BAli-Phy User's Guide v4.3

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1. Introduction

BAli-Phy runs on Windows, macOS, and Linux. It is a command-line program: you type commands in a terminal to analyze data and summarize results, rather than using menus and buttons. The arguments and options in a command specify the input data and the evolutionary model to use.

An analysis has two phases. First, `bali-phy` generates *posterior samples* of trees, alignments, and model parameters and saves them in files. Then `bpy-summarize` summarizes these samples into trees, alignments, and parameter estimates, checks for signs of a lack of convergence, and creates a report you can view in a web browser. The Quick Start walks through both phases.

The program that reads and interprets commands in a terminal is called a *shell*. Unix shells such as Bash and Zsh are used on Linux and macOS, and inside WSL on Windows. Git Bash and MSYS2 provide Bash for native Windows; Windows also provides PowerShell and Command Prompt (`cmd.exe`). If command-line programs are unfamiliar, see the introductions to PowerShell or the Linux command line.

Commands in this guide apply to all these shells unless labelled otherwise. Where instructions differ, choose the panel for your shell; “Unix shell” includes Git Bash and MSYS2 Bash. Printed copies show all alternatives. Prompt markers such as `%` and `PS>` show where to type; do not copy them. A shared example shows just one prompt but works in each shell named. PowerShell examples for installation and the Quick Start work in both Windows PowerShell 5.1 and PowerShell 7; later examples note any additional requirements.

BAli-Phy also includes small command-line utilities such as `alignment-cat` and `trees-consensus` for processing alignments, assembling data sets, and summarizing MCMC results.

2. Installation

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2.1. Hardware requirements

We typically run BAli-Phy on workstations with at least 16 GB of RAM and 4 cores. More cores will allow you to run more MCMC chains at once, and more RAM will allow you to run larger data sets. However, it is often easier and faster to run BAli-Phy on a (Linux) computing cluster, if you have one available.

2.2. Upgrades

If you have previously installed bali-phy, you do not have to remove the old version before installing the new version. Simply follow the installation instructions for the new version. If you are manually adding the new version of bali-phy to your PATH, just make sure that the new version comes before the old version in the PATH, or remove the old version from the PATH.

To remove an older version, delete its `bali-phy-`*`oldversion`* directory and remove that version from `PATH`. User-installed packages and the compiled-module cache are stored separately, normally under `%LOCALAPPDATA%\bali-phy` on native Windows or `~/.local/share/bali-phy` on Unix-like systems. They are shared between installed versions and are not removed when you delete a program directory.

2.3. Install with a package manager

BAli-Phy is available through Bioconda, Homebrew, and Spack. On Windows, use these instructions inside WSL. Package versions may differ from the latest BAli-Phy release.

2.3.1. Bioconda

Install the Bioconda package using either Pixi or conda:

Pixi

If needed, install Pixi and complete its shell setup. Then run:

```
% pixi global install -c conda-forge -c bioconda bali-phy
% bali-phy --version
```

No environment activation is required.

Conda

If you don't already have conda, install it through Miniforge and complete its shell setup. Then run:

```
% conda create -n bali-phy --override-channels \
  -c conda-forge -c bioconda --strict-channel-priority bali-phy
% conda activate bali-phy
% bali-phy --version
```

In each new terminal, run `conda activate bali-phy` before using BAli-Phy.

Bioconda includes programs used by `bpy-summarize`, including R. See the Bioconda package page for available versions and platforms.

2.3.2. Homebrew

On macOS, install Apple's Command Line Tools if needed:

```
% xcode-select --install
```

Check the Homebrew installation requirements, install Homebrew, and complete its shell setup. Then run:

```
% brew install brewsci/bio/bali-phy
% bali-phy --version
```

Homebrew uses a prebuilt package when available; otherwise, it builds from source. No additional BAli-Phy-specific PATH setting is needed. If the formula lags behind a release you need, use the archives for macOS or Linux.

2.3.3. Spack

Spack is a package manager for scientific software, designed for computing clusters and also usable on individual computers.

If needed, follow Spack's getting-started instructions, including shell setup, and its compiler configuration instructions. Installation may compile BAli-Phy and its dependencies. Then run:

```
% spack install bali-phy
% spack load bali-phy
% bali-phy --version
```

`spack load bali-phy` makes the installed programs available in the current shell. In each new terminal, initialize Spack and run this command again before using BAli-Phy.

See the Spack package recipe for available versions, dependencies, and options.

Continue with *Section 2.8, "Test the installed software"* after installation.

2.4. Install on Windows

Choose between installing inside Windows Subsystem for Linux (WSL2, preferred) and installing the native Windows package. Inside WSL2, follow the Linux installation instructions. For terminals outside WSL2, including Git Bash and MSYS2 Bash, use the native Windows package below.

2.4.1. Install inside WSL2 (preferred)

Install WSL2 and open its Linux terminal. Follow the Linux installation instructions inside WSL2, using a package manager or release archive. Continue using that terminal for the Linux commands throughout this guide.

Programs installed inside WSL2 use Linux paths such as `/home/user/data.fasta`. The native Windows package instead uses Windows paths such as `C:\Users\user\data.fasta`. Choose one installation route for an analysis rather than mixing commands and paths from the two environments.

2.4.2. Install the native Windows package

If you use PowerShell, installing the latest stable PowerShell improves handling of quoted arguments and redirected program output. Start it with `pwsh`; `powershell` still starts Windows PowerShell 5.1. Upgrading is optional for the installation checks and Quick Start below.

Download `bali-phy-4.3-win64.tar.gz` from the BAli-Phy release page. In your chosen terminal, extract it into a versioned directory under your home directory and check that it runs:

PowerShell

```
PS> New-Item -ItemType Directory -Force "$HOME\Applications"
PS> tar -xf "$HOME\Downloads\bali-phy-4.3-win64.tar.gz" -C "$HOME\Applications"
PS> & "$HOME\Applications\bali-phy-4.3\bin\bali-phy.exe" --version
```

Command Prompt

```
C:\> if not exist "%USERPROFILE%\Applications" mkdir "%USERPROFILE%\Applications"
C:\> tar -xf "%USERPROFILE%\Downloads\bali-phy-4.3-win64.tar.gz" -C "%USERPROFILE%
\Applications"
C:\> "%USERPROFILE%\Applications\bali-phy-4.3\bin\bali-phy.exe" --version
```

Git Bash / MSYS2 Bash

```
% mkdir -p "$HOME/Applications"
% tar -xf "$(cygpath "$USERPROFILE")/Downloads/bali-phy-4.3-win64.tar.gz" -C "$HOME/
Applications"
% "$HOME/Applications/bali-phy-4.3/bin/bali-phy" --version
```

The archive is read from your Windows Downloads directory and extracted under `$HOME`, your shell's home directory. In MSYS2, this can differ from your Windows user directory. `cygpath` converts the Windows user-directory path to the Unix-style path used by these commands.

If your browser downloaded the archive somewhere other than `Downloads`, replace that path with the location of the downloaded file. The archive contains all required non-system runtime DLLs for the included executables, including `draw-tree`. You do not need to install MSYS2 to run the programs in the binary package.

Add the BAli-Phy `bin` directory to the current terminal's command search path:

PowerShell

```
PS> $env:Path = "$HOME\Applications\bali-phy-4.3\bin;$env:Path"
```

Command Prompt

```
C:\> set "PATH=%USERPROFILE%\Applications\bali-phy-4.3\bin;%PATH%"
```

Git Bash / MSYS2 Bash

```
% export PATH="$HOME/Applications/bali-phy-4.3/bin:$PATH"
```

These settings affect only the current terminal. See *Section 2.7.1, "Windows"* to make the setting permanent.

The compiled BAli-Phy programs do not require Python. The `bpy-summarize` and `bali-phy-pkg` commands are Python scripts, however, and require Python 3. You can install Python 3 for Windows. When using the Python installer, select the option that makes Python available from the command line. In the terminal you will use for BAli-Phy, check `py -3 --version`. If `py` is not found, check `python --version` instead; it must report Python 3. Run the scripts as `bpy-summarize` and `bali-phy-pkg` in all these shells; no filename extension is needed.

R is optional. When it is installed and available on `Path`, `bpy-summarize` uses it to generate additional plots. Without it, it still produces the main HTML report, summary trees, alignments, and diagnostics, and reports which optional figures were omitted.

Continue with *Section 2.8, "Test the installed software"* after installing Python and adding BAli-Phy to `Path`.

2.5. Install on macOS

Install BAli-Phy with Bioconda or Homebrew, or download a release archive as described below.

2.5.1. Install from a release archive

Open a window in the Terminal app to access the UNIX command line. Follow the instructions below to download and extract the executables.

Important: Removing the quarantine attribute

If you download the archive from the official BAli-Phy release page using a web browser, macOS marks it as quarantined. Remove the quarantine attribute before extracting the archive so macOS will allow the installed programs to run. Otherwise, the extracted files inherit the quarantine attribute. Only do this for an archive that you trust. For an archive saved in Downloads, run the command for your processor:

Apple Silicon:

```
% xattr -d com.apple.quarantine "$HOME/Downloads/bali-phy-4.3-mac-arm64.tar.gz"
```

Intel:

```
% xattr -d com.apple.quarantine "$HOME/Downloads/bali-phy-4.3-mac-intel64.tar.gz"
```

If you saved the archive elsewhere, adjust the path. After removing quarantine, extract the archive and move the resulting `bali-phy-4.3` directory into `~/Applications`. Then add its `bin` directory to your PATH as described in *Section 2.7, "Add BAli-Phy to your `PATH`"*.

The `curl` commands below do not normally add the quarantine attribute.

If you have an Apple Silicon computer, type:

```
% mkdir -p ~/Applications
% cd ~/Applications
% curl -LO https://github.com/bredelings/BAli-Phy/releases/download/4.3/bali-phy-4.3-mac-
```

```
arm64.tar.gz
% tar -zxf bali-phy-4.3-mac-arm64.tar.gz
```

If you have an older Intel computer, type:

```
% mkdir -p ~/Applications
% cd ~/Applications
% curl -LO https://github.com/bredelings/BAli-Phy/releases/download/4.3/bali-phy-4.3-mac-
intel64.tar.gz
% tar -zxf bali-phy-4.3-mac-intel64.tar.gz
```

Check that the executable runs:

```
% ~/Applications/bali-phy-4.3/bin/bali-phy --version
```

You still need to add it to your PATH as described in *Section 2.7, "Add BAli-Phy to your `PATH`"*.

2.5.2. Install programs used by `bpy-summarize` using Homebrew

You can install R via Homebrew:

```
% brew install r
```

2.5.3. Install programs used for viewing the results

Programs for viewing trees, alignments, and MCMC traces are optional. Install them from their project websites as needed; see *Section 2.9, "Optional programs for inspecting results"*.

2.6. Install on Linux

Install BAli-Phy with Bioconda, Homebrew, or Spack, or use a release archive or distribution package as described below.

2.6.1. Install from a release archive

First install `wget`. If you have Debian or Ubuntu Linux, type:

```
% sudo apt-get install wget
```

Then download and extract the executables. For Ubuntu 24.04 on x86-64, type:

```
% mkdir -p ~/Applications
% cd ~/Applications
% wget https://github.com/bredelings/BAli-Phy/releases/download/4.3/bali-phy-4.3-
ubuntu-24.04.tar.gz
% tar -zxf bali-phy-4.3-ubuntu-24.04.tar.gz
```

For Ubuntu 26.04 on x86-64, type:

```
% mkdir -p ~/Applications
% cd ~/Applications
% wget https://github.com/bredelings/BAli-Phy/releases/download/4.3/bali-phy-4.3-
ubuntu-26.04.tar.gz
% tar -zxf bali-phy-4.3-ubuntu-26.04.tar.gz
```

Second, check that the executable runs:

```
% ~/Applications/bali-phy-4.3/bin/bali-phy --version
```

Third, add BAli-Phy to your PATH as described in *Section 2.7, "Add BAli-Phy to your `PATH`"*.

Fourth, test the software as described in *Section 2.8, "Test the installed software"*.

2.6.2. Install BAli-Phy using `apt-get` (alternative)

BAli-Phy packages exist for Ubuntu and for Debian (testing and unstable). However, these may not be up-to-date.

```
% sudo apt-get install bali-phy
```

Check that the executable runs:

```
% bali-phy --version
```

If you install with `apt-get`, you don't need to do anything extra to put bali-phy in your PATH.

2.6.3. Install programs used by `bpy-summarize`

On Debian or Ubuntu Linux, install R with:

```
% sudo apt-get install r-base
```

2.6.4. Install programs used to view the results

Programs for viewing trees, alignments, and MCMC traces are optional. Install them from their project websites as needed; see *Section 2.9, "Optional programs for inspecting results"*.

2.7. Add BAli-Phy to your `PATH`

The `PATH` environment variable lists the directories in which your terminal searches for commands. Once the BAli-Phy `bin` directory is on `PATH`, you can type `bali-phy`, `bpy-summarize`, and the other command names without specifying their complete locations.

2.7.1. Windows

The Windows installation instructions above show how to add BAli-Phy to the current terminal session. For PowerShell and Command Prompt, make the change permanent by searching for "environment variables" in Windows Settings, editing your user `Path`, and adding:

```
%USERPROFILE%\Applications\bali-phy-4.3\bin
```

For Git Bash and MSYS2 Bash, add this line to `~/.bashrc`:

```
export PATH="$HOME/Applications/bali-phy-4.3/bin:$PATH"
```

If you installed BAli-Phy somewhere else, use the `bin` directory from that location instead. Open a new terminal and check the result:

```
% bali-phy --version
```

If a new Git Bash or MSYS2 Bash terminal does not pick up the setting, make sure its login startup file reads `~/.bashrc`. Bash uses the first existing file among `~/.bash_profile`, `~/.bash_login`, and `~/.profile`; if none exists, create `~/.bash_profile`. Add this line if it is not already present, then open a new terminal and repeat the check:

```
if [ -f ~/.bashrc ]; then . ~/.bashrc; fi
```

2.7.2. Linux, macOS, and WSL2

If you extracted BAli-Phy under `~/Applications`, add its `bin` directory to the current `sh`, `bash`, or `zsh` shell:

```
% export PATH="$HOME/Applications/bali-phy-4.3/bin:$PATH"
```

For `csh` or `tcsh`, use:

```
% setenv PATH $HOME/Applications/bali-phy-4.3/bin:$PATH
```

To apply the setting in future interactive terminals, add the corresponding command above to the file for your shell:

Shell	Startup file
Bash	`~/.bashrc`
Zsh	`~/.zshrc`
Csh	`~/.cshrc`
Tcsh	`~/.tcshrc`

Open a new terminal and check the result:

```
% bali-phy --version
```

If a new Bash terminal does not pick up the setting, see Bash's startup-file documentation.

If you installed BAli-Phy somewhere else, substitute the corresponding `bin` directory. Package managers such as Homebrew and apt normally configure `PATH` automatically. With Spack, use `spack load bali-phy` as described in *Section 2.3.3, "Spack"*.

2.8. Test the installed software

First copy an example alignment into a new working directory:

Unix shell
including Git Bash and MSYS2 Bash

```
% cp "$HOME/Applications/bali-phy-4.3/share/doc/bali-phy/examples/5S-rRNA/25.fasta" .
```

PowerShell

```
PS> cp "$HOME\Applications\bali-phy-4.3\share\doc\bali-phy\examples\5S-rRNA\25.fasta" .
```

Command Prompt

```
C:\> copy "%USERPROFILE%\Applications\bali-phy-4.3\share\doc\bali-phy\examples\5S-
rRNA\25.fasta" .
```

If a package manager installed BAli-Phy elsewhere, copy `examples/5S-rRNA/25.fasta` from its documentation directory instead. For Spack, use:

```
% cp "$(spack location -i bali-phy)/share/doc/bali-phy/examples/5S-rRNA/25.fasta" .
```

Then run these commands in the same terminal:

```
% bali-phy --version
% draw-tree --help
% bpy-summarize --help
% bali-phy-pkg --help
% bali-phy 25.fasta --iterations=200
% bali-phy 25.fasta --iterations=200
% bpy-summarize 25-1 25-2
```

Then check that the file `Results/index.html` exists and can be opened in a web browser.

2.9. Optional programs for inspecting results

The main output from `bpy-summarize` is the report `Results/index.html`. Open this report in a web browser first: it contains summary trees and alignments, mixing diagnostics, and links to the underlying result files. The programs below are optional and are useful when you want to inspect those files more closely.

Task	Recommended program	When to use it
Inspect scalar MCMC traces	Tracer	Load all the `C1.log` files from an analysis at once to compare traces, marginal distributions, and effective sample sizes. The `statreport` command and the HTML report provide non-interactive summaries of the same numerical parameters.
View a tree locally	TreeViewer	A cross-platform viewer for exploring, styling, and exporting trees. It reads BAli-Phy consensus trees and also recognizes structured attributes in bare Newick trees.
View a tree locally	FigTree	A long-established general-purpose viewer for Newick and NEXUS trees, with controls for formatting and exporting tree figures. It is widely used with BEAST output.
View and annotate a tree online	iTOL	A web application with extensive annotation and figure-export options. It accepts Newick and NEXUS trees and understands NHX and MrBayes-style metadata. Using it requires uploading the tree to a third-party service.

Task	Recommended program	When to use it
Quickly view or edit an alignment	SeaView	A lightweight, cross-platform alignment editor that reads FASTA and other common alignment formats.
Quickly view or edit an alignment	AliView	A lightweight, cross-platform alignment editor that reads FASTA and other common alignment formats. Handles large alignments particularly well.
Explore an alignment in more detail	Jalview	A cross-platform alignment viewer, editor, and analysis workbench with more annotation and analysis facilities than a simple alignment viewer.
Create scripted tree figures	ggtree and treeio	R packages for reproducible tree figures and for importing annotated tree formats. These are most appropriate when the figure should be generated by a script rather than edited interactively.

