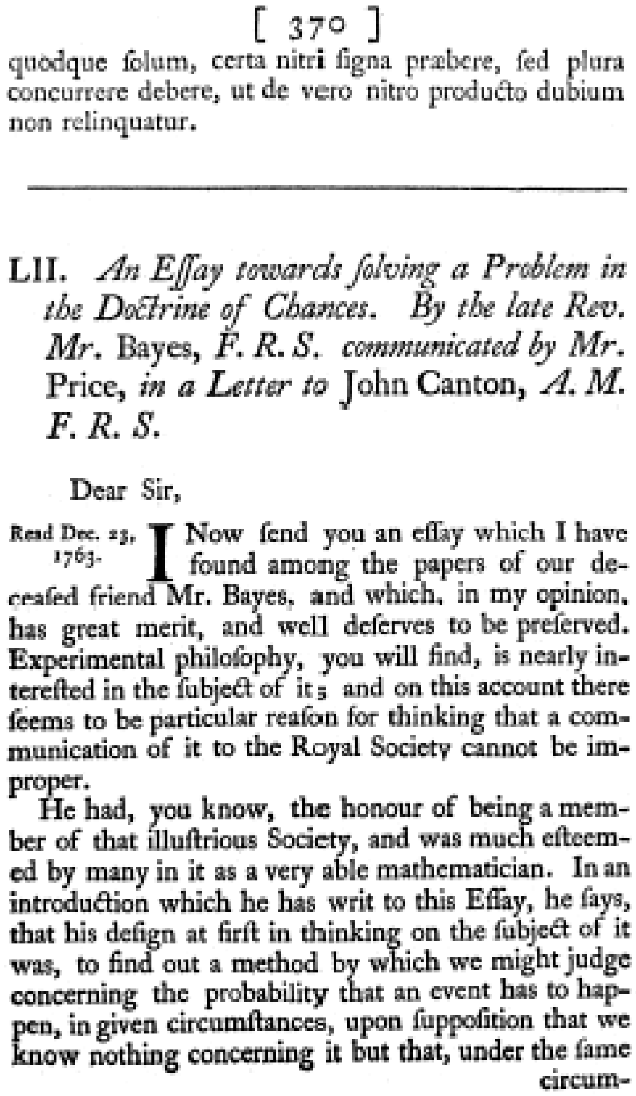
Phylogenetic tree building methods

method of building trees	type of data	
	distances	DNA sequences or other characters
clustering algorithm	UPGMA neighbor-joining tree	
optimality criterion	minimum evolution tree	parsimony maximum likelihood → Bayesian analysis

Bayesian analysis

Bayes, T. 1763. An essay towards solving a problem in the doctrine of chances. Phil. Trans. Roy. Soc. London 53: 370-418.



[370]

quodque ſolum, certa nitri ſigna præbere, ſed plura concurrere debere, ut de vero nitro producto dubium non relinquatur.

LII. *An Eſſay towards ſolving a Problem in the Doctrine of Chances. By the late Rev. Mr.* Bayes, *F. R. S. communicated by Mr.* Price, *in a Letter to* John Canton, *A. M. F. R. S.*

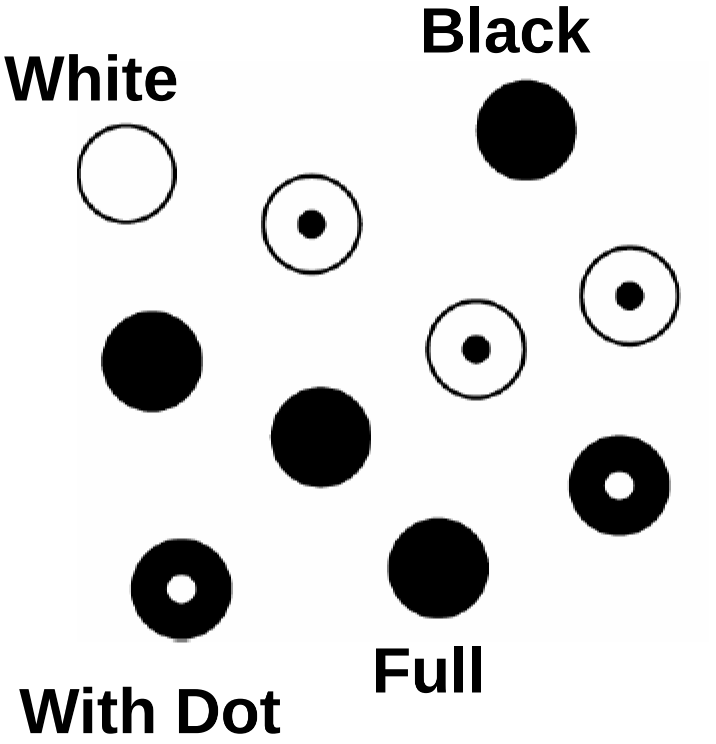
Dear Sir,

Read Dec. 23, 1763.

I Now ſend you an eſſay which I have found among the papers of our deceaſed friend Mr. Bayes, and which, in my opinion, has great merit, and well deſerves to be preſerved. Experimental philoſophy, you will find, is nearly intereſted in the ſubject of it; and on this account there ſeems to be particular reaſon for thinking that a communication of it to the Royal Society cannot be improper.

He had, you know, the honour of being a member of that illuſtrious Society, and was much eſteemed by many in it as a very able mathematician. In an introduction which he has writ to this Eſſay, he ſays, that his deſign at firſt in thinking on the ſubject of it was, to find out a method by which we might judge concerning the probability that an event has to happen, in given circumſtances, upon ſuppoſition that we know nothing concerning it but that, under the ſame circum-

Joint probability



Pr(W) = 0,4
Pr(B) = 0,6
Pr(D) = 0,5
Pr(F) = 0,5

Pr(◉) = Pr(B,D) = 0,2

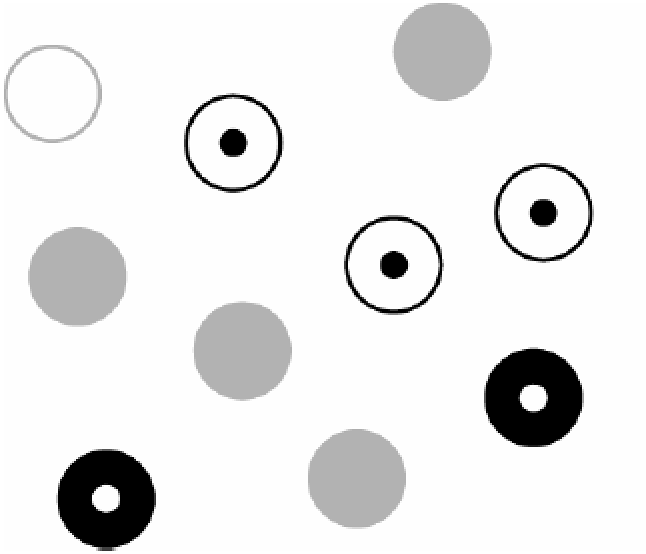
Pr(●) = Pr(B,F) = 0,4

Pr(⊙) = Pr(W,D) = 0,3

Pr(○) = Pr(W,F) = 0,1

Paul O. Lewis, Woods Hole Molecular Evolution Workshop, 2006

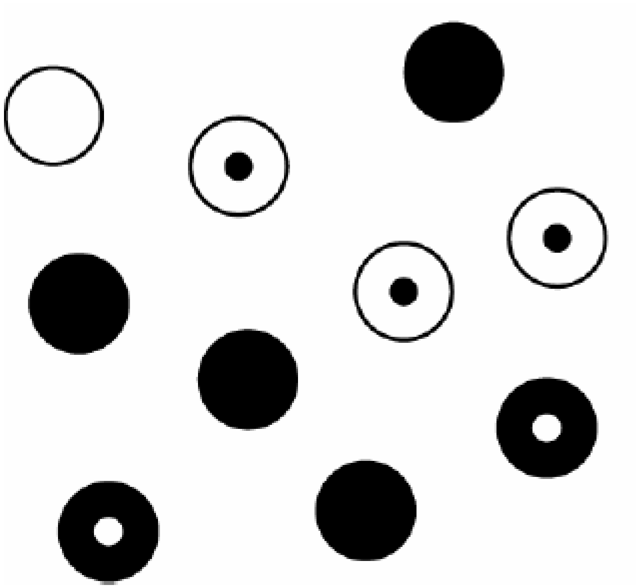
Conditional probability



Pr(B|D) = 2/5 = 0,4

Probability of B given
that D is true
(i.e. excluding all F)

Bayes rule (theorem)



$\Pr(B,D) = 0{,}2 =$

$= \Pr(D) \times \Pr(B|D) = \Pr(B) \times \Pr(D|B)$

$1/2 \times 2/5 = 3/5 \times 1/3$

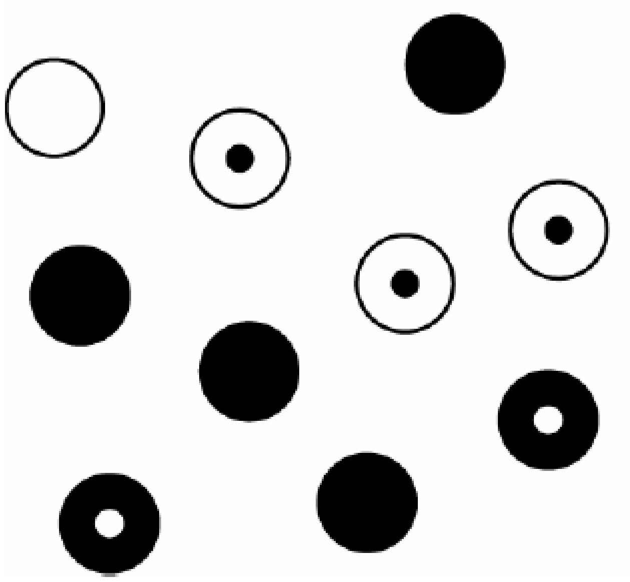
$$\Pr(B|D) = \frac{\Pr(B) \times \Pr(D|B)}{\Pr(D)}$$

$$= \frac{3/5 \times 1/3}{1/2} = 2/5$$

Marginal (prior) probability - probabilities of observing D and F, respectively without any given conditions

$$\Pr(B|D) = \frac{\Pr(B) \times \Pr(D|B)}{\Pr(D)}$$

$$\Pr(W|D) = \frac{\Pr(W) \times \Pr(D|W)}{\Pr(D)}$$



$$\Pr(D) = \Pr(W,D) + \Pr(B,D) = [\Pr(W) \times \Pr(D|W)] + [\Pr(B) \times \Pr(D|B)]$$

$$1/2 \quad = \quad 3/10 \quad + \quad 1/5 \quad = \quad [4/10 \times 3/4] \quad + \quad [3/5 \times 1/3]$$

Bayes rule (theorem)

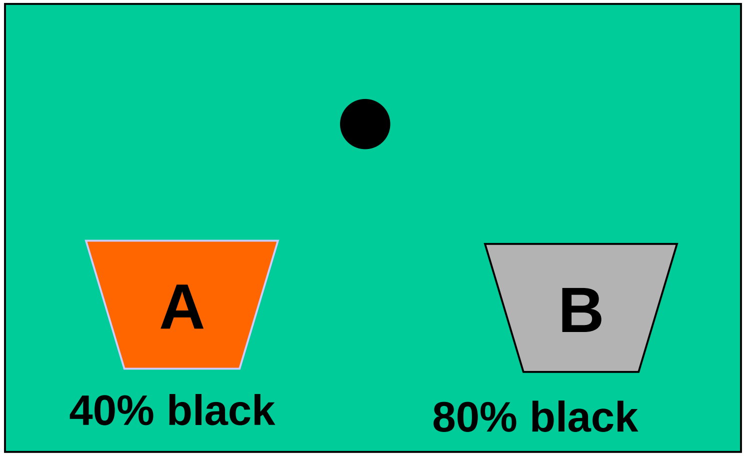
$$\Pr(\text{tree} \mid \text{data}) = \frac{\Pr(\text{data} \mid \text{tree}) \times \Pr(\text{tree})}{\Pr(\text{data})}$$

$$\Pr(\text{hypothesis} \mid \text{data}) = \frac{\Pr(\text{data} \mid \text{hypothesis}) \times \Pr(\text{hypothesis})}{\Pr(\text{data})}$$

$\Pr(\text{tree} \mid \text{data})$	= posterior probability
	= probability that the tree is correct
$\Pr(\text{data} \mid \text{tree})$	= likelihood of the tree
$\Pr(\text{tree})$	= prior probability of the tree
$\Pr(\text{data})$	= probability of data (a normalizing constant, which ensures that the sum of the posterior probabilities is 1.)

Provides the possibility of calculating probabilities of various hypotheses given existing data.

Example: black and white marbles in urns



Pr(D,H) = Pr(D)Pr(H|D)

= Pr(H)Pr(D|H)

Pr(H|D) = Pr(H) Pr(D|H) / Pr(D)

Data – we selected black marble

Hypotheses – from which urn it was taken?

Pr(H|D) = posterior probability, i.e. probability of the hypothesis given the data

Pr(D|H) = likelihood of hypothesis, i.e. probability of the data given the hypothesis

Pr(H) = prior probability of hypothesis - unconditional probability of the hypothesis, specified without reference to the data

Pr(D) = unconditional probability of the data, which can be obtained, using the law of total probability, by calculating the sum of the product Pr(H)Pr(D|H) for all possible values of H

Lewis, P. O. 2001. Phylogenetic systematics turns over a new leaf. Trends Ecol. Evol. 16: 30-37.

Likelihood

is simply the probability that a single marble is black, given a particular urn hypothesis

The likelihood for urn A is 0.4
The likelihood for urn B is 0.8
The prior probability of each urn is 0.5

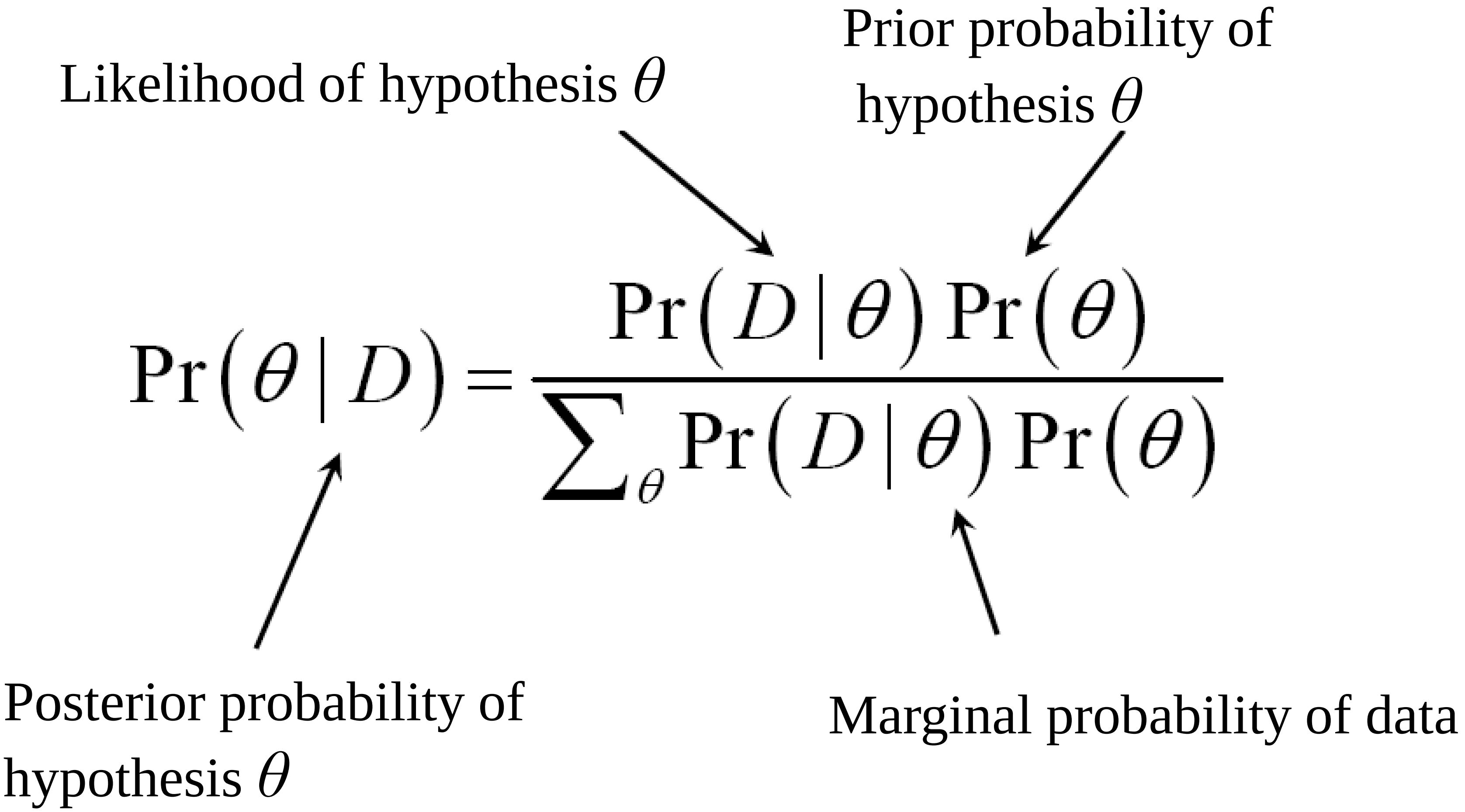
Pr (black marble was drawn)
= (0.5)(0.4) + (0.5)(0.8) = 0.6 - the sum of the product Pr(H)Pr(D|H) for all values of H (urn A and urn B)

Posterior probability

Pr (urn A / black marble was drawn)
= (0.5)(0.4) / 0.6 = 1/3
Pr (urn B / black marble was drawn)
= (0.5)(0.8) / 0.6 = 2/3

Thus, the probability that the black marble came from urn B, given the datum, is 2/3, In this case, the posterior distribution (0.33, 0.67) represents an updated version of the prior distribution (0.5, 0.5)

The previous example dealt with hypotheses with discrete values, in Bayesian analysis we mostly use continuous parameters. In such cases probabilities of discrete hypotheses are replaced by functions of probability densities.



Likelihood of hypothesis θ

Prior probability of hypothesis θ

$$\Pr(\theta \mid D) = \frac{\Pr(D \mid \theta)\Pr(\theta)}{\sum_{\theta}\Pr(D \mid \theta)\Pr(\theta)}$$

Posterior probability of hypothesis θ

Marginal probability of data

Prior probability density

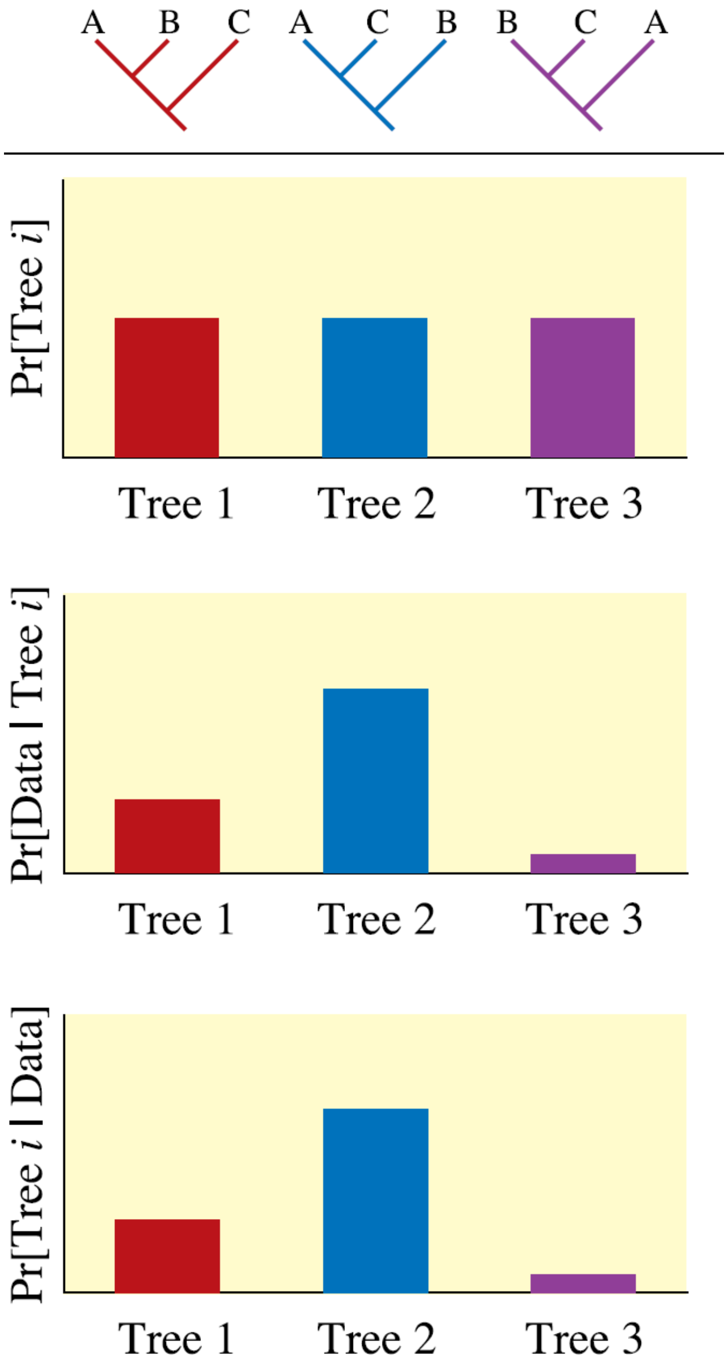
Likelihood

$$f\left(\theta \mid D\right) = \frac{f\left(D \mid \theta\right) f\left(\theta\right)}{\int_{\theta} f\left(D \mid \theta\right) f\left(\theta\right) d\theta}$$

Posterior probability density

Marginal probability of data

Huelsenbeck, J. P., F. Ronquist, R. Nielsen & J. P. Bollneck. 2001. Bayesian inference of phylogeny and its impact on evolutionary biology. Science 294: 2310-2314.



The prior probability – probability of the tree before observationas are taken, mostly all trees are considered equally probable (but some trees may have higher probability, e.g., based on taxonomic knowledge).

The likelihood – proportional to the probability of observations (e.g., DNA alignment) conditional on the tree topology; it is calculated using models of character evolution (requires making specific assumptions about the processes generating the observations.

The posterior probability of a tree – is the probability of the tree conditional on the observatioins; it is obtained by combining the prior and likelihood for each tree using the Bayes' Formula.

