1.0 (`prset shapepr=exponential(1.0)`). Note that the mean of the exponential distribution is the inverse of the rate, so if, for example, changing the rate of the exponential distribution to 10.0, will move the mean from 1.0 to $1/10.0 = 0.1$. The sampled values of the shape parameter are found under the column heading **alpha** in the `.p` file(s).

6.6.2 Autocorrelated Gamma Model

In this model, rates vary across sites according to an autocorrelated gamma model where the rate at each site depends to some extent on the rates at adjacent sites (Yang, 1995). The spatial autocorrelation is measured by the ρ (rho) parameter, which ranges from -1 (negative autocorrelation, that is, adjacent sites tend to have wildly different rates) to 1 (adjacent sites have very similar rates). The default prior probability for rho is a uniform distribution covering the entire interval $(-1, 1)$.

In the worst case, a small symmetric tree, the extra computational complexity incurred by invoking the autocorrelated gamma model instead of the gamma model is comparable to a doubling of the number of taxa in the analysis. In more typical cases, moderate to large data sets, the additional computational cost is negligible and equivalent to adding a single taxon. As with the gamma model, the autocorrelated gamma distribution is approximated with a number of discrete rate categories determined by `lset ngammacat`.

As described by Yang (Yang, 1995), protein-coding sequences tend to have a three-position offset in their autocorrelation. That is, the first codon position sites tend to have rates that are correlated with the adjacent first-position sites, second-position site rates are correlated with adjacent second-position rates, etc. You can take this effect into account by partitioning your sites into the three

codon positions and then applying a separate autocorrelated gamma model to each of the categories.

You might want to invoke an autocorrelated gamma model with the same correlation coefficient for a dataset consisting of several concatenated genes. If so, it is necessary to inform MrBayes about the break points between different genes. Otherwise, the rates at the first site of each gene except the first one will be erroneously compared to the rates at the last site in the preceding gene. The command **atabreaks** is provided for this purpose. For instance, if there are only two genes in your data set, the first with 960 sites, you would specify the break between them with the statement **atabreaks 960**. Note that you specify the break by giving the last sequence site before the break. The **atabreaks** command is only needed when you invoke a single autocorrelated gamma model for a multigene dataset. The **atabreaks** command cannot be used to partition a data set.

6.6.3 Lognormal-distributed rates across sites

LNorm — Lognormal-distributed rates across sites. The rate at a site is drawn from a lognormal distribution. the lognormal distribiton has a single parameter, sigma (SD) that describes how much rates vary (mean fixed to $\log(1.0) = 0.0$).

6.6.4 Site rates from a mixture with k categories

Kmixture — Site rates come from a mixture with k categories. Category rates are drawn from an ordered flat Dirichlet distribution with mean rather than sum equal to 1.0.

6.6.5 Proportion of Invariable Sites

A completely different approach to rate variation is to allow a proportion of sites to be invariable. This model is invoked using **lset rates=propinv**. The proportion of invariable sites is referred to as **pinvar**; it can vary from 0 (no

invariable sites) to 1 (all sites are invariable). The default prior is a uniform distribution on the interval $(0, 1)$; change it using `prset pinvarpr`.

The proportion of invariable sites model can be combined with the gamma model using `lset rates=invgamma`. Although this model is slightly better than the simple gamma model for many data sets, it sometimes results in a bimodal or ridge-like posterior probability distribution. In particular, it is not uncommon to see two peaks in the posterior, one with a low proportion of invariable sites and significant rate variation in the gamma distribution (low alpha value) and the other with a high proportion of invariable sites and moderate amounts of rate variation in the gamma distribution (moderately high alpha value). If you have a posterior of this kind, you should not be surprised if Metropolis-coupling results in rapid (instantaneous) shifts from one mode to the other during the stationary phase of the analysis. The reason for this is that different chains are likely to explore different peaks in the posterior, and successful swapping involving the cold chain is likely to result in mode-jumping. Also, you should consider presenting the entire distribution of the sampled `alpha` and `pinvar` parameters since simple point estimates of each parameter would be misleading.