To check the viewers with the tutorial output, open `25-1/C1.log` and `25-2/C1.log` together in Tracer, `Results/c50.PP.tree` in a tree viewer, and `Results/P1.consensus.pd-wsum.fasta` in an alignment viewer.

Consensus trees produced by `bpy-summarize`, such as `Results/c50.PP.tree`, can be opened directly in TreeViewer, FigTree, or iTOL.

Caution: Preserve branch annotations

Be more careful with annotated input trees: some BAli-Phy analyses use comments such as `[&foreground=1]` to identify particular branches. TreeViewer can read these annotations from a bare Newick tree, but its ordinary Newick export omits them. FigTree reads metadata comments from NEXUS trees, but not from bare Newick trees. Keep the original annotated tree, and check any copy saved by a viewer before using it as input to BAli-Phy.

3. Running the program

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A BAli-Phy analysis uses two programs. `bali-phy` samples trees, alignments, and model parameters. `bpy-summarize` summarizes those samples and produces a report with estimates and convergence diagnostics. You can update the report while the analysis is running.

By default, BAli-Phy estimates both the alignment and the tree:

```
% bali-phy sequences.fasta
```

BAli-Phy can also estimate trees and model parameters while keeping the input alignment fixed:

```
% bali-phy sequences.fasta -I none
```

Run `bali-phy --help` for commonly used options. The Quick Start below shows how to run several independent analyses and inspect their results.

3.1. Quick Start

This example uses the 25-taxon 5S-rRNA data set. Create a new working directory and change into it. Using a new directory ensures that the four runs below save their results in directories named `25-1` through `25-4`. Copy the example data into this directory:

Unix shell
including Git Bash and MSYS2 Bash

```
% cp "$HOME/Applications/bali-phy-4.3/share/doc/bali-phy/examples/5S-rRNA/25.fasta" .
```

PowerShell

```
PS> cp "$HOME\Applications\bali-phy-4.3\share\doc\bali-phy\examples\5S-rRNA\25.fasta" .
```

Command Prompt

```
C:\> copy "%USERPROFILE%\Applications\bali-phy-4.3\share\doc\bali-phy\examples\5S-
rRNA\25.fasta" .
```

If a package manager installed BAli-Phy elsewhere, copy `examples/5S-rRNA/25.fasta` from its documentation directory instead.

Start four independent chains:

Unix shell
including Git Bash and MSYS2 Bash
Run this command *four times* in the same terminal. The trailing `&` lets the chains run simultaneously:

```
% bali-phy 25.fasta -S "GTR +> ASRV.Free +> Covarion.Huelsenbeck02" --iterations=1000 &
```

PowerShell / Command Prompt
Open four terminals in the directory containing `25.fasta`. Make sure BAli-Phy is on `Path` in each terminal, and run this command once in each:

```
PS> bali-phy 25.fasta -S "GTR +> ASRV.Free +> Covarion.Huelsenbeck02" --iterations=1000
```

Once the runs have produced some samples, you can summarize their results while they are still running. Use an available terminal in the directory containing `25.fasta`, opening another terminal if necessary:

```
% bpy-summarize 25-1 25-2 25-3 25-4
```

You can rerun this command as more samples accumulate. Open or reload `Results/index.html` in a web browser to inspect the updated report. The report contains tree and alignment summaries, convergence warnings, mixing diagnostics, and links to the underlying result files.

These short runs demonstrate how to run an analysis and summarize its results. They stop after 1,000 iterations, but reaching that limit does not establish convergence. Check the report's warnings and diagnostics before interpreting the estimates. See *Section 12, “Convergence and Mixing: Is it done yet?”* for help deciding whether longer runs are needed. That section also explains how to compare chains using tools such as Tracer.

See *Section 4.2, “Posterior summaries”* for details of the output files and *Section 2.9, “Optional programs for inspecting results”* for optional programs to inspect trees, alignments, and parameter traces.

3.2. Input

When estimating the alignment, BAli-Phy accepts unaligned sequences: you do not need to align them first. If the input already contains an alignment, BAli-Phy removes the gaps and estimates a new alignment as part of the analysis.

When keeping the alignment fixed with `-I none`, provide aligned sequences of equal length, including gaps. Their columns define the alignment used throughout the analysis.

BAli-Phy can read in sequences and alignments in both FastA and PHYLIP formats. Filenames for FastA files should end in `.fasta`, `.mpfa`, `.fna`, `.fas`, `.fsa`, or `.fa`. Filenames for PHYLIP files should end in `.phy`. If one of these extensions is not used, then BAli-Phy will attempt to guess which format is being used.

FASTA format prefixes sequence names with ">":

FASTA example

```
>human       this is a comment and is not part of the sequence name
CTGACTCCTGAGGAGAAGTCTGCCGTTACTGCCCTGTGGGGCAAGGTGAACGTGGATGAA
GTTGGTGGTGAGGCCCTGGGCAGGCTGCTGGTGGTCTACCCTTGGACCCAGAGGTTCTTT
>tarsier     this is also a comment
CTGACTGCTGAAGAGAAGGCCGCCGTCACTGCCCTGTGGGGCAAGGTAGACGTGGAAGAT
GTTGGTGGTGAGGCCCTGGGCAGGCTGCTGGTCGTCTACCCATGGACCCAGAGGTTCTTT
>bushbaby
CTGACTCCTGATGAGAAGAATGCCGTTTGTGCCCTGTGGGGCAAGGTGAATGTGGAAGAA
GTTGGTGGTGAGGCCCTGGGCAGGCTGCTGGTTGTCTACCCATGGACCCAGAGGTTCTTT
>hare
CTGTCCGGTGAGGAGAAGTCTGCGGTCACTGCCCTGTGGGGCAAGGTGAATGTGGAAGAA
GTTGGTGGTGAGACCCTGGGCAGGCTGCTGGTTGTCTACCCATGGACCCAGAGGTTCTTC
```

If the sequence-name line contains a space, BAli-Phy treats everything after the space as a comment.

To analyze only part of the input, append a range to the filename:

```
% bali-phy sequences.fasta:1-30,90-100
```

This selects positions 1–30 and 90–100 in the input sequences, counting from 1 and including both endpoints. For aligned input, these positions are alignment columns. Selection takes place before any gaps are removed for alignment estimation.

3.3. Command line options

Sensible defaults are supplied for command line options that are not specified. For example, if `sequences.fasta` contains DNA sequences, then

```
% bali-phy sequences.fasta
```

is equivalent to

```
% bali-phy sequences.fasta -A DNA -S TN93 -I RS07
```

Default values that are used will always be displayed on the screen and in the output files so that you do not have to guess. You can specify a more complex substitution model using the `-S` option. You will generally need to write the substitution model inside quotes unless it is just a single word. The double quotes in this example work in all the shells covered here. See *Section 3.4, "Shell differences"* for expressions containing quoted strings.

```
% bali-phy sequences.fasta -S "LG +> ASRV.Gamma +> Inv"
```

Every short option like `-S` has an equivalent long option like `--smodel`. Long option names must be written in full. For example, use `--iterations` (or `-i`), not the old abbreviation `--iter`.

Use `bali-phy --help` or `bali-phy help` for commonly used options. For more specialized options, use `bali-phy help advanced`, `bali-phy help expert`, or `bali-phy help developer`. Each level includes the options from the preceding levels.

3.4. Shell differences

The same BAli-Phy options, such as `-S` and `--iterations`, work in all the shells covered here. In PowerShell and Command Prompt, native Windows also accepts slash forms such as `/iterations:1000`, but this guide uses the shared forms.

Put quotes around model expressions containing spaces, parentheses, or square brackets. Single quotes work in Unix shells and PowerShell; in Command Prompt, use double quotes instead. For example:

Unix shell / PowerShell
including Git Bash and MSYS2 Bash

```
% bali-phy sequences.fasta -S 'HKY85(kappa=2)'
```

Command Prompt

```
C:\> bali-phy sequences.fasta -S "HKY85(kappa=2)"
```

Models containing double-quoted strings are easier to specify in a configuration file. For DNA data, save this line in `model.config`:

model.config

```
:smodel GTR(pi={"A":0.1, "C":0.2, "G":0.3, "T":0.4})
```

Then use the same command in any shell:

```
% bali-phy sequences.fasta -c model.config
```

The configuration file contains the model expression itself, without outer shell quotes. See *Section 3.5, "Configuration files"* for other configuration options.

For these expressions on the command line, Unix shells and PowerShell 7.3 or later with its default native argument handling preserve double quotes inside single quotes. Windows PowerShell 5.1 and earlier PowerShell 7 releases can lose those inner quotes when passing arguments to a program; use the configuration-file form there or in Command Prompt. To check your PowerShell version, run `$PSVersionTable.PSVersion`.

To view a report in any environment, open `Results/index.html` in your web browser. For interactive PowerShell commands on Windows, `cp` is a short form of `Copy-Item`, and `start` is a short form of `Start-Process`. PowerShell and Command Prompt also provide these shortcuts:

PowerShell

```
PS> start Results\index.html
```

Command Prompt

```
C:\> start "" Results\index.html
```

Put paths containing spaces inside double quotes. Keep double quotes around PowerShell paths using `$HOME` or other variables so their values are expanded. Invoking an executable by a quoted path in PowerShell also requires `&` before the path, as in the installation instructions.

3.4.1. Pipelines and redirected output

The native-program pipelines (`|`) and output redirections (`>`) shown later work in Unix shells, Command Prompt, and PowerShell 7.4 or later. Older PowerShell versions convert program output to text; Windows PowerShell 5.1 also writes UTF-16 files through `>`, which these analysis tools do not expect. For those examples in an older PowerShell, enter `cmd` to open Command Prompt in the same directory, run the commands there, then enter `exit` to return to PowerShell.

PowerShell does not support `<` for input redirection. The subsampling example below includes a PowerShell alternative that asks Command Prompt to handle that one command.

3.5. Configuration files

In addition to using the command line, you may specify options and model-language definitions in a configuration file. Configuration options use the long form of command-line options. Each option is given on its own line using the syntax "`:option value`" instead of "`--option value`". The value can be blank if the option does not take an argument. The `align` option indicates se-

quence files. Other non-comment lines are combined into model-language definitions and may occur before, between, or after option lines. Lines whose first non-whitespace character is `#` are comments, and blank lines are ignored.

For example, consider the following configuration file:

config.txt

```
# sequence data for 3 genes/partitions
:align ITS1.fasta
:align 5.8S.fasta
:align ITS2.fasta

# linked substitution model for 1st and 3rd partition
:smodel 1,3:TN93  +>  ASRV.Free(n=3)

# substitution model for 2nd partition
:smodel 2:TN93

# indel model for second partition
:imodel 2:none

# linked scale for 1st and 3rd partition
:scale 1,3:

# choose a name for output directories
:name ITS-analysis1
```

Configuration files are specified with the `-c configuration_file` option. For example:

```
% bali-phy -c config.txt
```

A configuration file belongs to the default inference mode and cannot be used with a named command. Scalar options given on the command line override values given in the configuration file. For example, this command overrides the analysis name:

```
% bali-phy -c config.txt --name ITS-analysis1b
```

Repeated options supplied in both places are combined, with command-line values first. This allows a command line to add values for options such as `--smodel` or `--variables` rather than replacing all values from the file.

BAli-Phy does not read an implicit configuration file from your home directory; pass each configuration file explicitly with `--config`.

3.6. Running on computing clusters

Running `bali-phy` on a computing cluster is not necessary, but can speed up the analysis dramatically. This is because a cluster allows you to run several *independent* MCMC chains simultaneously and pool the resulting samples. You can run multiple chains simultaneously simply by starting several different instances of `bali-phy`. Each instance of bali-phy runs only one chain and does not require using MPI or special command-line options.

This approach to parallel computation is sometimes more efficient than MCMCMC-based parallelism involving heated chains. It is equivalent to running MCMCMC with no temperature difference between chains, with the exception that it allows results from *all* chains to be used, instead of just results from the single "cold" chain. Thus, if you run 10 independent chains in parallel, then you may gather samples 10 times faster than a single chain.

3.7. Is my data set too large?

Bayesian inference programs must run for many iterations to complete an analysis. A data set is considered "too large" if waiting for it to complete takes "too long".

3.7.1. Too many sequences?

Bayesian phylogenetics analyses require more iterations to converge as the number of sequences increases. Additionally, the computing time for each iteration increases with the number of sequences.

BAli-Phy has been successfully used to compute the full posterior with up to 150 sequences. Additionally, it has been used with up to 500 sequences to obtain alignment estimates that are more accurate than alignments from other software, but without measures of uncertainty.

If you have many sequences, we recommend using the tool alignment-thin with the `--down-to=`*n* option to construct a preliminary data set of 30-60 sequences. It is described in section *Section 13, “Alignment utilities: brief overview”* . Analyzing such a data set can complete much more quickly. You can then increase the size of your data set until a balance between speed and usefulness is reached.

3.7.2. Sequences too long?

Aligning just a pair of sequences takes $O(L^2)$ time and memory, where L represents the sequence length. Therefore sequences longer than (say) 1000 letters become increasingly slow.

One solution to this problem is to divide a long gene into multiple partitions. Dividing a long gene into *n* partitions will be roughly *n* times as fast as a single partition. The downside of this approach is that it requires performing a preliminary alignment, perhaps with a different aligner, in order to identify the partition boundaries.

When the multiple partitions can be categorized as introns or exons, then this approach allows treating intron and exon regions differently. It is possible to link all the intron partitions and link all the exon partitions, so that introns have one evolutionary rate and exons have another. Likewise, it is possible to fix the alignment for the exons, but infer the alignment for the introns. A similar approach can be taken with RNA stem and loop regions.

4. Output

In this section

4.1. Posterior samples 24
4.2. Posterior summaries 27
4.3. Posterior summaries (Advanced) 28

BAli-Phy analyses have two phases. (This structure is common to all Bayesian analyses.) First the `bali-phy` program generates *posterior samples* of trees, alignments and parameters. Second, the `bpy-summarize` script creates *posterior summaries* that collapse the collection of posterior samples down to single trees, alignments, and parameter estimates. It also diagnoses *lack of convergence*.

4.1. Posterior samples

4.1.1. Output directories

BAli-Phy creates a new directory to store its output files each time it is run. By default, the directory name is the name of the sequence file, with a number added on the end to make it unique. BAli-Phy first checks if there is already a directory called *`file`*`-1/`, and then moves on to *`file`*`-2/`, etc. until it finds an unused directory name.

You can specify a different name to use instead of the sequence-file name by using the `--name` option.

4.1.2. Output files

BAli-Phy writes the following output files inside the directory that it creates:

C1.Pn.fastas

Sampled alignments for partition n including ancestral sequences.

C1.Pn.initial.fasta

The initial alignment for partition n.

C1.log

Numeric parameters: indel and substitution rates, etc.

(*One sample per line.*)

C1.log.column-map.json

A JSON object mapping every complete scalar field name to the shorter column name used in `C1.log`.

C1.Pn.site-property-samples.jsonl

Sampled character properties for partition n, when property logging is enabled.

C1.trees

Tree samples in Newick format.

(*One sample per line*.)

C1.run.json

JSON file containing information about the command line, models, hostname, start time, etc.

4.1.3. Field names in `C1.log`

This section explains the meaning of the various field names in the file `C1.log`.

prior

The log prior probability.

likelihood

The log likelihood.

posterior

The log of the posterior probability.

(*The posterior probability is the product of the prior and the likelihood*).

prior_A

The log-probability of the alignments in all partitions.

|A|

The total number of alignment columns across all partitions.

#indels

The total number of indel events across all partitions.

(*Adjacent indels that occur on the same branch are merged*).

|indels|

The total length of indel events across all partitions.

(*Adjacent indels that occur on the same branch are merged*).

#substs

The total unweighted parsimony score for substitutions across all partitions.

Pn/likelihood

The substitution log-likelihood for partition n.

Pn/prior_A

The log-probability of the alignment for partition n.

Pn/|A|

The length of the alignment in the nth partition.

Pn/#indels

The number of indel events in partition n, if we group adjacent indels that occur on the same branch.

Pn/|indels|

The length of indel events in partition n, if we group adjacent indels that occur on the same branch.

Pn/#substs

The unweighted parsimony score for substitutions in partition n.

|T|

The *unscaled* tree length. (This will probably be around 1.0).

scale$n$

The factor that multiplies every branch length in the shared tree for partitions in the nth scale group.

scale$n$ * |T|

The scaled tree length for partitions in the nth scale group: the expected number of substitutions per site summed over the entire tree.

scale

The alignment-length-weighted mean of the scale factors for all partitions. For a single partition this equals `scale1`.

scale * |T|

The overall scale multiplied by the unscaled tree length: the alignment-length-weighted mean of the scaled tree lengths for all partitions.

The "prior" field includes the probability of the alignment, since the alignment is not observed.

The likelihood is the probabilistic analogue to summed mismatch penalties.

The prior_A is the probabilistic analogue to summed gap penalties.

Parameters belonging to a model are generally named after the model and parameter, such as `TN93:kappaPur` or `RS07:rate`. Run `bali-phy help` *`model`*, for example `bali-phy help TN93`, to see the meaning of that model's parameters. The precise field names may be disambiguated when an analysis contains multiple copies of a model, so the header of `C1.log` is authoritative.

See `bali-phy help scale` for more information about scale groups, linking scales between partitions, and setting their priors.

