

Befi-BaTS – Bayesian Tip-association Significance testing

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Version 0.1.1 Documentation

INTRODUCTION	2
LICENSE & DISCLAIMER	3
<i>License</i>	3
<i>Disclaimer</i>	3
CHANGES IN THIS VERSION	4
WHAT IS BEFI-BATS?.....	5
WHAT CAN IT DO?.....	6
SYSTEM REQUIREMENTS	7
<i>Java</i>	7
<i>Hardware</i>	7
INSTALLING BEFI-BATS	8
USING BEFI-BATS: INPUT FILE REQUIREMENTS.....	9
<i>Preconditions</i>	9
<i>Burnin period</i>	9
<i>Format</i>	9
USING BEFI-BATS: RUNNING AN ANALYSIS	11
<i>Versions</i>	11
<i>Usage: SingleBefi-BaTS</i>	11
<i>Usage: BatchBefi-BaTS</i>	11
USING BEFI-BATS: INTERPRETING ANALYSES.....	12
<i>The test statistics</i>	12
<i>The null hypothesis</i>	12
<i>Output</i>	13
USING BEFI-BATS: CAVEATS AND WARNINGS.....	14
<i>Computational constraints:</i>	14
<i>Biological constraints</i>	15
FAQ.....	16
CONTACT	17
REFERENCES & BIBLIOGRAPHY	18

Introduction

Welcome to the documentation for this version of `Befi-BaTS`, Version 0.1.1. This is the first publically-available version to be released. I hope you find `Befi-BaTS` accessible and of use to you in your research.

`Befi-BaTS` was essentially conceived in response to a specific problem encountered in my own studies and although a number of changes have been incorporated in this (beta) release that allow a wider range of problems to be addressed, it remains a fairly inflexible tool, both in terms of technical requirements and logical problems that can be solved. Of course, over time, other researchers may yet find `Befi-BaTS` useful in situations as-yet unthought of by us.

I would gratefully like to acknowledge Oli Pybus and Andrew Rambaut, my supervisors, whose direction and input brought `Befi-BaTS` to this point, and Aris Katzourakis, Rob Belshaw, Philippe Lemey, Simon Ho and Beth Shapiro for advice and help.

For the specific Bayesian approach to quantifying phylogeny-trait associations, as well as an exploration of the three statistics and discussion of their merits, users are encouraged to read Parker *et al*, (2008) which should also be used as the preferred reference when citing `Befi-BaTS`:

Parker J, Rambaut A, Pybus OG. (2008). Correlating Viral Phenotypes With Phylogeny: Accounting for Phylogenetic Uncertainty. *Infect Genet Evol.* 8(3):239-46.

License & Disclaimer

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Changes in this version

Date	Version	Changes
2010-11-23	0.1.1	Added support for lowercase #NEXUS file block parsing (<i>e.g.</i> 'begin states' or 'Begin STaTes' as well as 'BEGIN STATES')
2010-05-29	0.1 (‘First Beta’)	First public beta release of Befi-BaTS: • Core code and topology-based correlation algorithms (<i>AI</i>, <i>PS</i> & <i>MC</i>) from BaTS v1.0 • Additional distance-based correlation algorithms (full phylogeny) included – <i>NTI/NRI</i>, <i>PD</i> and <i>UniFrac</i> IMPORTANT NOTE: Trees with hard polytomies (<i>e.g.</i> (mouse:0.1,rat:0.12,human:0.11):0.6,chicken:0.5) are no longer supported in Befi-BaTS due to current constraints on the PD algorithm. Such trees will cause a <code>HardPolytomyException</code> at runtime if input to Befi-BaTS. This problem will be addressed in a future release. Users can still perform analyses using the topological statistics released in Befi-BaTS’ predecessor, BaTS v1.0. If you need to use BaTS v1.0 for this reason and cannot obtain it from evolve.zoo.ac.uk/software, please contact the author.

What is Befi-BaTS?

This software aims to provide a method by which the degree to which phenotypic traits seen in a phylogeny are associated with ancestry are correlated. In other words, where a set of character states are seen on the tip of a phylogenetic tree, is any given taxon more likely to share a character state with a sister taxon than we would expect due to chance?