6.6.6 Partitioned (Site Specific) Rate Model

For protein-coding nucleotide sequences, a site-specific rate model is often used, allowing each codon position (first, second and third codon position sites) to have its own rate. This results in a model with three rates, two of which are free to vary (since the average rate is 1.0 by definition). More generally, we might have different character divisions (separate genes, morphology, etc) which potentially evolve at very different rates.

In MrBayes 3, we provide a general mechanism for setting up these models based on partitioning the data set and then unlinking parameters across the partitions. Assume for instance that we want to set up a site specific rate model for a data set with one sequence. We first set up the codon site partitioning scheme using the following lines in a MrBayes block:

```
charset pos1 = 1-.\3;
```

```
charset pos2 = 2-.\3;  
charset pos3 = 3-.\3;  
partition by_codon = 3:pos1,pos2,pos3;  
set partition = by_codon;
```

The character sets are first defined using the dot sign (.) to mark the last character in the data set and the \3 sequence to include every third character in the specified range. Then a partitioning scheme called `by_codon` is defined using the previously named character sets. Finally, the partitioning scheme called `by_codon` is invoked using the `set` command.

When we process these commands in MrBayes using the `execute` command, the characters are divided into three sets corresponding to the codon positions. By default, however, all model parameters including the rate will be shared across partitions. To allow the rates to differ across partitions, we need to change the prior for rates using `prset`. Specifically, `prset ratepr=variable` invokes partition-specific rates. The partition-specific rate parameter is referred to as `ratemult`, and the individual rates are labeled `m{1}`, `m{2}`, etc for the rate (multiplier) of character division 1, 2, etc. See below for more information on how to set up partitioned models.

6.6.7 Inferring Site Rates

When you are allowing rate variation across sites, you may be interested in inferring the rates at each individual site. By default, the site rates are not sampled during a MCMC run. You need to request the sampling of these values using `report siterates=yes`. The rates will be referred to as `r(<site number>)`. For instance, `r(45)` is the inferred rate at site (character) 45 of your data set.

6.7 Rate Variation Across the Tree

6.7.1 The Covarion Model

For both nucleotide sequence and amino-acid data, MrBayes allows rates to change across the tree under a covarion-like model (Tuffley and Steel (1998); Huelsenbeck (2002); see also Galtier (2001)). Specifically, the covarion-like model assumes that a site is either “on” or “off”. When it is on, it evolves under a standard four-by-four nucleotide or 20 by 20 amino acid model but when it is off, it does not change at all. The switching between on and off states is controlled by two rate parameters, s_{01} (from off to on) and s_{10} (from on to off). The instantaneous rate matrix of the nucleotide variant (also referred to as the covarion model), assuming a GTR model for the on state, is

$$Q = \begin{bmatrix} & [A_{off}] & [C_{off}] & [G_{off}] & [T_{off}] & [A_{on}] & [C_{on}] & [G_{on}] & [T_{on}] \\ [A_{off}] & - & 0 & 0 & 0 & s_{01} & 0 & 0 & 0 \\ [C_{off}] & 0 & - & 0 & 0 & 0 & s_{01} & 0 & 0 \\ [G_{off}] & 0 & 0 & - & 0 & 0 & 0 & s_{01} & 0 \\ [T_{off}] & 0 & 0 & 0 & - & 0 & 0 & 0 & s_{01} \\ [A_{on}] & s_{10} & 0 & 0 & 0 & - & \pi_C r_{AC} k & \pi_G r_{AG} k & \pi_T r_{AT} k \\ [C_{on}] & 0 & s_{10} & 0 & 0 & \pi_A r_{AC} k & - & \pi_G r_{CG} k & \pi_T r_{CT} k \\ [G_{on}] & 0 & 0 & s_{10} & 0 & \pi_A r_{AG} k & \pi_C r_{CG} k & - & \pi_T r_{GT} k \\ [T_{on}] & 0 & 0 & 0 & s_{10} & \pi_A r_{AT} k & \pi_C r_{CT} k & \pi_G r_{GT} k & - \end{bmatrix}$$

where k is a scaling constant determined by the proportion of time the sites spend in the on state. The matrix can be simplified into

$$Q = \begin{bmatrix} R_1 & R_2 \\ R_3 & kR_4 \end{bmatrix}$$

where each R element is a four by four matrix: R_1 contains the rates in the off state (all rates are 0), R_2 and R_3 describe the switching process (the diagonal elements are either s_{01} or s_{10}), and R_4 is the chosen model for the evolution in the on state.

