

UC Davis

UC Davis Previously Published Works

Title

Species Tree Inference with BPP Using Genomic Sequences and the Multispecies Coalescent

Permalink

<https://escholarship.org/uc/item/1ht060bz>

Journal

Molecular Biology and Evolution, 35(10)

ISSN

0737-4038

Authors

Flouri, Tomáš

Jiao, Xiyun

Rannala, Bruce

et al.

Publication Date

2018-10-01

DOI

10.1093/molbev/msy147

Copyright Information

This work is made available under the terms of a Creative Commons Attribution License, available at <https://creativecommons.org/licenses/by/4.0/>

Peer reviewed

Species Tree Inference with BPP Using Genomic Sequences and the Multispecies Coalescent

Tomáš Flouri,¹ Xiyun Jiao,¹ Bruce Rannala,^{*,2} and Ziheng Yang^{*,1}

¹Department of Genetics, Evolution and Environment, University College London, London, United Kingdom

²Department of Ecology and Evolution, University of California, Davis, CA

*Corresponding authors: E-mails: brannala@ucdavis.edu; z.yang@ucl.ac.uk.

Associate editor: Anne D. Yoder

Abstract

The multispecies coalescent provides a natural framework for accommodating ancestral genetic polymorphism and coalescent processes that can cause different genomic regions to have different genealogical histories. The Bayesian program BPP includes a full-likelihood implementation of the multispecies coalescent, using transmodel Markov chain Monte Carlo to calculate the posterior probabilities of different species trees. BPP is suitable for analyzing multilocus sequence data sets and it accommodates the heterogeneity of gene trees (both the topology and branch lengths) among loci and gene tree uncertainties due to limited phylogenetic information at each locus. Here, we provide a practical guide to the use of BPP in species tree estimation. BPP is a command-line program that runs on LINUX, MACOSX, and WINDOWS. This protocol shows how to use both BPP 3.4 (<http://abacus.gene.ucl.ac.uk/software/>) and BPP 4.0 (<https://github.com/bpp/>).

Key words: BPP, MCMC, multispecies coalescent, species tree inference.

Introduction

In the past decade, it has become evident that different genes or genomic regions may have different evolutionary histories (gene trees), due to several important biological processes, including the coalescent process in ancestral species, gene duplication, and horizontal gene transfer (introgression) (Maddison 1997; Nichols 2001; Edwards 2009). Gene tree heterogeneity due to the coalescent process is universal as it is the natural consequence of polymorphism and genetic drift in ancestral species, or the stochastic nature of the coalescent process. However, the magnitude of the differences depends on population genetic parameters. Conflicts between gene trees and the species tree caused by the coalescent processes in ancestral species are often referred to as incomplete lineage sorting (ILS). ILS is most prominent if the species arose in a rapid succession of speciation events (a species radiation), creating short internal branches on the species tree, and if the ancestral species had large population sizes. For such challenging phylogenetic problems, traditional phylogenetic methods, which concatenate the sequences across loci and infer a common phylogeny using maximum likelihood or Bayesian inference, may be statistically inconsistent and converge to an incorrect phylogeny when the number of loci increases (Kubatko and Degnan 2007; Edwards et al. 2016). Similarly conducting a separate phylogenetic analysis at each locus and using the most common gene tree as the species tree estimate can also be inconsistent (Degnan and Rosenberg 2006, 2009).

The multispecies coalescent (MSC) has emerged as the natural framework to account for genealogical

heterogeneity across the autosomal genome due to ILS (Rannala and Yang 2003; Edwards 2009; Xu and Yang 2016). The MSC lies at the interface of population genetics and molecular phylogenetics (Rannala and Yang 2003; see also Takahata et al. 1995; Yang 2002). It differs from models of population structure and subdivision in population genetics in that it accounts for the history of species/population divergences. It differs from traditional phylogenetic models in that it accounts for the coalescent process and the resulting genealogical heterogeneity across the genome. Because it accounts for the coalescent process in both extant and extinct ancestral species, the MSC naturally accommodates ILS (fig. 1).