4.2. Posterior summaries

The `bpy-summarize` script summarizes the posterior samples to create posterior summaries for the alignment, tree, and parameters. It creates an HTML page `Results/index.html` that summarizes the posterior distribution.

You may run `bpy-summarize` inside the output directory, like this:

```
% bpy-summarize --skip=iterations
```

You may also run it with one or more output directories as arguments, like this:

```
% bpy-summarize --skip=iterations directory-1/ directory-2/
```

In this case, output from multiple runs will be used to assess convergence and mixing, as well as to increase the precision of the estimates.

Note: Inspecting the summary commands

All the commands that are executed by `bpy-summarize` will be logged to `Results/commands.log`. You can also see these commands as they are executed by supplying the `--verbose` option:

```
% bpy-summarize --skip=iterations --verbose
```

4.2.1. Meaning of generated files

The `Results/` directory will contain the following useful files:

Report
: A summary of numerical parameters: credible intervals and mixing.

consensus
: A summary of supported splits (clades).

c-levels.plot
: The number of splits (clades) supported at each LOD level.

c50.tree
: The majority consensus topology + branch lengths (Newick format)

c50.PP.tree
: The majority consensus topology + branch lengths + Posterior Probabilities (Newick format)

MAP.tree

An estimate of the MAP topology + branch lengths (Newick format)

The following files will be generated to summarize alignment uncertainty, unless the analysis uses a fixed alignment.

P*p*.consensus.pd-*score*.fasta

A posterior-decoding consensus alignment for partition *p*. The *score* is `multiply`, `wsum`, or `sum`, producing relatively short, medium, or long alignments, respectively.

P*p*.consensus.pd-*score*-AU.html

An AU plot of the corresponding posterior-decoding alignment for partition *p* (AA/DNA color scheme).

The following files describe convergence and mixing:

partitions.bs

Confidence intervals on the support for partitions, generated using a block bootstrap.

partitions.SRQ

A collection of SRQ plots for the supported partitions.

c50.SRQ

An SRQ plot for the majority consensus tree.

The SRQ plots can be viewed by typing "`plot 'file' with lines`" in gnuplot.

4.2.2. `Results/partitions.bs`: partition mixing

This file reports the quality of estimates of support for each partition in terms of the posterior probability (PP) and log-10 odds (LOD). It also reports the auto-correlation time (ACT), the effective sample size (Ne), the number of samples that support (1) or do not support (0) the partition, and the number of regenerations. Only partitions with PP > 0.1 are shown by default.

4.3. Posterior summaries (Advanced)

This section is primarily about summarizing the posterior to extract estimates from posterior samples, not about assessing convergence. See *Section 12, “Convergence and Mixing: Is it done yet?”* for methods of determining effective sample sizes, and for checking mixing and convergence.

4.3.1. Finding the majority consensus tree

To compute the majority consensus tree, do the following. A program such as TreeViewer allows you to view the resulting tree graphically; see *Section 2.9, "Optional programs for inspecting results"* for alternatives.

```
% trees-consensus dir-1/C1.trees dir-2/C1.trees > c50.PP.tree
```

By default, the first 10% of tree samples are skipped as burn-in (`--skip=10%` or `-s 10%`) and every generation is included (`--subsample=1` or `-x 1`). To discard the first 1000 tree samples and include every 10th sample:

```
% trees-consensus -s 1000 -x 10 dir-1/C1.trees dir-2/C1.trees > c50.PP.tree
```

By default, splits are included in the consensus tree if they have a PP greater than 0.5. You can specify a more stringent level (e.g. 0.66) by adding the option `--consensus=0.66` as follows:

```
% trees-consensus -s20% -x10 --consensus=0.66 dir-1/C1.trees dir-2/C1.trees > c66.PP.tree
```

You may also make the program write directly to the output file (e.g. `c66.PP.tree`) by using the more general form `--consensus=0.66:c66.PP.tree`. Leaving off the "`:c66.PP.tree`" part (as we did above) or specifying "`:-`" sends the output to the standard output (e.g. the terminal, if not redirected).

```
% trees-consensus -s20% -x10 dir-1/C1.trees dir-2/C1.trees --consensus=0.66:c66.PP.tree
```

You can supply multiple levels and filenames separated by commas. This is faster than running the program multiple times with different consensus levels.

```
% trees-consensus -s20% -x10 dir-1/C1.trees dir-2/C1.trees --consensus=0.5:c50.PP.tree,0.66
:c66.PP.tree
```

The consensus trees contain posterior probabilities as internal node labels. To remove these labels, use `tree-tool`:

```
% tree-tool c50.PP.tree --strip-internal-names > c50.tree
```

See `trees-consensus --help` for a complete list of options.

4.3.2. Finding the greedy consensus tree

The greedy consensus tree may be used instead of a majority-consensus tree when a fully resolved (e.g. bifurcating) tree is required. When the topology has many tips and each topology may be sampled only once, the greedy consensus should be higher quality than the estimate of the MAP topology. To obtain a fully resolved tree, the greedy consensus strategy starts with the majority consensus and then adds the highest-supported split that does not conflict.

To compute the *greedy consensus* tree, run:

```
% trees-consensus --skip=burnin dir-1/C1.trees dir-2/C1.trees --greedy-consensus=greedy.tre
e
```

4.3.3. Finding the M.A.P. tree

To compute the *maximum a posteriori* tree do:

```
% trees-consensus --skip=burnin dir-1/C1.trees dir-2/C1.trees --map-tree=MAP.tree
```

When the tree has many tips, each topology may be sampled only once, leading to low quality estimates of the MAP topology. As a result, when you need a bifurcating tree you should probably use the greedy consensus instead.

4.3.4. Checking topology convergence

```
% trees-bootstrap dir-1/C1.trees dir-2/C1.trees
```

This command computes the effective sample size for the posterior probability of each split. It also computes the Average Standard Deviation of Split Frequencies (ASDSF) between two or more independent runs.

See *Section 12, “Convergence and Mixing: Is it done yet?”* for more information.

4.3.5. Summarizing numerical parameters

This command gives a median and confidence interval, ESS, and a stabilization time:

```
% statreport dir-1/C1.log dir-2/C1.log > Report
```

When multiple runs are summarized, this command gives PSRF and joint ESS values. Tracer allows you to inspect the same numerical parameters interactively; see *Section 2.9, “Optional programs for inspecting results”*.

See *Section 12, “Convergence and Mixing: Is it done yet?”* for more information.

4.3.6. Computing an alignment using posterior decoding

To construct an alignment estimate via posterior decoding, select any tree file *tree* that corresponds to your alignment. It does not need to be fully resolved.

```
% cut-range dir-1/C1.Pp.fastas dir-2/C1.Pp.fastas --skip=burn-in | alignment-chop-internal
--tree tree | alignment-max > Pp-max.fasta
```

You can optionally replace `--tree tree` with `-N n_sequences`, where *n_sequences* is the number of non-ancestral sequences in your alignment.

You can use SeaView to view the alignment graphically; see *Section 2.9, “Optional programs for inspecting results”* for alternatives.

4.3.7. Create an Au (Alignment Uncertainty) plot

To annotate a specific alignment *alignment*.fasta, choose a fully resolved tree estimate *tree*:

```
% cut-range dir-1/C1.Pp.fastas dir-2/C1.Pp.fastas --skip=burn-in | alignment-chop-internal
--tree tree  | alignment-gild alignment.fasta tree  > alignment-AU.prob
% alignment-draw alignment.fasta alignment-AU.prob > alignment-AU.html
```

The majority consensus tree is usually not fully resolved, so we recommend using the greedy consensus instead.

5. Substitution models

In this section

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5.1. Non-reversible models and rooted trees

The default tree distribution for BAli-Phy is unrooted. This works fine for reversible substitution models, but if you specify a non-reversible substitution model then you will get an error:

```
bali-phy: Error! phyloCTMC: the tree is unrooted, but the model is not reversible!
```

In this case you will need to specify a rooted tree distribution. The simplest approach is to add the following to the command line:

```
--tree '~UniformRootedTree(taxa)'
```

This distribution creates rooted trees by sampling an unrooted tree and choosing one of the nodes as the root. So the root will *not* be in the middle of a branch. BAli-Phy will then estimate the root location.

5.2. DNA and RNA models

The default substitution model for DNA and RNA is TN93.

5.2.1. Substitution rates

All the DNA models are special cases of the GTR model.

Model	d.f.	Summary
`JC69`	0	Equal rates and equal base frequencies. (Jukes and Cantor, 1969)

Model	d.f.	Summary
`K80`	1	Unequal transition & transversion rates, equal base frequencies. (Kimura, 1980)
`F81`	3	Equal exchangeabilities, unequal frequencies. (Felsenstein, 1981)
`HKY85`	4	Unequal Transition & transversion rates, unequal base frequencies. (Hasegawa, Kishino, and Yano, 1985)
`TN93`	5	Unequal rates for transitions (purines), transitions (pyrimidines) and transversions, unequal base frequencies. (Tamura and Nei, 1993)
`GTR`	8	Unequal exchangeabilities, unequal frequencies. (Tavare, 1986)
`nonRev`	11	Unequal rates. Requires a rooted tree.
`nonEq`	14	Unequal rates, unequal initial frequencies. Requires a rooted tree.

5.2.2. Frequencies

Frequencies are estimated by default. Frequencies can be fixed by setting the `pi` parameter to a constant value, if the model allows unequal frequencies.

Constant frequencies are specified as a map associating each letter with its frequency:

```
GTR(pi={"A":0.1, "C":0.2, "T":0.3, "G":0.4})
```

Frequencies can also be specified using functions:

```
GTR(pi=Frequencies.uniform)
```

Model	d.f.	Summary
`Frequencies.uniform`	0	Equal frequencies

5.3. Protein models

The default substitution model for proteins is LG.

5.3.1. Substitution rates

Model	d.f.	mixture components	Summary
`JC69`	0	1	Equal rates and equal frequencies. (Jukes and Cantor, 1969)
`F81`	19	1	Equal exchangeabilities, unequal frequencies. (Felsenstein, 1981)
`JTT +> F`	19	1	Empirical exchange rates, all proteins. (Jones, Taylor, and Thornton, 1992)
`WAG +> F`	19	1	Empirical exchange rates, all proteins. (Whelan and Goldman, 2001)
`LG +> F`	19	1	Empirical exchange rates, all proteins. (Le and Gascuel, 2008)
`LG4M`	1	4	Four empirical rate matrices whose category rates follow a discrete gamma distribution with an estimated shape parameter. (Le, Dang, and Gascuel, 2012)
`LG4X`	6	4	Four empirical rate matrices with freely estimated category rates and frequencies. (Le, Dang, and Gascuel, 2012)
`UL2`	0	2	Empirical exchange rates, all proteins. (Le, Gascuel, and Lartillot, 2008)
`C10`	0	10	Empirical profile mixture model, 10 categories (Le, Gascuel, and Lartillot, 2008)
`C20`	0	20	Empirical profile mixture model, 20 categories (Le, Gascuel, and Lartillot, 2008)

Model	d.f.	mixture components	Summary
`C30`	0	30	Empirical profile mixture model, 30 categories (Le, Gascuel, and Lartillot, 2008)
`C40`	0	40	Empirical profile mixture model, 40 categories (Le, Gascuel, and Lartillot, 2008)
`C50`	0	50	Empirical profile mixture model, 50 categories (Le, Gascuel, and Lartillot, 2008)
`C60`	0	60	Empirical profile mixture model, 60 categories (Le, Gascuel, and Lartillot, 2008)
`Empirical(file) +> F`	19	1	
`GTR`	208	1	Unequal exchangeabilities, unequal frequencies. (Tavare, 1986)
`nonRev`	379	1	Unequal rates. Requires a rooted tree.
`nonEq`	398	1	Unequal rates, unequal initial frequencies. Requires a rooted tree.

5.3.2. Frequencies

Frequencies are estimated by default. Frequencies can be fixed by setting the `pi` parameter to a constant value, if the model allows unequal frequencies.

Constant frequencies are specified as a map associating each letter with its frequency:

```
WAG +> F({"A":0.047, "R":0.19,...})
```

Frequencies can also be specified using functions:

```
WAG +> F(pi=Frequencies.uniform)
```

Model	d.f.	Summary
`Frequencies.uniform`	0	Equal frequencies

Model	d.f.	Summary
`WAG_freq`	0	The constant amino-acid frequencies from the WAG paper.
`LG_freq`	0	The constant amino-acid frequencies from the LG08 paper.

The `+> FE` model is shorthand for `+> F(pi=Frequencies.uniform)`:

```
WAG +> FE
```

5.4. Doublet models (RNA stems)

The doublets alphabet consists of 16 RNA dinucleotides. It is used to model RNA stems, where two nucleotides matched in the RNA secondary structure are highly correlated.

The default substitution model for doublets is `TN93_sym +> x2_sym +> F`.

5.4.1. Doublet data

BAli-Phy does not allow specifying which nucleotides are paired either with a string like `((.))` or with a "pairs" file. Instead you must manually extract the paired nucleotides and put them in their own partition (for stems), and then manually extract each loop and put it in its own partition.

The stems should be arranged so that paired nucleotides are adjacent. For example, suppose the sequence `AGGCT` was paired according to `((.))`. Then the input file for the stems should contain a sequence of doublets that looks like `ATGC`, where `AT` is the first pair, and `GC` is the second pair.

5.4.2. Substitution rates

Model	d.f.	Summary
`nuc_model` `+> x2`	df(nuc_model)	The same as *nuc_model*, but on dinucleotides instead of nucleotides. Simultaneous changes of both letters are *not* allowed. Dinucleotide frequencies are the product of independent nucleotide frequencies.

Model	d.f.	Summary
`nuc_model +> x2 +> MutSel`	df(nuc_model)+15	Mutation-selection model: neutral mutation follows *nuc_model* and scaled selection coefficients 2Ns on dinucleotides. Simultaneous changes of both letters are *not* allowed.
`nuc_model +> x2_sym +> F`	df(nuc_model)+15	This model has separate frequencies for each dinucleotide. Simultaneous changes of both letters are *not* allowed.
`RNA.m16a`	19	This model has separate frequencies for each dinucleotide, and distinguishes between transitions and transversion between match states (including GU/UG). Simultaneous changes of both letters *are* allowed, but only between match states. (Savill et al., 2001)
`GTR`	134	Unequal exchangeabilities, unequal frequencies. It is unlikely that you would want to use this model, since it has so many parameters. (Tavare, 1986)
`nonRev`	239	Unequal rates. Requires a rooted tree.
`nonEq`	254	Unequal rates, unequal initial frequencies. Requires a rooted tree.

5.4.3. Frequencies

Frequencies are estimated by default. Frequencies can be fixed by setting the `pi` parameter to a constant value, if the model allows unequal frequencies.

Constant frequencies are specified as a map associating each letter with its frequency.

```
HKY85(pi={"A":0.1, "C":0.2, "T":0.3, "G":0.4}) +> x2
```

```
HKY85_sym +> x2_sym +> F({"AA":0.01, "AC":0.01, "AG":0.01, "AU":0.22, "CA":0.01, "CC":0.0
1, "CG":0.22, "CU":0.01, "GA":0.01, "GC":0.22, "GG":0.01, "GU":0.01, "UA":0.22,
"UC":0.01, "UG":0.01, "UU":0.01})
```

Frequencies can also be specified using functions:

Model	d.f.	Summary
`Frequencies.uniform`	0	Equal frequencies on dinucleotides

5.4.4. Branch lengths

BAli-Phy interprets branch lengths for doublet models as 1/2 the number of substitutions per doublet. Thus, they should be comparable to branch lengths under DNA/RNA nucleotide models.

5.5. Triplet models

The triplets alphabet is similar to the codons alphabet, except that stop codons are included. Unlike the codons alphabet, the triplets alphabet has no knowledge of the genetic code.

The default substitution model for triplets is TN93 +> x3.

5.5.1. Substitution rates

Model	d.f.	Summary
nuc_model `+> x3_sym +> F`	df(*nuc_model*)+63	GY94-style rate matrix constructed from nucleotide exchangeability matrix.
nuc_model `+> x3`	df(*nuc_model*)	MG94-style rate matrix constructed from nucleotide rate matrix. This model should give the same likelihood as *nuc_model* on triplets, but not on codons.
nuc_model `+> x3 +> MutSel`	df(*nuc_model*)+63	Mutation-selection model with neutral mutation following *nuc_model* and scaled selection coefficients 2Ns *for each codon*.

5.5.2. Frequencies

Frequencies are estimated by default. Frequencies can be fixed by setting the `pi` parameter to a constant value, if the model allows unequal frequencies.

Constant frequencies are specified as a map associating each letter with its frequency.

```
HKY85(pi={"A":0.1, "C":0.2, "T":0.3, "G":0.4}) +> x3
```

Frequencies can also be specified using functions:

```
HKY85_sym +> x3_sym +> F(pi=F1x4)            // nucleotide frequencies are estimated
```

Model	d.f.	Summary
`Frequencies.uniform`	0	Equal frequencies
`F1x4`	3	Constructs triplet frequencies from independent nucleotide frequencies.
`F3x4`	9	Constructs triplet frequencies from independent nucleotide frequencies for each codon position.

The `+> FE` model is shorthand for `+> F(pi=Frequencies.uniform)`:

```
HKY85_sym +> x3_sym +> FE
```

Branch lengths for triplet models measure expected nucleotide substitutions per nucleotide site, as for DNA/RNA models. A mutation changing two or three nucleotides counts as two or three substitutions, respectively.

5.6. Codon models

The default substitution model for codons is GY94.