This problem has been posed in a variety of contexts over the last three decades, particularly molecular epidemiology and phylogeography. A number of approaches have been developed over the years, of which the method of Slatkin & Maddison (1989) is perhaps the best known.

Befi-BaTS uses two established statistics (the Association Index, AI (Wang *et al.*, 2001), and Fitch parsimony score, PS) as well as a third statistic (maximum exclusive single-state clade size, MC) introduced by us in the Befi-BaTS citation, where the merits of each of these are discussed. What sets Befi-BaTS aside from previous methods, however, is that we incorporate uncertainty arising from phylogenetic error into the analysis through a Bayesian framework. While other many other methods obtain a null distribution for significance testing through tip character randomization, they rely on a single tree upon which phylogeny-trait association is measured for any observed or expected set of tip characters.

To improve on this basic approach we use posterior sets of trees (PSTs), obtained through earlier Bayesian MCMC analysis of the data, that integrate over all likely phylogenies and incorporate the phylogenetic uncertainty arising from the data. Although a Bayesian MCMC analysis is therefore a precondition to using Befi-BaTS, we do not feel that this is likely to deter potential users since these analyses are increasingly common.

What can it do?

`Befi-BaTS` can estimate phylogeny-trait associations for n different states of a single character at a time using the AI, PS and MC statistics, and provide 95% confidence intervals and significance estimation for these. `Befi-BaTS` is also capable of performing batch analyses of large numbers of datasets.

System requirements

Java

Befi-BaTS is written and compiled for Java 1.5.0, ("J2SE 5.0"). You will need a computer capable of running this version of Java or higher. For most platforms it is sufficient to download the required version of Java directly from java.sun.com Mac OS X users should note however that on versions 10.4.5 and lower the procedure for upgrading to Java 1.5.0 (from 1.4.2, the default on these systems) differs. They should consult

http://www.apple.com/downloads/macosx/apple/macosx_updates/index_abc.html for further instructions. If unsure, typing ' `java -version` ' from a Terminal session will tell you which version of Java is currently used by the operating system.

Hardware

We have not identified any specific minimum hardware requirements; these in any case scale with the number of taxa in the tree, number of possible states observed and number of null sets used to form the null distribution. Generally speaking, at least 256 Mb of system memory (physical RAM, not virtual memory or swap file cache) should be available for each separate instance of Befi-BaTS running. Note that in some cases this may not be sufficient and users will need to increase the amount of memory available to the Java Virtual Machine (JVM) using the `-Xms` command; for more information type ' `man java` ' from the command-line or see

<http://edocs.bea.com/wls/docs70/perform/JVMTuning.html>

Installing Befi-BaTS

Given the correct JVM (1.5.0 or higher) is available, installation of Befi-BaTS is simple. The complete Befi-BaTS package is hosted at http://www.lonelyjoeparker.com/wp/?page_id=274 for download as an archived jarfile. Simply download the jarfile to some memorable location on your hard drive.

Using Befi-BaTS: input file requirements

Preconditions

Because `Befi-BaTS` uses the PST from a Bayesian MCMC analysis to integrate over all credible trees to account for error in the phylogenetic signal, users must first use an appropriate package to produce a PST. This is a single treefile containing many trees from the posterior set, weighted by the MCMC sampler so that the most likely topologies are sampled more often. `MrBayes` (mrbayes.csit.fsu.edu) and `BEAST` (evolve.zoo.ox.ac.uk/software) are ideal packages available for this task, and produce these treefiles automatically.

Burnin period

Before they begin to efficiently sample from the posterior likelihood distribution of interest, MCMC samplers typically require an initial period of optimisation during which they arrive in the vicinity of highest-likelihood and tune weighting parameters, etc. During this initial ‘burnin’ period the posterior likelihood fluctuates wildly; once the likelihood becomes more stable the MCMC chain can be said to be sampling efficiently. It is common to discard the initial burnin, but currently no burnin is automatically discarded from treefiles in `Befi-BaTS`. Users **must** therefore decide on an appropriate burnin period using external data analysis software such as `Tracer` (evolve.zoo.ox.ac.uk/software) and remove these trees from the start of their treefile accordingly.