The basic MSC model for a species tree of s species involves two types of parameters: $s-1$ species divergence times (τ s) and up to $2s-1$ population size parameters for the populations on the species tree (θ s) (fig. 1). Both τ and θ parameters are measured by the sequence distance or the expected number of mutations/substitutions per site. The parameter $\theta = 4N\mu$ is the average distance between two sequences sampled at random from a population with effective population size N , where μ is the mutation rate per site per generation. For the example, for the human species, $\theta \approx 0.0006$, meaning that two genomic sequences from the species have on average 0.6 differences per kb. The parameter τ is the age of an internal node (species divergence event) in the species tree, measured in units of expected number of mutations per site. For example, in the species tree, ((A, B), C), there are two species divergence times (τ_{AB} and τ_{ABC}) and five population sizes (θ_A , θ_B , θ_C , θ_{AB} , and θ_{ABC}). Note that at least two sequences are needed to calculate a distance (or to estimate θ); if only

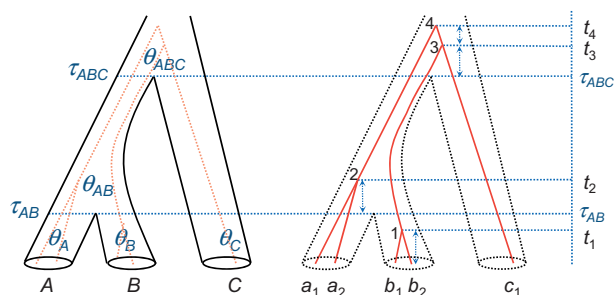


Fig. 1. A species tree for three species (A, B, and C) to illustrate the parameters of the MSC model, with a gene tree for five sequences (a_1 and a_2 sampled from species A, b_1 and b_2 from species B, and c_1 from species C) running inside the species tree. Within each species/population, sequences coalesce at random at the rate determined by the population size (or θ parameter), generating a gene tree with branch lengths (coalescent times), conditioned on the species tree. Note that θ_C is not estimable if there is at most one sequence from species C at each locus.

one sequence is available for an extant (contemporary) species at every locus, θ for that species cannot be estimated.

Two kinds of methods are often used to estimate the species tree under the MSC: the summary and full-likelihood methods (Edwards et al. 2016; Xu and Yang 2016). The summary methods typically have two steps: 1) estimating the gene tree at each locus using phylogenetic methods and 2) treating the estimated gene trees as observed data to infer the species tree. They tend to have reduced statistical efficiency but are computationally fast. The full likelihood methods operate on sequence alignments and have the strength of accommodating uncertainties in gene trees. By combining information across many loci, those methods can produce a confident and reliable species tree estimate even if there are few variable or informative sites at each locus so that the information at every locus is weak (Xu and Yang 2016; Shi and Yang 2018).

In this protocol, we assume some familiarity with Bayesian MCMC and molecular phylogenetics, or experience with phylogenetic programs such as MrBayes. For an introduction to Bayesian MCMC algorithms, see Nascimento et al. (2017). For detailed reviews of the MSC model and Bayesian inference under the MSC, see Yang (2014: Chapter 9), Rannala (2015), Edwards et al. (2016), Mallo and Posada (2016), and Xu and Yang (2016).

Species Tree Inference Using BPP

The BPP implementation of the MSC model uses the Bayesian model-selection framework to evaluate different models of species delimitations and species phylogenies. The basic assumptions include the following: 1) no recombination within a locus, 2) free recombination between loci, 3) no migration (gene flow) between species, 4) neutral evolution, and 5) clock-like evolution. These assumptions suggest that certain properties are desirable for data sets to be analyzed using BPP. To satisfy assumption i short genomic segments (e.g., 500 to 1,000 bp)

should be used (called loci); this insures that recombination within a locus is rare. To satisfy assumption ii the different loci should be physically distant from one another in the genome; this insures that recombination between them is common, allowing the loci to have approximately independent histories. To satisfy assumption iii the populations for analysis should not be experiencing significant ongoing gene flow; this assumption can be tested using preliminary population genetic analyses (Reich et al. 2010; Dalquen et al. 2017). To satisfy assumption iv, the loci should be evolving neutrally, implying that their gene trees are not affected to a significant extent by natural selection. Despite this assumption, protein-coding genes appear to be useable for BPP analysis even if they show obvious evidence of purifying selection. Most proteins perform similar functions in closely related species and the main effect of purifying selection on nonsynonymous mutations is a reduction of the neutral mutation rate for the locus. Studies comparing species trees inferred using exons and using introns or noncoding DNA gave highly consistent results between the two kinds of data (Shi and Yang 2018). Nevertheless, it is prudent to analyze noncoding and coding regions of the genome as separate data sets. Assumption v arises because BPP currently uses the JC69 model (Jukes and Cantor 1969) to correct for multiple mutations/substitutions at a site with a constant rate over time (the molecular clock). The program is thus suitable for analyzing closely related species with sequence divergences below $\sim 10\%$.