The covarion-like model can be described as a general case of the proportion of invariable sites model (Huelsenbeck, 2002). As the switching rates go to zero, the proportion of these rates represented by the switch to the off state (s_{10}) becomes identical to the proportion of invariable sites. When the switching rates are zero, there is no exchange between the on and off states and the characters in the off state remain off throughout the tree; in other words, they are invariable sites.

Note that the covarion-like model implemented in MrBayes differs from the original covarion model in that sites switch completely independently of each other between the on and off states. To invoke the covarion-like model, simply use `lset covarion=yes` and then choose the desired nucleotide or amino acid model using the other `lset` and the `prset` options. The covarion-like model can be combined with the gamma model of rate variation across sites.

6.7.2 Relaxed Clock Models

6.8 Topology and Branch Length Models

The topology and branch length models in MrBayes are set using the `prset topologypr` options, which deal with the tree topology prior, and the `prset brlenspr` options, which deal with the branch lengths.

6.8.1 Unconstrained and Constrained Topology

There are two choices for the prior probability distribution on topology: uniform or constrained. By default, topologies are not constrained in the prior (`prset topologypr=uniform`), resulting in equal prior probability being associated with all possible labeled trees (unless a different topology prior is induced by the branch length model, see below). There are two instances in which you might want to constrain the topology: (1) when you want to contrast a hypothesis of monophyly for a group with the more general hypothesis with no topological constraints; and (2) when you want to infer ancestral states for a particular node in the tree. In both cases, you specify the constraint(s) first by listing the taxa

that should form a monophyletic group. For instance, if you wanted to constrain taxa 4, 5 and 6 to be monophyletic, you would use

```
constraint my_constraint -1 = 4 5 6;
```

This defines a constraint called “my_constraint” forcing taxa 4, 5 and 6 to form a monophyletic group in all trees that are sampled from the chain. In future versions of MrBayes, the value following the name of the constraint (-1 here), will give the relative probability of trees having the constrained partition. A negative number will force the constraint to always be present in the sampled trees; a positive number will specify how many times more likely the trees with the constraint are compared to the trees not having it. In version 3.2, however, MrBayes ignores this value and always treats the constraint as absolute.

When you define constraints, make sure that you have the outgroup selected correctly. By default, MrBayes uses the first taxon in the data matrix as the outgroup. You can change this by using the outgroup command. For instance, if you want taxon number 7 called “My_taxon” to be the outgroup, either use **outgroup 7** or **outgroup My_taxon**. MrBayes 3.2 only allows a single taxon as the outgroup.

Before the constraints take effect, you have to invoke them by using **prset topologypr=constraints(<comma-separated list of constraints>)**. For instance, to enforce the constraint my_constraint defined above, use **prset topologypr=constraints(my_constraint)**.

6.8.2 Non-clock (Standard) Trees

If you do not want to enforce a molecular clock, you choose an unconstrained branch length prior. Actually, you do not have to do anything because unconstrained branch lengths are the default. You can associate unconstrained branch lengths with either a uniform prior from 0 to some arbitrary value or an exponential prior. The default is an exponential distribution with parameter 10 (**Exponential(10)**); it has an expectation of 0.1(= 1/10) but (in principle) it allows branch lengths to vary from 0 to infinity. The exponential distribution apparently puts a lot more probability on short branches than on long branches.

However, because transition (substitution) probabilities change rapidly at small branch lengths but only very slowly at long branch lengths, the exponential prior is actually closer to an uninformative prior than the uniform distribution. We advise against using a uniform prior on branch lengths because of the large prior probability it puts on long branches and their close-to-random substitution probabilities.

To change the prior on unconstrained branch lengths you use `prset brlenspr`. For instance, assume you wanted to use an exponential prior with parameter 1 instead of the default prior. This prior is set by typing `prset brlenspr=unconstrained:exponential(1)`.