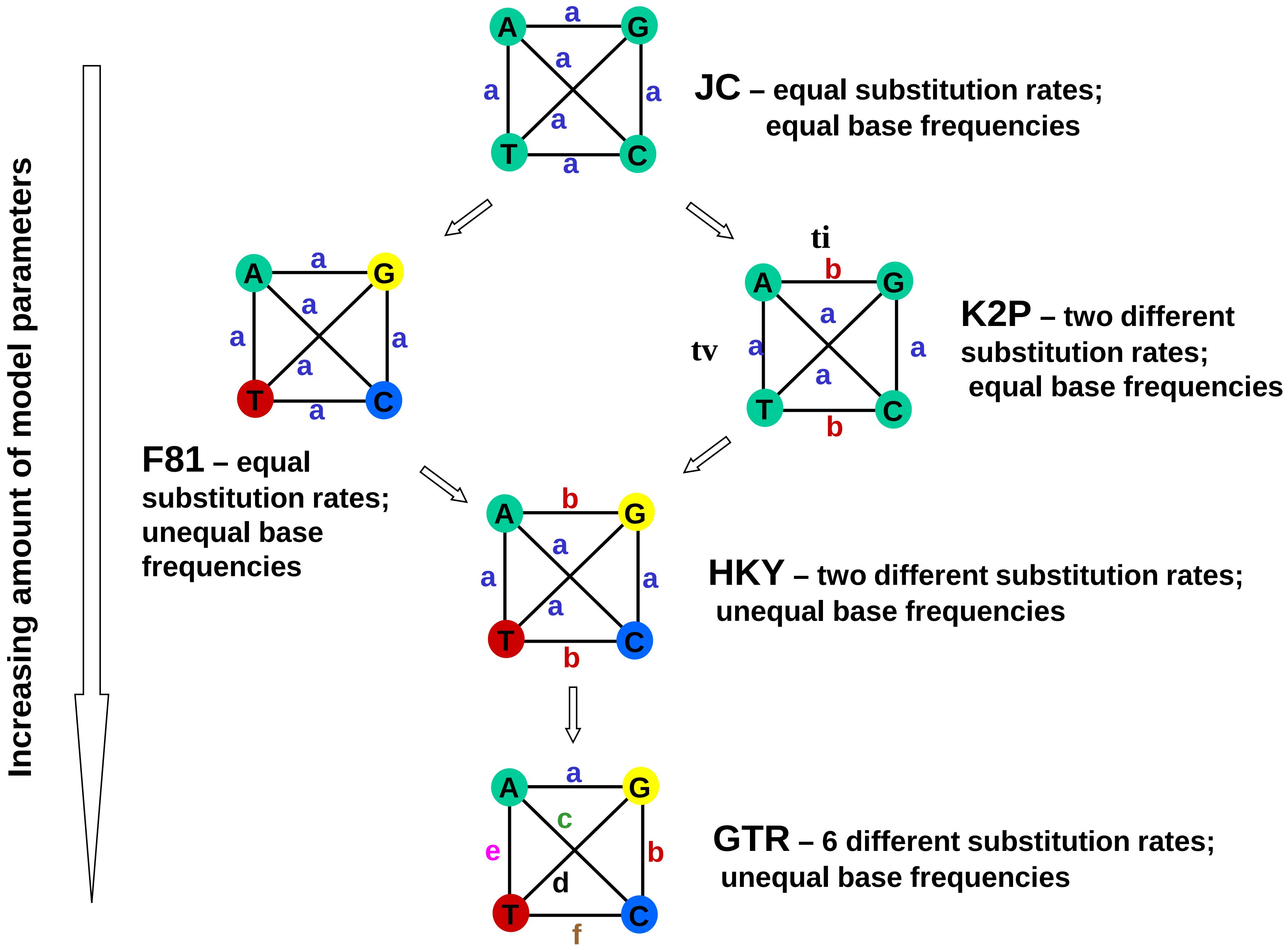
When calculating posterior probabilities using Bayes’ rule we face the problem that we need to summarise information on all trees, for all trees all branch length parameters and also for all parameters of substitution models.

Therefore we use Markov chain, which moves in the space defined by the model parameters.

Each step in Markov chain involves random modification of the tree topology, branch length or parameter in substitution model. If the posterior probability for such modified tree is higher this step is accepted.

If the posterior probability for such modified tree is lower, then we use the function that is based on the ratio of new and old posterior probability. Decission whether the given step is accepted or not depends on the value of this ratio.

Substitution models



Increasing amount of model parameters

JC – equal substitution rates;
equal base frequencies

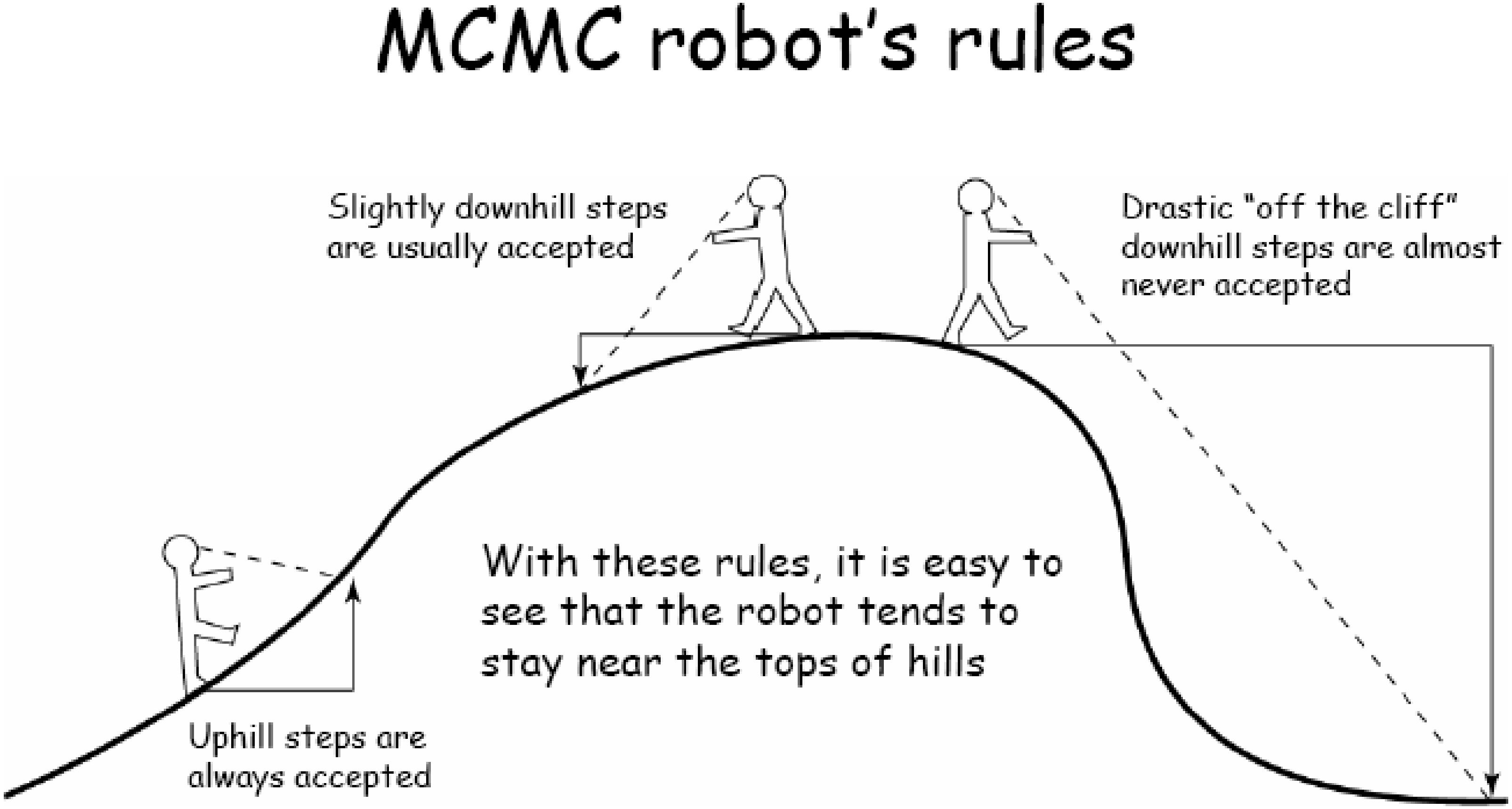
K2P – two different substitution rates;
equal base frequencies

F81 – equal substitution rates;
unequal base frequencies

HKY – two different substitution rates;
unequal base frequencies

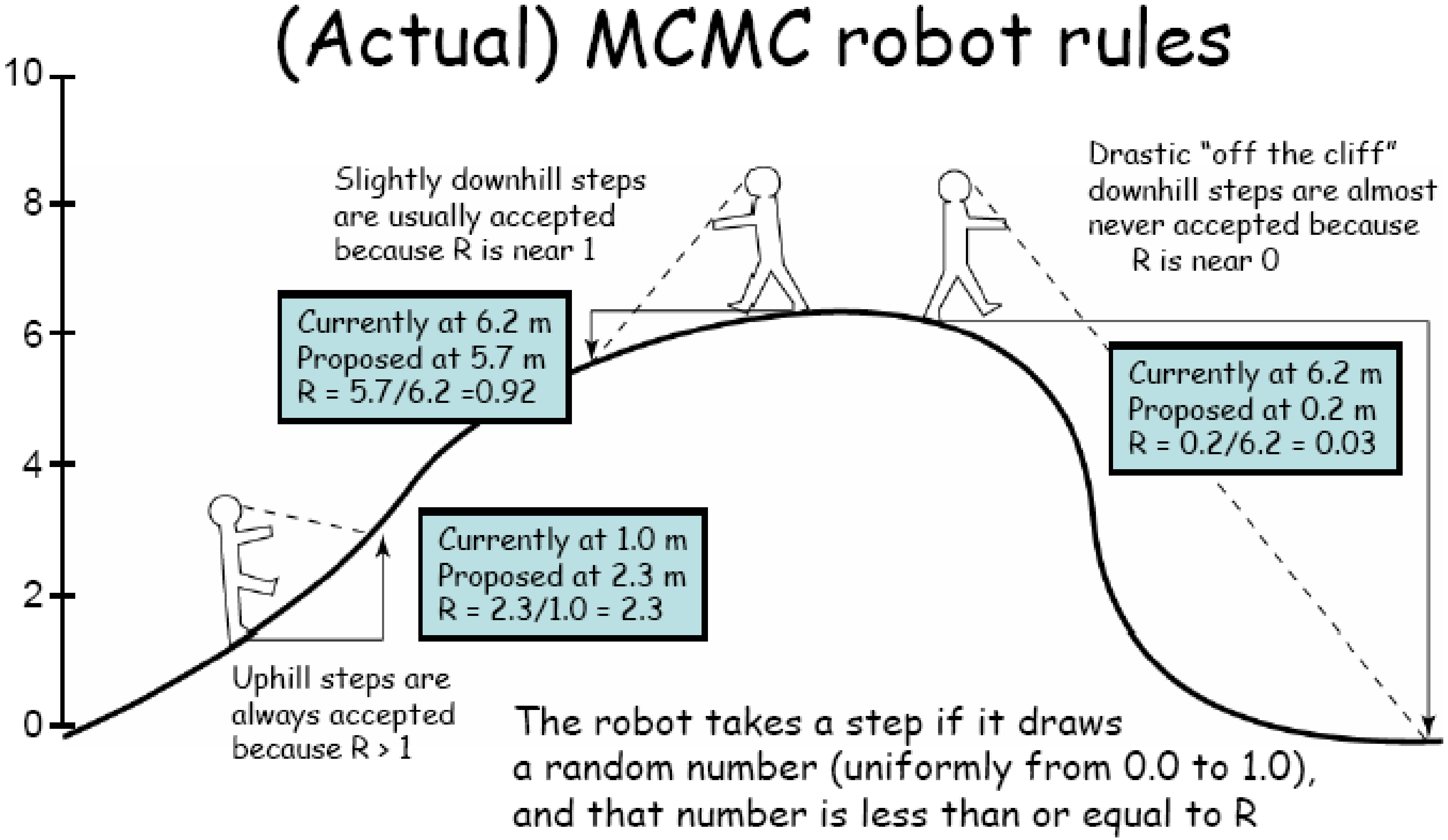
GTR – 6 different substitution rates;
unequal base frequencies

MCMC – Markov chain Monte Carlo method



Lewis, P. O. 2001. Phylogenetic systematics turns over a new leaf. Trends Ecol. Evol. 16: 30-37.
Paul O. Lewis, Woods Hole Molecular Evolution Workshop, 2006

MCMC – Markov chain Monte Carlo method



(Actual) MCMC robot rules

Slightly downhill steps are usually accepted because R is near 1

Currently at 6.2 m
Proposed at 5.7 m
R = 5.7/6.2 =0.92

Drastic "off the cliff" downhill steps are almost never accepted because R is near 0

Currently at 6.2 m
Proposed at 0.2 m
R = 0.2/6.2 = 0.03

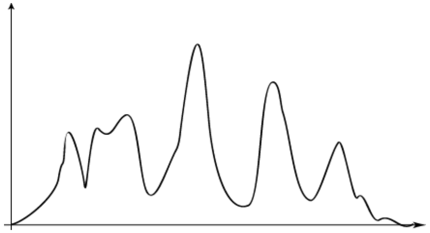
Currently at 1.0 m
Proposed at 2.3 m
R = 2.3/1.0 = 2.3

Uphill steps are always accepted because R > 1

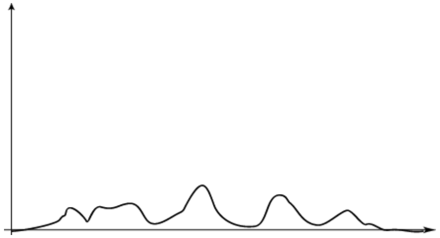
The robot takes a step if it draws a random number (uniformly from 0.0 to 1.0), and that number is less than or equal to R

MCMCMC –
Metropolis coupled Markov chain Monte Carlo method

Cold landscape,
high hills and deep valleys



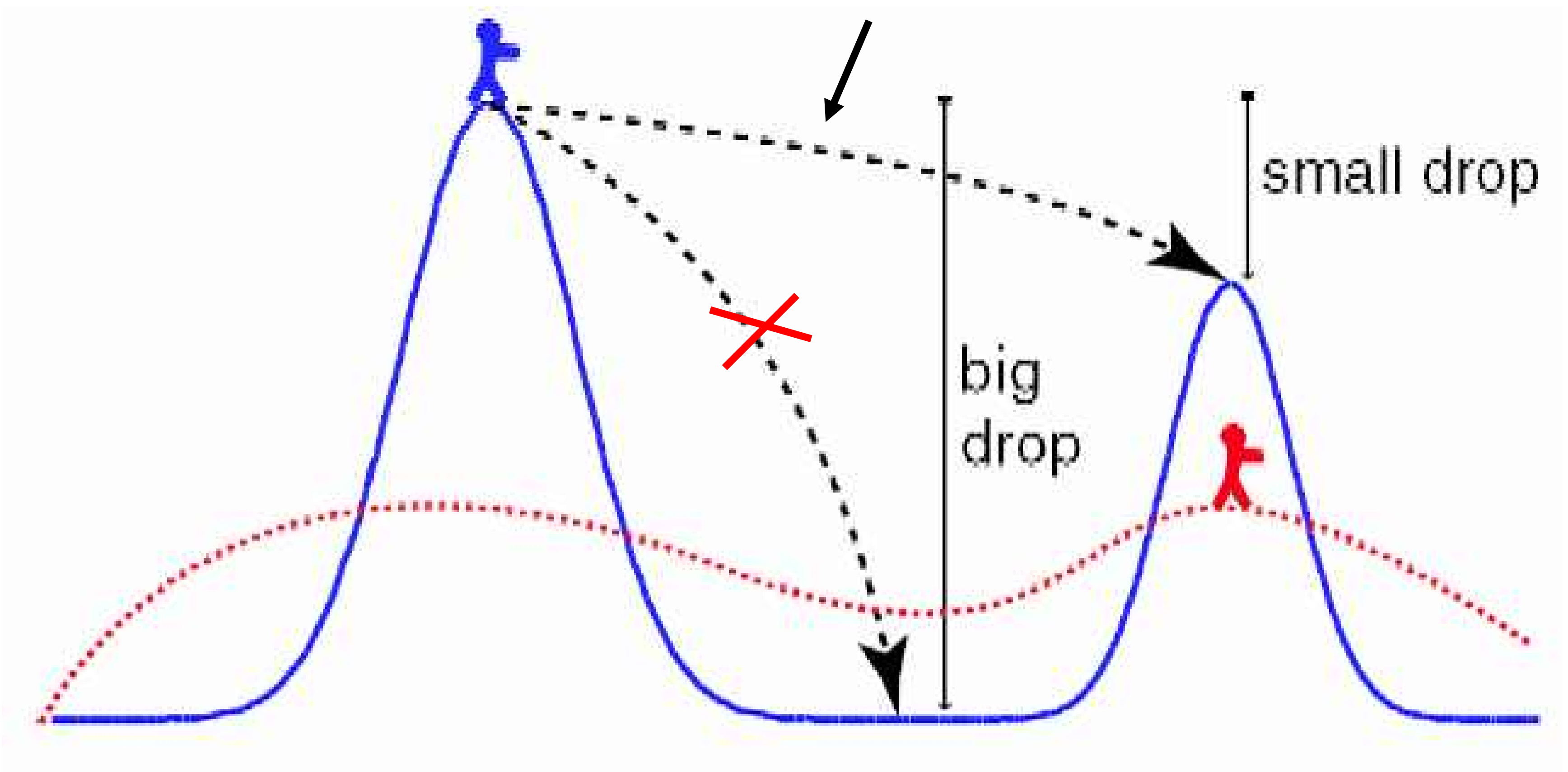
Heated landscape,
low hills and shallow valleys



MCMCMC –
Metropolis coupled Markov chain Monte Carlo method

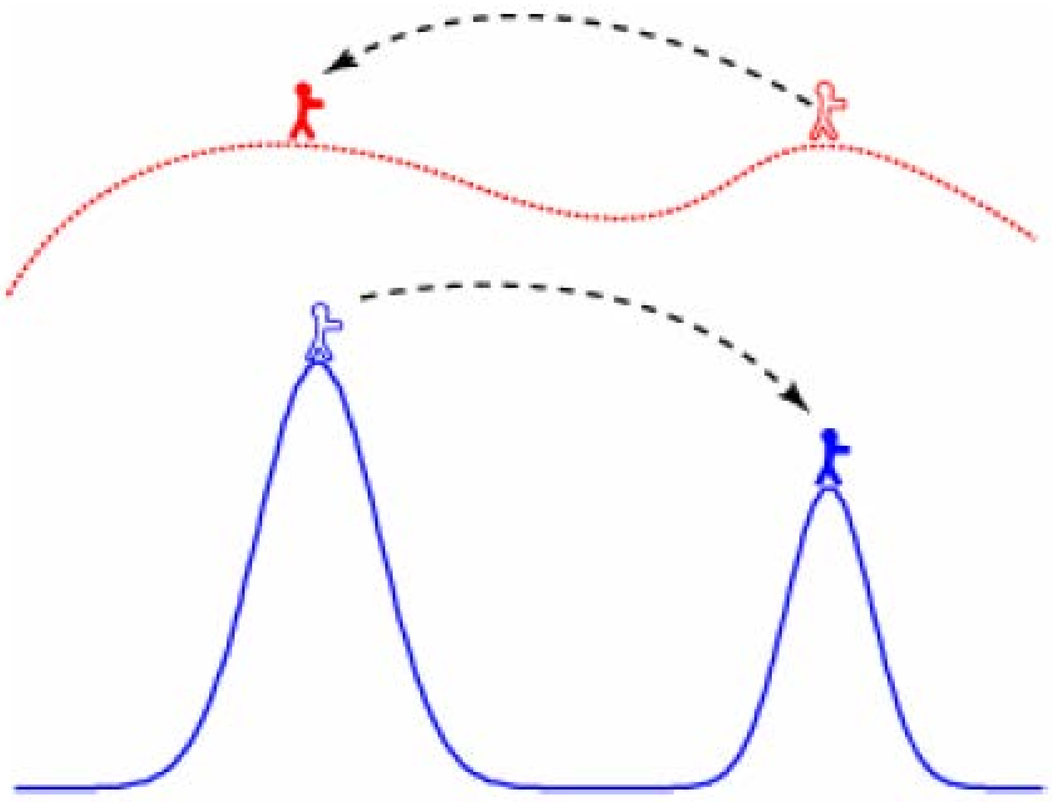
The heated chain serves to the cold chain aa a scout

Step proposed by the scout



MCMCMC –
Metropolis coupled Markov chain Monte Carlo method

In each generation there is a certain probability that the heated and cold chain exchange their positions and thus the cold chain can reach the higher hill