BPP is a Bayesian Markov chain Monte Carlo (MCMC) program for analyzing multilocus sequence data under the MSC. It can be used for four kinds of inference problems or analyses (Yang 2015):

- A00 (specified by `speciesdelimitation = 0` and `speciestree = 0`): estimation of parameters under the MSC (including species divergence times and population sizes) on a fixed species phylogeny (Yang 2002; Rannala and Yang 2003; Burgess and Yang 2008);
- A01 (`speciesdelimitation = 0` and `speciestree = 1`): estimation of species phylogeny when species assignment and delimitation is given (Yang and Rannala 2014; Rannala and Yang 2017);
- A10 (`speciesdelimitation = 1` and `speciestree = 0`): comparison of species delimitation models induced on a given “guide” tree (Yang and Rannala 2010; Rannala and Yang 2013);
- A11 (`speciesdelimitation = 1` and `speciestree = 1`): joint comparison of species delimitation/assignment and species tree models (Yang and Rannala 2014; Rannala and Yang 2017).

In this protocol, we focus on analysis A01: species tree estimation.

Protocol

Here, we describe the procedure for installing or compiling and running BPP from the command line in either Linux,

Windows, or Mac OSX. If you have not used the command line before, please work through one of the following short tutorials first:
<http://abacus.gene.ucl.ac.uk/software/CommandLine.Windows.pdf>
<http://abacus.gene.ucl.ac.uk/software/CommandLine.MACosx.pdf>

Obtaining and Compiling the BPP Program

The protocols presented here use both BPP versions 3.4 and 4.0. Both programs are written in the C language and can be compiled to run on LINUX, MacOSX, and WINDOWS. BPP 3.4 is available at <http://abacus.gene.ucl.ac.uk/software/>, and the manual (BPPDOC.pdf) is included in the release, which details the format of the data files, the screen output, as well as the interpretation of the output. BPP 4 is a highly optimized reimplementation, available at <https://github.com/bpp/>. On-line documentation is available for BPP 4 at <https://github.com/bpp/wiki>. Precompiled WINDOWS and MAC OS X executables are available for BPP 3.4 and 4 from their respective websites. For LINUX you may need to compile the programs yourself to generate the executable file `bpp` (optionally you can manually compile the program executable for Mac OS X as well if you have the X Code compiler system installed).

The program compilation needs to be done only once. Here, it is assumed that you have uncompressed the distribution file into a subdirectory `bpp`. On Linux, for example, the following commands use the `gcc` compiler to compile the program and move the generated executable file (`bpp`) into the `bin/` folder.

BPP3.4	BPP4.0
cd bpp	cd bpp
mkdir bin	mkdir bin
cd src	cd src
gcc -o bpp -O3 bpp.c tools.c -lm	make
mv bpp ../bin	mv bpp ../bin

Running BPP

BPP takes three input data files: 1) a control file that specifies the model and the priors and effectively “drives” the analysis (fig. 2), 2) a sequence alignment file that contains the sequence data for all loci, and 3) an individual-to-population map file (Imap file) that assigns each individual to a population (fig. 3). All those are plain text files and can be prepared using any text editor. Here, we will use a data set of five nuclear loci from the East Asia brown frogs (Zhou et al. 2012), previously analyzed by Yang (2015). The three input files are in the folder `frogs` in the release. We will run each analysis twice in two folders, `frogs/r1/` and `frogs/r2/`. Start two command-line terminals. Then change directory to `r1` (or `r2`), and run the program as follows.