6.8.3 Strict Clock Trees

MrBayes implements three strict clock models: the simple (uniform) model, the birthdeath model, and the coalescence model. The birth-death model and coalescence models both have additional parameters describing the tree-generating process, whereas the simple model does not.

In the *birth – death model* (see Yang and Rannala (1997), for a Bayesian implementation), trees are generated according to a birth-death model with a speciation and an extinction rate. The model, as implemented in MrBayes, can also be associated with a sampling probability of terminal lineages. The priors for these three parameters are set using the `speciationpr`, `extinctionpr`, and `sampleprob` parameters of the `prset` command.

In the *coalescence model*, the tree generating process is looked at from the opposite perspective, backward in time. Instead of lineages branching, this model sees them as coalescing into fewer and fewer ancestral lineages. This process occurs at a rate determined by the θ (theta) parameter; it is also affected by the ploidy of the data (haploid or diploid). The prior for the theta parameter is set using `prset thetapr`. The ploidy level is set using `lset ploidy`.

The *simple clock* model is invoked by `prset brlenspr=clock:uniform`. There is only one additional parameter in the simple clock model, namely the total tree

height. The prior for this parameter is set by `prset treeheightpr`. The default prior is an exponential distribution with parameter 1.0 (`Exponential(1.0)`).

6.8.4 Relaxed Clock Trees

Relaxed clock models and functions for dating are not implemented in MrBayes 3.1. They are available in version 3.2.

6.9 Partitioned Models

MrBayes provides great flexibility in setting up partitioned models. By default, the characters are divided into partitions based on the data type. If there is only one data type in the matrix, then all characters will be in a single partition. The default partitioning scheme is called `default`. For information on how to set up a file with mixed data types, see the example file `cynmix.nex` and the tutorial in section 3 of this manual.

You can easily set up partitioning schemes that divide the characters up further than the default partition does using the `partition` command. The most convenient way of partitioning data is to define character sets first using the `charset` command. For instance, assume that you have concatenated nucleotide sequences from three genes in your data set of length 1962, 1050, and 2082 sites, respectively. Then you create character sets for those three genes using

```
charset gene1 = 1-1962;  
charset gene2 = 1963-3012;  
charset gene3 = 3013-.;
```

in a MrBayes block. Note the use of the dot (.) as a synonym of the last site. You can also use the “backslash n” sequence to include every *n*th character in the preceding range of characters (see the description of the site specific rate model above). Once the character sets are defined, the partitioning scheme based on the genes is defined with the `partition` command and selected using the `set` command:

```
partition by_gene = 3:gene1,gene2,gene3;
set partition=by_gene;
```

Here, `by_gene` is the name we chose for the partitioning scheme. The name is followed by an equal sign, the number of partitions, and then a comma-separated list of characters to include in each partition. Note that MrBayes requires the partitioning scheme to include all characters. Say, for instance, that you wanted to run an analysis with only gene 1 and gene 2. Then define a two-partition scheme and exclude the characters represented by gene 3:

```
partition gene1&2 = 2:gene1,gene2 gene3;
exclude gene3;
set partition=gene1&2;
```

If the only purpose of the partition `gene1&2` is to allow exclusion of gene 3, then gene 3 can of course be included in either of the two partitions before being excluded.

Once the partitions have been correctly set up, MrBayes allows you to set models for individual partitions using the `lset applyto` and `prset applyto` mechanism. For instance, assume that we have two partitions, a standard data partition (partition 1) and a nucleotide partition (partition 2), and want to apply a GTR model to the nucleotide data, gamma-shaped rate variation to both partitions, and allow the partition rates to be different. Then we would use the commands

```
lset applyto=(2) nst=6;
lset applyto=(all) rates=gamma;
prset applyto=(all) ratepr=variable;
```

By default, all model parameters that are identical and have the same prior probability distribution associated with them, are linked across partitions (they are assumed to be one and the same parameter). To unlink parameters, use the `unlink` command. For instance, assume that we want to unlink the shape parameter across the partitions discussed above (after all, why should the standard data and the molecular data have the same distribution of rates across sites?). This would be achieved using

```
unlink shape=(all);
```

If you unlink parameters by mistake, they can be linked again using the `link` command. All of the commands mentioned above and given as they would appear in a MrBayes block in a Nexus file can of course be entered from the command line as well (without the trailing semicolon). However, it is often more convenient to have them in either your data file or in a separate Nexus file that you process after you have read in your data. MrBayes will keep the data set in memory until you read in a new data block, so you can have your different MrBayes blocks pertaining to the same data file distributed over as many separate Nexus files as you like.