MCMCMC –
Metropolis coupled Markov chain Monte Carlo method

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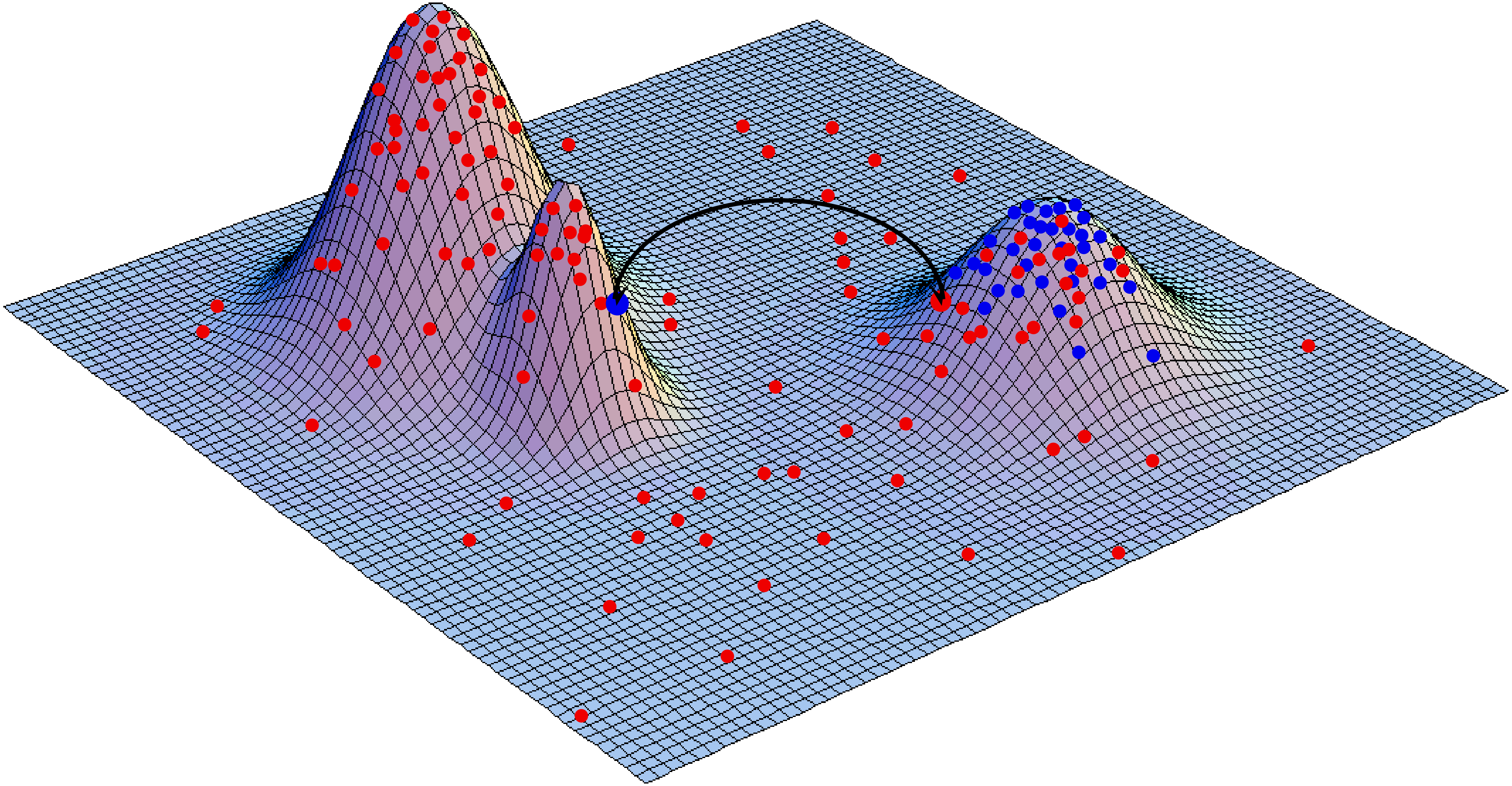
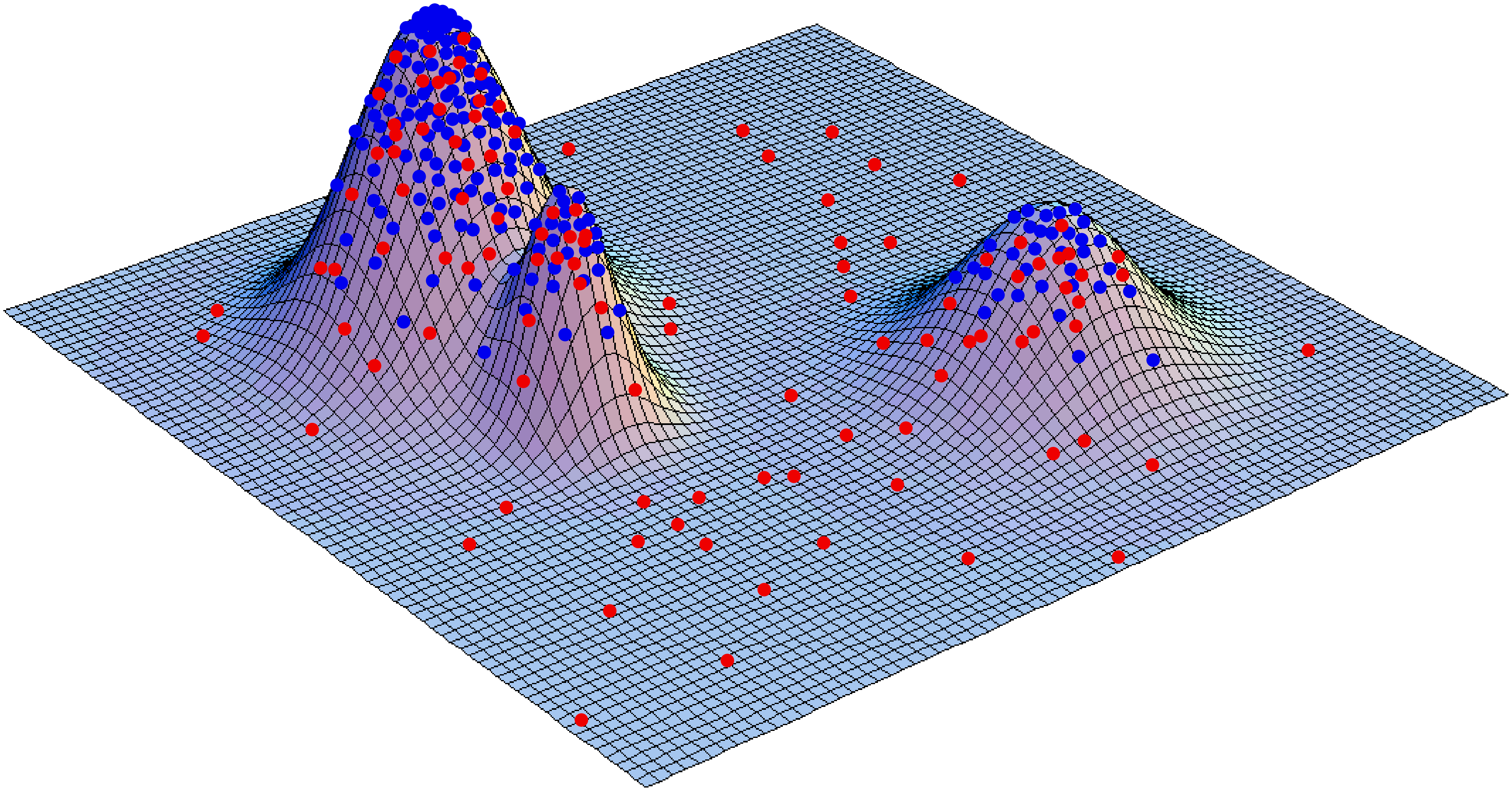
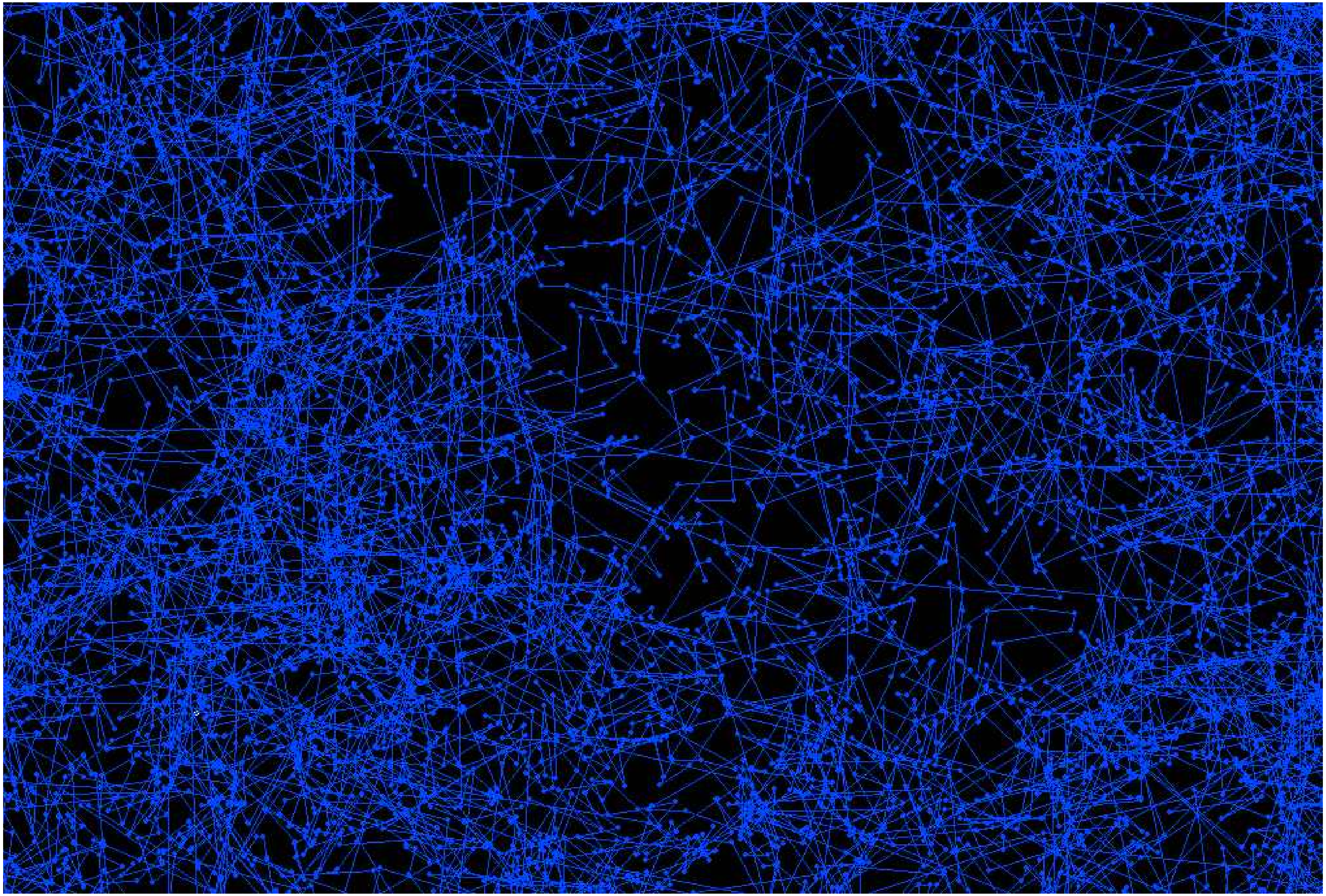
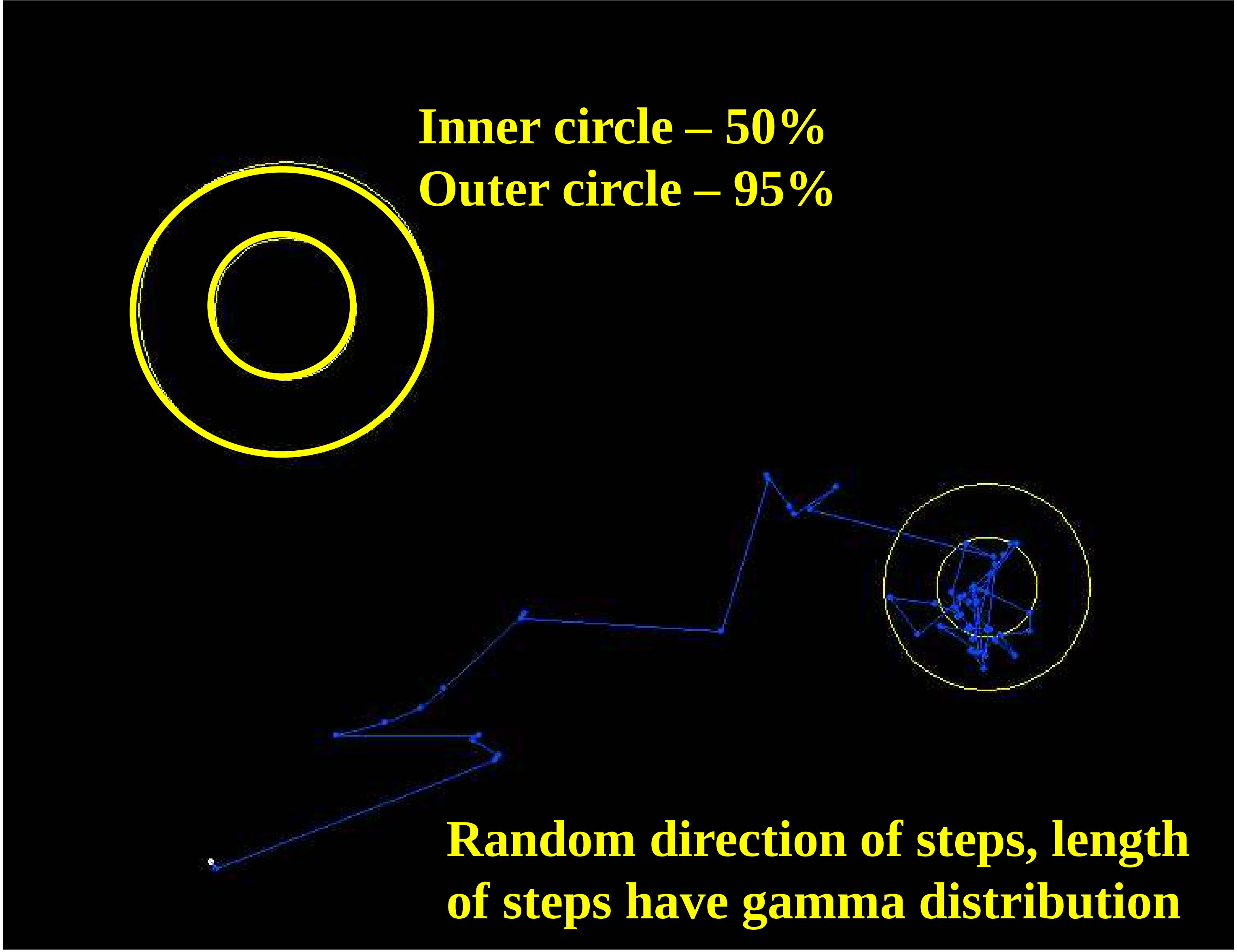




MCMC – Markov chain Monte Carlo method



Robot makes only random steps

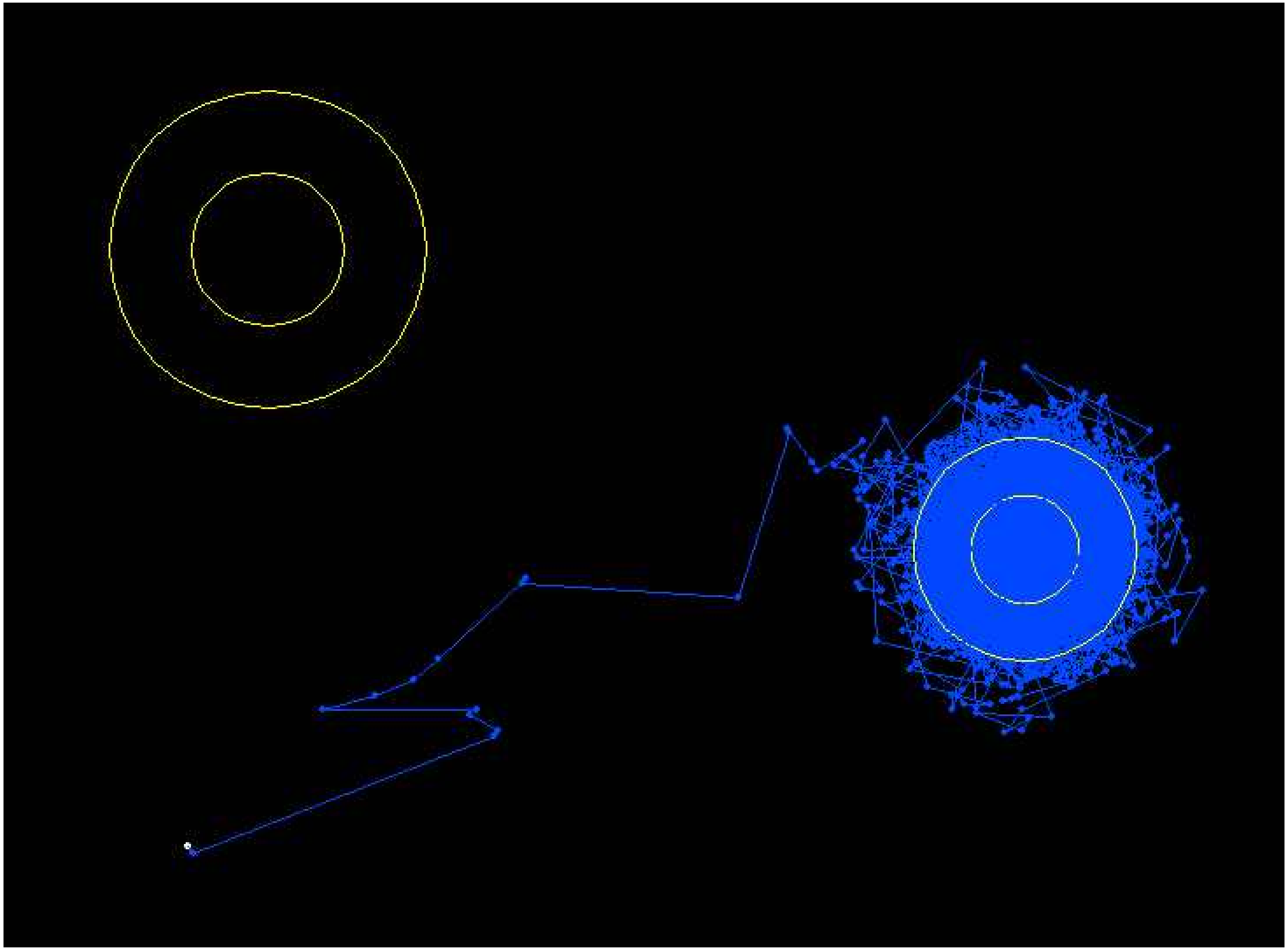


Inner circle – 50%
Outer circle – 95%

Random direction of steps, length of steps have gamma distribution

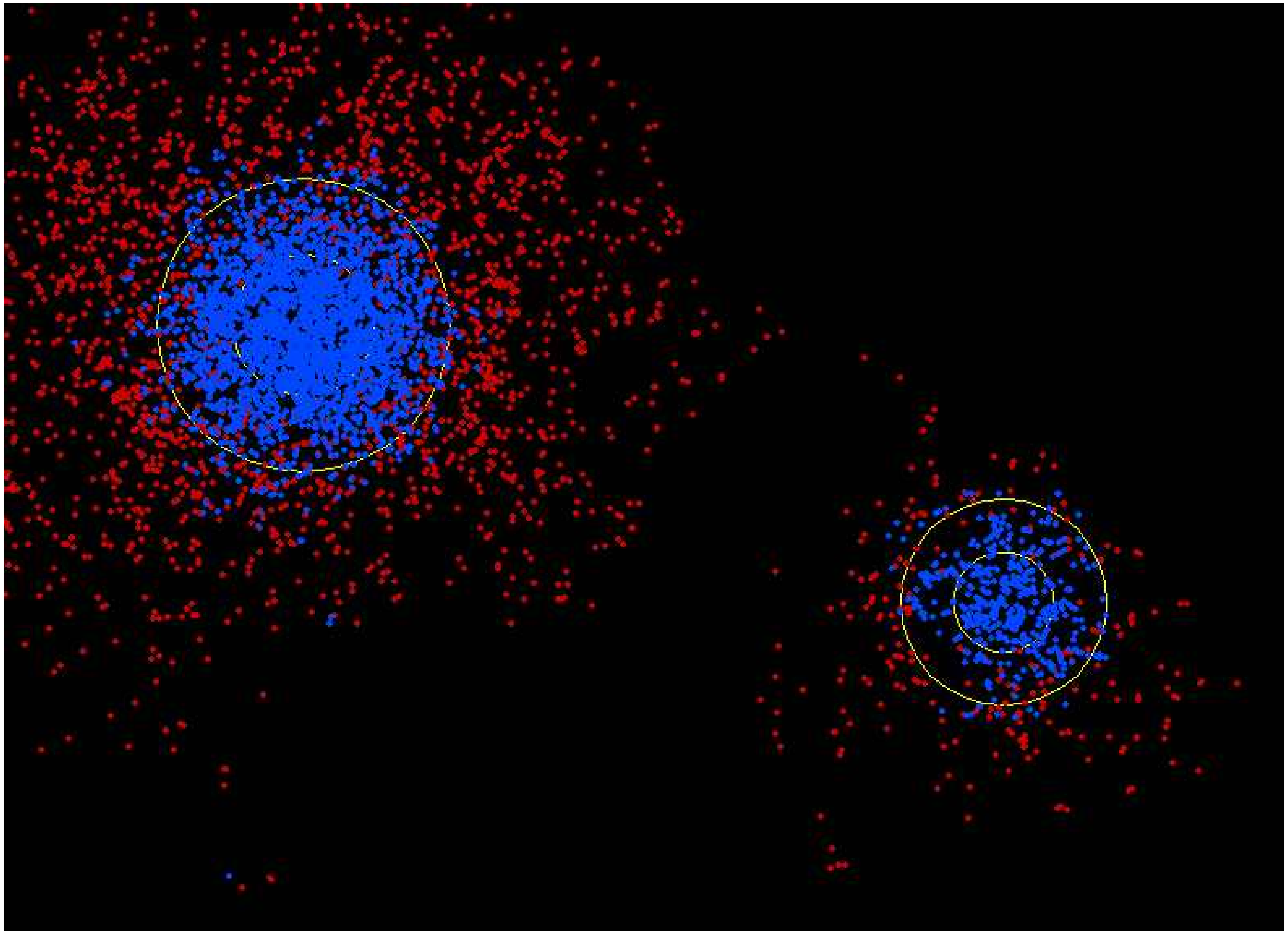
There are two hills in the landscape and the robot after several steps (burn-in period) reaches the top of of the hills ...

MCMC – Markov chain Monte Carlo method



... where he stays, although there is also higher hill in the landscape

MCMCMC –
Metropolis coupled Markov chain Monte Carlo method



Heated chain **(steps of the robot that serves as a scout), gives the** **cold chain** **(another robot) the reach higher hill ...**

MCMC – Markov chain Monte Carlo metóda

Landscape – evolution trees with certain length of branches and other parameters

Height of the hill – posterior probability density that is a product of:

likelihood – probability of data given certain tree and its parameters (lengths of branches, substitution parameters, speed of changes on certain positions)

prior probability density

MCMC – Markov chain Monte Carlo method

Steps:

We start with the randomly chosen tree and arbitrarily chosen length of branches and substitution model parameters

Each generation is represented by one randomly chosen possibility of the following two ones:

Another tree is chosen and it is accepted or not

Another model parameter is chosen and it is accepted or not

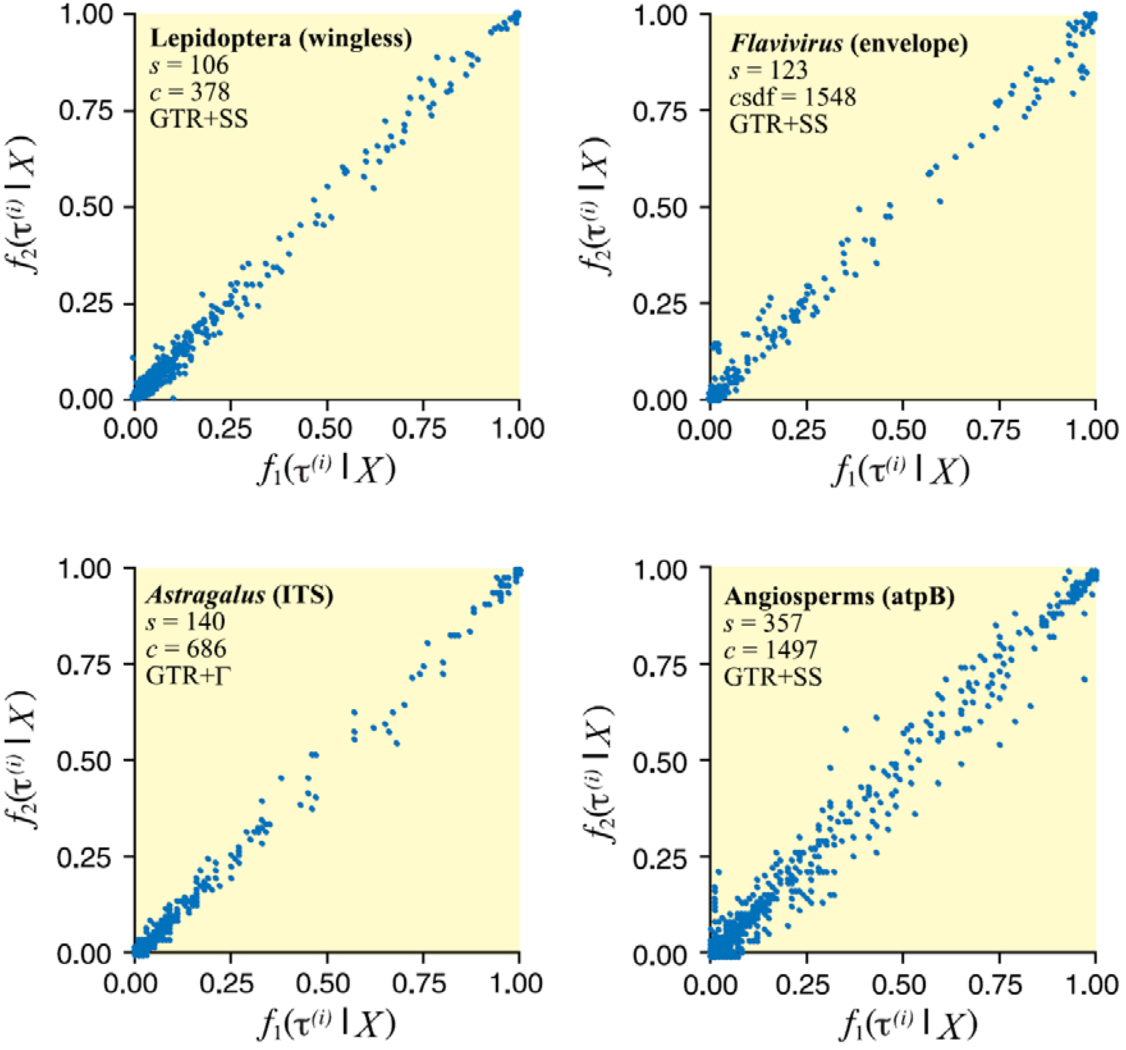
Each *k* generations (usually 100) the tree is recorded with its branch lengths and all model parameters

After *n* generations the Markov chain reaches stable equilibrium when certain topologies appear in stable frequency. This frequency is a convenient approximation of their posterior probabilities.

In the equilibrium stage the likelihood values are more-less stable.

From the acquired trees in the equilibrium stage we can make consensus tree.

The number of times the respective clade appears on the trees in this tree selection is essentially equivalent to the bootstrap value in the ML analysis.



The posterior probabilities of individual "clades" are highly correlated between two independent chains - Metropolis coupled MCMC

Huelsenbeck, J. P., F. Ronquist, R. Nielsen & J. P. Bollneck. 2001. Bayesian inference of phylogeny and its impact on evolutionary biology. Science 294: 2310-2314.

Salemi, M. & Vandamme, A-M., eds. 2003. The Phylogenetic handbook. Cambridge University Press, Cambridge.

Hall, B. G. 2004. Phylogenetic trees made easy. Ed. 2. Sinauer Associates, Sunderland.

Felsenstein, J. 2004. Inferring phylogenies. Sinauer Associates, Sunderland.