5.6.1. Standard models

Model	d.f.	Summary
`GY94`	62	Model of dN/dS with a separate frequency for each codon. Rate for changing a nucleotide depends on neighboring nucleotides. (Goldman and Yang, 1994)
`GY94(pi=F1x4)`	5	The GY94 model with codon frequencies constructed from nucleotide frequencies. (Goldman and Yang, 1994)

Model	d.f.	Summary
`GY94(pi=F3x4)`	11	The GY94 model with codon frequencies constructed from nucleotide frequencies for each codon position. (Goldman and Yang, 1994)
`GY94_ext(`*nucModel*`)`	df(*nucModel*)+61	GY94 model extended with a generic nucleotide exchangeability matrix. (Goldman and Yang, 1994)
`MG94`	4	Model of dN/dS with F81 as the neutral model. Rate for changing a nucleotide depends only on that nucleotide. (Muse and Gaut, 1994)
`MG94K`	5	Model of dN/dS with HKY85 as the neutral model. (Muse and Gaut, 1994)
`MG94_ext(`*nucModel*`)`	df(*nucModel*)+1	Model of dN/dS with *nucModel* as the neutral model. (Muse and Gaut, 1994)
`FMutSel`	69	MG94-like model with fitnesses for each codon. (Yang and Nielsen, 2008)
`FMutSel0`	28	MG94-like model with fitnesses for each amino-acid. (Yang and Nielsen, 2008)
`BUSTED`	13	MG94-like model where each branch and site has a randomly chosen dN/dS category. (Murrell et al, 2015)
`BUSTED_S`	14	BUSTED model with synonymous rate variation. (Wisotsky et al, 2020)

Parameter counts use the default settings and the standard genetic code. For tests, counts describe the selection hypothesis and exclude the hypothesis indicator. Some parameters are unused under the null hypothesis.

Branch lengths for codon models measure expected nucleotide substitutions per nucleotide site, as for DNA/RNA models. A mutation changing two or three nucleotides counts as two or three substitutions, respectively.

5.6.2. Constructing and tweaking codon models

Sometimes the researcher wants to use an existing codon model, but with a slight tweak. For example, you might want to use an M3 model that is based on GTR instead of HKY. This section provides building blocks to construct codon models from nucleotide models. This allows you to change the underlying nucleotide model or add other modifications.

The mutation-selection models FMutSel and FMutSel0 (Yang and Nielsen, 2008) assign fitnesses to codons and amino acids, respectively. FMutSel0 gives synonymous codons the same fitness. In BAli-Phy, these names denote complete models. MutSel and MutSelAA instead denote modifiers: components applied to an input rate matrix to incorporate those fitnesses. Their default full-model constructions appear below. Exposing the components lets you change the nucleotide model or replace `x3` with `MNM` to extend the model to multinucleotide mutations.

Unconstrained ω parameters default to `LogNormal(0,1)`, giving equal prior probability below and above one. To restrict ω below one, specify `omega~Uniform(0,1)`, for example `GY94(omega~Uniform(0,1))`. Conserved categories in mixture models retain their constrained priors.

Here are some examples for how to construct classic codon models piecewise:

- `MG94` is equivalent to `F81 +> x3 +> dNdS`.
- `MG94K` is equivalent to `HKY85 +> x3 +> dNdS`.
- `GY94` is equivalent to `HKY85_sym +> x3_sym +> F +> dNdS`.
- FMutSel with default arguments is equivalent to `GTR +> x3 +> MutSel +> dNdS`.
- FMutSel0 with default arguments is equivalent to `GTR +> x3 +> MutSelAA +> dNdS`.
- `M3` is equivalent to `|w:GTR +> MNM +> MutSelAA +> dNdS(omega=w)| +> M3`.
- `M3_test` is equivalent to `|w:GTR +> MNM +> MutSelAA +> dNdS(omega=w)| +> M3_test`.
- `BUSTED` is equivalent to `|w:GTR +> x3 +> dNdS(omega=w)| +> M3_test(n=2) +> BranchSiteMixture`.
- `BUSTED_S` is equivalent to `|w:GTR +> x3 +> dNdS(omega=w)| +> M3_test(n=2) +> BranchSiteMixture +> ASRV.Gamma(n=3)`.

Model	d.f.	Summary
`nuc_model +> x3_sym +> F`	df(nuc_model)+60	GY94-style rate matrix constructed from nucleotide exchangeability matrix (dN/dS = 1). This model should give the same likelihood as *nuc_model* on codons only if the frequency of stop codons is zero.
`nuc_model +> x3`	df(nuc_model)	MG94-style rate matrix constructed from nucleotide rate matrix (dN/dS = 1). Constructs either a codon model or a triplet model, depending on the alphabet.
`nuc_model +> MNM`	df(nuc_model)+2	Like `x3`, but with multi-nucleotide mutations.
`codon_model +> dNdS(omega)`	df(*codon_model*)+1	Scales non-synonymous rates by *omega*.
`codon_model +> MutSel`	df(*codon_model*)+60	Mutation-selection model with neutral mutation following *codon_model* and scaled selection coefficients 2Ns *for each codon*.
`codon_model +> MutSelAA`	df(*codon_model*)+19	Mutation-selection model with neutral mutation following *codon_model* and scaled selection coefficients 2Ns *for each amino acid*.
`codon_mixture_model +> BranchSiteMixture`	df(*codon_mixture_model*)	Codon model where the rate matrix for each branch and site is chosen from the mixture distribution.

5.6.3. Frequencies

Frequencies are estimated by default. Frequencies can be fixed by setting the `pi` parameter to a constant value, if the model allows unequal frequencies.

Constant frequencies are specified as a map associating each letter with its frequency.

```
GY94(pi={"AAA":0.01, "AAC":0.02,...})
MG94(pi={"A":0.1, "C":0.2, "T":0.3, "G":0.4})
```

Frequencies can also be specified using functions:

```
GY94(pi=F1x4)              // nucleotide frequencies are estimated
```

Model	d.f.	Summary
`Frequencies.uniform`	0	Equal frequencies
`F1x4`	3	Constructs codon frequencies from independent nucleotide frequencies.
`F3x4`	9	Constructs codon frequencies from independent nucleotide frequencies for each codon position.

5.6.4. Genetic Codes

When using a codon-based substitution model like `GY94`, you may select the genetic code by specifying `-A "Codons(,genetic-code)"`. Available genetic codes are:

Name	Number	Description
standard	1	Standard
mt-vert	2	Mt: Vertebrate
mt-yeast	3	Mt: Yeast
mt-protozoa	4	*: Mold, Protozoan and Coelenterate Mitochondrial Code and Mycoplasma/Spiroplasma
mt-invert	5	Mt: Invertebrate
nuc-ciliate	6	Nuc: Ciliate, Dasycladacean and Hexamita
mt-echinoderm	9	Mt: Echinoderm and Flatworm
nuc-euplotid	10	Nuc: Euplotid
bacteria	11	*: Bacterial, Archaeal and Plant Plastid
nuc-yeast-alt	12	Nuc: Alternative Yeast
mt-ascidian	13	Mt: Ascidian
mt-flatworm-alt	14	Mt: Alternative Flatworm
nuc-blepharisma	15	Nuc: Blepharisma Nuclear Code

Name	Number	Description
mt-chlorophycean	16	Mt: Chlorophycean
mt-trematode	21	Mt: Trematode
mt-scenedesmus-obliquus	22	Mt: Scenedesmus obliquus
mt-thraustochytrium	23	Mt: Thraustochytrium
mt-rhabdopleuridae	24	Mt: Rhabdopleuridae
bacteria-sr1	25	*: Candidate Division SR1 and Gracilibacteria
nuc-pachysolen-tannophilus	26	Nuc: Pachysolen tannophilus
nuc-karyorelict	27	Nuc: Karyorelict
nuc-condylostoma	28	Nuc: Condylostoma
nuc-mesodinium	29	Nuc: Mesodinium
nuc-peritrich	30	Nuc: Peritrich
nuc-blastocrithidia	31	Nuc: Blastocrithidia
mt-cephalodiscidae	33	Mt: Cephalodiscidae UAA-Tyr

Genetic codes may be specified by name or by `code`*n* where *n* is the code number. For example `code1` is the standard code. If the genetic code is not specified, then the standard code is used:

```
% bali-phy sequence-file -S GY94 -A Codons
% bali-phy sequence-file -S GY94 -A "Codons(RNA)"
```

These examples specify the vertebrate mitochondrial code:

```
% bali-phy sequence-file -S GY94 -A "Codons(DNA,mt-vert)"
% bali-phy sequence-file -S GY94 -A "Codons(,mt-vert)"
```

5.6.5. Heterogeneous dN/dS and tests for positive selection

The M1a, M2a, M3, M7, M8, and M8a families, including their test variants, use `GTR +> MNM +> MutSelAA` as the default background. Their category structures act on an ω multiplier of nonsynonymous rates after accounting for amino-acid fitnesses. Thus a category called neutral (ω=1) retains those fitness effects. These names identify category structures; the background is supplied by the `submodel` argument.

For concepts, commands, and interpretation of the reports, see *Section 10, “Positive selection”*.

Model	d.f.	Summary
`M1a`	df(*submodel*)+2	A mixture of conserved and neutral sites. (Wong et al., 2004)
`M2a`	df(*submodel*)+4	A mixture of conserved, neutral, and positively-selected sites. (Wong et al., 2004)
`M2a_test`	df(*submodel*)+4	A Bayesian test for positive selection that compares M2a with M1a. (Wong et al., 2004)
`M3`	df(*submodel*)+2*n-1	An free mixture of n categories of conserved dN/dS values. (Yang et al., 2000)
`M3_test`	df(*submodel*)+2*n+1	A Bayesian test for positive selection based on the M3 model extended with an extra category of either neutral of positively-selected sites.

Model	d.f.	Summary
`M7`	df(*submodel*)+2	The M7 model places a beta distribution on dN/dS. The beta component is approximated by Gauss-Jacobi quadrature, with ten categories by default and generally unequal category weights. M8, M8a, and M8a_test use the same beta component; their additional category is not counted in `n`. The beta mean `mu` and normalized variance `v` have independent `Uniform(0,1)` default priors. The normalized variance is the fraction of the maximum possible variance at that mean: $$\begin{aligned}\texttt{v} &= \frac{\mathrm{Var}(\texttt{omega})}{\texttt{mu}(1-\texttt{mu})} \\ \texttt{concentration} &= 1/\texttt{v} - 1 \\ \texttt{alpha} &= \texttt{concentration} \cdot \texttt{mu} \\ \texttt{beta} &= \texttt{concentration} \cdot (1-\texttt{mu})\end{aligned}$$ The shape parameters are logged as computed values `a` and `b`. For a proper beta distribution, both `mu` and `v` lie strictly between zero and one. With an interior mean, `v=0` uses the concentrated limit at that mean; `v=1` does not define a proper beta distribution and produces an unavailable likelihood. The same priors and parameterization apply to the beta component of M8, M8a, and M8a_test. (Yang et al., 2000)
`M8a`	df(*submodel*)+3	The M8a model adds a category of *neutral* sites to the M7 model. (Swanson et al., 2003)
`M8`	df(*submodel*)+4	The M8 model adds a category of *positively-selected* sites to the M7 model. (Yang et al., 2000)
`M8a_test`	df(*submodel*)+4	A Bayesian test for positive selection that compares the M8 to the M8a model. (Swanson et al., 2003)

Model	d.f.	Summary
`BranchSite`	df(*submodel*)+4	A Bayesian test for positive selection at unknown sites on designated foreground branches. See *Section 10.6, "Selection at some sites on designated branches"* for instructions. (Zhang et al., 2005)

5.7. Heterogeneous Rates across Sites

Complex substitution models in BAli-Phy are constructed as mixtures of reversible CTMC models that run at different rates (e.g. $\Gamma_4 + Inv$) or have different parameters (e.g. an M2a codon model).

Model	d.f.	Summary
`submodel +> ASRV.Gamma`	df(*submodel*)+1	Site rates follow a discrete approximation to the Gamma distribution (Yang, 1994)
`submodel +> ASRV.LogNormal`	df(*submodel*)+1	Site rates follow a discrete approximation to the log-normal distribution
`submodel +> ASRV.Free`	df(*submodel*)+2(n-1)	Sites fall in one of n categories. Each category has its own rate. (Yang, 1995)
`submodel +> MultiRate(dist)`	df(*submodel*)+df(*dist*)	Site rates follow a discrete approximation to the distribution *dist*.
`submodel +> Inv`	df(*submodel*)+1	Some fraction Inv:pInv of sites are invariable.

5.8. Heterotachy models

These models attempt to model the fact that evolutionary rates may change over time within a single column. These models are sometimes called "covarion" models, based on the idea that changes in rate might be caused by changes in an unspecified covarying site.

These models are "Markov modulated" models that create multiple different states for each letter by augmenting each letter with some unobserved hidden state. They attempt to model the fact that substitution processes might not be Markov on the letters, but might become more Markov given the hidden state.

Model	d.f.	Summary
`Q +> Covarion.TuffleySteel98`	df(*submodel*)+2	Each state in rate matrix Q is split into an ON and OFF variant. Models burstiness. (Tuffley and Steel, 1998)
`Q +> ASRV.Gamma +> Covarion.Huelsenbeck02` `submodel +> Covarion.Huelsenbeck02`	df(*Q+ASRV.Gamma*) +2 df(*submodel*)+2	Combines Gamma (or other) rate heterogeneity with the Tuffley-Steel model. (Huelsenbeck, 2002)
`Q +> ASRV.Gamma +> Covarion.Galtier01` `submodel +> Covarion.Galtier01`	df(*Q+ASRV.Gamma*) +2 df(*submodel*)+2	Allows switching between Gamma (or other) rate classes over time. Models changes in conservation. (Galtier, 2001)
`Q +> ASRV.Gamma +> Covarion.Wang07` `submodel +> Covarion.Wang07`	df(*Q+ASRV.Gamma*) +4 df(*submodel*)+4	Allows switching between ON/OFF states and *also* between Gamma (or other) rate classes over time. Models both burstiness and changes in conservation. (Wang et al., 2007)

Caution: Combining covarion models with rate variation

The obvious way to combine the Tuffley-Steel model with rate heterogeneity is wrong:

- `Q +> Covarion.TuffleySteel98 +> ASRV.Gamma`: This is incorrect. Under this model, sites with faster substitution rates will switch between the ON/OFF states faster.
- `Q +> ASRV.Gamma +> Covarion.Huelsenbeck02`: This is correct. Sites switch between ON/OFF states independent of the speed of substitution.

6. Insertion/deletion models

Each of these models is a probability distribution on pairwise alignments. The probability distribution on multiple sequence alignments $\Pr(A|T,\tau,\Lambda)$ is constructed by factoring the multiple sequence alignment into pairwise alignments along each branch of the tree, as described in Redelings and Suchard (2005).

The default insertion/deletion model is `RS07`.

Model	d.f.	Summary
`RS05`	3	A symmetric insertion-deletion model with geometrically-distributed indel lengths. Indels occur on all branches with the same probability, regardless of branch length. (Redelings and Suchard, 2005)
`RS07`	2	A symmetric insertion-deletion model with geometrically-distributed indel lengths. Longer branches have more indels. (Redelings and Suchard, 2007)
`none`		No indel model for the partition, indels uninformative. Fixed alignment for the partition.

The user can specify priors and parameters for indel models (See section *Section 7, "Models and Priors"*):

```
RS07(rate~LogLaplace(-4,0.707),meanLength=2)
```

7. Models and Priors

In this section

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7.1. Models and distributions are functions

Models, probability distributions, and functions are treated the same in BAli-Phy because all of them have parameters or arguments. Parameters have names in BAli-Phy. Parameter values are specified using parentheses as follows:

```
HKY85(kappa=2)            // model
log(x=2)                  // function
Normal(mean=0,sigma=1)    // probability distribution
```

It is possible to specify parameter values by position instead of by name:

```
HKY85(2)
log(2)
Normal(0,1)
```

It is even possible to mix positional and named arguments, as long as all the positional arguments come before all the named arguments:

```
Normal(0,sigma=1)   // OK
Normal(mean=0,1)    // not OK
```

The order and type of parameters for a function can be found with the `help` command. For example,

```
% bali-phy help HKY85
```

A value must be given for each parameter, unless the parameter has a default value (See *Section 7.4, "Default values and default priors"*).

Note: Quote model expressions on the command line

Put quotes around terms with parentheses or square brackets on the command line. These examples use single quotes for Unix shells and PowerShell. In Command Prompt, use double quotes instead (see *Section 3.4, "Shell differences"*):

```
% bali-phy file.fasta -S 'HKY85(kappa=2)'
% bali-phy file.fasta -S 'mixture([TN93,HKY85(2)])'
```

If you do not add quotes, the shell will try to interpret the parentheses or square brackets and give an error message without running bali-phy. For example, "`-bash: syntax error near unexpected token `('`" (for `bash`) or "`Badly placed ()'s`" (for `csh`) or "`zsh: no matches found: mixture([TN93,HKY85(2)])`" (for `zsh`).

7.2. Model stacking and `+>` notation

Models in phylogenetics literature are often combined using `+`. For example, the model `WAG + F + G4 + I` starts with the WAG amino-acid model, and places several modifiers, like " + G4" on the right.

BAli-Phy follows this convention by treating `A +> B` as an abbreviation for `B(A)`. When there are multiple '`+>`' symbols they associate to the left, so that `A +> B +> C` is understood to mean `(A +> B) +> C`, which is equivalent to `C(B(A))`. For example:

```
HKY85 +> ASRV.Gamma              // rewritten to ASRV.Gamma(HKY85)
HKY85 +> Inv                      // rewritten to Inv(HKY85)
WAG +> F                          // rewritten to F(WAG)
WAG +> F +> ASRV.Gamma +> Inv    // rewritten to Inv(ASRV.Gamma(F(WAG)))
```

This allows a simple method for combining models, when one model is an argument to another model.