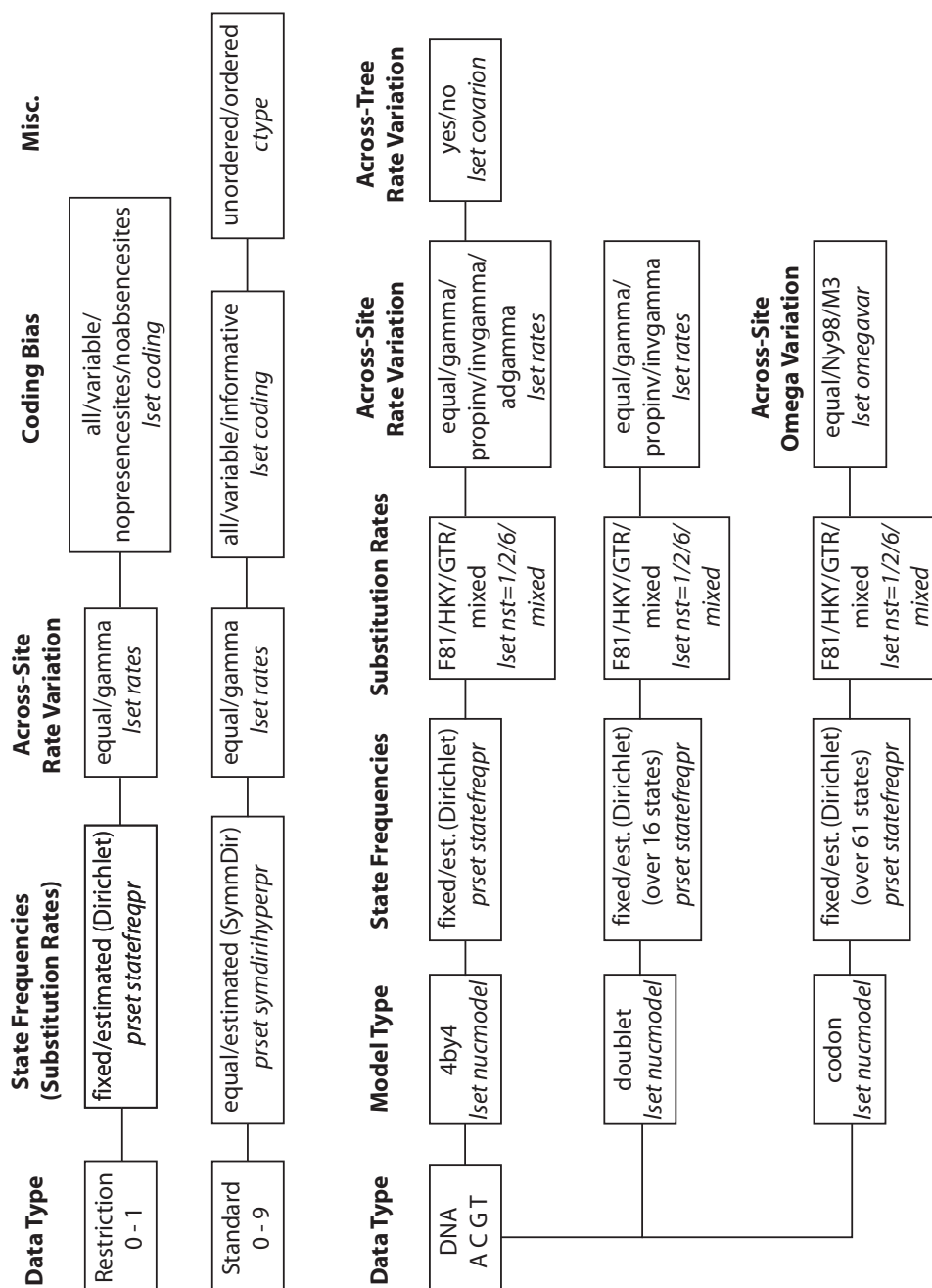
We recommend that, before you run your analysis, you check the current model settings using the `showmodel` command. This command will list all the active parameters and how they are linked across partitions, as well as the priors associated with each parameter.