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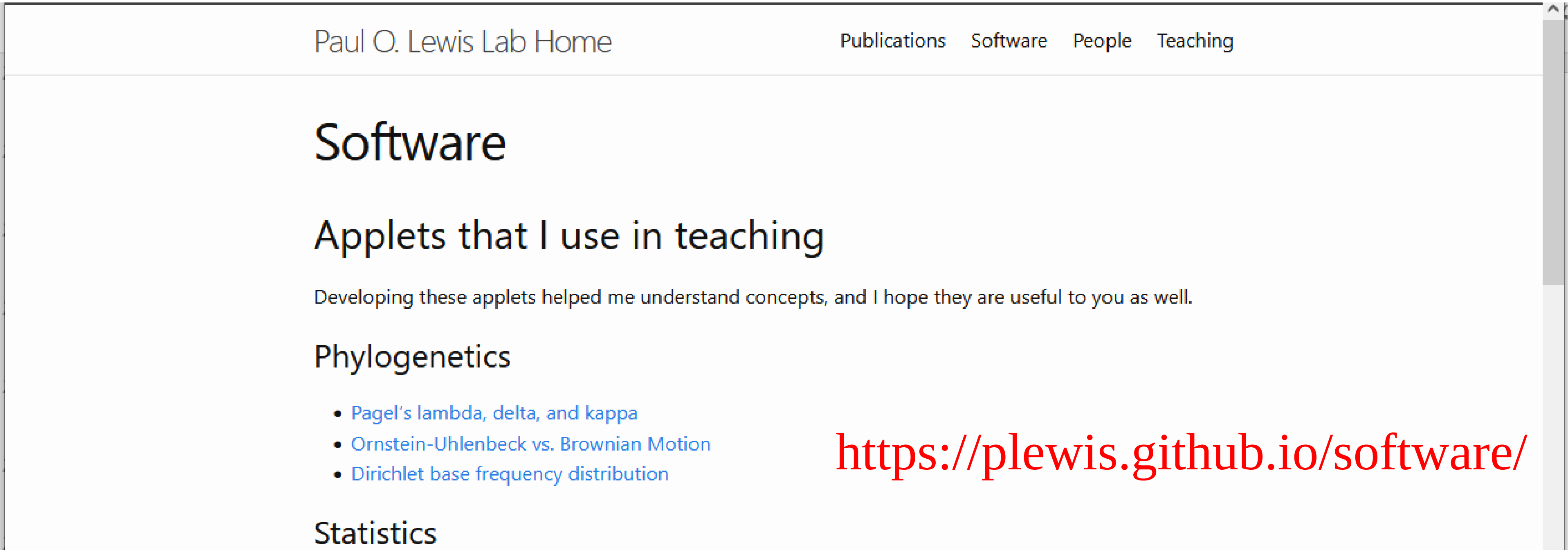


Paul O. Lewis Lab Home

Publications Software People Teaching

Software

Applets that I use in teaching

Developing these applets helped me understand concepts, and I hope they are useful to you as well.

Phylogenetics

- Pagel's lambda, delta, and kappa
- Ornstein-Uhlenbeck vs. Brownian Motion
- Dirichlet base frequency distribution

Statistics

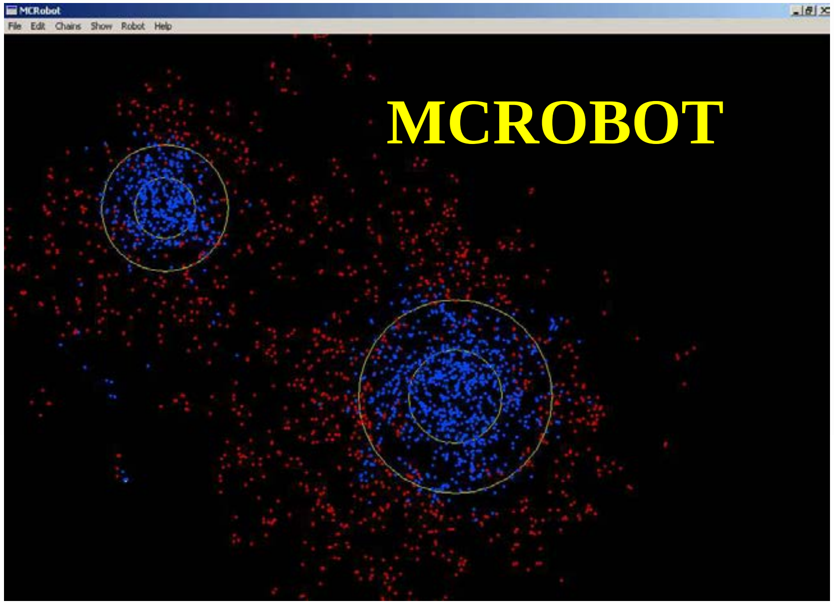
- Density Rain
- MCMC
- Dirichlet Process Prior

Natural Selection

- Natural selection ball-drop game
- Biston betularia (1848-1898)
- Biston betularia (1959-2003)

Population Genetics

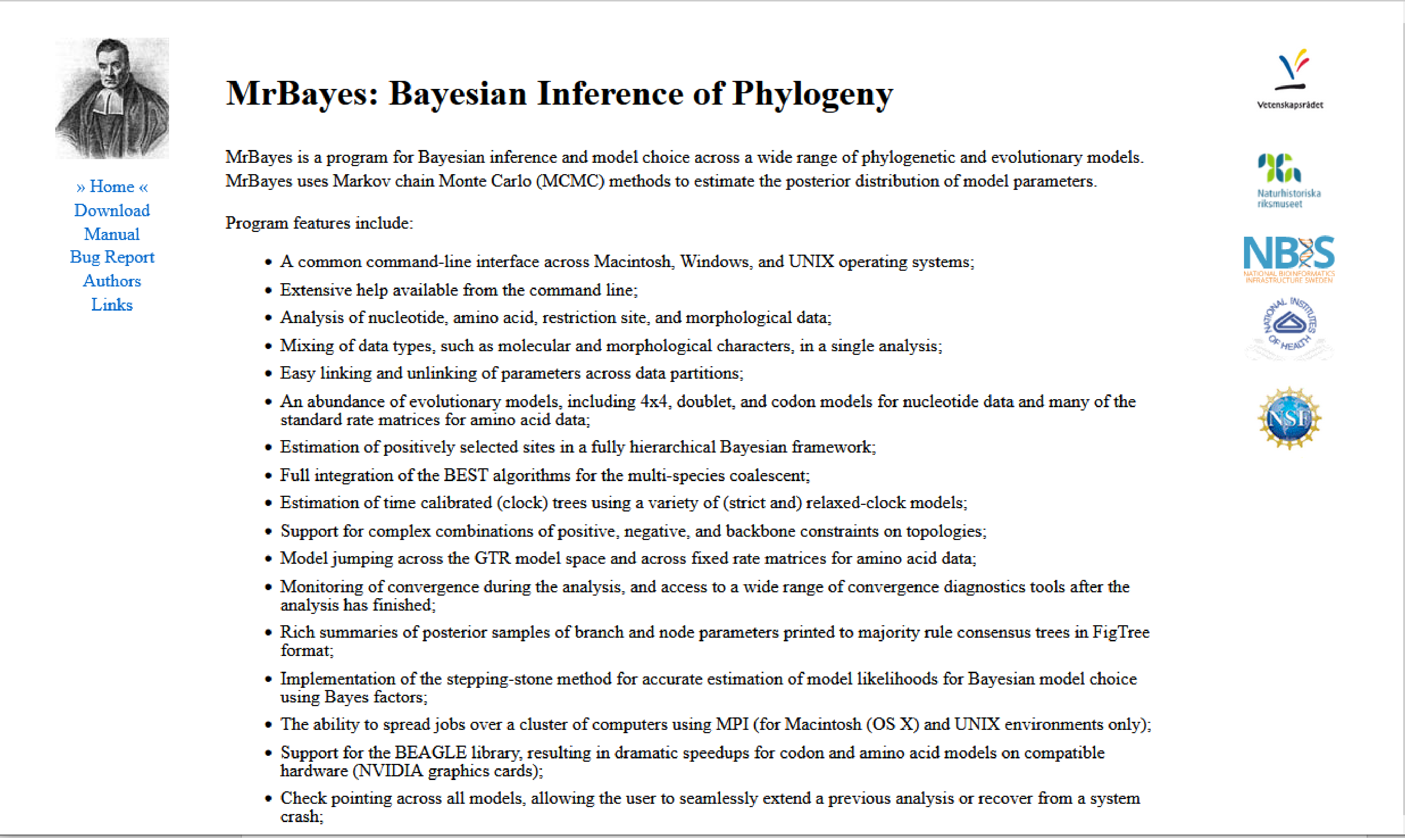
https://plewis.github.io/software/



MCROBOT

program MrBayes

http://nbisweden.github.io/MrBayes/

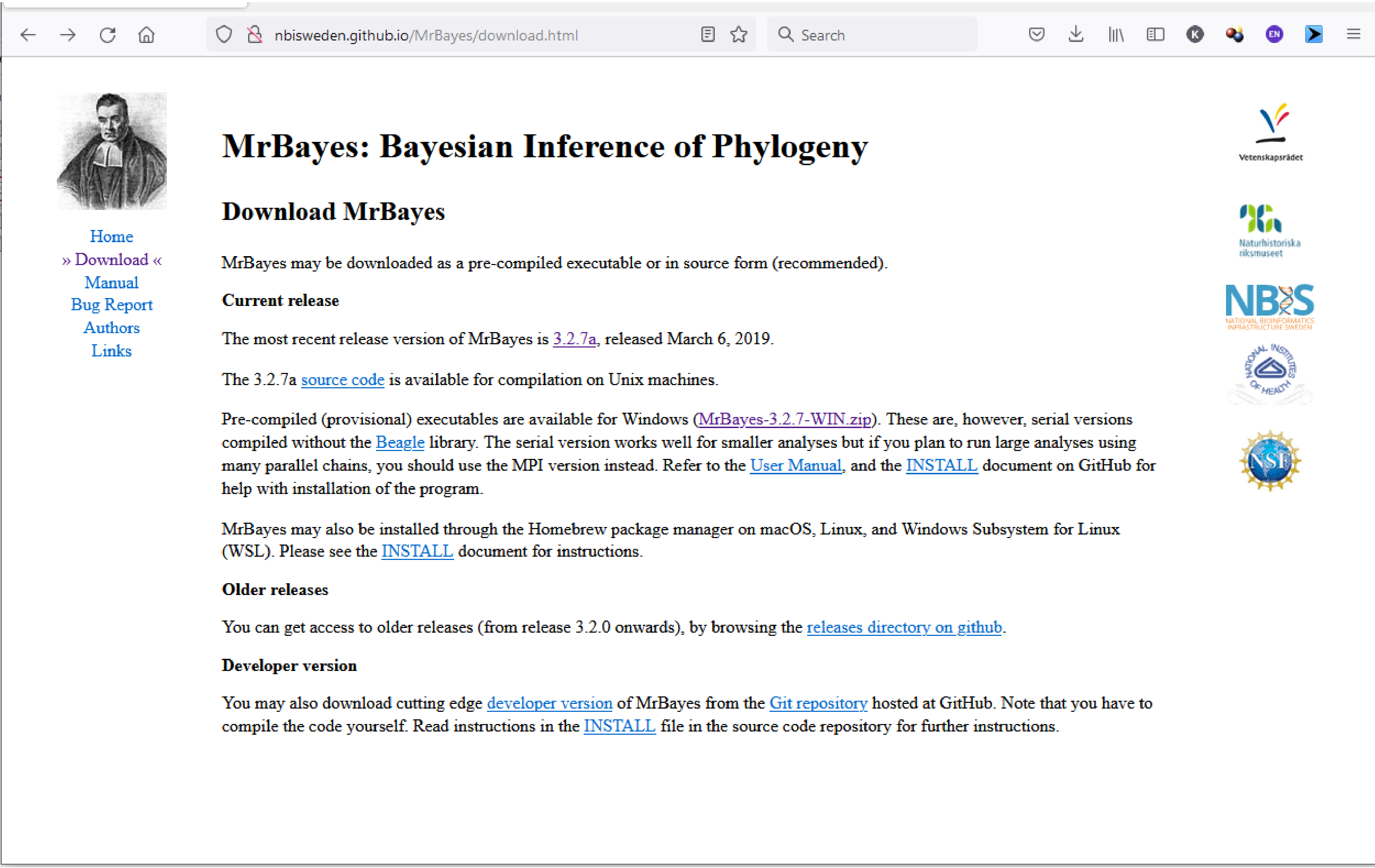


MrBayes: Bayesian Inference of Phylogeny

MrBayes is a program for Bayesian inference and model choice across a wide range of phylogenetic and evolutionary models. MrBayes uses Markov chain Monte Carlo (MCMC) methods to estimate the posterior distribution of model parameters.

Program features include:

- A common command-line interface across Macintosh, Windows, and UNIX operating systems;
- Extensive help available from the command line;
- Analysis of nucleotide, amino acid, restriction site, and morphological data;
- Mixing of data types, such as molecular and morphological characters, in a single analysis;
- Easy linking and unlinking of parameters across data partitions;
- An abundance of evolutionary models, including 4x4, doublet, and codon models for nucleotide data and many of the standard rate matrices for amino acid data;
- Estimation of positively selected sites in a fully hierarchical Bayesian framework;
- Full integration of the BEST algorithms for the multi-species coalescent;
- Estimation of time calibrated (clock) trees using a variety of (strict and) relaxed-clock models;
- Support for complex combinations of positive, negative, and backbone constraints on topologies;
- Model jumping across the GTR model space and across fixed rate matrices for amino acid data;
- Monitoring of convergence during the analysis, and access to a wide range of convergence diagnostics tools after the analysis has finished;
- Rich summaries of posterior samples of branch and node parameters printed to majority rule consensus trees in FigTree format;
- Implementation of the stepping-stone method for accurate estimation of model likelihoods for Bayesian model choice using Bayes factors;
- The ability to spread jobs over a cluster of computers using MPI (for Macintosh (OS X) and UNIX environments only);
- Support for the BEAGLE library, resulting in dramatic speedups for codon and amino acid models on compatible hardware (NVIDIA graphics cards);
- Check pointing across all models, allowing the user to seamlessly extend a previous analysis or recover from a system crash;



MrBayes: Bayesian Inference of Phylogeny

Download MrBayes

MrBayes may be downloaded as a pre-compiled executable or in source form (recommended).

Current release

The most recent release version of MrBayes is 3.2.7a, released March 6, 2019.

The 3.2.7a source code is available for compilation on Unix machines.

Pre-compiled (provisional) executables are available for Windows (MrBayes-3.2.7-WIN.zip). These are, however, serial versions compiled without the Beagle library. The serial version works well for smaller analyses but if you plan to run large analyses using many parallel chains, you should use the MPI version instead. Refer to the User Manual, and the INSTALL document on GitHub for help with installation of the program.

MrBayes may also be installed through the Homebrew package manager on macOS, Linux, and Windows Subsystem for Linux (WSL). Please see the INSTALL document for instructions.

Older releases

You can get access to older releases (from release 3.2.0 onwards), by browsing the releases directory on github.

Developer version

You may also download cutting edge developer version of MrBayes from the Git repository hosted at GitHub. Note that you have to compile the code yourself. Read instructions in the INSTALL file in the source code repository for further instructions.

Huelsenbeck JP and Ronquist F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754-755.

Huelsenbeck JP, Ronquist F, Nielsen R, Bollback JP. 2001. Bayesian inferemce of phylogeny and its impact on evolutionary biology. *Science* 294: 2310-2314.

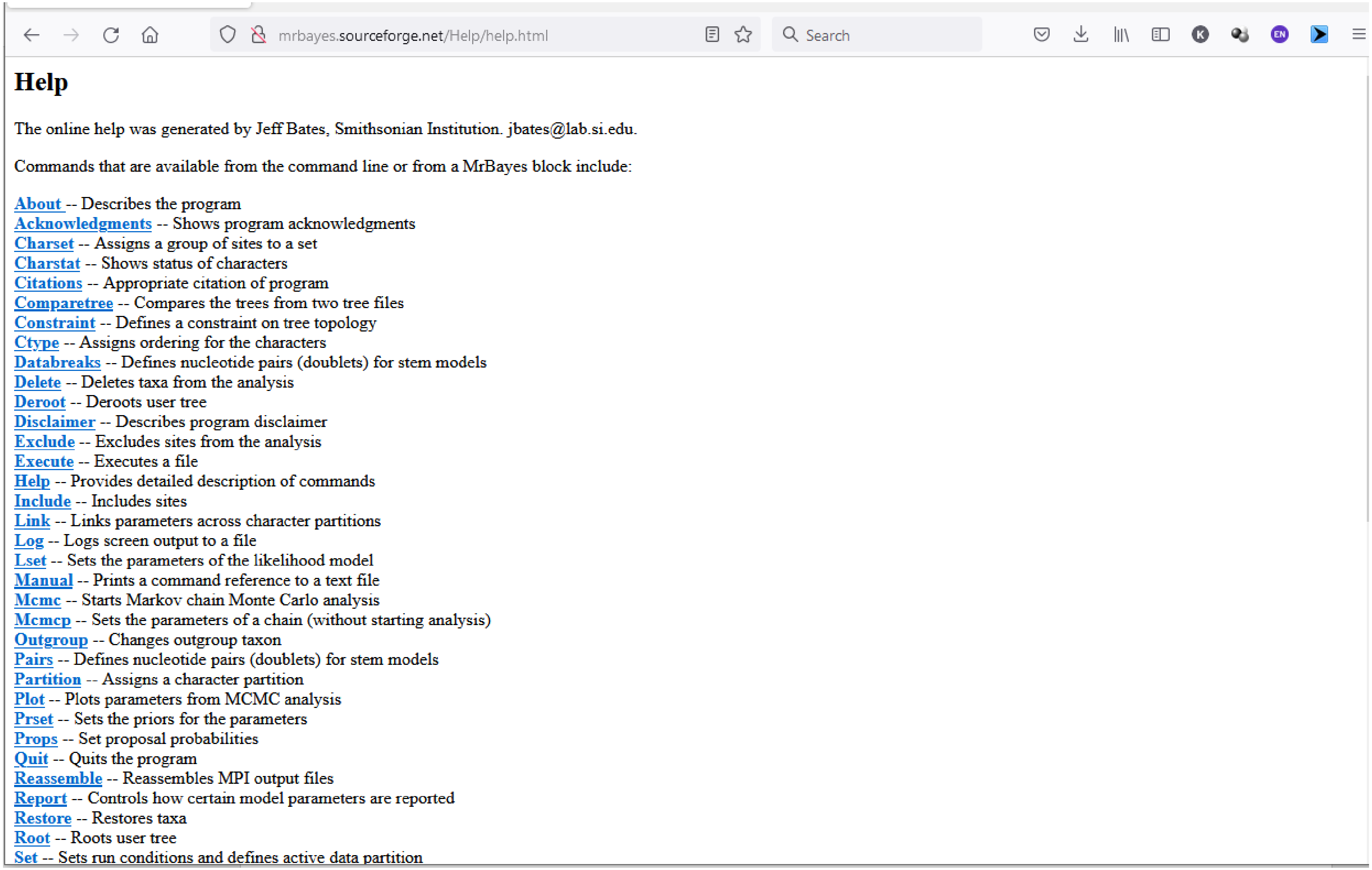
Archibald JK, Mort ME, Crawford DJ. 2003. Bayesian inference of phylogeny: a non-technical primer. *Taxon* 52: 187-191.

Ronquist F. and Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572-1574.

Ronquist F. 2004. Bayesian inference of character evolution. *Trends in ecology and Evolution* 19: 475-481.

Other References in the Manual (MrBayes version 3.2 Manual), which is downloadable as part of the installation package.

http://mrbayes.sourceforge.net/Help/help.html



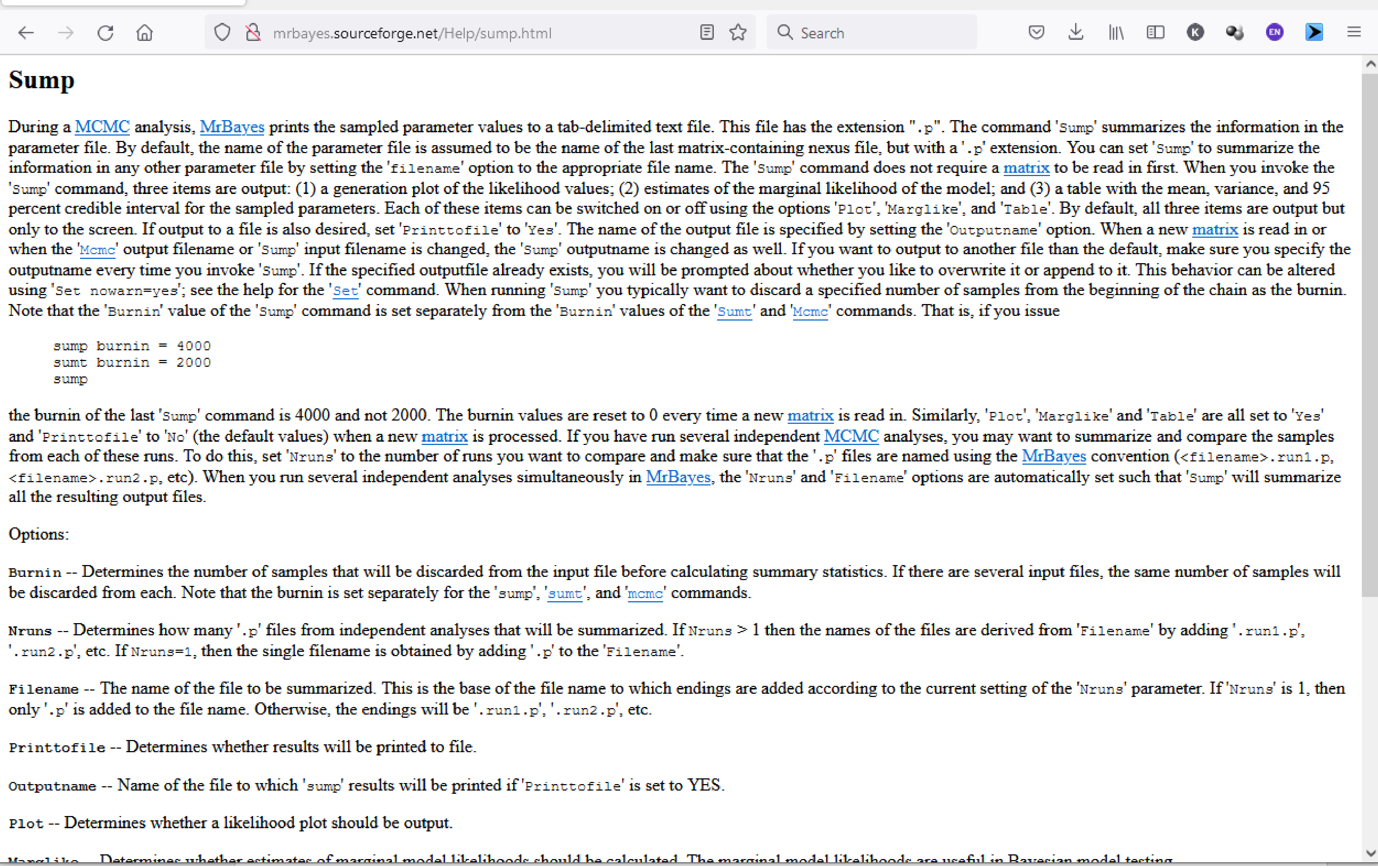
Help

The online help was generated by Jeff Bates, Smithsonian Institution. jbates@lab.si.edu.