7.3. Priors

7.3.1. Specifying priors

Priors on model parameters are specified by giving a random value. Random values can be obtained from distributions using the function `sample`. For example, this places a log-normal prior on the parameter `kappa` of the `HKY85` model:

```
HKY85(kappa=sample(LogNormal(1,1)))
```

You can write `~Dist` as a shorthand for `sample(Dist)`:

```
HKY85(kappa = ~LogNormal(1,1))
```

The `=~` can be further shortened to just `~`:

```
HKY85(kappa ~ LogNormal(1,1))
```

7.3.2. Random function arguments

It also is possible to use random values as inputs to other functions. For example:

```
1.0 + ~Exponential(10)
```

In such cases the parameter value should be specified with `=`, as in the following example:

```
RS07(meanLength=1.0 + ~Exponential(10))
```

7.3.3. Distributions are not random values

Random values and distributions have different types. For example, the following is of type `Distribution<Double>`:

```
Uniform(0,1)
```

In contrast, the following are both of type `Double`:

```
sample(Uniform(0,1))
~Uniform(0,1)
```

This is important when passing distributions as arguments to other distributions and functions. For example, the distribution `IID` is used to generate a specific number of samples from another distribution. Thus, it needs to receive a distribution as an argument:

```
~IID(4, Normal(0,1))      // OK    : 4 samples from the Normal(0,1) distribution
~IID(4, ~Normal(0,1))     // not OK: 4 samples from ... a random number?
```

(See *Section 7.5, "Argument and result types"*.)

7.4. Default values and default priors

Some function arguments have default values. For example, the `ASRV.Gamma` parameter `n` has a default value of 4. Thus the following are equivalent:

```
HKY85 +> ASRV.Gamma(n=4) +> Inv
HKY85 +> ASRV.Gamma +> Inv
```

When the default value is random, then the argument has a default prior. For example, the `kappa` parameter of `HKY85` has a default value of `~LogNormal(log(2),0.25)`, so the following are equivalent:

```
HKY85(kappa~LogNormal(log(2),0.25))
HKY85
```

This prior has median 2 and places approximately 95% of its probability between 1.23 and 3.26. A prior describes uncertainty about the parameter; it is not an optimization starting value. See LogNormal for the distribution's parameterization.

The website Function reference lists parameter meanings and default values or priors. The same help is available offline, for example:

```
% bali-phy help HKY85
```

Function help describes defaults; the analysis report's `Model and priors` section records the model and priors actually used.

7.5. Argument and result types

Every function has a *result type*, as well as an *argument type* for each argument. The argument type specifies what kind of arguments are acceptable, and the result type specifies what kind of result the function produces. Types include `Int` for integers, `Double` for double-precision floating point numbers, and `String` for text strings. Integer arguments are implicitly converted to `Double` when the argument type is `Double`.

Some types contain parameters. For example `List<Int>` indicates a list of integers and `List<Double>` indicates a list of real numbers. In order to indicate a list of unknown type, we use a *type variable* `a` and write `List<a>`. Type variables always begin with a lower-case letter. They are able to match any specific type, and their value is found by pattern-matching. For example, the function `x+y` takes two arguments of type `a` and has a result of type `a`. Thus:

```
1 + 2        // arguments are a=Int, so result is of type Int
1.0 + 2.0    // arguments are a=Double, so result is of type Double
```

`(a,b)` is a parameterized type that can be specialized to (for example) `(String,Double)` and `(Int,Int)`.

Types for components of substitution models are often parameterized by type of the alphabet. For example, HKY85 has a result type of `CTMC<a>`, where `a` could be `DNA` or `RNA`. The use of alphabet types in substitution models prevents combining substitution models with mismatched alphabets.

8. Partitioned data sets

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8.1. Partitions

You should analyze multiple genes under different evolutionary models by putting each one it its own data partition. Placing different genes in different partitions means that their alignments vary independently. It also prevents sequences in one gene from being aligned against sequences in another gene.

Different partitions share the same tree topology and a common set of unscaled branch lengths. However, branch lengths are scaled by a different factor in each partition, since some genes may evolve faster than others.

To put different genes in different partitions, you can place the sequences from each partition in a different FASTA or Phylip file. The sequence names in files for all partitions should be the same.

```
% bali-phy gene1.fasta gene2.fasta
```

You can also select different sites from a single larger file:

```
% bali-phy sequences.fasta:3-350 sequences.fasta:351-570
```

8.2. Unlinked models

By default, each partition will have its own substitution model, insertion/deletion model, and scaled tree length. For example, even if all partitions are assigned a `TN93` substitution model, their base frequencies will all be estimated independently. When parameters are estimated separately for two partitions, we say that the parameters for those partitions are "unlinked".

A substitution model or insertion-deletion model that is specified without qualification will apply to every partition. However, each partition will receive its own copy of each model with unlinked parameter values:

```
% bali-phy sequence-file1 sequence-file2 -S TN93 -I RS07
```

You can select partition-specific values for 4 options: `-S`, `-I`, `-A`, and `--scale`. For example, to specify different substitution models but the same alphabet:

```
% bali-phy sequence-file1 sequence-file2 -S 1:TN93 -S 2:GTR -A DNA
```

8.3. Fixing the alignment in some partitions

You can fix the alignment and ignore insertion/deletion information in one partition, while allowing the alignment to vary and using insertion/deletion information in another partition:

```
% bali-phy sequence-file1 sequence-file2 -I 2:none
```

Since alignments are estimated by default, the alignment will be estimated in the first partition, but fixed in the second partition.

Specifying `-I none` fixes the alignment in all partitions:

```
% bali-phy sequence-file1 sequence-file2 -I none
```

8.4. Linked models

You can also specify that two partitions share a single copy of a single substitution model or indel model. For example, if two partitions both have a `TN93` model, linking these models would force the partitions to have the same nucleotide frequencies and substitution rates. Linking partitions reduces the number of parameters that need to be estimated, and also pools information between the partitions:

```
% bali-phy sequence-file1 sequence-file2 -S 1,2:TN93 -I 1,2:RS07
```

By default each partition has a separate scale, but you can force groups of partitions to share a scale as follows:

```
% bali-phy sequence-file1 sequence-file2 --scale 1,2:
```

8.5. Linking models via the `link` command

The `--link` command is provided to allow specifying a model for each partition separately, and then afterwards choose which partitions to link.

```
% bali-phy sequence-file1 sequence-file2 -S 1:TN93 -S 2:TN93 --link=1,2 -t
% bali-phy sequence-file1 sequence-file2 -S TN93             --link=1,2 -t
```

If the linked partitions are given different models, BAli-Phy will give an error and refuse to run:

```
% bali-phy sequence-file1 sequence-file2 -S 1:TN93 --link=1,2 -t
bali-phy: Error! Partitions 1 and 2 cannot be linked because they have differing values
'TN93' and ''
```

You can also specify which of the 3 attributes "smodel", "imodel", and "scale" are being linked:

```
% bali-phy sequence-file1 sequence-file2 --link=1,2:smodel,scale -t
```

This links the substitution model and scale, leaving the indel models separate.

9. Character properties

Character properties describe quantities such as the substitution rate of each observed letter. For codon models, a letter is a whole codon; properties can include its ω value and probability of positive selection. Which properties are available depends on the model.

Properties belong to letters, not alignment columns. A letter keeps its identity as the alignment changes: its sequence name and position in the ungapped sequence identify it. Property estimates account for the sampled alignments. Letters displayed in the same column can therefore have different estimates. The alignment used to display the results need not have been held fixed during the analysis.

For example, run a model with variation in rate among sites:

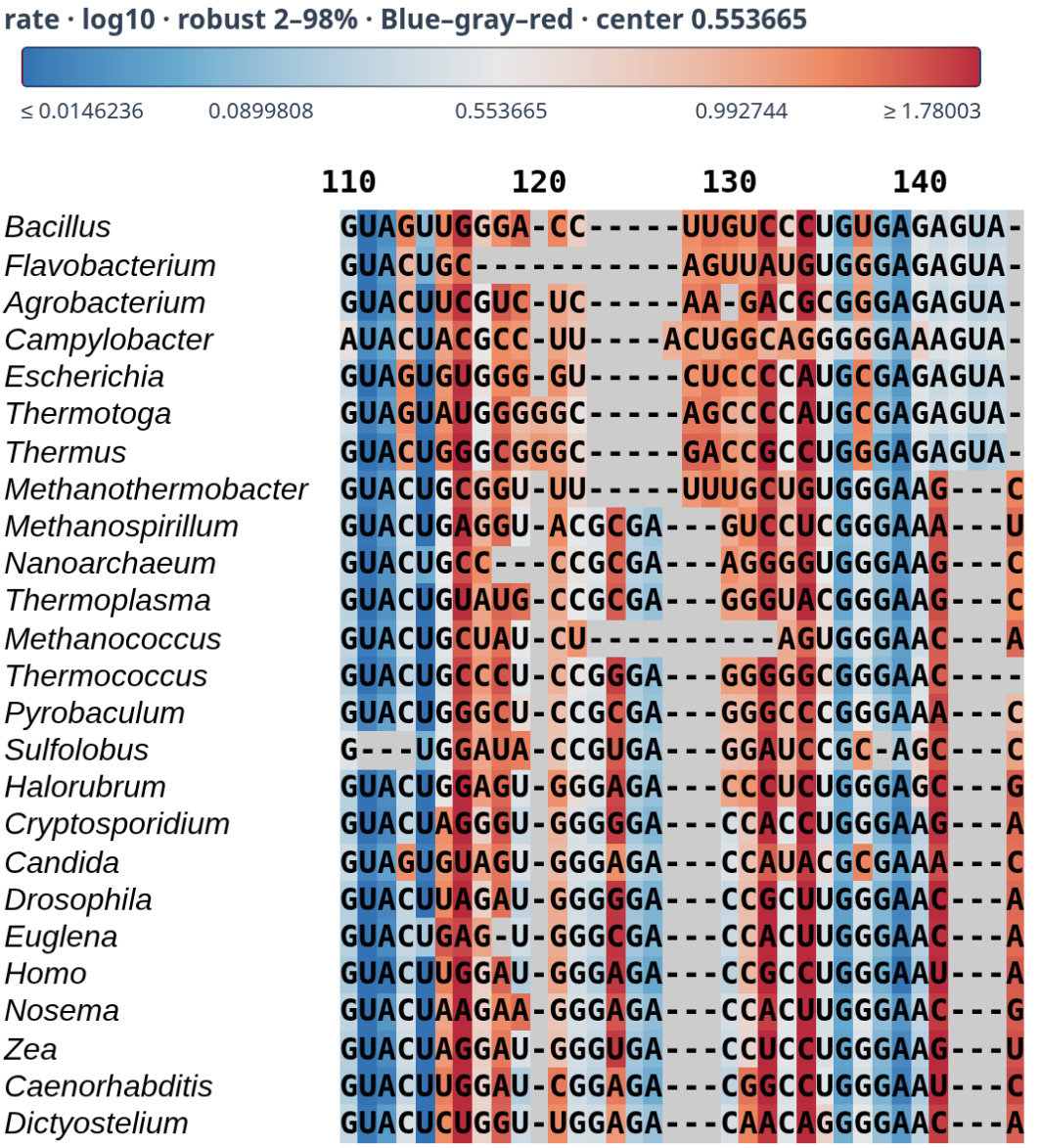
```
% bali-phy sequences.fasta -S 'GTR +> ASRV.Gamma'
```

Run independent chains and check their mixing and convergence (*Section 12, "Convergence and Mixing: Is it done yet?"*). Summarize their output directories after discarding the first *burnin* iterations of each run:

```
% bpy-summarize --skip=burnin run-1/ run-2/
```

Open `Results/index.html` and follow a partition's alignment link. Choose `rate` under `Color by` and clear `Original colors`. Colors show the posterior mean rate of each letter. Point to a letter to inspect its estimates. The standard deviation measures uncertainty about the property's value, not the sampling error of its estimated mean.

Heterotachy models allow a site's rate to change along the tree. The example below uses `GTR +> ASRV.Gamma(4) +> Inv +> Covarion.Huelsenbeck02` for 25 RNA sequences.



Letters in the same alignment column can have different inferred rates. Compare the colors among sequences around columns 130–140: blue indicates lower posterior mean rates and red higher rates. These estimates average over sampled alignments and are displayed here on the AMA alignment. The colors summarize rate variation under the covarion model; they do not provide a formal test for heterotachy.

Figure 1. Inferred rates for individual letters

To explore this example, open the interactive alignment. Select `rate`, clear `Original colors`, and choose `Log10`, `Blue–gray–red`, and `Robust (2–98%)`. The Log10 scale retains rate values as legend labels. The robust range assigns the lowest and highest 2% of values to the endpoint colors.

Open `Column summaries` to inspect or sort the numerical table, then select a column number to locate it in the alignment. Summaries start collapsed for both rates and positive-selection prop-

erties. In AU views, `Fade by AU` fades colors according to alignment uncertainty; leave it off to compare rates alone.

Clear `Show letters` to compare background colors without the sequence letters. You can still point to cells to inspect their estimates.

Supported properties are logged automatically in `C1.P1.site-property-samples.jsonl` for partition 1, and similarly for other partitions. To export a table using an alignment of your choice, use `character-properties`:

```
% character-properties summarize run-1/C1.P1.site-property-samples.jsonl \
  run-2/C1.P1.site-property-samples.jsonl --skip=burnin > properties.json
% character-properties columns properties.json alignment.fasta rate --format=tsv >
rates.tsv
```

This table summarizes the per-letter rate estimates within each alignment column. Sequence names and ungapped sequences must match the analyzed data. Sequence positions identify letters; column numbers refer only to the alignment used for display. See `character-properties columns --help` for column-report options, or `character-properties letters --help` to select and report representative letters.

10. Positive selection

In this section

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Codon models distinguish synonymous changes, which preserve the amino acid, from nonsynonymous changes, which alter it. The parameter ω (often called dN/dS) multiplies the nonsynonymous rates in a codon model. Values below one reduce these rates; values above one increase them and represent diversifying positive selection. Here positive selection means an excess of amino-acid changes relative to the model's background rates, not evidence that a particular mutation is advantageous.

Use homologous protein-coding sequences in the correct reading frame. Remove terminal stop codons and check for internal stops or frameshifts. The commands below assume the standard genetic code. By default, BAli-Phy estimates the alignment along with selection; add `-I none` to hold the input alignment fixed. Alignment uncertainty is included in the character summaries (*Section 9, "Character properties"*). An alignment model does not correct sequencing errors, incorrect gene boundaries, or recombination.

Choose an analysis according to the question you want to answer:

Question	Analysis
What is the overall dN/dS?	Single-ω analysis
Is there evidence of positively selected sites?	Site test
Does dN/dS differ between branch groups?	Branch test
Is there evidence of selection at some sites on specified branches?	Branch-site test

A branch test detects differences in ω, which need not involve positive selection. After assessing evidence for selection in the gene, you can locate codons with evidence of selection and display their probabilities on an alignment.

10.1. Bayesian tests in BAli-Phy

BAli-Phy uses Bayesian model comparison to assess support for competing hypotheses. Its tests include those hypotheses within a single analysis. During MCMC sampling, the analysis can move between them. A variable called the *model indicator* records which hypothesis is currently active. Its posterior distribution tells us how much probability the analysis assigns to each hypothesis, given the data and priors.

The site models, `BranchModel`, `BranchModel_test`, and `BranchSite` share the default background `GTR +> MNM +> MutSelAA`. Their ω values scale nonsynonymous rates relative to that background. A category called neutral (ω=1) retains amino-acid fitness effects; positive selection refers to excess nonsynonymous change beyond that background. The mutation and fitness parameters are shared across categories, branch groups, and competing hypotheses.

Site and branch-site tests compare a hypothesis that allows positive selection with one that does not. Branch tests compare hypotheses about how ω varies among branches; a difference in ω need not involve positive selection. Unlike CODEML's likelihood-ratio tests, these comparisons do not require fitting the competing models separately. Support is summarized using posterior probabilities and Bayes factors.

For parameters and default priors, consult the M8a_test function reference or run `bali-phy help M8a_test`. Other models have corresponding help pages.

The following subsections explain which models to run and where to find their results in the reports generated by `bpy-summarize`. Bayes factors describe how strongly the data shift support toward one hypothesis over another. See *Section 10.7, "Interpreting support for competing hypotheses"* for their interpretation and their relationship to posterior probabilities.

10.2. One ω for the whole sequence

Start with a model that uses one ω for all sites and branches:

```
% bali-phy coding.fasta -A Codons -S 'GTR +> MNM +> MutSelAA +> dNdS'
```

`GTR` describes nucleotide mutation. `MNM` allows mutations to change one, two, or three nucleotides within a codon. `MutSelAA` estimates amino-acid preferences shared across sites, and `dNdS` adds the ω multiplier. Here ω describes nonsynonymous change beyond the effect of those preferences; it is not a raw ratio of observed changes. Setting ω to one leaves those preferences intact, rather than removing all selection.

`MutSelAA` assigns equal fitness to synonymous codons. To allow separate codon preferences, use `MutSel` instead:

```
% bali-phy coding.fasta -A Codons -S 'GTR +> MNM +> MutSel +> dNdS'
```

Both models estimate preferences shared across sites. The examples below use `MutSelAA` because `MutSel` currently mixes substantially more slowly and thus needs longer runs for reliable estimates. See *Section 10.9, "Codon frequencies and the background model"* and *Section 10.11, "Multinucleotide mutations"* for the reasons behind these background-model choices.