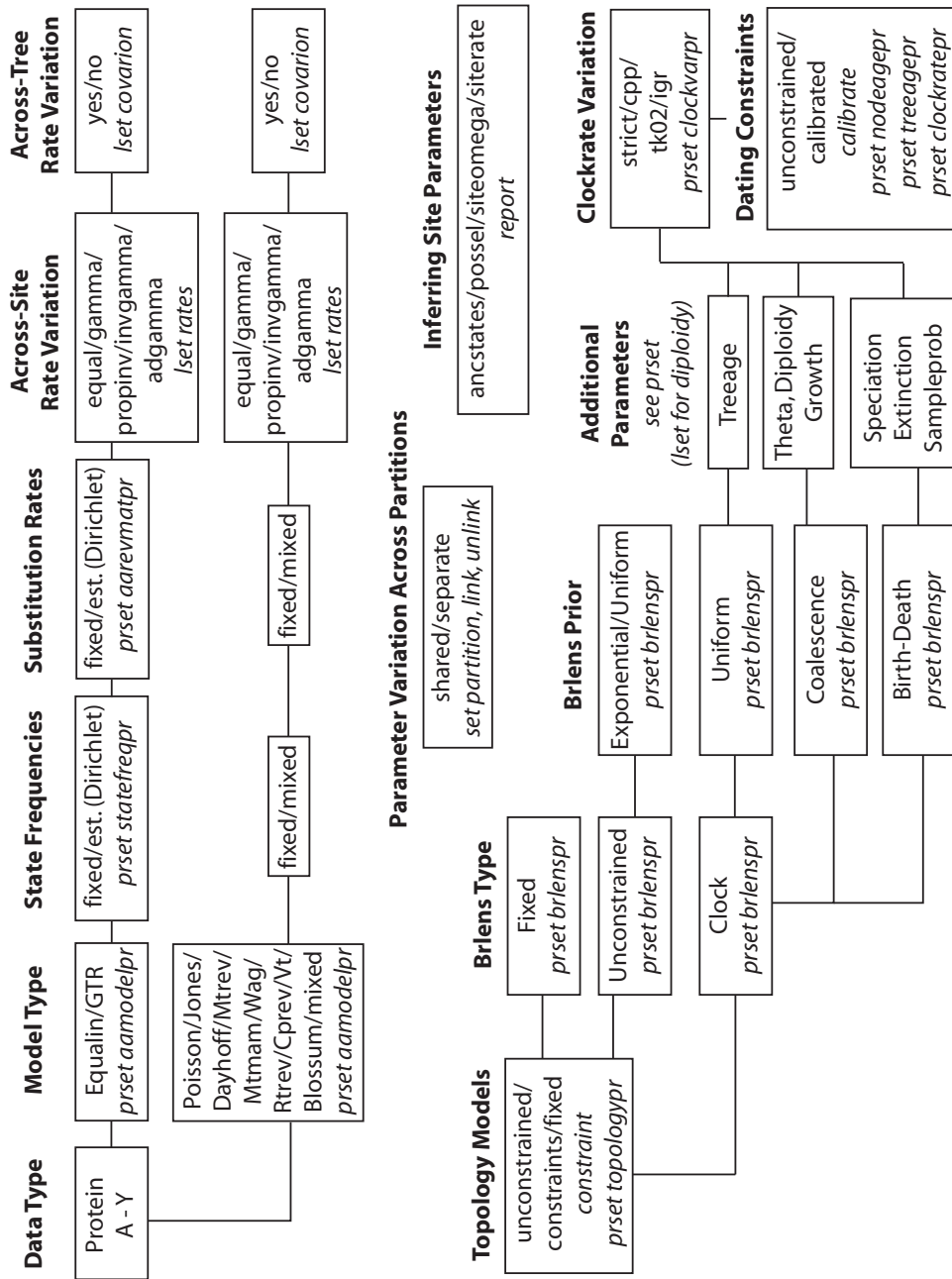
Finally, we want to give you a warning. Even though MrBayes allows you to easily set up extremely complex and parameter-rich models, and the Bayesian MCMC approach is good at handling such models, think carefully about the parameters you introduce in your model. There should be at least some reasonable chance of estimating the parameters based on your data. For instance, a common mistake is to use a separate GTR model for a partition with so few substitutions that there is not a single observation for several rate categories. Making sure there are at least some observations allowing you to estimate each parameter is good practice. Over-parameterized models often result in problems with convergence in addition to the excessive variance seen in the parameter estimates.