Format

Input files for `Befi-BaTS` follow the popular `NEXUS` file format, with a small modification: Instead of the normal ‘`taxa`’ and ‘`translate`’ blocks, a single ‘`states`’ block is present. The formatting for this is shown below. It is currently necessary to format these by hand; we are working on a graphical interface to parse these input files, please check for updates at evolve.zoo.ox.ac.uk/software

Typical NEXUS format:

```
#NEXUS

Begin taxa;
  Dimensions ntax=8;
  Taxlabels
    HIV_env_JP2000a
    HIV_env_JP2000b
    HIV_env_JP2001
    HIV_env_JP2002
    HIV_env_JP2003
    HIV_env_JP2003b
    HIV_env_JP2005
    HIV_env_JP2005b
  ;
End;

Begin trees;
  Translate
    1 HIV_env_JP2000a
    2 HIV_env_JP2000b
    3 HIV_env_JP2001
    4 HIV_env_JP2002
    5 HIV_env_JP2003
    6 HIV_env_JP2003b
    7 HIV_env_JP2005
    8 HIV_env_JP2005b
  ;
  tree STATE_0 = [&R] (((((20:35.3479176569581,((
tree STATE_11000 = [&R] (((((24:19.959266963075
tree STATE_22000 = [&R] (((((12:80.83442567419
tree STATE_33000 = [&R] (((((9:13.267831008023
tree STATE_44000 = [&R] (((((3:55.6268027053323
```

Befi-BaTS format for the same data:

```
#NEXUS

begin states;
1 island
2 island
3 main
4 island
5 main
6 main
7 main
8 main
End;

begin trees;
tree STATE_1011000 = [&R] (((((((8:1.442671720049141
tree STATE_1022000 = [&R] (((((((4:2.19177960366
tree STATE_1033000 = [&R] (((((((8:1.77960366104
tree STATE_1044000 = [&R] (((((((8:1.85759597599
```

The formatting is relatively simple. The key difference in the Befi-BaTS-formatted example is that the numbered taxon labels found in the trees no longer correspond, through the 'translate' block, to individual taxon names. Instead, through the 'begin states' block, each taxon is assigned a character state. Here for instance two states are present, 'island' and 'main'. The state coding used, number of states, and their biological nature are all irrelevant to Befi-BaTS; furthermore, all states are weighted to be equally different to each other in state reconstruction.

Note that no whitespace characters (spaces or tabs) appear at the start of any line within the 'begin states' block.

Using Befi-BaTS: running an analysis

Versions

Two configurations of Befi-BaTS are available: the `SingleBefi-BaTS` estimates significance values for a single dataset and also provides 95% CIs as well as p -values; while the `BatchBefi-BaTS` batch-analyses datafiles a directory at a time and only provides a summary set of p -values. This version is useful for analysing a very large number of datasets, for example those derived by simulation.

Usage: SingleBefi-BaTS

To use `SingleBefi-BaTS` from the command-line, type:

```
java -jar Befi-BaTS_beta_build2.jar single <treefile_name> <reps> <states>
```

Where:

<treefile_name> is the name and full location of the treefile or directory to be analysed,

<reps> is the number (an integer > 1, typically 100 at least) of state randomizations to perform to yield a null distribution, and

<states> is the number of different states seen.

Usage: BatchBefi-BaTS

To use `BatchBefi-BaTS` from the command-line, type:

```
java -jar Befi-BaTS_beta_build2.jar batch <dataset_dir> <reps> <states>
```

where

<dataset_dir> is the name and full location of the directory of treefiles to be analysed (`Befi-BaTS`) will attempt to analyse all files with the extension `.trees` in that directory,

<reps> is the number (an integer > 1, typically 100 at least) of state randomizations to perform to yeild a null distribution, and

<max_states> is the maximum number of different states seen in any one treefile.