Commands that are available from the command line or from a MrBayes block include:

About -- Describes the program
Acknowledgments -- Shows program acknowledgments
Charset -- Assigns a group of sites to a set
Charstat -- Shows status of characters
Citations -- Appropriate citation of program
Comparetree -- Compares the trees from two tree files
Constraint -- Defines a constraint on tree topology
Ctype -- Assigns ordering for the characters
Databreaks -- Defines nucleotide pairs (doublets) for stem models
Delete -- Deletes taxa from the analysis
Deroot -- Deroots user tree
Disclaimer -- Describes program disclaimer
Exclude -- Excludes sites from the analysis
Execute -- Executes a file
Help -- Provides detailed description of commands
Include -- Includes sites
Link -- Links parameters across character partitions
Log -- Logs screen output to a file
Lset -- Sets the parameters of the likelihood model
Manual -- Prints a command reference to a text file
Mcmc -- Starts Markov chain Monte Carlo analysis
Mcmcp -- Sets the parameters of a chain (without starting analysis)
Outgroup -- Changes outgroup taxon
Pairs -- Defines nucleotide pairs (doublets) for stem models
Partition -- Assigns a character partition
Plot -- Plots parameters from MCMC analysis
Prset -- Sets the priors for the parameters
Props -- Set proposal probabilities
Quit -- Quits the program
Reassemble -- Reassembles MPI output files
Report -- Controls how certain model parameters are reported
Restore -- Restores taxa
Root -- Roots user tree
Set -- Sets run conditions and defines active data partition

http://mrbayes.sourceforge.net/Help/help.html



Sump

During a MCMC analysis, MrBayes prints the sampled parameter values to a tab-delimited text file. This file has the extension "`.p`". The command '`Sump`' summarizes the information in the parameter file. By default, the name of the parameter file is assumed to be the name of the last matrix-containing nexus file, but with a '`.p`' extension. You can set '`Sump`' to summarize the information in any other parameter file by setting the '`filename`' option to the appropriate file name. The '`Sump`' command does not require a matrix to be read in first. When you invoke the '`Sump`' command, three items are output: (1) a generation plot of the likelihood values; (2) estimates of the marginal likelihood of the model; and (3) a table with the mean, variance, and 95 percent credible interval for the sampled parameters. Each of these items can be switched on or off using the options '`Plot`', '`Marglike`', and '`Table`'. By default, all three items are output but only to the screen. If output to a file is also desired, set '`Printtofile`' to '`Yes`'. The name of the output file is specified by setting the '`Outputname`' option. When a new matrix is read in or when the '`Mcmc`' output filename or '`Sump`' input filename is changed, the '`Sump`' outputname is changed as well. If you want to output to another file than the default, make sure you specify the outputname every time you invoke '`Sump`'. If the specified outputfile already exists, you will be prompted about whether you like to overwrite it or append to it. This behavior can be altered using '`Set nowarn=yes`'; see the help for the '`Set`' command. When running '`Sump`' you typically want to discard a specified number of samples from the beginning of the chain as the burnin. Note that the '`Burnin`' value of the '`Sump`' command is set separately from the '`Burnin`' values of the '`Sumt`' and '`Mcmc`' commands. That is, if you issue

```
   sump burnin = 4000
   sumt burnin = 2000
   sump
```

the burnin of the last '`Sump`' command is 4000 and not 2000. The burnin values are reset to 0 every time a new matrix is read in. Similarly, '`Plot`', '`Marglike`' and '`Table`' are all set to '`Yes`' and '`Printtofile`' to '`No`' (the default values) when a new matrix is processed. If you have run several independent MCMC analyses, you may want to summarize and compare the samples from each of these runs. To do this, set '`Nruns`' to the number of runs you want to compare and make sure that the '`.p`' files are named using the MrBayes convention (`<filename>.run1.p`, `<filename>.run2.p`, etc). When you run several independent analyses simultaneously in MrBayes, the '`Nruns`' and '`Filename`' options are automatically set such that '`Sump`' will summarize all the resulting output files.

Options:

`Burnin` -- Determines the number of samples that will be discarded from the input file before calculating summary statistics. If there are several input files, the same number of samples will be discarded from each. Note that the burnin is set separately for the '`sump`', '`sumt`', and '`mcmc`' commands.

`Nruns` -- Determines how many '`.p`' files from independent analyses that will be summarized. If `Nruns` > 1 then the names of the files are derived from '`Filename`' by adding '`.run1.p`', '`.run2.p`', etc. If `Nruns=1`, then the single filename is obtained by adding '`.p`' to the '`Filename`'.

`Filename` -- The name of the file to be summarized. This is the base of the file name to which endings are added according to the current setting of the '`Nruns`' parameter. If '`Nruns`' is 1, then only '`.p`' is added to the file name. Otherwise, the endings will be '`.run1.p`', '`.run2.p`', etc.

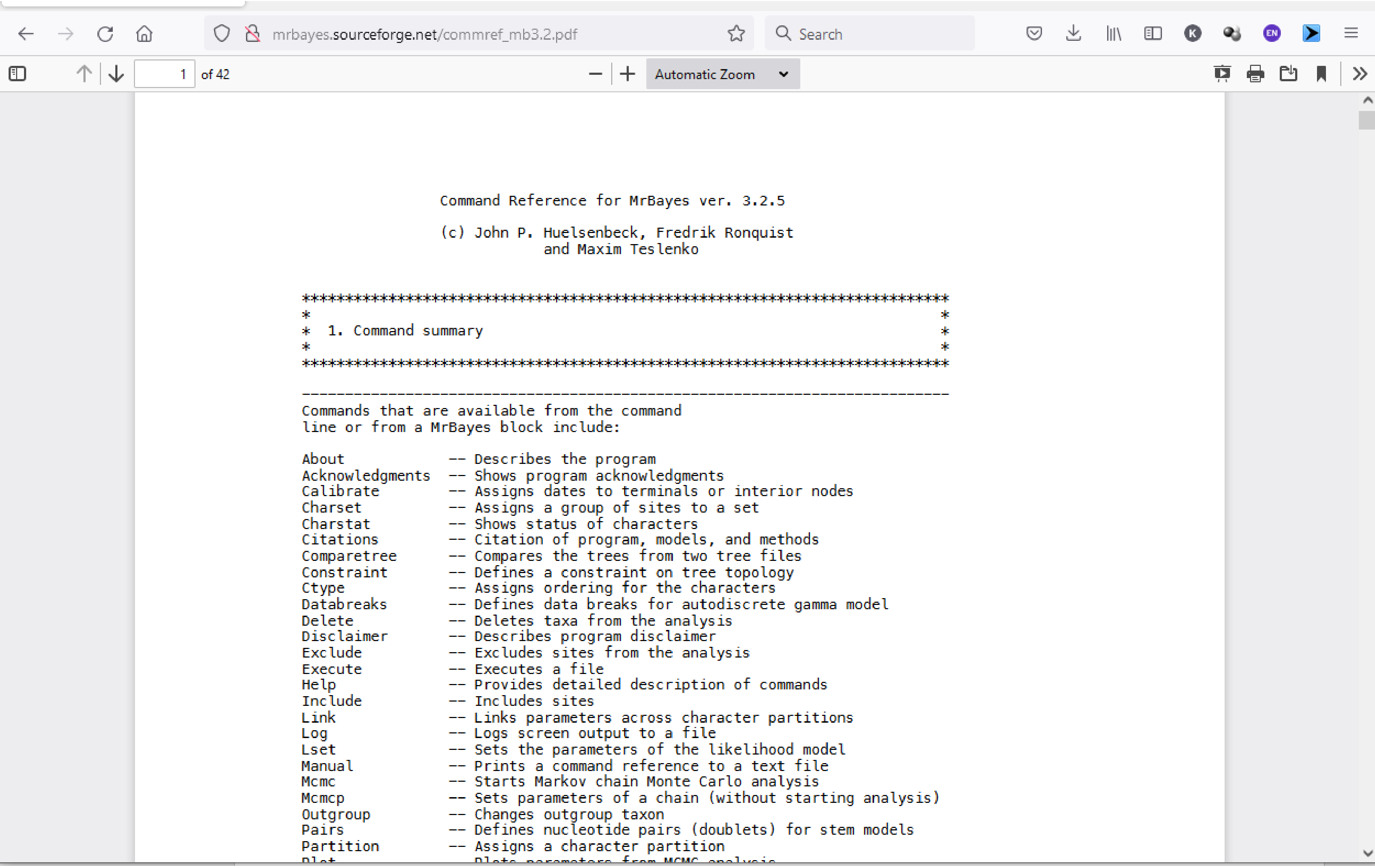
`Printtofile` -- Determines whether results will be printed to file.

`Outputname` -- Name of the file to which '`sump`' results will be printed if '`Printtofile`' is set to YES.

`Plot` -- Determines whether a likelihood plot should be output.

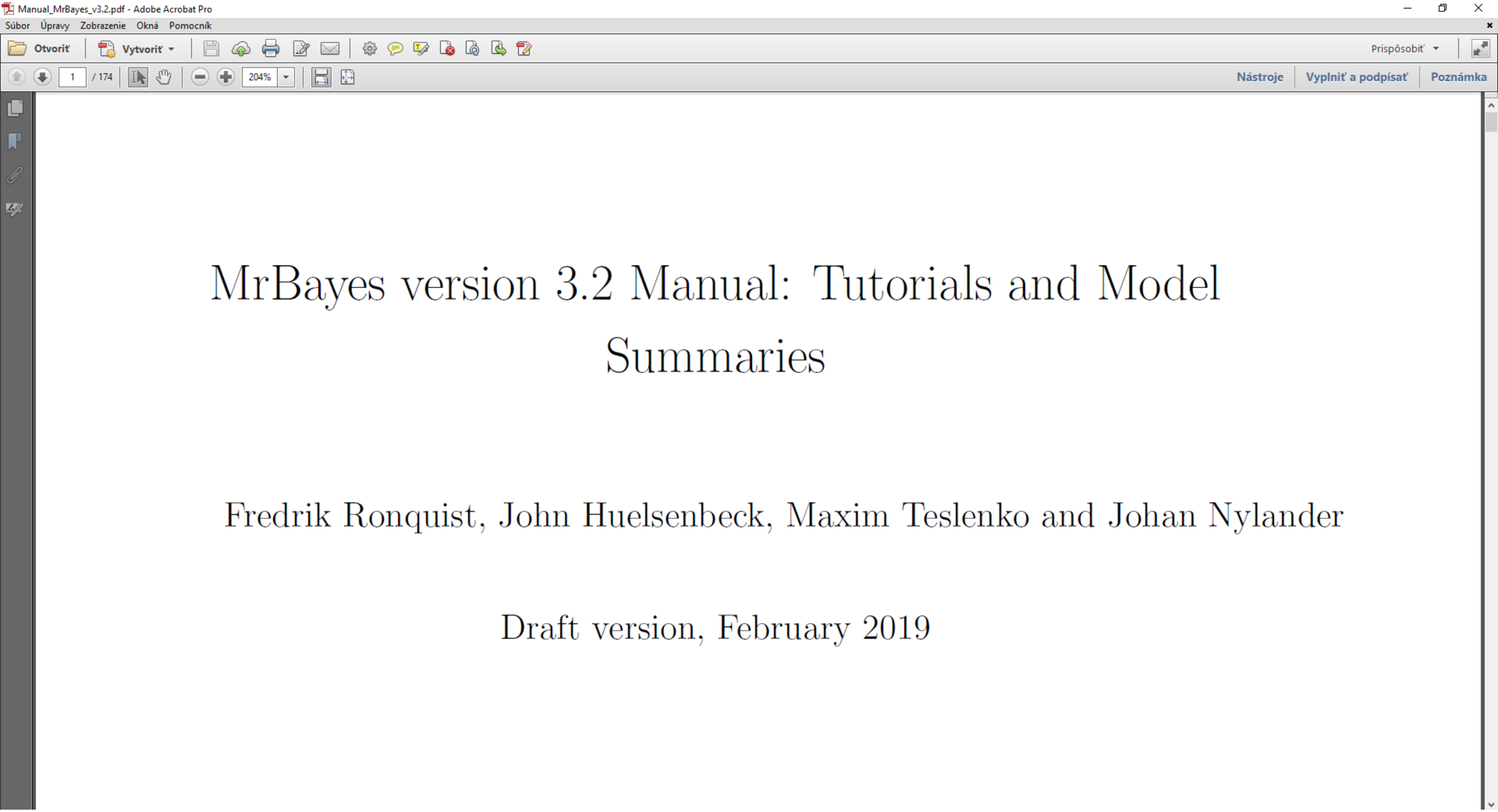
`Marglike` -- Determines whether estimates of marginal model likelihoods should be calculated. The marginal model likelihoods are useful in Bayesian model testing.

http://mrbayes.sourceforge.net/commref_mb3.2.pdf



```
                    Command Reference for MrBayes ver. 3.2.5

                    (c) John P. Huelsenbeck, Fredrik Ronquist
                                and Maxim Teslenko


   ***************************************************************************
   *                                                                         *
   *  1. Command summary                                                     *
   *                                                                         *
   ***************************************************************************

   ---------------------------------------------------------------------------
   Commands that are available from the command
   line or from a MrBayes block include:

   About                -- Describes the program
   Acknowledgments      -- Shows program acknowledgments
   Calibrate            -- Assigns dates to terminals or interior nodes
   Charset              -- Assigns a group of sites to a set
   Charstat             -- Shows status of characters
   Citations            -- Citation of program, models, and methods
   Comparetree          -- Compares the trees from two tree files
   Constraint           -- Defines a constraint on tree topology
   Ctype                -- Assigns ordering for the characters
   Databreaks           -- Defines data breaks for autodiscrete gamma model
   Delete               -- Deletes taxa from the analysis
   Disclaimer           -- Describes program disclaimer
   Exclude              -- Excludes sites from the analysis
   Execute              -- Executes a file
   Help                 -- Provides detailed description of commands
   Include              -- Includes sites
   Link                 -- Links parameters across character partitions
   Log                  -- Logs screen output to a file
   Lset                 -- Sets the parameters of the likelihood model
   Manual               -- Prints a command reference to a text file
   Mcmc                 -- Starts Markov chain Monte Carlo analysis
   Mcmcp                -- Sets parameters of a chain (without starting analysis)
   Outgroup             -- Changes outgroup taxon
   Pairs                -- Defines nucleotide pairs (doublets) for stem models
   Partition            -- Assigns a character partition
   Plot                 -- Plots parameters from MCMC analysis
```

Manual is included in the installation package MrBayes-3.2.7-WIN



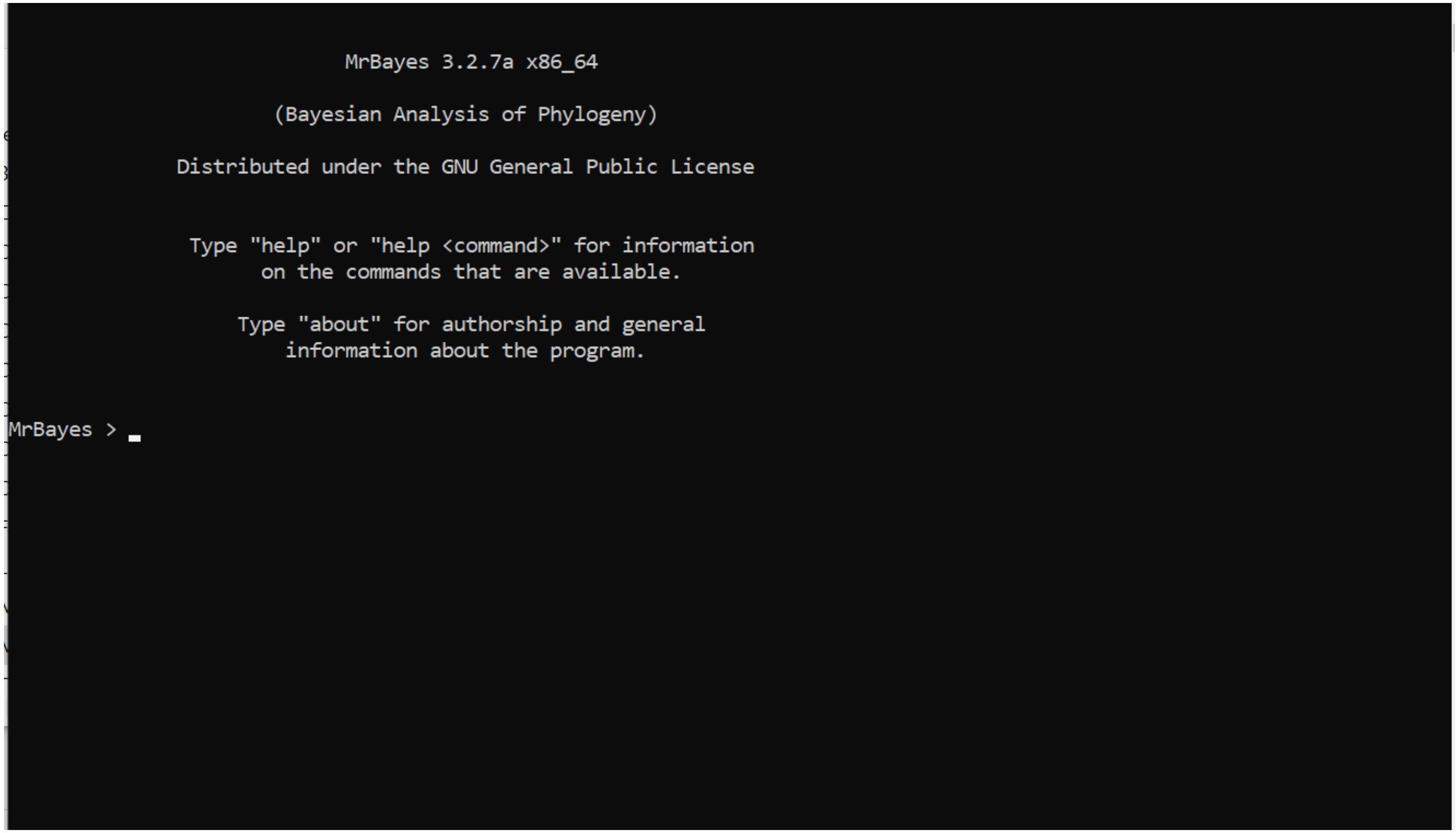
MrBayes version 3.2 Manual: Tutorials and Model Summaries

Fredrik Ronquist, John Huelsenbeck, Maxim Teslenko and Johan Nylander

Draft version, February 2019

MrBayes analysis - stepwise tpsubmission of commands

- commands are included in the *nexus* file

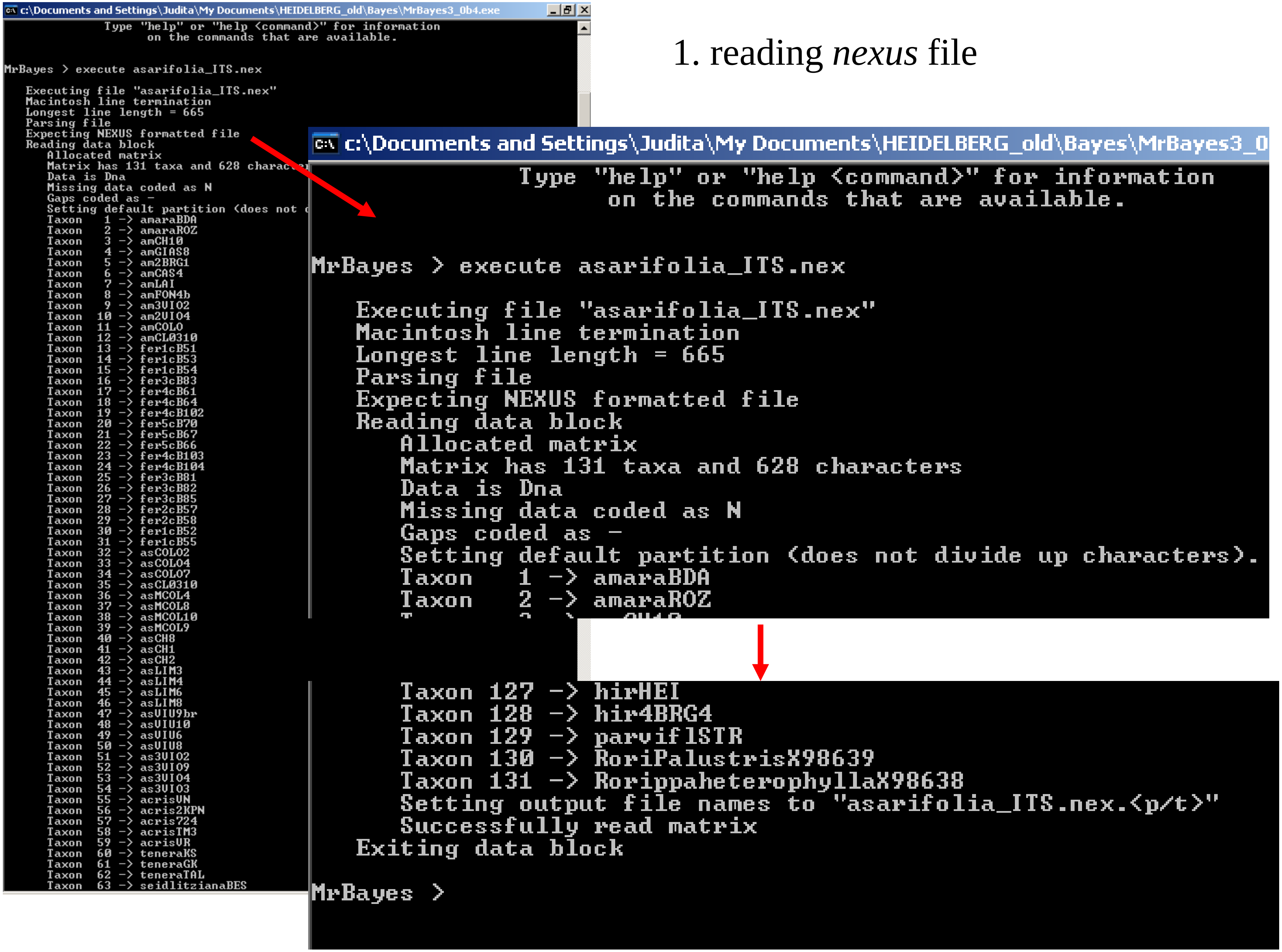


```
                        MrBayes 3.2.7a x86_64

                   (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: examples of commands and outputs are from the previous version of the program, current version will be used in practicals

```
MrBayes > execute asarifolia_ITS.nex
```

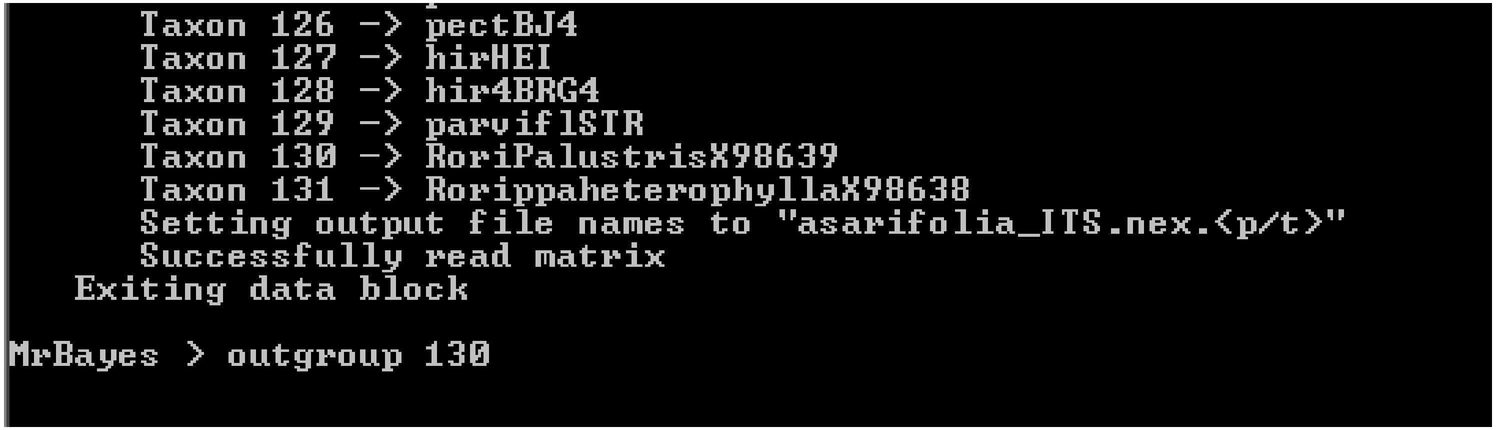
1. reading *nexus* file



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


MrBayes > execute asarifolia_ITS.nex

   Executing file "asarifolia_ITS.nex"
   Macintosh line termination
   Longest line length = 665
   Parsing file
   Expecting NEXUS formatted file
   Reading data block
      Allocated matrix
      Matrix has 131 taxa and 628 charac
      Data is Dna
      Missing data coded as N
      Gaps coded as -
      Setting default partition (does not
      Taxon   1 -> amaraBDA
      Taxon   2 -> amaraROZ
      Taxon   3 -> amCH10
      Taxon   4 -> amGIAS8
      Taxon   5 -> am2BRG1
      Taxon   6 -> amCAS4
      Taxon   7 -> amLAI
      Taxon   8 -> amFON4b
      Taxon   9 -> am3VIO2
      Taxon  10 -> am2VIO4
      Taxon  11 -> amCOLO
      Taxon  12 -> amCL0310
      Taxon  13 -> fer1cB51
      Taxon  14 -> fer1cB53
      Taxon  15 -> fer1cB54
      Taxon  16 -> fer3cB83
      Taxon  17 -> fer4cB61
      Taxon  18 -> fer4cB64
      Taxon  19 -> fer4cB102
      Taxon  20 -> fer5cB70
      Taxon  21 -> fer5cB67
      Taxon  22 -> fer5cB66
      Taxon  23 -> fer4cB103
      Taxon  24 -> fer4cB104
      Taxon  25 -> fer3cB81
      Taxon  26 -> fer3cB82
      Taxon  27 -> fer3cB85
      Taxon  28 -> fer2cB57
      Taxon  29 -> fer2cB58
      Taxon  30 -> fer1cB52
      Taxon  31 -> fer1cB55
      Taxon  32 -> asCOLO2
      Taxon  33 -> asCOLO4
      Taxon  34 -> asCOLO7
      Taxon  35 -> asCL0310
      Taxon  36 -> asMCOL4
      Taxon  37 -> asMCOL8
      Taxon  38 -> asMCOL10
      Taxon  39 -> asMCOL9
      Taxon  40 -> asCH8
      Taxon  41 -> asCH1
      Taxon  42 -> asCH2
      Taxon  43 -> asLIM3
      Taxon  44 -> asLIM4
      Taxon  45 -> asLIM6
      Taxon  46 -> asLIM8
      Taxon  47 -> asVIU9br
      Taxon  48 -> asVIU10
      Taxon  49 -> asVIU6
      Taxon  50 -> asVIU8
      Taxon  51 -> as3VIO2
      Taxon  52 -> as3VIO9
      Taxon  53 -> as3VIO4
      Taxon  54 -> as3VIO3
      Taxon  55 -> acrisVN
      Taxon  56 -> acris2KPN
      Taxon  57 -> acris724
      Taxon  58 -> acrisTM3
      Taxon  59 -> acrisVR
      Taxon  60 -> teneraKS
      Taxon  61 -> teneraGK
      Taxon  62 -> teneraTAL
      Taxon  63 -> seidlitzianaBES
```