Appendix A

Overview of Models and Moves

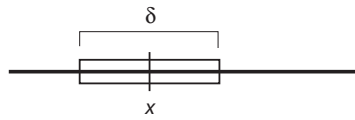
Models supported by MrBayes 3 (simplified)





The most common proposal types used by MrBayes 3

Sliding Window Proposal



New values are picked uniformly from a sliding window of size δ centered on x .

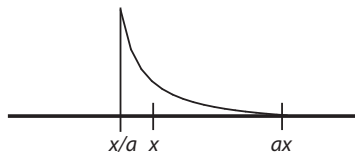
Tuning parameter: δ

Bolder proposals: increase δ

More modest proposals: decrease δ

Works best when the effect on the probability of the data is similar throughout the parameter range

Multiplier Proposal



New values are picked from the equivalent of a sliding window on the log-transformed x axis.

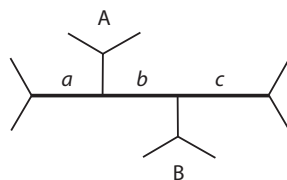
Tuning parameter: $\lambda = 2 \ln a$

Bolder proposals: increase λ

More modest proposals: decrease λ

Works well when changes in small values of x have a larger effect on the probability of data than changes in large values of x . Example: branch lengths.

LOCAL



Three internal branches - a , b , and c - are chosen at random. Their total length is changed using a multiplier with tuning parameter λ .

One of the subtrees A or B is picked at random.

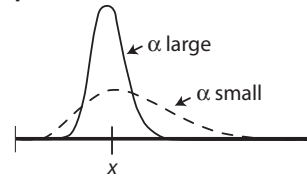
It is randomly reinserted on $a + b + c$ according to a uniform distribution

Bolder proposals: increase λ

More modest proposals: decrease λ

Changing λ has little effect on the boldness of the proposal

Dirichlet proposal



New values are picked from a Dirichlet (or Beta) distribution centered on x .

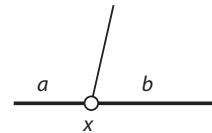
Tuning parameter: α

Bolder proposals: decrease α

More modest proposals: increase α

Works well for proportions, such as revmat and statefreqs.

Node Slider



Two adjacent branches a and b are chosen at random

The length of $a + b$ is changed using a multiplier with tuning parameter λ

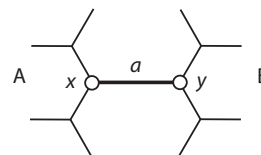
The node x is randomly inserted on $a + b$ according to a uniform distribution

Bolder proposals: increase λ

More modest proposals: decrease λ

The boldness of the proposal depends heavily on the uniform reinsertion of x , so changing λ may have limited effect

Extending TBR



An internal branch a is chosen at random

The length of a is changed using a multiplier with tuning parameter λ

The node x is moved, with one of the adjacent branches, in subtree A, one node at a time, each time the probability of moving one more branch is p (the extension probability).

The node y is moved similarly in subtree B.

Bolder proposals: increase p

More modest proposals: decrease p

Changing λ has little effect on the boldness of the proposal.

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