Using Befi-BaTS: interpreting analyses

The test statistics

Befi-BaTS currently includes implementation of three test statistics used to quantify the strength of phylogeny-trait association. These are the parsimony score ('PS') statistic of Slatkin & Maddison (1989), association index ('AI') of Wang *et al* (2001) and a new measure introduced by Parker *et al* (2007, submitted), the maximum monophyletic clade ('MC') size. On a single tree these measures all use the tree node structure and a Fitch (1967b) parsimony algorithm to reconstruct internal node trait values {...} For a full discussion of the statistics, see Parker *et al* (2008.)

Release note: Befi-BaTS v0.1.1 includes the Phylogenetic Diversity ('PD') statistic of Hudson *et al* (1992), the Nearest Taxa and Net Relatedness indices ('NTI' & 'NRI') of Webb *et al* (2000; 2002) and the Unique Fraction ('UniFrac') index of Lozupone *et al* (2005.) These indices also include branch length information as well as tree topology, hence weighting related clades by the strength of their relatedness.

The null hypothesis

The null hypothesis under test is one of random phylogeny-trait association; that is, that

"No single tip bearing a given character trait is any more likely to share that trait with adjoining taxa than we would expect due to chance"

As implemented in Befi-BaTS, each statistic is scored on the PST and a null distribution generated against which the true posterior statistic distribution is compared. The p-value reported is the proportion of trees from the null distribution equal to, or more extreme than, the median posterior estimate of the statistic from the PST. By convention therefore, we reject the null hypothesis at the desired level of significance α where $p \leq \alpha$, e.g. $p \leq 0.05$ for a significance level of 0.05. We leave it to the user to decide what level of significance is appropriate. Computationally, the p-value is stored, manipulated and printed to output as a Java `float`, a 32-bit floating-point number of variable precision. Because of the way Java handles and rounds floating-point numbers, it is possible that, for instance, a number reported as:

`0.0500`

might actually represent the number:

0.0500953...

in the analysis. For this reason we advise that users accept decimal numbers to at least 3 decimal places; preferably more.

Output

The `Befi-BaTS` output varies depending on whether a Single or Batch analysis has been carried out.

Single `Befi-BaTS` analyses output a table of information with rows corresponding to posterior estimates of observed and expected values for the PS, AI and MC metrics respectively. Regardless of the number of character states (trait values) only one row of information is presented for the PS and AI metric, but there will be as many MC metric rows as trait values. The MC metrics appear in the order in which they occur in the input `.trees` file. Columns in the table correspond to the mean, median, and upper and lower 95% HPD intervals of the observed values of the metric, followed by those of the expected (null set), then a p-value. This is the proportion of trees in the observed set equal to, or more extreme than, the median value of the expected (null) set.

Batch `Befi-BaTS` analyses are not conducted any differently to single analyses. However they are intended to support large-scale analysis, such as simulation or automated sequence analysis, where the investigator is more interested in the behaviour of a set of metadata than individual datasets. To this end, in order to reduce computation time where possible and simplify output data, only p-values are recorded. The output takes the form of a table with one row per dataset where columns give the p-values for PS, AI and `MC{0..n}` statistics respectively. If you intend to carry out a large scale `Batch Befi-BaTS` analysis it is recommended that you first carry out a number of trial `Single Befi-BaTS` analyses of a random selection of the input datasets to check parsing of the input files is correct and that posterior observed and expected values are approximately in line with those predicted by other methods or *a priori* expectation.

Note that the expected (null) distributions generated are a function of the PST and terminal taxon trait values in combination; even if the same taxa are used the null distributions `Befi-BaTS` generates are not valid for PSTs generated under a different tree model, nor for different tip labels. If you reanalyse your data under a different model, or change your tip labelling scheme, you *must* re-analyse the data.

Using Befi-BaTS: caveats and warnings

Computational constraints:

Users should be aware that although `Befi-BaTS` has been rigorously tested on our development machines, this is the first public release of the package. As such, the real-world performance of the core packages (which in any case are highly dependent on hardware architecture, and software architecture to a degree) is unknown at this point; in fact, we would appreciate any feedback concerning performance.