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


MrBayes > execute asarifolia_ITS.nex

   Executing file "asarifolia_ITS.nex"
   Macintosh line termination
   Longest line length = 665
   Parsing file
   Expecting NEXUS formatted file
   Reading data block
      Allocated matrix
      Matrix has 131 taxa and 628 characters
      Data is Dna
      Missing data coded as N
      Gaps coded as -
      Setting default partition (does not divide up characters).
      Taxon   1 -> amaraBDA
      Taxon   2 -> amaraROZ

      Taxon 127 -> hirHEI
      Taxon 128 -> hir4BRG4
      Taxon 129 -> parviflSTR
      Taxon 130 -> RoriPalustrisX98639
      Taxon 131 -> RorippaheterophyllaX98638
      Setting output file names to "asarifolia_ITS.nex.<p/t>"
      Successfully read matrix
   Exiting data block

MrBayes >
```

2. Selection of *outgroup* for rooting the tree

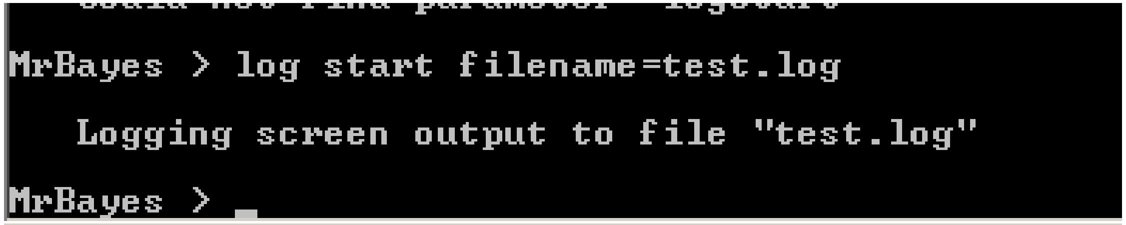


```
      Taxon 126 -> pectBJ4
      Taxon 127 -> hirHEI
      Taxon 128 -> hir4BRG4
      Taxon 129 -> parvif1STR
      Taxon 130 -> RoriPalustrisX98639
      Taxon 131 -> RorippaheterophyllaX98638
      Setting output file names to "asarifolia_ITS.nex.<p/t>"
      Successfully read matrix
   Exiting data block

MrBayes > outgroup 130
```

```
MrBayes > outgroup 130

   Setting outgroup to taxon "RoriPalustrisX98639"

MrBayes >
```

3. Saving the output into the *log* file

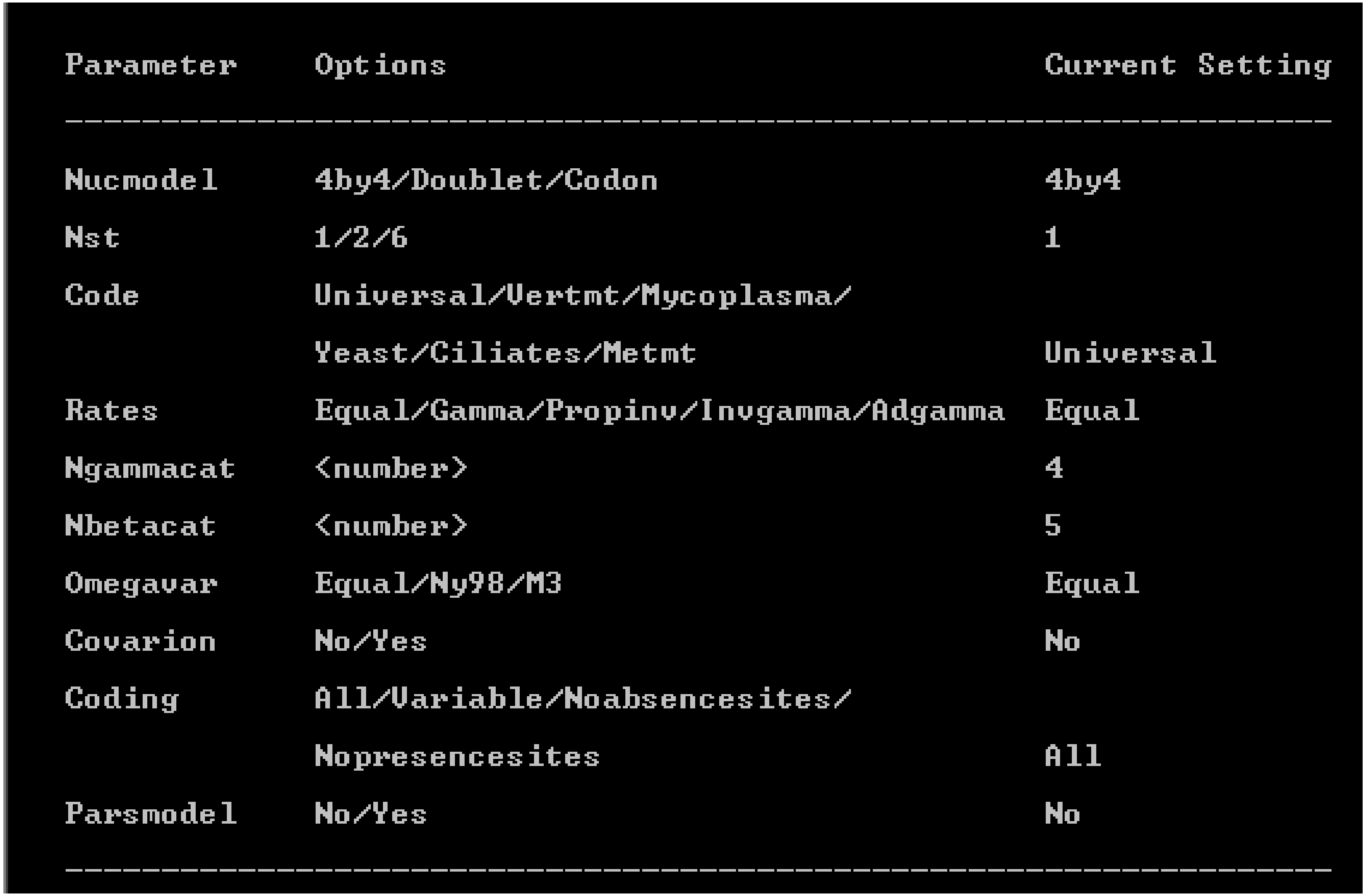
```
MrBayes > log start filename=test.log
```



```
MrBayes > log start filename=test.log

   Logging screen output to file "test.log"

MrBayes >
```

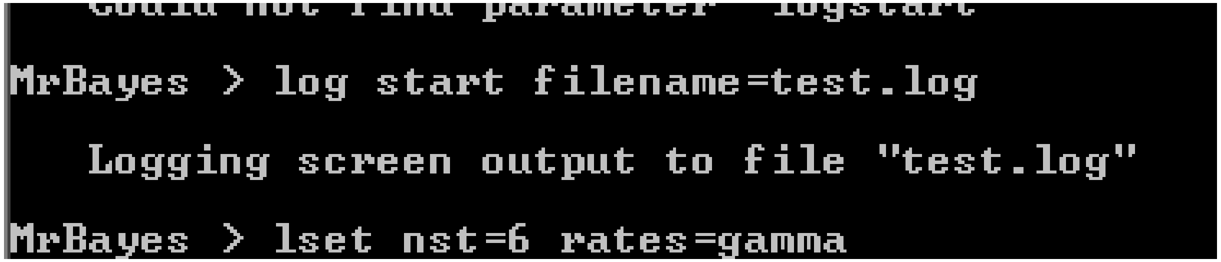
4. Setting the model parameters

help lset...display of possible parameters

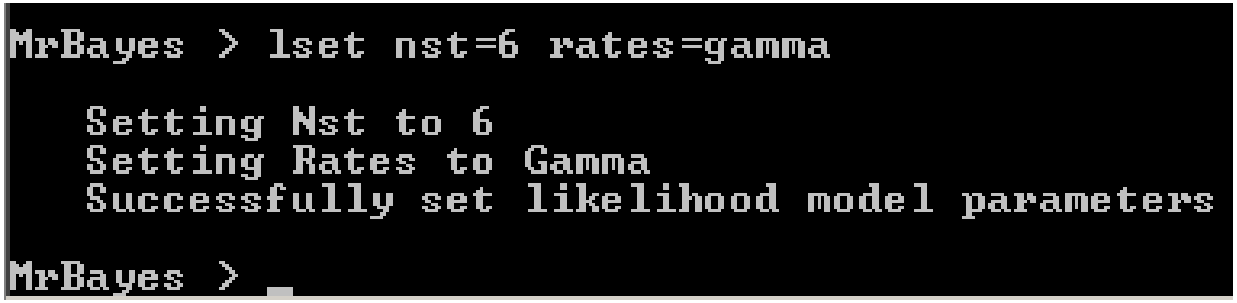


```
Parameter    Options                                Current Setting
-------------------------------------------------------------------
Nucmodel     4by4/Doublet/Codon                     4by4
Nst          1/2/6                                  1
Code         Universal/Vertmt/Mycoplasma/
             Yeast/Ciliates/Metmt                   Universal
Rates        Equal/Gamma/Propinv/Invgamma/Adgamma   Equal
Ngammacat    <number>                               4
Nbetacat     <number>                               5
Omegavar     Equal/Ny98/M3                          Equal
Covarion     No/Yes                                 No
Coding       All/Variable/Noabsencesites/
             Nopresencesites                        All
Parsmodel    No/Yes                                 No
-------------------------------------------------------------------
```

4. Setting the model parameters
1 = F81 model, 6 = GTR model



```
   Could not find parameter "logstart"

MrBayes > log start filename=test.log

   Logging screen output to file "test.log"

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



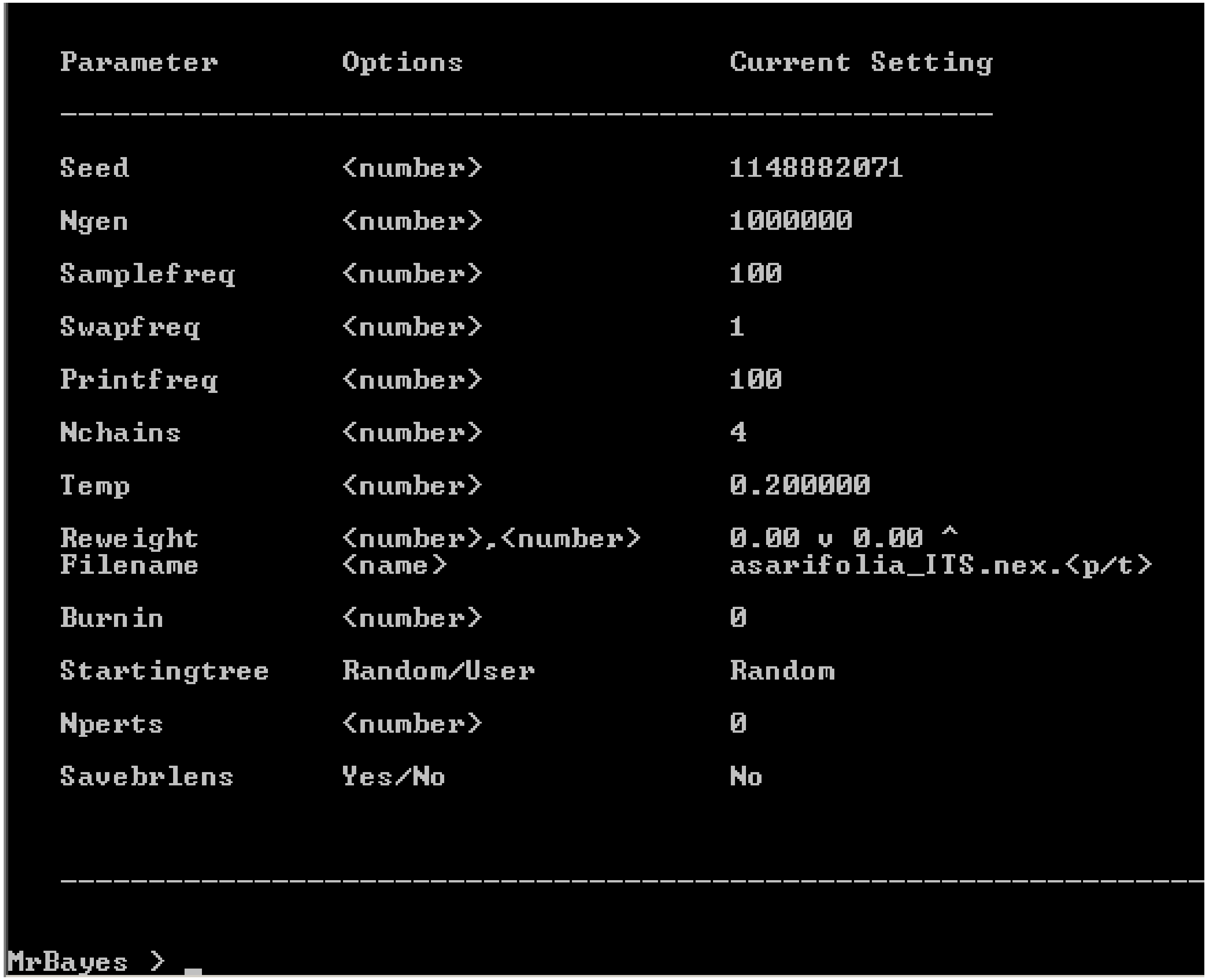
```
MrBayes > lset nst=6 rates=gamma

   Setting Nst to 6
   Setting Rates to Gamma
   Successfully set likelihood model parameters

MrBayes > _
```

5. setting the “prior settings” – setting of parameters based on prior specifications

help prset...display of parameters and possible settings

6. Setting the analysis parameters

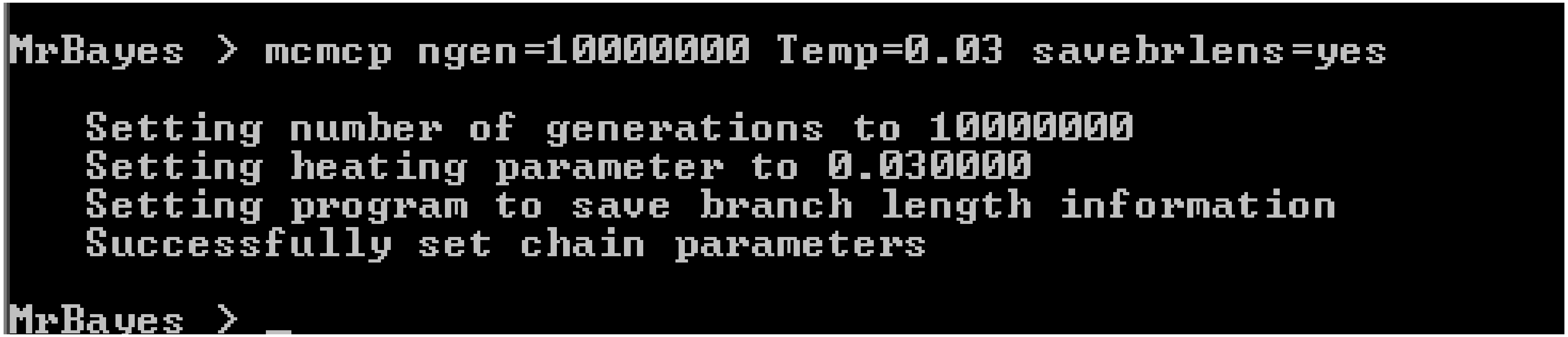
help mcmc...display of possible settings



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

   Seed            <number>               1148882071

   Ngen            <number>               1000000

   Samplefreq      <number>               100

   Swapfreq        <number>               1

   Printfreq       <number>               100

   Nchains         <number>               4

   Temp            <number>               0.200000

   Reweight        <number>,<number>      0.00 v 0.00 ^
   Filename        <name>                 asarifolia_ITS.nex.<p/t>

   Burnin          <number>               0

   Startingtree    Random/User            Random

   Nperts          <number>               0

   Savebrlens      Yes/No                 No


   ------------------------------------------------------------------

MrBayes > _
```

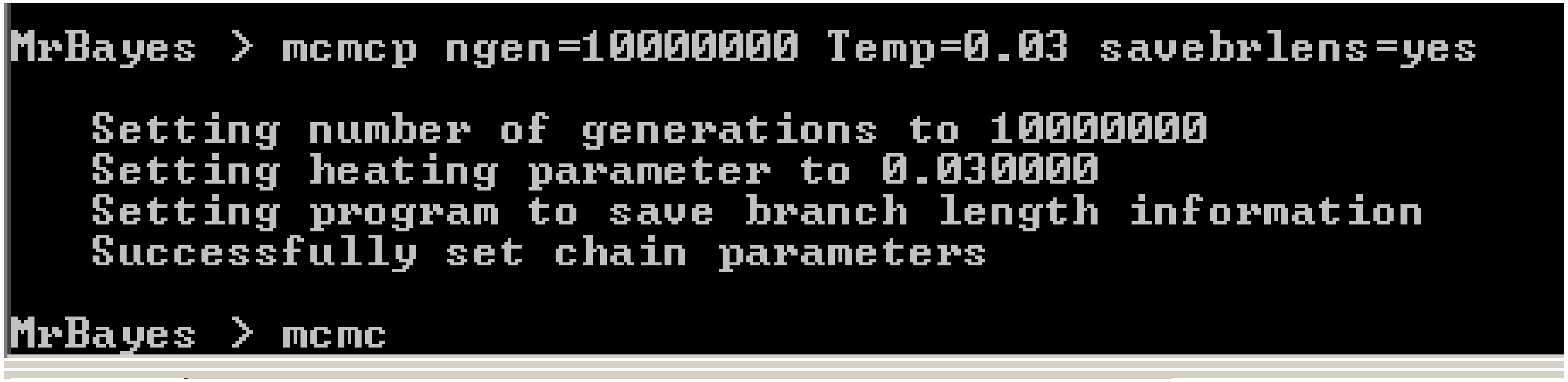
6. Setting the analysis parameters – number of generations, temperature

```
MrBayes > mcmcp ngen=10000000 Temp=0.03 savebrlens=yes
```



```
MrBayes > mcmcp ngen=10000000 Temp=0.03 savebrlens=yes

   Setting number of generations to 10000000
   Setting heating parameter to 0.030000
   Setting program to save branch length information
   Successfully set chain parameters

MrBayes > _
```

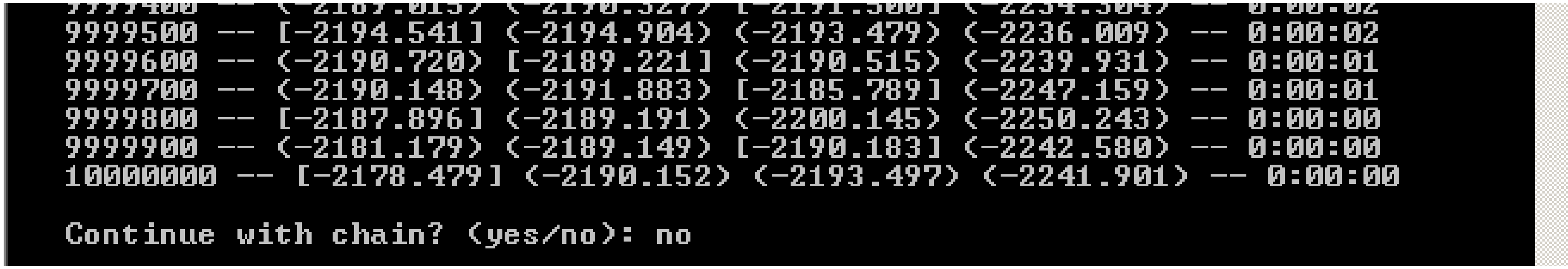
7. Start of the analysis



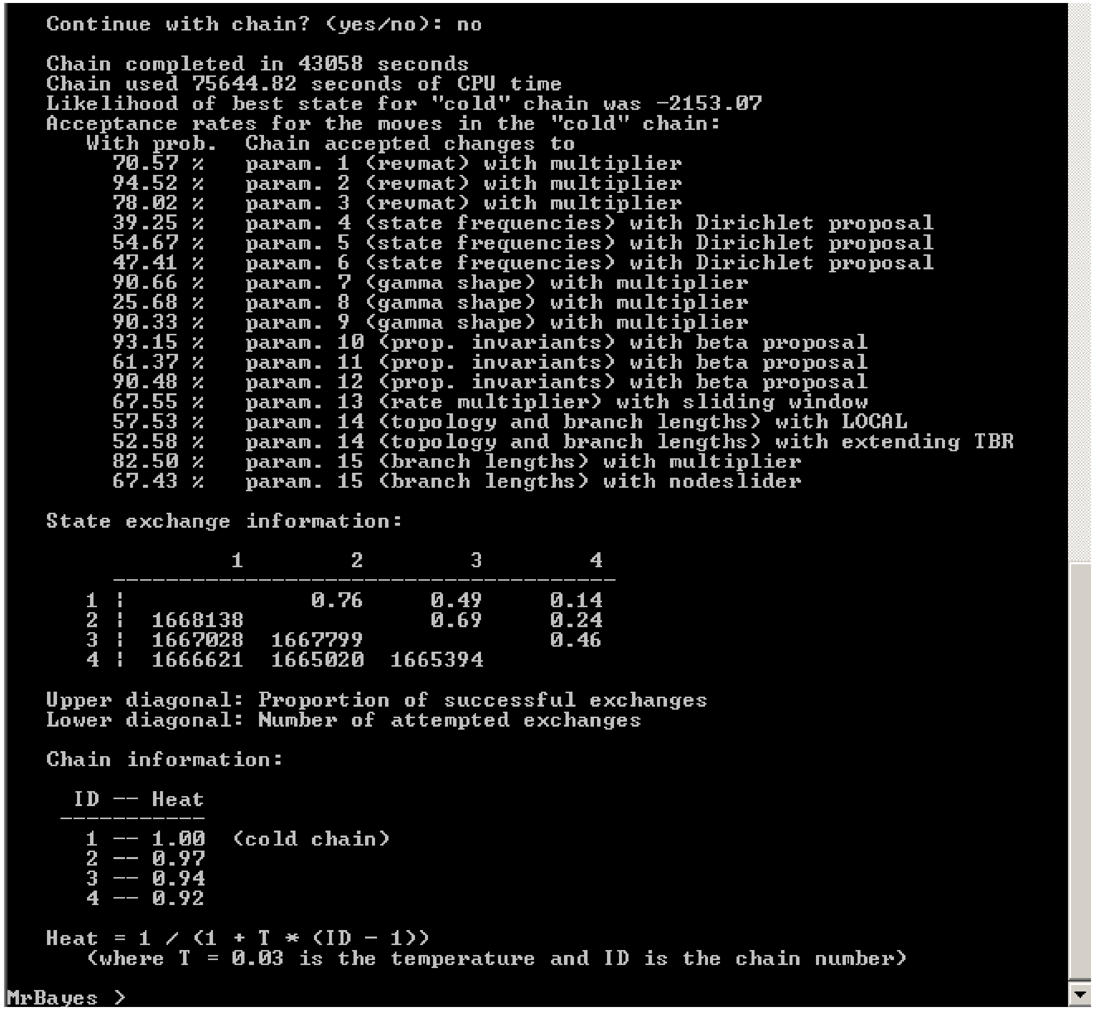
```
MrBayes > mcmcp ngen=10000000 Temp=0.03 savebrlens=yes

   Setting number of generations to 10000000
   Setting heating parameter to 0.030000
   Setting program to save branch length information
   Successfully set chain parameters

MrBayes > mcmc
```

Analysis is going on

```
MrBayes > mcmc

   Running Markov chain
   MCMC stamp = 9889988200
   Model settings:

         Datatype  = DNA
         Nucmodel  = 4by4
         Nst       = 6
                     Substitution rates, expressed as proportions
                     of the rate sum, follow a Dirichlet
                     (1.00,1.00,1.00,1.00,1.00,1.00)
         Covarion  = No
         # States  = 4
                     State frequencies have a Dirichlet prior
         Rates     = Gamma
                     Gamma shape parameter is uniformly dist-
                     ributed on the interval (0.05,50.00).
                     Gamma distribution is approximated using 4 categories.