The default `LogNormal(0,1)` prior gives equal probability to ω below and above one. To restrict ω to values below one, use `dNdS(omega=~Uniform(0,1))`. Run independent chains, check convergence, and summarize them as in *Section 4.2, "Posterior summaries"*. Read the ω estimate and credible interval in the parameter report. A single ω cannot identify selected sites, and averaging across the sequence can hide selection affecting only a few sites.

10.3. Different ω values among sites

A site model allows ω to differ among codon sites. Each site belongs to an unknown category with its own ω, which applies on every branch. The analysis estimates the category proportions, any unknown ω values, and each site's probability of belonging to each category.

M1a has two categories: ω below one and ω equal to one. M2a adds a category with ω above one. M7 uses a beta distribution for ω between zero and one, approximated by a finite set of categories. M8a adds a category with ω equal to one; M8 instead adds one with ω above one.

Use `M2a_test` to compare M1a with M2a, or `M8a_test` to compare M8a with M8. In each comparison, the selection hypothesis includes a category with ω above one; the null hypothesis does not. Each command allows the chain to move between the two hypotheses:

```
% bali-phy coding.fasta -A Codons -S M2a_test
% bali-phy coding.fasta -A Codons -S M8a_test
```

For example, `M8a_test` expands to:

```
|w:GTR +> MNM +> MutSelAA +> dNdS(omega=w)| +> M8a_test
```

The expression `|w:...|` builds the codon model for a supplied ω value. The site model supplies these values and their priors, rather than using the single-ω default prior. To include positive selection throughout the analysis without comparing hypotheses, replace `M2a_test` with `M2a`, or `M8a_test` with `M8`.

See *Section 10.7.1, "Reading the summary report"* for reading model support in the report. The following section explains how to examine individual codons.

10.4. Locating positively selected codons

After assessing evidence for positive selection in the gene (*Section 10.7, "Interpreting support for competing hypotheses"*), examine the site table in the `bpy-summarize` positive-selection re-

port. It gives probabilities that individual codons have ω above one. These estimates account for alignment uncertainty by following each codon across sampled alignments (*Section 9, "Character properties"*).

`Overall posterior` averages over both the selection and no-selection hypotheses. `Posterior with selection` is conditional on the selection hypothesis. For example, a codon with conditional probability 0.95 has overall probability 0.19 if the selection hypothesis has posterior probability 0.20. Mean `dNdS` estimates ω; it is not a selection probability.

To display these probabilities on an alignment, choose `posSelection` in the alignment viewer. There, the overall and conditional summaries are labelled `Model-averaged` and `Conditional on positiveSelectionInModel`, respectively.

When the alignment is uncertain, codons in the same displayed column can occupy different columns in sampled alignments, and therefore have different selection probabilities. The site table represents each column by the codon with the highest conditional probability, or the highest overall probability if conditional estimates are unavailable. Its sequence name and ungapped position identify the observed codon; the displayed letter is not an inferred ancestral or consensus codon.

The table shows up to 20 columns that pass the display threshold. `All rows (TSV)` exports every column, regardless of the threshold. The threshold controls the display; it is not a significance test.

Conditional estimates may be imprecise when few samples were drawn under the selection hypothesis. With no such samples, these estimates are unavailable.

10.5. Different ω values among branches

A branch model assigns branches to numbered categories, each with one ω shared across all sites. Several branches can share a category. Fix the topology and label branches in a Newick tree containing all your sequences. Save it as `branches.tree`. This four-sequence example is an unrooted tree and labels the branch separating A and B from C and D:

`branches.tree`

```
((A,B):[&foreground=1],C,D);
```

The label follows a colon because it belongs to the branch, not the node. This assigns that branch to category 1; unlabelled branches use category 0. Branch lengths are still estimated:

```
% bali-phy coding.fasta -A Codons --fix topology=branches.tree \
  -S '|w:GTR +> MNM +> MutSelAA +> dNdS(omega=w)| +> BranchModel'
```

The `Branch models` report summarizes the category ω values. A category number identifies a shared parameter, not a particular branch. Different branch groups can have different ω values while all remain below one.

To test whether the labelled branch needs a separate ω, change its label to `[&foreground={0,1}]` and replace `BranchModel` with `BranchModel_test` in the command. In this example, hypothesis 0 uses category 0 everywhere; hypothesis 1 uses category 1 on that branch.

Each entry in the label gives that branch's category under a hypothesis: the first entry is for hypothesis 0, the second for hypothesis 1, and so on. Thus, `[&foreground={1,0}]` reverses the assignments on that branch. Unlabelled branches use category 0 under every hypothesis.

The report gives the probability of each hypothesis and separate ω summaries using only samples from that hypothesis. These tables omit unused categories. Summaries across all hypotheses include prior draws whenever a category is unused. This tests whether ω differs among branches, not specifically whether it exceeds one. See *Section 10.7.1, "Reading the summary report"* for reading support for the hypotheses in the report.

10.6. Selection at some sites on designated branches

A branch-site model distinguishes branches of interest, called foreground branches, from the remaining background branches. Some sites can have ω at or below one on background branches but ω above one on foreground branches. `BranchSite` compares this with a null hypothesis in which those sites have ω equal to one on the foreground branches. The analysis estimates which sites are affected and the ω shared by these sites on foreground branches.

Use a fixed topology with `[&foreground=1]` on foreground branches, as in the first tree above, not the `{0,1}` label used for `BranchModel_test`. Leave background branches unlabelled:

```
% bali-phy coding.fasta -A Codons --fix topology=branches.tree \
  -S '|w:GTR +> MNM +> MutSelAA +> dNdS(omega=w)| +> BranchSite'
```

Read model support as in *Section 10.7.1, "Reading the summary report"* and the two posterior views as in *Section 10.4, "Locating positively selected codons"*. In the alignment viewer, `foreground-posSelection` gives selection probabilities and `foreground-dNdS` gives ω estimates on foreground branches. `background-posSelection` and `background-dNdS` describe the same sites on background branches. Fixing the topology does not fix the alignment. Choose foreground branches from the biological question before examining selection results. Searching many branch sets and reporting only the strongest result can exaggerate the evidence.

10.7. Interpreting support for competing hypotheses

Posterior probabilities describe support for the competing hypotheses given the data and priors. Each probability refers to a hypothesis as a whole. In a branch test, the hypotheses describe different ways of assigning ω values to branches.

For a two-hypothesis selection test, a posterior probability of 0.9 for the selection hypothesis means a probability of 0.1 for the no-selection hypothesis. These probabilities describe support for the competing models, rather than selection at a particular codon. See *Section 10.4, "Locating positively selected codons"* for interpreting individual-codon probabilities.

The odds in favor of one hypothesis over another are the ratio of their probabilities. A Bayes factor measures how the data change those odds: it is the posterior odds divided by the prior odds. For hypotheses A and B:

$$\mathrm{BF}_{A:B} = \frac{\Pr(A \mid D) / \Pr(B \mid D)}{\Pr(A) / \Pr(B)}$$

Here D denotes the data. The site and branch-site tests give their two alternatives equal prior probability by default, so posterior odds equal the Bayes factor. In the example above, posterior probabilities of 0.9 and 0.1 give a Bayes factor of 9 in favor of selection. With unequal prior probabilities, divide the posterior odds by the prior odds even when comparing only two models.

The same formula applies to a pair of alternatives when there are more than two. To compare one alternative with all the others together, sum the others' probabilities in both the posterior and prior odds. Even with equally probable alternatives, the prior odds of one against the rest are not one unless there are only two alternatives.

Model support averages over uncertainty in parameters and, when the alignment is estimated, over alignments. Parameter priors therefore matter as well as the prior probabilities of the models. See *Section 10.13, "Comparing with CODEML"* for how this differs from likelihood-ratio tests.

10.7.1. Reading the summary report

Open `Results/index.html`, generated by `bpy-summarize`. For site and branch-site tests, find `Support for positive selection in the model` under `Positive selection`. The `Posterior probability` column gives support for the selection hypothesis; `Log odds` compares it with the no-selection hypothesis. For the site-level tables below it, see *Section 10.4, "Locating positively selected codons"*.

For branch tests, find `Hypothesis support` under `Branch models`. Each row gives a hypothesis's `Posterior probability` and `Posterior log odds` against all other hypotheses combined. `Used categories` lists the ω categories used under that hypothesis. Hypotheses have equal prior probability by default.

Both tables report posterior log odds, not log Bayes factors. Subtract the corresponding prior log odds to obtain a log Bayes factor, using the comparisons described above.

These tables are especially useful when one hypothesis has very little support. Counting how often each hypothesis was sampled can give a probability estimate of exactly zero or one if the rare hypothesis never appears. For example, with posterior log odds of 50, the less probable hypothesis has probability approximately e^{-50}. Roughly e^{50} independent samples would be needed merely to expect one occurrence, and more would be needed for a reliable frequency estimate.

The support tables use conditional probabilities calculated at each MCMC sample, rather than relying only on which hypothesis was active. They can therefore retain information about a hypothesis even when the indicator never takes that value. Calculating with logged conditional odds also avoids losing this information when ordinary probabilities round to zero or one. The analysis must still explore the relevant parameter distributions adequately.

Related quantities can also appear under `Scalar variables`. When present, their means have the following interpretations:

Quantity	Meaning of its mean
`posSelection`	Fraction of retained samples in which selection is active. This can be exactly zero or one when one hypothesis is rarely sampled.
`PrPosSelection`	Estimates posterior selection probability using conditional probabilities, rather than only counting indicator values.
`PrBranchHypothesis[i]`	Estimates posterior support for hypothesis `i` using conditional probabilities.
`hypothesis`	Average of numerical hypothesis labels; not generally a hypothesis probability.
`LogOddsPosSelection`, `LogOddsBranchHypothesis[i]`	Average conditional log odds, not the posterior log odds shown in the dedicated support tables.

Use the dedicated support tables to interpret model evidence. Even means of conditional probabilities can round to zero or one; the log-odds summaries retain information that those rounded probabilities cannot show.

10.7.2. Text output and logged quantities

`bpy-summarize` uses `statreport` to produce these summaries. To inspect a text summary directly, you can also run:

```
% statreport --skip=burnin run-1/C1.log run-2/C1.log
```

`statreport` uses the logged conditional odds to estimate posterior probabilities and log odds. It combines the probabilities of a hypothesis and its alternatives separately, calculating on the log scale to preserve very small values. Do not average the logged odds directly: the mean of conditional log odds is not the log odds of the posterior hypothesis probability. For branch tests, the reported log odds compare each hypothesis with all other hypotheses combined.

10.8. Model assumptions and false positives

A false positive is an inference of positive selection when it is absent. Evidence for selection depends on which changes the model expects without it. If the background model assigns those changes too little probability, increasing ω can partly compensate for the error. This can improve the fit without correctly explaining the process: ω increases only nonsynonymous rates, rather than correcting the background model.

A better background can strengthen or weaken evidence for selection. The following subsections explain the background choices in the examples, how alignment uncertainty is handled, and how to add synonymous rate variation. These choices improve robustness to particular sources of systematic error; they do not prevent all false positives.

10.9. Codon frequencies and the background model

Goldman–Yang (GY) models weight a change by the frequency of the destination codon. Muse–Gaut (MG) models instead use the frequency of the nucleotide being introduced. For example, for AGA → AGT, a GY model uses the frequency of AGT, whereas an MG model uses the frequency of T. With codon frequencies calculated as products of nucleotide frequencies, the GY rate therefore includes factors for the unchanged A and G as well. These different backgrounds can lead to different conclusions about selection, including false positives or missed selection (Yap et al. 2010).

The mutation–selection models used here separate nucleotide mutation biases from amino-acid or codon preferences. Rates depend on mutation rates and fitness differences, rather than directly multiplying by the destination-codon frequency. This separates two causes of unequal codon usage and motivates using `MutSelAA` or `MutSel` in the examples. It does not remove all assumptions about the background process.

10.10. Alignment error and uncertainty

Aligning unrelated codons can create apparent amino-acid substitutions and spurious evidence of selection. BAli-Phy jointly estimates alignment and selection, allowing each hypothesis to be evaluated over plausible alignments instead of treating one alignment as certain. In branch-site simulations, this removed the excess false positives caused by alignment error while detecting selection nearly as often as when the true alignment was known (Redelings 2014).

The examples above already sample alignments. Omit `-I none` to retain this behavior. Fixing the topology for a branch or branch-site analysis does not fix the alignment. See *Section 9, "Character properties"* for interpreting site results across sampled alignments.

10.11. Multinucleotide mutations

Two or three differences within a codon can arise from one mutation. A model allowing only single-nucleotide mutations must explain them through several events, potentially exaggerating the evidence for positive selection. This effect has been demonstrated for branch-site tests (Venkat et al. 2018).

`MNM`, already included in the examples, estimates rates of double and triple mutations as part of the background model. Likelihood evaluation has the same computational cost as for single-nucleotide mutations; estimating the two additional parameters may increase the total computation required for inference. It allows these events within codons, not across codon boundaries. Replace it with `x3` to restrict mutations to single-nucleotide events, as in *Section 10.13, "Comparing with CODEML"*.

10.12. Synonymous rate variation

Codons can differ in their baseline substitution rates, affecting synonymous as well as nonsynonymous changes. This is distinct from variation in ω. Ignoring baseline-rate variation can distort evidence for selection (Rubinstein et al. 2011). For comparable nonsynonymous change, a lower inferred baseline rate can strengthen evidence for selection; a higher baseline can weaken it.

Append `ASRV.Gamma` to add a gamma distribution of rates among codons:

```
% bali-phy coding.fasta -A Codons \
  -S '|w:GTR +> MNM +> MutSelAA +> dNdS(omega=w)| +> M8a_test +> ASRV.Gamma'
```

Each codon now has a rate category as well as an ω category. Its rate multiplier scales synonymous and nonsynonymous rates together without changing ω, and applies on every branch. Append ASRV after the completed selection model, outside `|w:...|`, as shown. The same distribution of rate multipliers applies to all ω categories and both hypotheses. This construction works with the other models above.

`ASRV.Gamma` approximates the gamma distribution with four categories by default. Alternatively, `ASRV.Free` estimates category rates and proportions without requiring a gamma shape. Start with two categories:

```
% bali-phy coding.fasta -A Codons \
  -S '|w:GTR +> MNM +> MutSelAA +> dNdS(omega=w)| +> M8a_test +> ASRV.Free(n=2)'
```

The number of categories is fixed by `n`, not inferred. If the two estimated rates are clearly separated, try `n=3` and check whether the data distinguish a third rate and mixing remains adequate. The default of four free-rate categories introduces more parameters. Both ASRV extensions increase computation by combining rate categories with selection categories, so they are optional rather than part of the baseline examples.

10.13. Comparing with CODEML

A typical CODEML likelihood-ratio test fits competing models separately and compares their maximized log likelihoods. BAli-Phy's Bayesian tests include the competing models in each analysis: you do not need separate fits or maximized log likelihoods.

CODEML likelihood-ratio workflow	**BAli-Phy Bayesian-test workflow**
Fit competing models separately	Include competing models in each analysis
Compare maximized log likelihoods	Summarize posterior model probabilities and Bayes factors
Optimize model parameters	Average over parameter uncertainty under specified priors
Condition on a supplied alignment	Fix the alignment or average over alignments

See *Section 10.1, "Bayesian tests in BAli-Phy"* for the Bayesian testing workflow, and *Section 10.7, "Interpreting support for competing hypotheses"* for Bayes factors and their interpretation. Bayes factors are neither likelihood-ratio statistics nor p-values.

The shared-ω versus foreground/background comparison in *Section 10.5, "Different ω values among branches"* corresponds to CODEML's M0 (`model=0, NSsites=0`) versus a labelled branch model (`model=2, NSsites=0`). The default `BranchSite` compares branch-site model A (`model=2, NSsites=2, fix_omega=0`) with its null model fixing ω_2 to one (`fix_omega=1, omega=1`). These identify the hypotheses; BAli-Phy compares them using Bayesian model support.

To request the traditional GY94 background explicitly, override `submodel`:

```
% bali-phy coding.fasta -A Codons -I none --fix topology=tree.tree \
  -S 'M8a_test(submodel=(|w:GY94(omega=w)|))'
```

The model families above correspond to analyses available in PAML's CODEML program, but the background choices differ. Many earlier analyses used GY94-type models. To compare with these, use `GY94` and match their codon-frequency specification. For example, `GY94(pi=F3x4)` uses separate nucleotide frequencies at each codon position. In a site model, supply ω explicitly:

```
% bali-phy coding.fasta -A Codons -I none --fix topology=tree.tree \
  -S '|w:GY94(omega=w,pi=F3x4)| +> M8a_test'
```

The Álvarez-Carretero et al. (2023) tutorial instead uses FMutSel (`CodonFreq=7`) with observed codon frequencies (`estFreq=0`). The command below uses the same mutation-selection construction, but infers fitness preferences rather than fixing observed frequencies. It replaces GTR with HKY85, MNM with x3, and MutSelAA with MutSel:

```
% bali-phy coding.fasta -A Codons -I none --fix topology=tree.tree \
  -S 'HKY85 +> x3 +> MutSel +> dNdS'
```

Using `MutSelAA` instead gives the FMutSel0 fitness structure (`CodonFreq=6`); see *Section 5.6.2, "Constructing and tweaking codon models"* for the full-model names and their components. For these CODEML models, `estFreq=1` estimates frequencies by maximum likelihood instead of using observed frequencies. This still differs from Bayesian inference of preferences under priors.

CODEML's `hkyREV=0` selects HKY-like mutation rates and `hkyREV=1` selects GTR-like rates. Its codon models restrict instantaneous changes to a single nucleotide; they do not include the MNM extension. See the PAML manual for CODEML settings.