As a guide, a `GeneralizedSingleBefi-BaTS` of 32 taxa with a binary (2-value) trait on a PST of 10,000 trees with expected distributions assembled from 100 replicates per tree typically takes approximately 5 minutes on a 1.25 GHz Apple PowerPC machine under Java 1.5.0 / Mac OS 10.4.10.

As usual, physical factors increasing compute time performance will include:

- Slower system CPUs
- Slower system bus speeds
- Slower system RAM access (in particular, because `Befi-BaTS` uses a lot of memory at present heavy reliance on virtual memory coupled with a slow hard drive is likely to adversely affect performance)

Problem parameters that will slow the analysis include:

- Number of replicates to construct expected posterior distribution. We have not specified a default value, since we prefer that users take responsibility for this key parameter; however we have not noticed significantly better power or Type I performance when the number of replicates increases above 100 and typically use this value. Increasing the number of replicate sets dramatically increases both compute time and memory use. We're not sure by how much (depends on platform) but it is expected to be a linear increase at best (e.g. 1000 replicates will take at least 10 times as long, and use 10 times as much memory as 100 replicates)
- Number of taxa. Increasing numbers of taxa will increase compute time and memory usage.
- Increasing sizes of PST. Longer tree sets linearly increase compute time, though memory usage increases by a lesser amount since most of the memory allocated to each tree is re-used for subsequent ones. We have often found it useful to downsample PSTs obtained from long MCMC chains (e.g. to 1000 trees from 90,000 states following a 10,000 state discard as burnin from an original 100,000 state chain); this should be done at regular (not random) intervals.

- Large numbers of different trait values may slow performance (e.g. an analysis with 20 rather than 2 trait values may suffer) because more tree traversals are required. We have not collected substantial data on this effect though, and would particularly welcome feedback.

Biological constraints

We have developed this method to analyse multi-state data on a single character. While there is no computational reason why large numbers of states cannot be analysed, there seems little point in, say, analysing the phylogeny-trait association of a character with 20 discrete states on a 25-taxon tree; any association seen may be as due to sampling error as genuine data signal. Users must use their judgement as to whether `Befi-BaTS` is an appropriate way to analyse their data, but are encouraged to contact the author for help.

The MCMC requirements given at the start of this documentation must be obeyed, since the analysis depends on an accurately estimated PST. In particular, users must have confidence in the MCMC, which should have reached stationarity and have no limits, priors or transformations on tree topology or branch lengths, except where there is a good *a priori* reason to apply them.

Hard polytomies (e.g. `(mouse:0.1,rat:0.12,human:0.11):0.6,chicken:0.5)` etc) are **not currently supported in `Befi-BaTS`** due to limitations on the PD algorithm. Users can still perform analyses using the topological statistics released in `Befi-BaTS`' predecessor, `BaTS v1.0`. If you need to use `BaTS v1.0` for this reason and cannot obtain it from evolve.zoo.ac.uk/software, please contact the author.

Lastly, the method uses unweighted parsimony reconstruction. This assumes that transitions between states are all equally likely, totally reversible, and independent of branch length. If your data does not obey these criteria then unfortunately a `Befi-BaTS` analysis should not be performed (though future versions of `Befi-BaTS` using the PD, NTI/NRI and UniFrac indices will include branch length information.)

FAQ

Note: this is just a preliminary list of FAQs; for any persistent problems, or if you have any other comments, please contact the author.

Why don't my analyses run?

Try running the sample datasets included in this package. If the sample files don't run either you may well have the wrong version of java installed; check and install the correct version. If they run but your data does not, it is likely you have parsed the input files incorrectly. Refer to the 'Input File Requirements' section of this documentation. If problems persist, contact the author.

Why do I get an error message saying I've run out of memory?

Typically this will manifest itself with a message such as

```
"Exception in thread 'main': java.lang.OutOfMemoryError: java heap space"
```

This error arises then the JVM doesn't have enough system memory ('RAM') available to hold all the data it needs to. Unfortunately increasing your computer's virtual memory allocation will not solve this problem. You can try increasing the default amount of RAM allocated to the JVM with the '-Xms' command (see the 'System Requirements > Hardware' section of this documentation for details.)