   Active parameters:

      Parameters
      ------------------
      Revmat           1
      Statefreq        2
      Shape            3
      Topology         4
      Brlens           5
      ------------------

      1 --  Parameter  = Revmat
            Prior      = Dirichlet(1.00,1.00,1.00,1.00,1.00,1.00)
      2 --  Parameter  = Statefreq
            Prior      = Dirichlet
      3 --  Parameter  = Shape
            Prior      = Uniform(0.05,50.00)
      4 --  Parameter  = Topology
            Prior      = All topologies equally probable a priori
      5 --  Parameter  = Brlens
            Prior      = Branch lengths are Unconstrained:Exponential(10.0)

   Number of taxa = 131
   Number of characters = 628
   Compressing data matrix for division 1
   Division 1 has 243 unique site patterns
   The chain will use the following moves:
      With prob.  Chain will change
         3.70 %   param. 1 (revmat) with multiplier
         3.70 %   param. 2 (state frequencies) with Dirichlet proposal
         3.70 %   param. 3 (gamma shape) with multiplier
        55.56 %   param. 4 (topology and branch lengths) with LOCAL
        11.11 %   param. 4 (topology and branch lengths) with extending TBR
        11.11 %   param. 5 (branch lengths) with multiplier
        11.11 %   param. 5 (branch lengths) with nodeslider
   Creating parsimony (bitset) matrix for division 1
   Initializing conditional likelihoods for terminals
   Initializing conditional likelihoods for chains
   Initial log likelihoods:
      Chain 1 -- -10168.043333
      Chain 2 -- -10389.666714
      Chain 3 -- -10361.807983
      Chain 4 -- -10319.898048

   Chain results:

      1 -- [-10168.043] (-10389.667) (-10361.846) (-10318.655)
    100 -- [-9726.668] (-10074.274) (-10088.676) (-9946.870) -- 27:46:39
    200 -- [-9534.361] (-9882.615) (-9864.944) (-9716.929) -- 27:46:38
```



```
9999500 -- [-2194.541] (-2194.904) (-2193.479) (-2236.009) -- 0:00:02
9999600 -- (-2190.720) [-2189.221] (-2190.515) (-2239.931) -- 0:00:01
9999700 -- (-2190.148) (-2191.883) [-2185.789] (-2247.159) -- 0:00:01
9999800 -- [-2187.896] (-2189.191) (-2200.145) (-2250.243) -- 0:00:00
9999900 -- (-2181.179) (-2189.149) [-2190.183] (-2242.580) -- 0:00:00
10000000 -- [-2178.479] (-2190.152) (-2193.497) (-2241.901) -- 0:00:00

Continue with chain? (yes/no): no
```



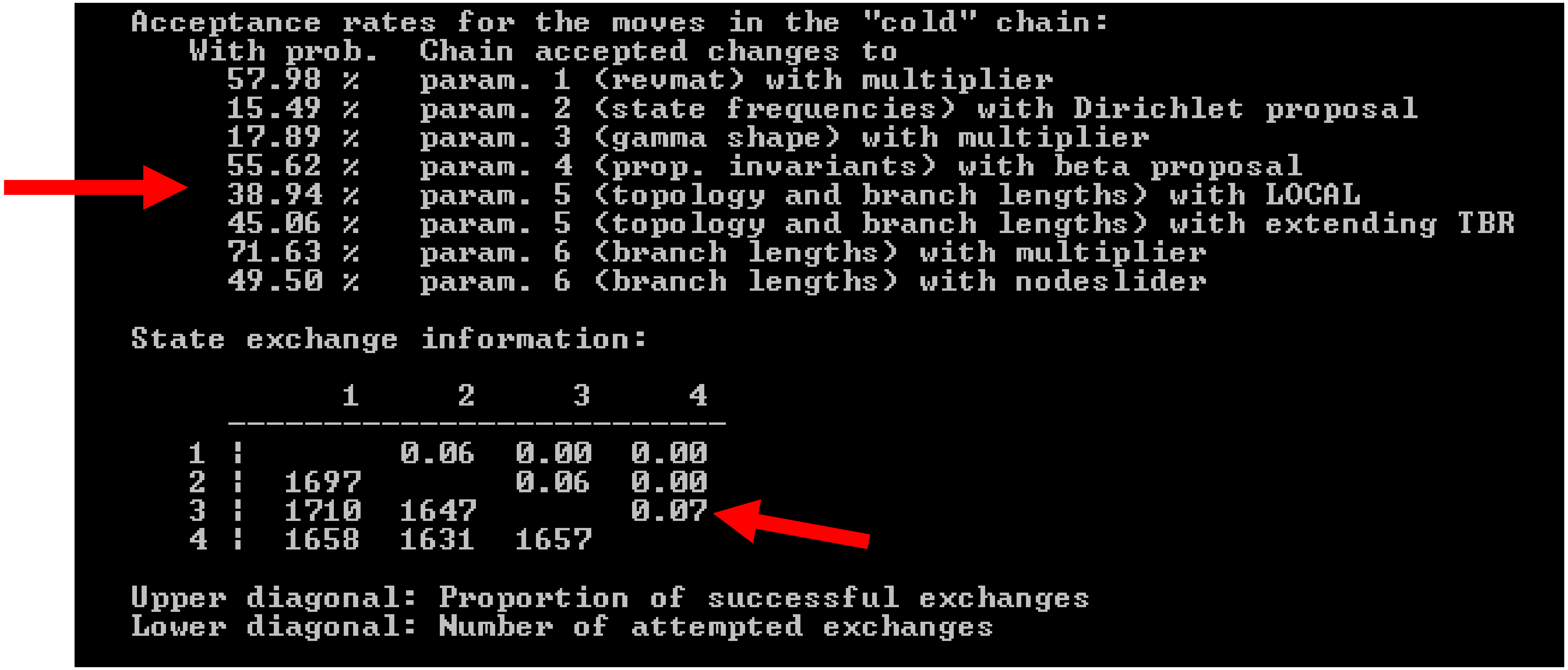
```
   Continue with chain? (yes/no): no

   Chain completed in 43058 seconds
   Chain used 75644.82 seconds of CPU time
   Likelihood of best state for "cold" chain was -2153.07
   Acceptance rates for the moves in the "cold" chain:
      With prob.  Chain accepted changes to
        70.57 %   param. 1 (revmat) with multiplier
        94.52 %   param. 2 (revmat) with multiplier
        78.02 %   param. 3 (revmat) with multiplier
        39.25 %   param. 4 (state frequencies) with Dirichlet proposal
        54.67 %   param. 5 (state frequencies) with Dirichlet proposal
        47.41 %   param. 6 (state frequencies) with Dirichlet proposal
        90.66 %   param. 7 (gamma shape) with multiplier
        25.68 %   param. 8 (gamma shape) with multiplier
        90.33 %   param. 9 (gamma shape) with multiplier
        93.15 %   param. 10 (prop. invariants) with beta proposal
        61.37 %   param. 11 (prop. invariants) with beta proposal
        90.48 %   param. 12 (prop. invariants) with beta proposal
        67.55 %   param. 13 (rate multiplier) with sliding window
        57.53 %   param. 14 (topology and branch lengths) with LOCAL
        52.58 %   param. 14 (topology and branch lengths) with extending TBR
        82.50 %   param. 15 (branch lengths) with multiplier
        67.43 %   param. 15 (branch lengths) with nodeslider

   State exchange information:

                 1        2        3        4
        --------------------------------------
      1 |              0.76     0.49     0.14
      2 |  1668138              0.69     0.24
      3 |  1667028  1667799              0.46
      4 |  1666621  1665020  1665394

   Upper diagonal: Proportion of successful exchanges
   Lower diagonal: Number of attempted exchanges

   Chain information:

     ID -- Heat
    -----------
      1 -- 1.00  (cold chain)
      2 -- 0.97
      3 -- 0.94
      4 -- 0.92

   Heat = 1 / (1 + T * (ID - 1))
      (where T = 0.03 is the temperature and ID is the chain number)

MrBayes >
```

Acceptance rates for the moves in the cold chain – thumb rule, according to which the values should be between 10-70%

State exchange information – in lower line of upper diagonale

Acceptance rates for the swaps between chains separated by only one *heating step* – thumb rule, according to which the values should be between 0.1-0.7

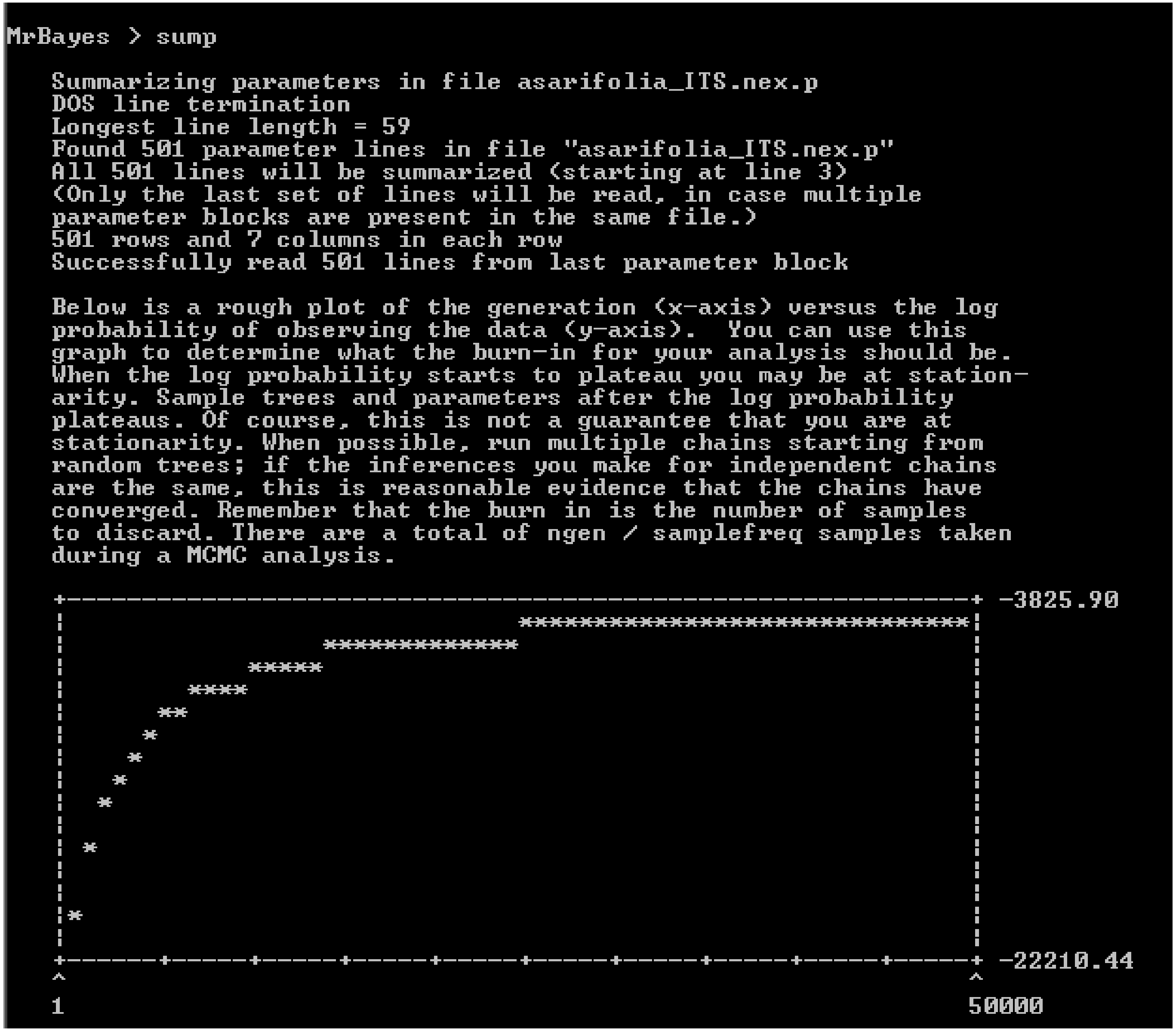
These parameters can be changed by changing temperature



```
   Acceptance rates for the moves in the "cold" chain:
      With prob.  Chain accepted changes to
        57.98 %   param. 1 (revmat) with multiplier
        15.49 %   param. 2 (state frequencies) with Dirichlet proposal
        17.89 %   param. 3 (gamma shape) with multiplier
        55.62 %   param. 4 (prop. invariants) with beta proposal
        38.94 %   param. 5 (topology and branch lengths) with LOCAL
        45.06 %   param. 5 (topology and branch lengths) with extending TBR
        71.63 %   param. 6 (branch lengths) with multiplier
        49.50 %   param. 6 (branch lengths) with nodeslider

   State exchange information:

              1      2      3      4
        --------------------------
      1 |          0.06   0.00   0.00
      2 |   1697          0.06   0.00
      3 |   1710   1647          0.07
      4 |   1658   1631   1657

   Upper diagonal: Proportion of successful exchanges
   Lower diagonal: Number of attempted exchanges
```

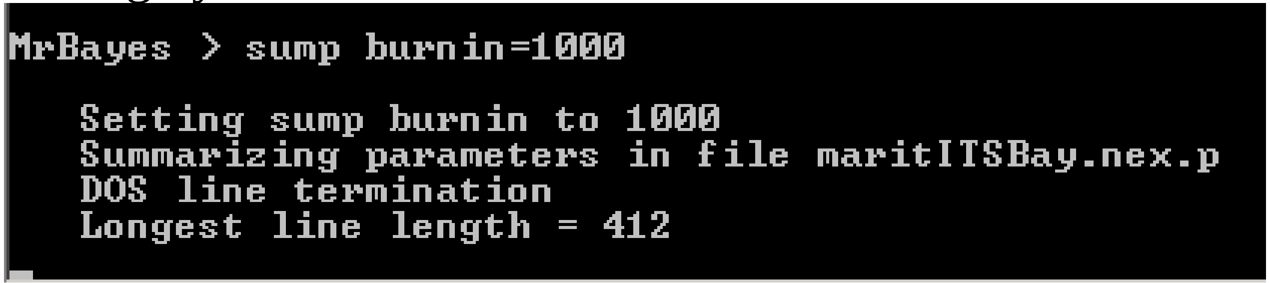
8. Summary of the output – *likelihood scores* of individual trees

sump

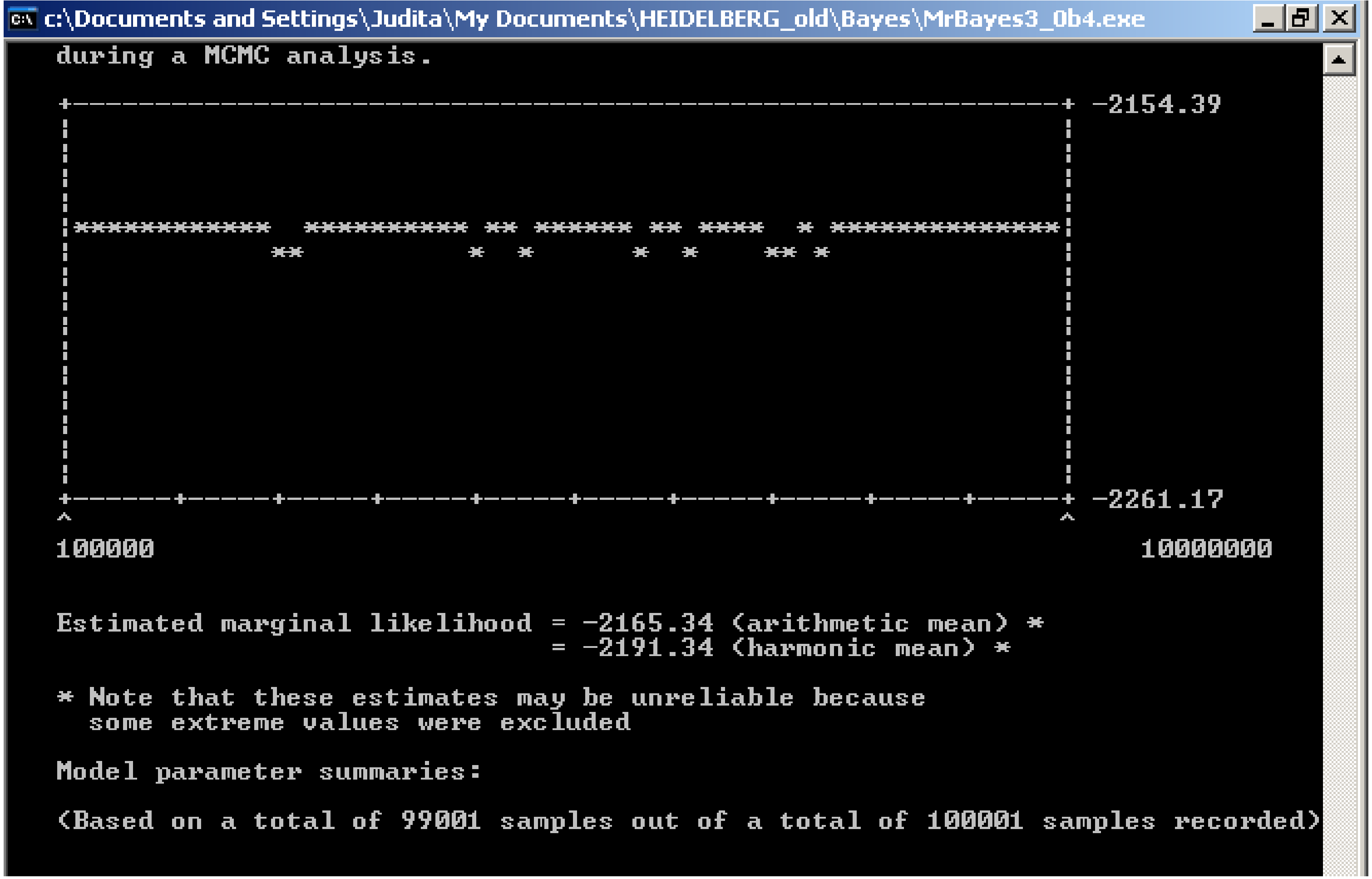


```
MrBayes > sump

   Summarizing parameters in file asarifolia_ITS.nex.p
   DOS line termination
   Longest line length = 59
   Found 501 parameter lines in file "asarifolia_ITS.nex.p"
   All 501 lines will be summarized (starting at line 3)
   (Only the last set of lines will be read, in case multiple
   parameter blocks are present in the same file.)
   501 rows and 7 columns in each row
   Successfully read 501 lines from last parameter block

   Below is a rough plot of the generation (x-axis) versus the log
   probability of observing the data (y-axis).  You can use this
   graph to determine what the burn-in for your analysis should be.
   When the log probability starts to plateau you may be at station-
   arity. Sample trees and parameters after the log probability
   plateaus. Of course, this is not a guarantee that you are at
   stationarity. When possible, run multiple chains starting from
   random trees; if the inferences you make for independent chains
   are the same, this is reasonable evidence that the chains have
   converged. Remember that the burn in is the number of samples
   to discard. There are a total of ngen / samplefreq samples taken
   during a MCMC analysis.