Use the same alignment, topology, genetic code, foreground branches, and retained sites in both programs. Omit ASRV if the CODEML analysis did not include it. Matching the model family does not make the analyses identical.

Specify the Beta category count explicitly with `M8a_test(n=10)` (the current default). The extra category is not included in `n`. BAli-Phy uses generally unequal quadrature weights, so matching category counts does not make the approximations identical; see *Section 5.6.5, "Heterogeneous dN/dS and tests for positive selection"*.

For the non-clock codon analyses compared here, CODEML reports branch lengths in expected nucleotide substitutions per codon; BAli-Phy uses expected nucleotide substitutions per nucleotide site. Divide CODEML lengths by three to compare units. For partitioned BAli-Phy analyses, use lengths scaled for the codon partition.

A CODEML result with $p < 0.05$ does not imply a posterior probability of positive selection above 95%. A p-value measures how unusual the test statistic would be under the hypothesis of no positive selection; it is not the probability that this hypothesis is true. A posterior probability measures support for a hypothesis given the data, model, and priors. Thus, CODEML can give a significant result when BAli-Phy reports posterior support below 95%, even with the same likelihood function. These are different criteria for assessing evidence, not equivalent thresholds.

For comparison with CODEML's site probabilities under the selection model, use `Posterior with selection`, not `Overall posterior` (*Section 10.4, "Locating positively selected codons"*). Both the BEB result and this conditional summary assume the selection model, rather than averaging over selection and no-selection models.

For some models, CODEML uses Bayes empirical Bayes (BEB) to account for uncertainty in the parameters describing variation in ω among sites when calculating site probabilities (Yang, Wong, and Nielsen 2005). Other parameters, such as branch lengths, remain fixed at their maximum-likelihood estimates. BAli-Phy averages over uncertainty in all parameters being inferred, for site probabilities and other estimates alike, so no separate BEB correction is needed. Quantities fixed by the user, such as the topology, remain fixed.

11. Ancestral sequence reconstruction

In this section

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11.1. Ancestral sequences with gaps

BAli-Phy can reconstruct ancestral sequences for all internal nodes. Unlike some programs, BAli-Phy explicitly infers the presence and absence of characters in ancestral sequences. This means that if the ancestral sequence has no character for a column, the reconstructed ancestor will have a gap there. BAli-Phy reconstructs ancestors for fixed-alignment partitions as well as variable-alignment partitions, but it won't write out fixed alignment samples unless you add the flag `--set write-fixed-alignments=true`. Additionally, if you have an ambiguous character such as `N` in an observed sequence BAli-Phy will impute this character.

11.2. Generating a consensus alignment with ancestral sequences

BAli-Phy can reconstruct ancestral sequences for a given tree topology and (leaf sequence) alignment. This is similar to the ancestor-reconstruction that is usually done for fixed-alignment analyses. However, it is not quite the same, because of uncertainty in the tree and the alignment. When computing the probability of an ancestral residue, this summary averages over uncertainty in the topology, the alignment, and the ancestral state itself.

This example uses the original run files and discards iterations 0–999 from each run. Replace 1000 with a burn-in appropriate for your runs. For older PowerShell versions, see *Section 3.4.1, "Pipelines and redirected output"* before running these pipelines. First, construct the tree topology to annotate:

```
% trees-consensus --skip=1000 dir-1/C1.trees dir-2/C1.trees | tree-tool - --strip-internal-
names --name-all-nodes > c50.tree
```

Construct the leaf-sequence alignment to annotate using posterior decoding:

```
% cut-range dir-1/C1.P1.fastas dir-2/C1.P1.fastas --skip=1000 | alignment-chop-internal --
tree c50.tree | alignment-max --out P1-max.fasta
```

Finally, reconstruct the ancestral sequences on the given tree and alignment:

```
% summarize-ancestors P1-max.fasta -A dir-1/C1.P1.fastas -T dir-1/C1.trees -A dir-2/
C1.P1.fastas -T dir-2/C1.trees --skip=1000 -n c50.tree > P1.ancestors.fasta
```

The default `--subsample=10` matches one saved alignment to every tenth tree before applying burn-in. Use `--until` to set the last retained iteration and `--thin` to thin the eligible pairs. Do not remove internal sequence names or tree labels from the original sample files.

For codon data, pass the same `--alphabet` option to `alignment-max` and `summarize-ancestors`, for example `--alphabet="Codons(DNA,standard)"`. Use the genetic code from your analysis.

BAli-Phy uses an alignment estimate (here, `P1-max.fasta`) as a template to construct a consensus alignment with ancestral sequences. BAli-Phy doesn't condition on the alignment columns, because (i) many columns occur only once in a posterior sample and (ii) conditioning on the column gives too much weight to the template alignment.

Because the alignment is uncertain, residues in the same column of the template alignment may end up in different columns in an MCMC sample. Therefore, in a given MCMC sample, different leaf residues in the same column may have different ancestors at the same internal node! However, in our ancestral reconstruction, a given column may only display a single ancestral residue.

BAli-Phy addresses this problem by averaging across the different ancestral residues in each column of the template alignment. When identifying the ancestral character for column C from a sampled alignment A, we randomly select a residue in C and use it to select a column from A. This procedure has the nice property that it will yield the traditional ancestral residue prediction if the alignment column is fixed.

11.3. Sampled alignments contain ancestral sequences

Ancestral sequences are written as part of the alignment matrix in each iteration. Ancestral sequences are given names starting with the letter `A`. For example, in the following alignment, the sequences `A5`, `A6`, and `A7` are reconstructed ancestors:

Sampled alignment excerpt — FASTA

```
>Halobacterium
-T-TAAGGCGGCCATAGCGGTGGGGTTACTCCCGTAC
>Pyrococcus
GG-TACGGCGGTCATAGCGGGGGGGCCACACCCGGTC
>Sulfolobus
GC-CCACCCGGTCACAGTGAGCGGGCAACACCCGGAC
>Homo
GTCTACGGC---CATACCACCCTGAACGCGCCCGATC
>Escherichia
TG-CCTGGCGGCCGTAGCGCGGTGGTCCCACCTGACC
```

```
>A5
GG-CAAGGCGGCCATAGCGGGGGGGCCACACCCGGCC
>A6
GT-CAAGGCGGCCATAGCGGGGGGGCTACACCCGGTC
>A7
GT-CAAGGCGGCCATAGCGGGGGGGCTACACCCGGTC
```

Sampled alignments for the *n*th partition are in the file. `C1.Pn.fastas`.

Ancestral states in these alignments are randomly sampled from their joint posterior and do *not* represent the most probable ancestral state. The alignment of ancestral sequences is also inferred, so these sequences may contain gaps. The length of ancestral sequences may vary between samples when the length of the ancestral sequence is uncertain.

11.4. Sampled alignments correspond to specific sampled trees

Each sampled alignment matrix corresponds to a tree in the file `C1.trees` that is written in the same iteration. This tree specifies the phylogenetic location of each ancestral sequence by labelling the internal nodes of the tree. For example, the tree below shows where the internal nodes `A5`, `A6`, and `A7` are located on the tree:

Sampled tree example — Newick

```
(Halobacterium:0.213240,((Escherichia:0.435762,Pyrococcus:0.122678)A5:0.114725,Sulfolobus
:0.427210)A6:0.042527,Homo:0.427026)A7;
```

While the tree is written every iteration, the alignment is only written every 10 iterations (by default) in order to save disk space. One method for extracting the trees that correspond to saved alignments is to extract every 10th tree with the program `bpy-subsample`:

Unix shell / Command Prompt
including Git Bash and MSYS2 Bash

```
% bpy-subsample 10 < C1.trees > C1.10.trees
```

PowerShell
Let Command Prompt handle the input and output redirection:

```
PS> cmd /c "bpy-subsample 10 < C1.trees > C1.10.trees"
```

11.5. Using the sampled alignments instead of a consensus

Instead of constructing consensus ancestral sequences, you can also summarize the sampled alignments and their ancestral sequences directly. This approach involves performing a downstream analysis on *each* sampled (alignment,tree) pair, yielding the posterior distribution of the downstream analysis. Averaging these results then yields the posterior mean analysis result.

When this approach is feasible, it is more statistically rigorous than analyzing the consensus ancestral sequence alignment. The consensus ancestral sequence alignment does not account for uncertainty in the ancestral sequences, and is not a joint reconstruction. In contrast, analyzing the posterior samples accounts for uncertainty in the ancestral sequences, the alignment, and the tree. Furthermore, it also summarizes joint reconstructions instead of a marginal reconstruction.

11.6. Tree uncertainty in ancestral sequence reconstruction.

In Bayesian phylogenetic analyses, the tree is not fixed. Therefore the internal node corresponding to the ancestral sequence you wish to reconstruct may not exist in every posterior sample. The standard Bayesian approach to tree uncertainty is to reconstruct the ancestor for each node by conditioning on the existence of that node in the tree. This allows the reconstructed ancestor for each node to average over uncertainty about the existence of other nodes.

BAli-Phy additionally allows the researcher to condition on branches, since a branch condition is less restrictive. BAli-Phy does not run a separate MCMC chain with a tree constraint for each node, but instead performs conditioning by selecting samples from a single run that satisfy the condition.

11.6.1. Extracting and naming sequences that satisfy a query

Note that the sequence names (e.g. A6) for internal nodes may change over time. Therefore, you cannot simply extract ancestral sequences with a given name. To extract ancestral sequences for a given node, you need to specify a method of identifying that node on a tree, and a name to give to the sequence at that node. This is called a *query*.

For example, you might specify how to identify the ancestor node of Eukaryotes, and the name "Eukaryotes" to use for the sequence there. You can then use the program `extract-ancestors` to extract ancestral sequences from the sampled trees and alignment, and label them with usable names. Prepare `post-1.fastas`, `post-1.trees`, the corresponding files for run 2, and `c50.tree` as in *Section 11.2, “Generating a consensus alignment with ancestral sequences”*:

```
% extract-ancestors -A post-1.fastas -T post-1.trees -A post-2.fastas -T post-2.trees --
subsample=1 -n c50.tree -g c50.tree > P1.ancestors.fastas
```

Here the options `-n c50.tree` and `-g c50.tree` specify node-based queries and branch-based queries.

11.6.2. Conditioning on a node: node-based queries

A node exists in a sampled tree if every branch connected to that node exists in the sampled tree. A node-based query asks for the reconstructed ancestral sequence only from samples where every branch connected to that node exists. A node-based query is more stringent than a branch-based query, since it requires multiple branches to exist.

BAli-Phy allows constructing node-based queries by passing in a Newick tree with labelled internal nodes. A node-based query is automatically constructed from each internal node that is labelled.

11.6.3. Conditioning on a branch: branch-based queries

A branch-based query requires only that a single branch exist in a sampled tree. The branch-based query asks for the reconstructed ancestral sequence on one endpoint of that (directed) branch. When the focus is on changes that occur on a particular branch, this makes more sense than a node-based query.

BAli-Phy allows constructing branch-based queries from a file where every line is either a Newick tree *or* a named group of taxa. For each line that contains a Newick tree, a branch-based query is automatically constructed from each branch where *both* endpoints are labelled. For a branch from `node1` to `node2`, the query is named `"node2<=node1"`.

Branch-based query files can also contain lines of the form

```
name = taxon1 taxon2 ... taxonN
```

This matches branches that separate the listed taxa from all other taxa, and points toward the listed taxa.

12. Convergence and Mixing: Is it done yet?

In this section

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12.2. Definition of Mixing 78
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When using Markov chain Monte Carlo (MCMC) programs like MrBayes, BEAST or BAli-Phy, it is hard to determine in advance how many iterations are required to give a good estimate. The number depends on the specific data set that is being examined. As a result, BAli-Phy relies on the user to summarize the output of a running chain periodically in order to determine when enough samples have been obtained. This section describes a number of techniques to diagnose when more samples must be taken.

Some of the better diagnostics for lack of convergence rely on running at least 2 independent copies of the Markov chain (preferably 4-10) from different random starting points to see if the sampled posterior distributions for each chain are the same. Unfortunately, when the distributions all seem to be the same, this doesn't *prove* that they have all converged to the equilibrium distribution. However, if the distributions are different then you can reject either convergence or good mixing.

12.1. Definition of Convergence

Convergence refers to the tendency of a Markov chain to "forget" its starting value and become typical of its equilibrium distribution. Note that convergence is a property of the Markov chain itself, not of individual runs of the Markov chain. Ideally a number of individual runs should be examined in order to determine how many initial iterations to discard as "burnin".

12.2. Definition of Mixing

In MCMC, each sample is not fully independent of previous samples. In fact, even after a Markov chain has converged, it can get "stuck" in one part of the parameter space for a long time, before jumping to an equally important part. When this happens, each new sample contributes very little new information, and we need to obtain many more samples to get good precision on our parameter estimates. In such a case, we say that the chain isn't "mixing" well.

12.3. Diagnostics: Variation in split frequencies across runs (ASDSF/MSDSF)

12.3.1. ASDSF and MSDSF

To calculate the ASDSF and MSDSF run:

```
% trees-bootstrap dir-1/C1.trees dir-2/C1.trees ... dir-n/C1.trees > partitions.bs
```

For each split, the SDSF value is just the standard deviation across runs of the Posterior Probabilities for that split. By averaging the resulting SDSF values across splits, we may obtain the ASDSF value (Huelsenbeck and Ronquist 2001). This is commonly considered acceptable if it is < 0.01.

However, it is also useful to consider the maximum of the SDSF values (MSDSF). This is the largest standard deviation of split frequencies across runs.

12.3.2. Split-frequency comparison plot

To generate the split-frequency comparison plot, you must have R installed. Locate the script `compare-runs.R`. Then run:

```
% trees-bootstrap dir-1/C1.trees dir-2/C1.trees ... dir-n/C1.trees --LOD-table=LOD-table >
partitions.bs
% R --slave --vanilla --args LOD-table compare-SF.pdf < compare-runs.R
```

Following Beiko et al. (2006), this displays the variation in estimates of split frequencies across runs. Splits are arranged on the x-axis in increasing order of Posterior Probability (PP), which is obtained by averaging over runs. We then plot a vertical bar from the minimum PP to the maximum PP.

12.4. Diagnostics: Potential Scale Reduction Factors (PSRF)

Potential Scale Reduction Factors check that different runs have similar posterior distributions. Only numerical variables may have a PSRF. To calculate the PSRF for each numerical parameter, you may run:

```
% statreport dir-1/C1.log dir-2/C1.log ... dir-n/C1.log > Report
```

The PSRF is a ratio of the width of the pooled distribution to the average width of each distribution, and should ideally be 1. The PSRF is customarily considered to be small enough if it is less than 1.01.

We compare the PSRF based on the length of 80% credible intervals (Brooks and Gelman 1998) and report the result as PSRF-80%CI. For integer-valued parameters, we add 1 to both the pooled and within-run interval widths before taking their ratio, since an integer interval from a to b contains $b - a + 1$ values.

We also report a new PSRF that is more sensitive for integer distributions. For each individual distribution, we find the 80% credible interval. We divide the probability of that interval (which may be more than 80%) by the probability of the same interval under the pooled distribution. The average of this measure over all distributions gives us a PSRF that we report as PSRF-RCF.

This convergence diagnostic gives a criterion for detecting when a parameter value has stabilized at different values in several independent runs, indicating a lack of convergence. This situation might occur if different runs of the Markov chain were trapped in different modes and failed to adequately mix between modes.

12.5. Diagnostics: Effective sample sizes (ESS)

12.5.1. ESS for numerical values

To calculate the ESS values for numerical parameters, run:

```
% statreport dir-1/C1.log dir-2/C1.log ... dir-n/C1.log > Report
```

We calculate effective sample sizes based on integrated autocorrelation times. This method has the nice property that simply duplicating every sample does not increase the ESS.

Tracer also computes ESS values and displays the numerical traces interactively; see *Section 2.9, “Optional programs for inspecting results”*.

12.5.2. ESS for split frequencies

As described in Gaya et al. (2011), we can also compute ESS values for splits on the tree:

```
% trees-bootstrap dir-1/C1.trees dir-2/C1.trees ... dir-n/C1.trees > partitions.bs
```

To compute the ESS for a split, we consider the presence or absence of a split in each iteration as a series of binary values. We compute the integrated autocorrelation time for this binary sequence, which leads to an ESS. This approach is similar to dividing the iterations into blocks and computing the ESS on the PP estimates in the blocks. It is also similar to estimating the variance reduction under a block bootstrap.

12.6. Diagnostics: Stabilization

12.6.1. Stabilization of numerical values

To obtain estimates of the stabilization time for each numerical parameter, you may run:

```
% statreport C1.log > Report
```

Each series of values is counted as having stabilized after the series crosses its upper and then lower 95% confidence bounds twice (if the initial value is below the median) or crosses its lower and then upper confidence bounds twice (if the initial value is above the median). The confidence bounds are those based on its equilibrium distribution as calculated from the last third of the values in the sequence.

12.6.2. Stabilization of tree topologies and tree distances

In addition to examining convergence diagnostics for continuous parameters, it is important to examine convergence diagnostics for the topology as well (Beiko et al., 2006).

It is also possible to assess stabilization of tree topologies using tools distributed with bali-phy by using commands like the following. Here, subsampling and burnin do not apply to the equilibrium tree files. Also, note that you need to manually construct the equilibrium samples, which we recommend should contain at least 500 trees; you might do this by subsampling using the BAli-Phy tool `bpy-subsample`.

1. To report the average distances within and between two tree samples:

   ```
   % trees-distances --skip=burnin --subsample=factor compare dir-1/C1.trees dir-2/
   C1.trees
   ```

2. To compute the distance from each tree in C1.trees to all trees equilibrium.trees, as a time series:

   ```
   % trees-distances --skip=burnin --subsample=factor convergence C1.trees equilibrium.t
   rees
   ```

3. To assess when the above time series stabilizes:

   ```
   % trees-distances --skip=burnin --subsample=factor converged C1.trees equilibrium.tre
   es
   ```

   The stabilization criterion is the same one described above for numerical values.