Why do I get an error message saying (ArrayList casting error)?

This is because you are using an earlier version of Java (below 1.5.0) that does not support the way this software handles `java.util.ArrayListS`. Check you have the correct version of Java installed.

Can I run Befi-BaTS on a bootstrapped set of trees?

No. This package and the discussion of statistics evaluated with it (Parker *et al*, 2007) is designed to be used in a Bayesian MCMC context. You can evaluate any set of trees you like as long as they are parsed properly, be they a bootstrapped set or some deliberately chosen set: but if you do, all assumptions about the power and behaviour of the statistics are invalid.

Which package should I use to produce a PST first?

Both `MrBayes` and `BEAST` produce acceptable output PSTs. We are unaware of any others.

Contact

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References & Bibliography

Parker, J., Rambaut, A.R. & Pybus, O.G. (2008) Correlating viral phenotypes with phylogeny: accounting for phylogenetic uncertainty. *MEEGID* **8**(3):239-246.

Recommended citation for Befi-BaTS.

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Cochrane, A., Searle, B., Hardie, A., Robertson, R., Delahooke, T., Cameron, S., Tedder, R.S., Dusheiko, G.M., de Lamballerie, X. & Simmonds, P. (2002). A genetic analysis of Hepatitis C Virus transmission between injection drug users. *J. Infect. Dis.* **186**:1212-21. **Example publication using phylogeny-trait association.**

Drummond, A.J. & Rambaut, A. (2003) BEAST v1.4.6, Available at <http://tree.bio.ed.ac.uk> **BEAST program for Bayesian MCMC phylogenetic estimation and analysis.**

Fitch, W.M. (1971b). Toward defining the course of evolution: Minimal change for a specific tree topology. *Syst. Zool.* **20**: 406-416. **The Fitch algorithm, used to implement trait value reconstruction in Befi-BaTS.**

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Holmes, E.C., Zhang, L.Q., Robertson, P., Cleland, A., Harvey, E., Simmonds, P. and Leigh Brown, A.J. (1995) The molecular epidemiology of HIV-1 in Edinburgh, Scotland. *J. Infect. Dis.* **171**: 45-53.

Holmes, E.C. (2004). The phylogeography of human viruses. *Molecular Ecology* **13**:745-756. **Example publication using phylogeny-trait association.**

Huelsenbeck, J.P., & Ronquist, F. (2001). MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* **17**:754-755. **MRBAYES application for Bayesian MCMC phylogeny estimation.**

Leigh Brown, A.J., Lobidel, D., Wade, C.M., Rebus, S., Philips, A.N., Brettle, R.P., France, A.J., Leen, C.S., McMenamin, J., McMillan, A., Maw, R.D., Mulcahy, F., Robertson, J.R., Sankar, K.N., Scott, G., Wyld, R. & Peutherer, J.F. (1997). The molecular epidemiology of human immunodeficiency virus Type 1 in six cities in Britain and Ireland. *Virology* **235**:166-177. **Example publication using phylogeny-trait association.**

Salemi, M., Lamers, S.L., Yu, S., de Oliveira, T., Fitch, W.M. & McGrath, M.S. (2005). Phylodynamic analysis of Human Immunodeficiency Virus Type 1 in distinct brain compartments provides a model for the neuropathogenesis of AIDS. *J. Virol* **79**(17): 11343-11352. **Example publication using phylogeny-trait association.**

Slatkin, M., & Maddison, W.P. (1989). A cladistic measure of gene flow measured from the phylogenies of alleles. *Genetics* **123**(3):603-613. **The Slatkin-Maddison implementation of the Fitch algorithm to detect phylogeny-trait association ('PS' statistic)**

Wang, T.H., Donaldson, Y.K., Brettle, R.P., Bell, J.E. & Simmonds, P. (2001). Identification of shared populations of Human immunodeficiency Virus Type 1 infecting microglia and tissue macrophages outside the central nervous system. *J. Virol.* **75** (23): 11686-11699. **The 'AI' statistic.**