   +------------------------------------------------------------+ -3825.90
   |                              ******************************|
   |                 *************                              |
   |            *****                                           |
   |        ****                                                |
   |      **                                                    |
   |     *                                                      |
   |    *                                                       |
   |   *                                                        |
   |  *                                                         |
   |                                                            |
   | *                                                          |
   |                                                            |
   |                                                            |
   |*                                                           |
   |                                                            |
   +------+-----+-----+-----+-----+-----+-----+-----+-----+-----+ -22210.44
   ^                                                            ^
   1                                                            50000
```

8. Summary of the output – deleting of ca. 10% (initial) trees, checking by *likelihood scores*



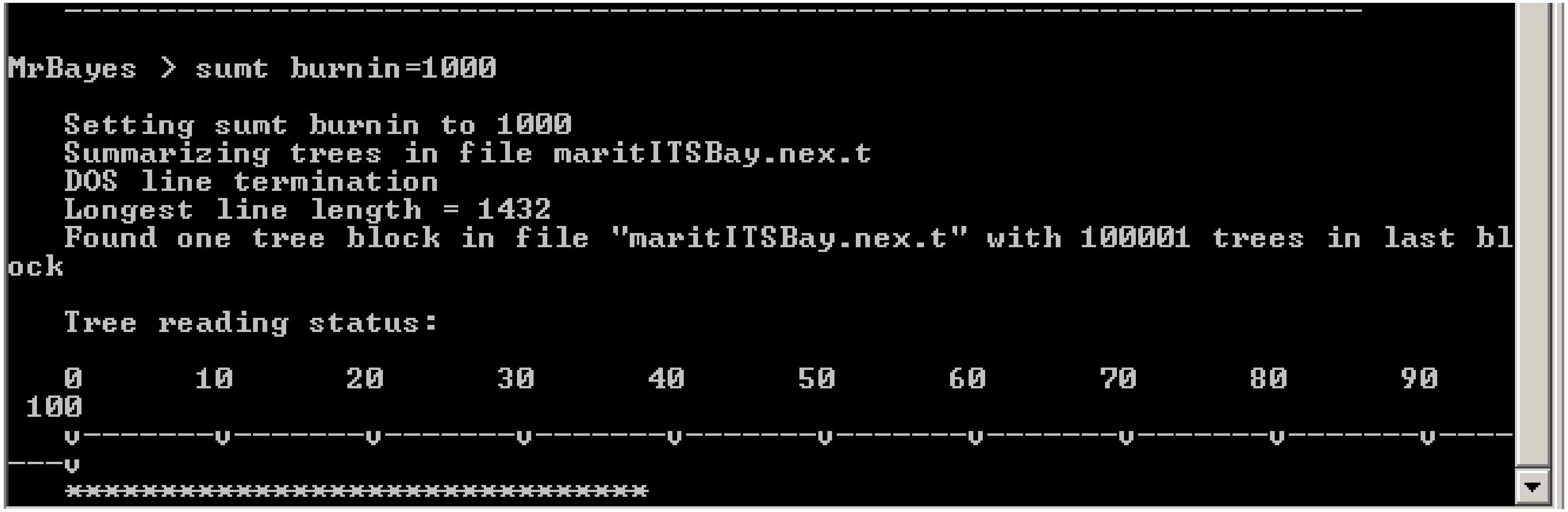
```
MrBayes > sump burnin=1000

   Setting sump burnin to 1000
   Summarizing parameters in file maritITSBay.nex.p
   DOS line termination
   Longest line length = 412
```



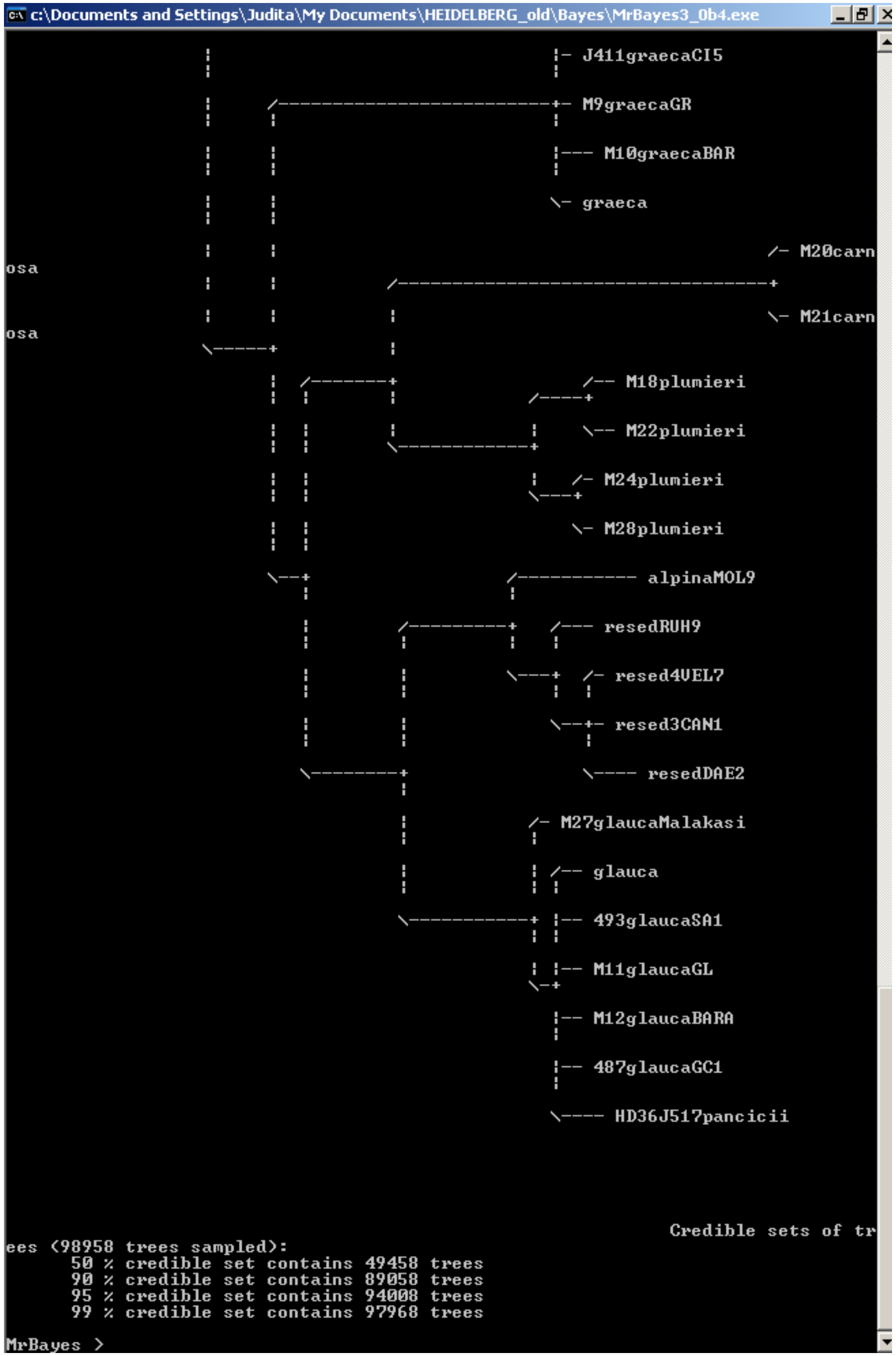
```
during a MCMC analysis.

+------------------------------------------------------------+ -2154.39
|                                                            |
|                                                            |
|                                                            |
|                                                            |
|************  ********** ** ****** ** ****  * **************|
|            **          *  *      *  *    ** *              |
|                                                            |
|                                                            |
|                                                            |
|                                                            |
|                                                            |
|                                                            |
|                                                            |
|                                                            |
+------+-----+-----+-----+-----+-----+-----+-----+-----+-----+ -2261.17
^                                                            ^
100000                                                            10000000


Estimated marginal likelihood = -2165.34 (arithmetic mean) *
                              = -2191.34 (harmonic mean) *

* Note that these estimates may be unreliable because
  some extreme values were excluded

Model parameter summaries:

(Based on a total of 99001 samples out of a total of 100001 samples recorded)
```

8. Summary of the output – deletion of ca. 10% initial trees, calculation of the consensus tree

sumt



```
   --------------------------------------------------------------------

MrBayes > sumt burnin=1000

   Setting sumt burnin to 1000
   Summarizing trees in file maritITSBay.nex.t
   DOS line termination
   Longest line length = 1432
   Found one tree block in file "maritITSBay.nex.t" with 100001 trees in last bl
ock

   Tree reading status:

   0      10      20      30      40      50      60      70      80      90
 100
   v-------v-------v-------v-------v-------v-------v-------v-------v-------v----
---v
   *******************************
```



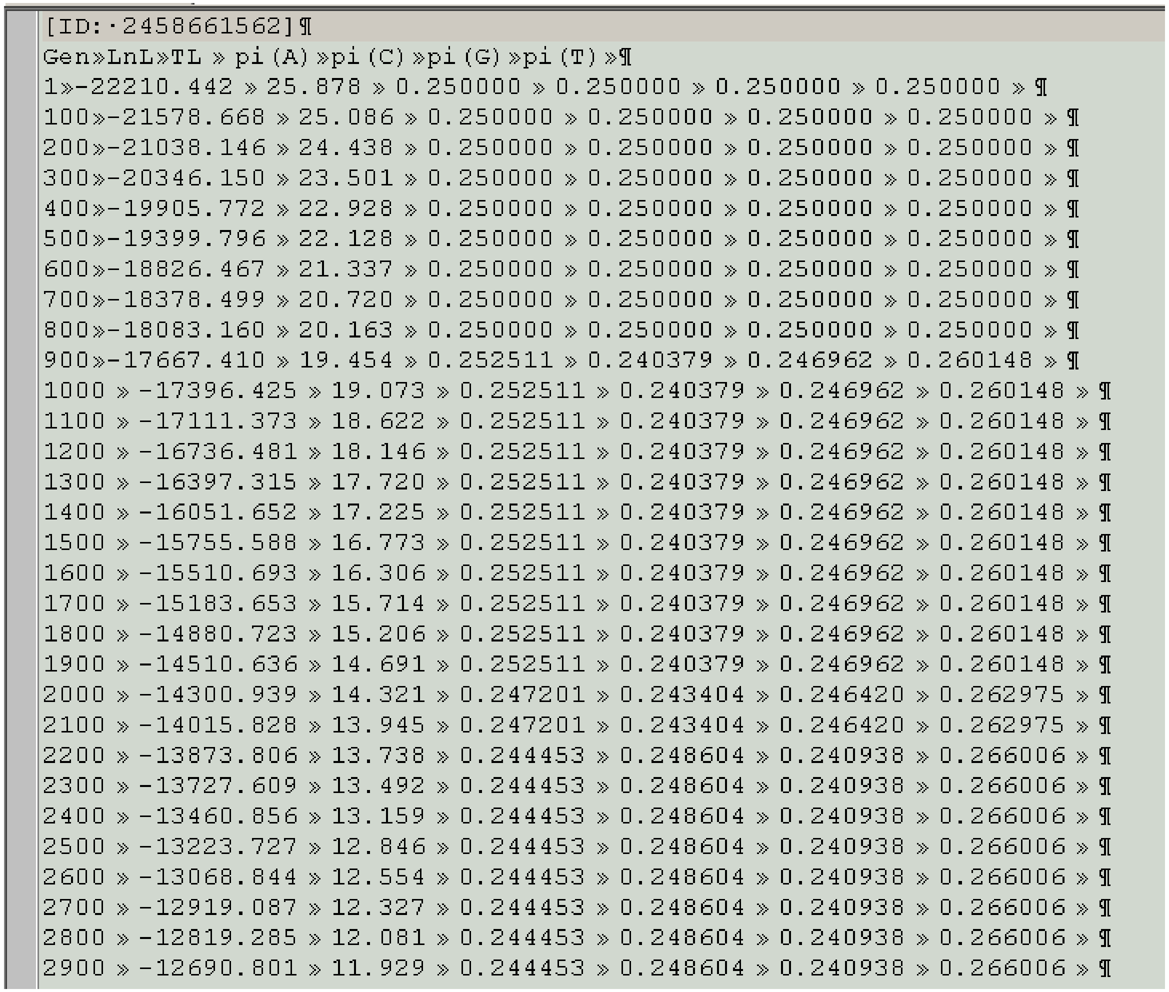
```
                  |                               |- J411graecaCI5
                  |                               |

                  |     /-------------------------+- M9graecaGR
                  |     |                         |

                  |     |                         |--- M10graecaBAR
                  |     |                         |

                  |     |                         \- graeca
                  |     |

                  |     |                                             /- M20carn
osa
                  |     |          /----------------------------------+

                  |     |          |                                  \- M21carn
osa
                  \-----+          |

                        |  /-------+                 /-- M18plumieri
                        |  |       |            /----+

                        |  |       |            |    \-- M22plumieri
                        |  |       \------------+

                        |  |                    |   /- M24plumieri
                        |  |                    \---+

                        |  |                        \- M28plumieri
                        |  |

                        \--+                  /----------- alpinaMOL9
                           |                  |

                           |        /---------+   /--- resedRUH9
                           |        |         |   |

                           |        |         \---+  /- resed4VEL7
                           |        |             |  |

                           |        |             \--+- resed3CAN1
                           |        |                |

                           \--------+                \---- resedDAE2
                                    |

                                    |           /- M27glaucaMalakasi
                                    |           |

                                    |           | /-- glauca
                                    |           | |

                                    \-----------+ |-- 493glaucaSA1
                                                | |

                                                | |-- M11glaucaGL
                                                \-+

                                                  |-- M12glaucaBARA
                                                  |

                                                  |-- 487glaucaGC1
                                                  |

                                                  \---- HD36J517pancicii






                                                             Credible sets of tr
ees (98958 trees sampled):
      50 % credible set contains 49458 trees
      90 % credible set contains 89058 trees
      95 % credible set contains 94008 trees
      99 % credible set contains 97968 trees

MrBayes >
```

Output of analyses – files:

***.p – recorded model parameters for particular generations**

*.t – recorded topology of the tree and branch lengths

*. con – recorded consensus tree with *posterior probabilities* for individual branches
 (-> program TreeView)



```
[ID:·2458661562]¶
Gen»LnL»TL » pi(A)»pi(C)»pi(G)»pi(T)»¶
1»-22210.442 » 25.878 » 0.250000 » 0.250000 » 0.250000 » 0.250000 » ¶
100»-21578.668 » 25.086 » 0.250000 » 0.250000 » 0.250000 » 0.250000 » ¶
200»-21038.146 » 24.438 » 0.250000 » 0.250000 » 0.250000 » 0.250000 » ¶
300»-20346.150 » 23.501 » 0.250000 » 0.250000 » 0.250000 » 0.250000 » ¶
400»-19905.772 » 22.928 » 0.250000 » 0.250000 » 0.250000 » 0.250000 » ¶
500»-19399.796 » 22.128 » 0.250000 » 0.250000 » 0.250000 » 0.250000 » ¶
600»-18826.467 » 21.337 » 0.250000 » 0.250000 » 0.250000 » 0.250000 » ¶
700»-18378.499 » 20.720 » 0.250000 » 0.250000 » 0.250000 » 0.250000 » ¶
800»-18083.160 » 20.163 » 0.250000 » 0.250000 » 0.250000 » 0.250000 » ¶
900»-17667.410 » 19.454 » 0.252511 » 0.240379 » 0.246962 » 0.260148 » ¶
1000 » -17396.425 » 19.073 » 0.252511 » 0.240379 » 0.246962 » 0.260148 » ¶
1100 » -17111.373 » 18.622 » 0.252511 » 0.240379 » 0.246962 » 0.260148 » ¶
1200 » -16736.481 » 18.146 » 0.252511 » 0.240379 » 0.246962 » 0.260148 » ¶
1300 » -16397.315 » 17.720 » 0.252511 » 0.240379 » 0.246962 » 0.260148 » ¶
1400 » -16051.652 » 17.225 » 0.252511 » 0.240379 » 0.246962 » 0.260148 » ¶
1500 » -15755.588 » 16.773 » 0.252511 » 0.240379 » 0.246962 » 0.260148 » ¶
1600 » -15510.693 » 16.306 » 0.252511 » 0.240379 » 0.246962 » 0.260148 » ¶
1700 » -15183.653 » 15.714 » 0.252511 » 0.240379 » 0.246962 » 0.260148 » ¶
1800 » -14880.723 » 15.206 » 0.252511 » 0.240379 » 0.246962 » 0.260148 » ¶
1900 » -14510.636 » 14.691 » 0.252511 » 0.240379 » 0.246962 » 0.260148 » ¶
2000 » -14300.939 » 14.321 » 0.247201 » 0.243404 » 0.246420 » 0.262975 » ¶
2100 » -14015.828 » 13.945 » 0.247201 » 0.243404 » 0.246420 » 0.262975 » ¶
2200 » -13873.806 » 13.738 » 0.244453 » 0.248604 » 0.240938 » 0.266006 » ¶
2300 » -13727.609 » 13.492 » 0.244453 » 0.248604 » 0.240938 » 0.266006 » ¶
2400 » -13460.856 » 13.159 » 0.244453 » 0.248604 » 0.240938 » 0.266006 » ¶
2500 » -13223.727 » 12.846 » 0.244453 » 0.248604 » 0.240938 » 0.266006 » ¶
2600 » -13068.844 » 12.554 » 0.244453 » 0.248604 » 0.240938 » 0.266006 » ¶
2700 » -12919.087 » 12.327 » 0.244453 » 0.248604 » 0.240938 » 0.266006 » ¶
2800 » -12819.285 » 12.081 » 0.244453 » 0.248604 » 0.240938 » 0.266006 » ¶
2900 » -12690.801 » 11.929 » 0.244453 » 0.248604 » 0.240938 » 0.266006 » ¶
```

Output of analyses – files:

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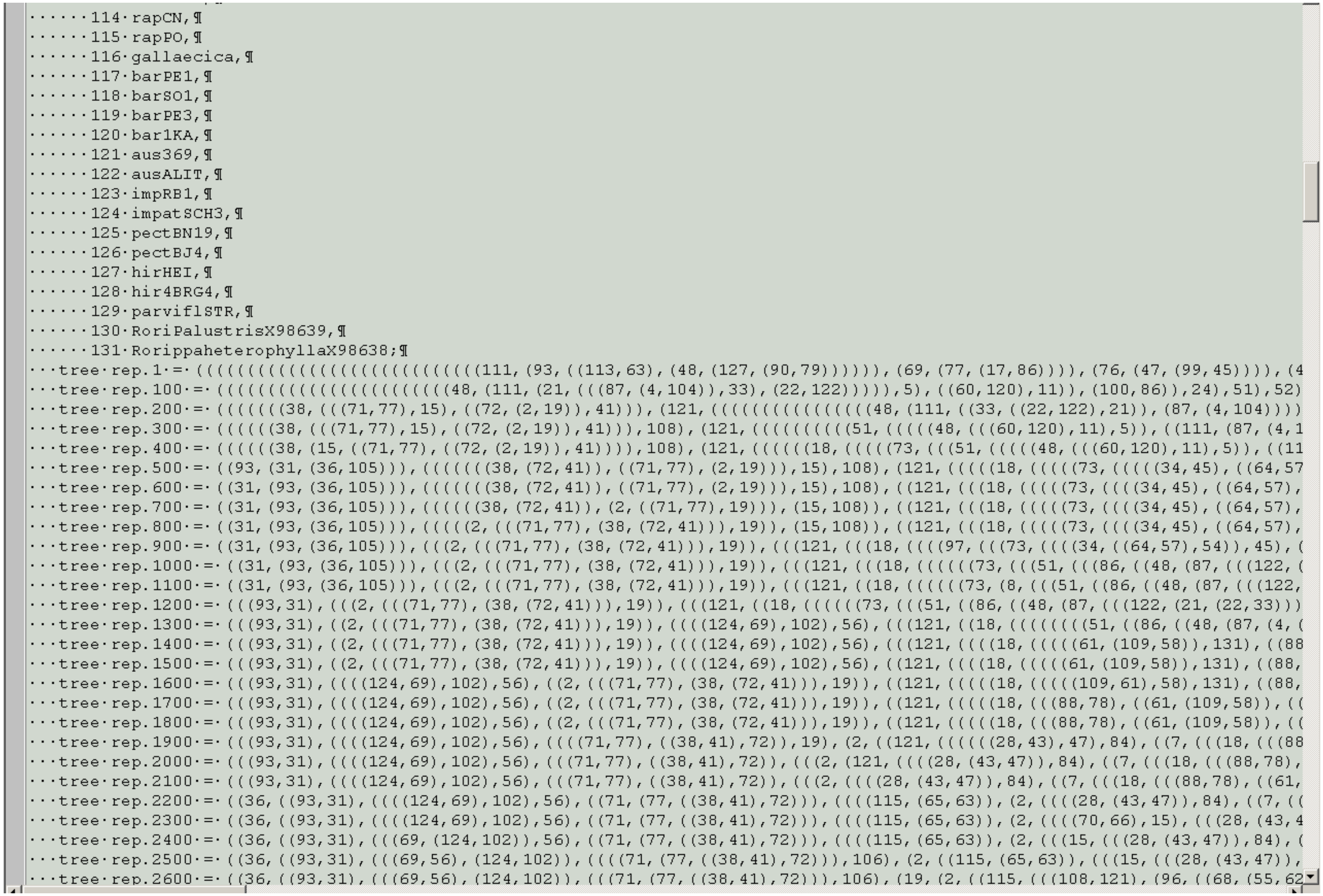
 (-> program TreeView)



```
······114·rapCN,¶
······115·rapPO,¶
······116·gallaecica,¶
······117·barPE1,¶
······118·barSO1,¶
······119·barPE3,¶
······120·bar1KA,¶
······121·aus369,¶
······122·ausALIT,¶
······123·impRB1,¶
······124·impatSCH3,¶
······125·pectBN19,¶
······126·pectBJ4,¶
······127·hirHEI,¶
······128·hir4BRG4,¶
······129·parviflSTR,¶
······130·RoriPalustrisX98639,¶
······131·RorippaheterophyllaX98638;¶
···tree·rep.1·=·((((((((((((((((((((((((((((111,(93,((113,63),(48,(127,(90,79)))))),(69,(77,(17,86)))),(76,(47,(99,45)))),(4
···tree·rep.100·=·(((((((((((((((((((((((48,(111,(21,(((87,(4,104)),33),(22,122))))),5),((60,120),11)),(100,86)),24),51),52)
···tree·rep.200·=·(((((((38,(((71,77),15),((72,(2,19)),41))),(121,(((((((((((((((48,(111,((33,((22,122),21)),(87,(4,104)))
···tree·rep.300·=·((((((38,(((71,77),15),((72,(2,19)),41))),108),(121,(((((((((51,((((48,(((60,120),11),5)),((111,(87,(4,1
···tree·rep.400·=·((((((38,(15,((71,77),((72,(2,19)),41)))),108),(121,((((((18,(((((73,(((51,((((48,(((60,120),11),5)),((11
···tree·rep.500·=·((93,(31,(36,105))),(((((((38,(72,41)),((71,77),(2,19))),15),108),(121,(((((18,(((((73,((((34,45),((64,57
···tree·rep.600·=·((31,(93,(36,105))),(((((((38,(72,41)),((71,77),(2,19))),15),108),((121,(((18,(((((73,((((34,45),((64,57),
···tree·rep.700·=·((31,(93,(36,105))),((((((38,(72,41)),(2,((71,77),19))),(15,108)),((121,(((18,(((((73,((((34,45),((64,57),
···tree·rep.800·=·((31,(93,(36,105))),(((((2,(((71,77),(38,(72,41))),19)),(15,108)),((121,(((18,(((((73,((((34,45),((64,57),
···tree·rep.900·=·((31,(93,(36,105))),(((2,(((71,77),(38,(72,41))),19)),(((121,(((18,((((97,(((73,((((34,((64,57),54)),45),(
···tree·rep.1000·=·((31,(93,(36,105))),(((2,(((71,77),(38,(72,41))),19)),(((121,(((18,((((((73,(((51,(((86,((48,(87,(((122,(
···tree·rep.1100·=·((31,(93,(36,105))),(((2,(((71,77),(38,(72,41))),19)),(((121,((18,((((((73,(8,(((51,((86,((48,(87,(((122,
···tree·rep.1200·=·(((93,31),(((2,(((71,77),(38,(72,41))),19)),(((121,((18,((((((73,(((51,((86,((48,(87,(((122,(21,(22,33)))
···tree·rep.1300·=·(((93,31),((2,(((71,77),(38,(72,41))),19)),((((124,69),102),56),(((121,((18,(((((((51,((86,((48,(87,(4,(
···tree·rep.1400·=·(((93,31),((2,(((71,77),(38,(72,41))),19)),((((124,69),102),56),(((121,((((18,(((((61,(109,58)),131),((88
···tree·rep.1500·=·(((93,31),((2,(((71,77),(38,(72,41))),19)),((((124,69),102),56),((121,((((18,(((((61,(109,58)),131),((88,
···tree·rep.1600·=·(((93,31),((((124,69),102),56),((2,(((71,77),(38,(72,41))),19)),((121,(((((18,(((((109,61),58),131),((88,
···tree·rep.1700·=·(((93,31),((((124,69),102),56),((2,(((71,77),(38,(72,41))),19)),((121,(((((18,(((88,78),((61,(109,58)),((
···tree·rep.1800·=·(((93,31),((((124,69),102),56),((2,(((71,77),(38,(72,41))),19)),((121,(((((18,(((88,78),((61,(109,58)),((
···tree·rep.1900·=·(((93,31),((((124,69),102),56),((((71,77),((38,41),72)),19),(2,((121,((((((28,43),47),84),((7,(((18,(((88
···tree·rep.2000·=·(((93,31),((((124,69),102),56),(((71,77),((38,41),72)),(((2,(121,((((28,(43,47)),84),((7,(((18,(((88,78),
···tree·rep.2100·=·(((93,31),((((124,69),102),56),(((71,77),((38,41),72)),(((2,((((28,(43,47)),84),((7,(((18,(((88,78),((61,
···tree·rep.2200·=·((36,((93,31),((((124,69),102),56),((71,(77,((38,41),72))),((((115,(65,63)),(2,((((28,(43,47)),84),((7,((
···tree·rep.2300·=·((36,((93,31),((((124,69),102),56),((71,(77,((38,41),72))),((((115,(65,63)),(2,((((70,66),15),(((28,(43,4
···tree·rep.2400·=·((36,((93,31),(((69,(124,102)),56),((71,(77,((38,41),72))),((((115,(65,63)),(2,(((15,(((28,(43,47)),84),(
···tree·rep.2500·=·((36,((93,31),(((69,56),(124,102)),((((71,(77,((38,41),72))),106),(2,((115,(65,63)),(((15,(((28,(43,47)),
···tree·rep.2600·=·((36,((93,31),(((69,56),(124,102)),(((71,(77,((38,41),72))),106),(19,(2,((115,(((108,121),(96,((68,(55,62
```