Note that the running time is the product of the number of trees in the two files. Therefore, comparing two complete tree samples without sub-sampling will take too long.

13. Alignment utilities: brief overview

In this section

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13.3. alignment-thin 83
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This section gives a brief overview showing *some* of the things that can be done with the included alignment utilities. It is intended to be helpful, but not exhaustive. To see the full set of options for each tool, give the argument "`--help`" on the command line.

13.1. alignment-info

Show basic information about the alignment:

```
% alignment-info file.fasta
% alignment-info file.fasta file.tree
```

13.2. alignment-cat

To select columns from an alignment:

```
% alignment-cat -c1-10,50-100,600- file.fasta > result.fasta
% alignment-cat -c5-250/3 file.fasta > first_codon_position.fasta
% alignment-cat -c6-250/3 file.fasta > second_codon_position.fasta
```

To concatenate two or more alignments:

```
% alignment-cat file1.fasta file2.fasta > all.fasta
```

13.3. alignment-thin

Remove columns without a minimum number of letters:

```
% alignment-thin --min-letters=5 file.fasta > file-thinned.fasta
```

Remove sequences by name:

```
% alignment-thin --remove=seq1,seq2 file.fasta > file2.fasta
```

Remove short sequences:

```
% alignment-thin --longer-than=250 file.fasta > file-long.fasta
```

Remove sequences with fewer than 5 directional mismatches from the closest other sequence:

```
% alignment-thin --cutoff=5 file.fasta > more-than-5-differences.fasta
```

Like `--cutoff`, but stop when we have the right number of sequences:

```
% alignment-thin --down-to=30 file.fasta > file-30taxa.fasta
```

Protect some sequences from being removed:

```
% alignment-thin --down-to=30 file.fasta --protect=seq1,seq2 > file-30taxa.fasta
```

Remove sequences that are missing conserved columns:

```
% alignment-thin --remove-gappy=10 file.fasta > file2.fasta
```

13.4. alignment-draw

Draw an alignment to HTML, optionally coloring residues by AU.

```
%  alignment-draw file.fasta --show-ruler --color-scheme=DNA+contrast > file.html
%  alignment-draw file.fasta file-AU.prob --show-ruler --color-
scheme=DNA+contrast+fade+fade+fade+fade > file-AU.html
```

13.5. alignment-find

Find the last (or first) FastA alignment in a file.

```
% alignment-find --first < file.fastas > first.fasta
% alignment-find < file.fastas > last.fasta
```

13.6. alignment-chop-internal

Remove ancestral sequences from each alignment, keeping sequences whose names occur as leaves in the specified tree.

```
% alignment-chop-internal --tree tree < file.fastas > file-chopped.fastas
```

14. Tree utilities: brief overview

In this section

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14.2. trees-bootstrap 86
14.3. trees-to-SRQ 86

This section gives a brief overview showing *some* of the things that can be done with the included tree utilities. It is intended to be helpful, but not exhaustive. To see the full set of options for each tool, give the argument "`--help`" on the command line.

14.1. trees-consensus

This program summarizes one or more tree samples. By default it writes the majority consensus topology, retaining relationships present in more than half the trees. Options select the MAP topology, supported partial partitions, and a detailed support report.

14.2. trees-bootstrap

Usage: trees-bootstrap *file1* [*file2* ...] [--predicates *predicate-file*] [OPTIONS]

This program summarizes the tree samples contained in *file1*, *file2*, etc. It reports support for splits selected from the samples, or for predicates supplied in *predicate-file*. With `--bootstrap`, it also estimates uncertainty in support using a block bootstrap of the sampled history.

Each predicate is the intersection of a set of partitions, and is specified as a list of partitions or (multifurcating) trees, one per line. Predicates are separated by blank lines.

14.3. trees-to-SRQ

Usage: trees-to-SRQ *predicate-file* [OPTIONS] *trees-file*

This program summarizes the tree samples contained in *trees-file*. It uses them to produce an SRQ plot for each predicate in *predicate-file*. Plots are produced in gnuplot format, with one point per line and with plots separated by a blank line.

If `--mode sum` is specified, then a "sum" plot is produced instead of an SRQ plot. With both `--no-scale-x` and `--no-scale-y`, the axes show the number of trees examined and the cumulative count of the event. The average slope is then the observed event frequency, an estimate of its posterior probability. By default, the axes are normalized independently, so a nonzero sum curve ends at (1,1); its average slope no longer estimates the event probability. If the `--invert` option is used, the curve counts trees in which the event is absent instead. With raw axes, its average slope estimates the probability of this inverse event. This is recommended if the proba-

bility of the event is near 1.0, because the sum plot does not distinguish variation in probabilities near 1.0 well.

15. Compiling BAli-Phy

In this section

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Compiling BAli-Phy is intended to be a relatively painless process. However, most people will want to use the pre-compiled binaries as described in the standard installation instructions at *Section 2, “Installation”* instead of compiling BAli-Phy themselves. You might want to compile BAli-Phy yourself if you want to

- run BAli-Phy on a non-Intel CPU (such as ARM64 or Alpha).
- run BAli-Phy on a computing cluster.
- test an unreleased version of bali-phy.
- change the optimization options used to compile BAli-Phy in the pre-compiled binaries.
- compile with debugging options to find the cause of a bug, and maybe fix it.
- modify the source code and submit a patch with new functionality.

Otherwise, the pre-compiled binaries will be fine.

15.1. Setup

In order to compile BAli-Phy, you need

- a C++23 compiler
- Meson (version >= 1.6)

Use the GNU C++ Compiler (GCC) version 14 or higher, or the Clang compiler version 20 or higher. GCC 14 and Clang 20 are the oldest versions of these compilers tested in CI. Linux Clang builds also need libstdc++ 14 or newer. The Cairo graphics library is optional, but if it is missing, the `draw-tree` tool that is used to draw consensus trees won't be built. See also *Section 2.9, “Optional programs for inspecting results”*.

15.1.1. Linux

On Debian and Ubuntu, you can type:

```
% sudo apt-get install g++-14 git libcairo2-dev pandoc libboost-all-dev libcli11-dev
% export CC=gcc-14 CXX=g++-14
```

If your version of Debian or Ubuntu is recent enough to contain meson version 1.6 or higher, you can install meson with apt-get:

```
% sudo apt-get install meson
% meson --version
1.6.0
```

On computing clusters, you might want to use miniconda to install the build tools.

```
% conda create -n devel -c conda-forge --strict-channel-priority
% conda activate devel
% conda install meson gxx boost-cpp cmake pkg-config cairo
% export BOOST_ROOT=$CONDA_PREFIX
```

Otherwise you can install meson through pip3:

```
% sudo apt-get install python3 python3-pip python3-venv ninja-build
% python3 -m venv meson
% source meson/bin/activate
% pip3 install meson
```

15.1.2. Mac

On macOS, the simplest way to get a compiler is to install Xcode version 16 (or newer) command line tools, which come with Clang.

```
% xcode-select --install
```

To get the other tools, first install Homebrew, and then type:

```
% brew install git meson cairo pandoc
```

15.1.3. Compile for native Windows using MSYS2

This section is for developers compiling BAli-Phy from source. Users of the native binary package do not need MSYS2. The MSYS2 project provides GCC/MinGW-w64 in its UCRT64 environment to create native Windows executables.

After installing MSYS2, open the MSYS2 UCRT64 shell and perform a full system update:

```
% pacman -Syu
```

If the update asks you to close the terminal, reopen the MSYS2 UCRT64 shell and run `pacman -Syu` again. Then install the compiler, build tools, and libraries used by BAli-Phy:

```
% pacman -S --needed \
  git \
  mingw-w64-ucrt-x86_64-boost \
  mingw-w64-ucrt-x86_64-cairo \
  mingw-w64-ucrt-x86_64-cereal \
  mingw-w64-ucrt-x86_64-eigen3 \
  mingw-w64-ucrt-x86_64-fmt \
  mingw-w64-ucrt-x86_64-gcc \
  mingw-w64-ucrt-x86_64-meson \
  mingw-w64-ucrt-x86_64-zstd
```

`pacman` installs the Python, Ninja, and pkg-config dependencies of the packaged UCRT64 version of Meson automatically. Cairo is included so that the `draw-tree` program is built. The UCRT64 shell refers to drives as `/c/` instead of `C:/`.

15.2. Clone, Configure, Compile

First check out the code using git:

```
% git clone https://github.com/bredelings/BAli-Phy.git
% cd BAli-Phy
```

Then run meson to configure the build process:

```
% meson setup build --prefix="$HOME/Applications/bali-phy-4.3/" --buildtype=release
```

Finally, build and install the software:

```
% ninja -C build test
% ninja -C build install
```

The command `bali-phy` and its associated tools should then be located in `~/Applications/bali-phy-4.3/bin/`. To install to another directory *dir*, specify --prefix=*dir* to `meson`.

15.3. Options: compiler and linker flags

You can select the C++ compiler by setting the CXX variable. A useful example of this is to use `g++-14` on systems where `g++` invokes a compiler that is too old:

```
% CXX=g++-14 meson setup build --prefix="$HOME/Applications/bali-phy-4.3" --
buildtype=release
```

You may also set compiler and linker options using the CPPFLAGS, CXXFLAGS, and LDFLAGS variables. For example, you can instruct the compiler to use all the features of your chip, instead of producing generic code that will run anywhere:

```
% CXXFLAGS="-mtune=native -march=native" meson setup build --prefix="$HOME/Applications/
bali-phy-4.3"
```

For example, you can set the CPPFLAGS and LDFLAGS variables to instruct the compiler where to look for libraries, such as cairo:

```
% CPPFLAGS="-I/usr/local/include" LDFLAGS="-L/usr/local/lib" meson setup build --
prefix="$HOME/Applications/bali-phy-4.3" --buildtype=release
```

16. Frequently Asked Questions (FAQ)

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16.1. Input files

Does BAli-Phy accept the wildcard characters "N" or "X"? How does it treat them?

Yes, BAli-Phy accepts the wildcard characters "N" (for DNA) and "X" (for proteins). These characters indicate that some letter is present (as opposed to a gap), but that you don't know *which* letter it is.

Does BAli-Phy accept "?" characters?

No. "?" characters are often used to indicate *either* letter presence (e.g. "N", "X") *or* absence (e.g. "-"). BAli-phy will insist that you replace each "?" with either "N"/"X" or "-" to indicate which one you mean.

(Most programs ignore indels and consider only substitutions, and in that case "N" and "-" have the same effect on the likelihood or parsimony score. However, since BAli-Phy takes indels into account, these two alternatives are quite different.)

Does BAli-Phy accept the characters "R" and "Y", etc.?

Yes. BAli-Phy accepts the characters Y, R, W, S, K, M, B, D, H, and V for DNA, RNA, and Codon alphabets. BAli-Phy also accepts the characters B, Z, and J for amino acids. These characters indicate partial knowledge about a letter. For example, R indicates that a nucleotide is present, and is a puRine (A or G). J indicates that an amino acid is present and is either I or L.

(Note that sequences sometimes contain such ambiguity codes because the DNA that was sequenced contains *both* values. This might occur when sequencing a heterozygote or when sequencing pooled DNA from several individuals. However, the model in BAli-Phy (and other phylogeny inference programs) is that only one letter is correct, but we do not know which one it is. This is probably not problematic when dealing with pooled sequences, but should be considered.)

16.2. Running `bali-phy`.

Can I fix the alignment and ignore indel information, like MrBayes, BEAST, PhyloBayes and other MCMC programs?

Yes. Add `-Inone` or `-I none` on the command line.

Can I fix the tree topology, while allowing the alignment to vary?

Yes. Add `--fix topology=`*`treefile`* on the command line.

Can I fix the tree topology and *absolute* branch lengths *in all data partitions*, while allowing the alignment to vary?

Yes. Add `--fix tree=`*`treefile`* on the command line.

Can I fix the tree topology and *relative* branch lengths, while allowing the alignment to vary?

Yes. Add `--fix tree=`*`treefile`* `'--scale=~Gamma(0.5,2)'` on the command line.

16.3. Run-time error messages

I tried to use `-S LG+>ASRV.Gamma(6)` and I got an error message "bali-phy: No match." What gives?

You are probably using the C-shell as your command line shell. It is trying to interpret `LG+>ASRV.Gamma(6)` as an array before running the command, and it is not succeeding. Therefore, it doesn't even run `bali-phy`.

To avoid this, put quotes around the substitution model, like this: `-S 'LG +> ASRV.Gamma(6)'`. This will keep the C-shell from interfering with your command. See *Section 3.4, "Shell differences"* for quoting in other shells.

16.4. Stopping `bali-phy`.

Why is `bali-phy` still running? How long will it take?

It runs until you stop it or it reaches its maximum number of iterations. The default maximum is 200,000 iterations; use `--iterations` to set a different limit.

The longer answer is that it is hard to predict how long MCMC will take to converge, since it depends on each data set in complex ways. Automatic rules for determining when to stop an MCMC chain can be difficult to get right. BAli-Phy does not contain an automatic stopping rule yet, so it relies on the user to run convergence diagnostics and determine when to stop the run.

How do I stop a `bali-phy` run on my personal computer?

Simply kill the process -- there is no special command to stop `bali-phy`. If you are running it on your personal workstation, then you can use the command `kill`. To do that, you need to find

the PID (process ID) of the running program. You can find this by examining the beginning of the file `C1.run.json`. For example:

```
% less 5d-1/C1.run.json
```

`C1.run.json` — excerpt

```
    ...
    "partitions": [
        {
            "alphabet": "DNA",
            "filename": "5d-muscle.fasta",
            "imodel": 0,
            "range": "",
            "scale": 0,
            "smodel": 0
        }
    ],
    "pid": 549319,
    "program": {
        "arch": "linux x86_64",
        "build-date": "Mar  2 2024 11:27:23",
        "compiler": "gcc 13.2.0 x86_64",
        "name": "bali-phy",
        "revision": "[HEAD -> master, origin/master, origin/HEAD commit d394a4fb6]  (Mar
02 2024 11:11:25)",
        "version": "4.0-beta9-preview"
    },
    ...
```

Here the PID is 549319. Therefore you can type:

```
% kill 549319
```

Warning: Stop all your BAli-Phy runs

On some operating systems you can also use the command below. It will terminate *all* of your `bali-phy` runs on that computer:

```
% killall bali-phy
```

How do I stop a `bali-phy` run on a computing cluster?

Simply terminate the submitted job. The specific command to terminate a job will depend on the queue manager that is installed on your cluster. Examine the documentation for your cluster, or ask your cluster support staff how to delete running jobs on your cluster.

As an example, if the SLURM software is used to submit jobs, then the command `squeue` should list your jobs and their job ID numbers (which is different than the process ID number). You can then use the command `scancel` to delete jobs by ID number. The SLURM documentation describes how to use these commands.

So, how can I know when to stop it?

You can stop when it has both converged and also run for long enough to give you >1000 effectively independent samples.

How can I tell when the chain has converged?

See section *Section 12, "Convergence and Mixing: Is it done yet?"*.

How can I check how many iterations the chain has finished?

Run `wc -l C1.log` inside the output directory, and subtract 2.

16.5. Running `bpy-summarize`.

Why does `bpy-summarize` say "Program 'draw-tree' not found. Tree pictures will not be generated"?

The program `draw-tree` is built only when the Cairo graphics library is available, so some installations omit it. This is not a fatal error message; it just means that a picture of the tree will not be generated automatically. You can still open the tree with TreeViewer or another program listed in *Section 2.9, "Optional programs for inspecting results"*.

Why does `bpy-summarize` say "Program 'R' not found. Some report plots will not be generated"?

R is not installed or is not on your command search path. The report will omit split-support plots, SRQ plots, between-run comparisons, and tree-distance plots. Numerical summaries are still produced.

Why is `bpy-summarize` stopping early, or failing to generate some files?

Look in the file `Results/commands.log`. This should contain the specific tool commands that were run, along with error message from these commands. Identify the first tool command that fails, and read the error message.

16.6. Interpreting the results.

How do I compute the clade support?

By default, BAli-Phy uses unrooted trees and reports bi-partition support. A bi-partition divides the taxa into two groups without specifying which contains the root. BAli-Phy also supports rooted tree models; see *Section 5.1, "Non-reversible models and rooted trees"*.

How do I compute the split/bi-partition support?

After you summarize the output (*Section 4.2, "Posterior summaries"*), the partition support is indicated in `Results/consensus` and in `Results/c50.PP.tree`.

16.7. How do I...

How do I concatenate alignments?

```
% alignment-cat filename1.fasta filename2.fasta > result.fasta
```

For older PowerShell versions, see *Section 3.4.1, "Pipelines and redirected output"*. The alignments must have the same sequence names, but the names need not be in the same order.

How do I select columns from an alignment?

You can select columns for analysis by specifying a range:

```
% bali-phy sequences.fasta:1-200,401-600 sequences.fasta:201-400
```

You can create a new alignment from selected columns using `alignment-cat`:

```
% alignment-cat -c1-10,50-100,600- filename.fasta > result.fasta
```

For older PowerShell versions, see *Section 3.4.1, "Pipelines and redirected output"*. The resulting alignment will contain the selected columns in the order you specified.

16.8. Installation

Why does an older Bioconda build fail to parse models on Apple Silicon?

Bioconda builds before BAli-Phy 4.2 have a model-parsing bug on Apple Silicon. Install BAli-Phy 4.2 or newer.