On Windows	On Linux/Unix/Mac OSX
To run BPP3.4	
cd frogs\r1	cd frogs/r1
..\..\bin\bpp	../bin/bpp
..\A01.bppctl	../A01.bppctl
To run BPP4.0	
cd frogs\r1	cd frogs/r1
..\..\bin\bpp -cfile=	../bin/bpp -cfile=
..\A01.bppctl	../A01.bppctl

Here, `A01.bppctl` is the control file for analysis A01 (fig. 2). The input data file names are specified in the control file. Note that in the control file, the data file is specified as `./frogs.txt` instead of `frogs.txt`, because the file is in the `frogs` folder while we run BPP in the `frogs/r1/` folder.

The run will produce an MCMC sample file (`mcmc.txt`), which is read and summarized by BPP to produce the output file (`out.txt`). Consistency across runs is an important indicator of MCMC convergence so we recommend running the same analysis multiple times. If the multiple runs produce similar posterior, MCMC samples from the multiple runs may be merged and then summarized: Append one MCMC sample file to the end of another (and remove the header line of the second file in the case of A00 analysis). Then run BPP with `print = -1`.

The Imap File

The Imap file assigns individuals or sequences to the populations or species. In the sequence data file, each sequence name has a tag (following the caret ‘’) which is interpreted as an individual ID and used in the Imap file to assign the sequence to a population (fig. 3). After this information is read, BPP uses the population IDs and ignores the individual IDs. In theory it would be sufficient to tag each sequence by its population ID without the need for the Imap file. The current two-layer design makes it easy to change the assignments, which involves editing the small Imap file without altering the much larger sequence file.

The Sequence Alignment File

Sequence alignments for multiple loci are in the PHYLIP/PAML format (fig. 3), with one alignment following the other, all in one file. The number of loci (or alignments) is specified by the variable `nloci` in the control file: BPP reads the specified number of loci and ignores the rest of the file.

The Control File

The variables in the control file (fig. 2) are described in detail in the program documentation. Here, we focus on those important to this protocol.

Analysis A01 (species tree estimation) is triggered by using `speciesdelimitation = 0`, `speciestree = 1`. BPP uses the subtree pruning and regrafting (SPR) and NodeSlider algorithms to change the species tree topology in the MCMC (Yang and Rannala 2014; Rannala and Yang


```

seed = -1
seqfile = ../frogs.txt
Imapfile = ../frogs.Imap.txt
outfile = out.txt
mcmcfile = mcmc.txt

speciesdelimitation = 0      # fixed species delimitation
speciestree = 1 0 0 0      # estimate species tree
speciesmodelprior = 1      # 0:uniform LH; 1:uniform rooted trees; 2:uniformSLH
3:uniformSRooted

species&tree = 4   K   C   L   H      # number of species and list of species labels
                  9   7 14   2      # max number of sequences from each species at a loci
                  ((K, C), (L, H));   # initial species phylogeny
diploid =         1   1   1   1      # 0: phased sequences; 1: diploid unphased sequences.

* checkpoint = 0          * 0: nothing; 1: save; 2: read
  usedata = 1             # 0: no data (prior); 1: seq like
  nloci = 5               # number of data sets to read in seqfile
  cleandata = 0           # remove sites with ambiguity data (1: yes, 0: no)

thetaprior = 3 0.002 e    # invgamma(a,b) for theta parameters
tauprior = 3 0.004        # invgamma(a,b) for root tau

* heredity = 1 4 4        # (0: no variation, 1: estimate, 2: from file)
* locusrate = 0 2.0       # (0: no variation, 1: estimate, 2: from file)

finetune = 1: .01 .01 .01 .01 .01 .01 .01 .01 # auto (0 or 1): MCMC step lengths

print = 1 0 0 0          # print MCMC samples, locusrate, heredity scalars, gene trees
burnin = 8000             # burn-In
sampfreq = 2              # frequency of sampling (sample every second MCMC iteration)
nsample = 100000          # total number of samples to log

```

Fig. 2. Sample control file A01 .bpp .ctl for species tree estimation (with speciesdelimitation = 0 and speciestree = 1). Lines starting with an asterisk are comments and the default values of speciesdelimitation and speciestree are 0.

frogs.txt		frogs.Imap.txt	
21 489			
^6	GGAGCCAACAGAGTTTAACGTTCTGTT		
^c66	GGAGCCAACAGAGTTTAACATTCTGTT		
^rd1	GGAGCCAACAGAGTTTAACGTTCTGTT		
^hn24	GGAGCCAACAGAGTTTAACGTTCTGTT		
^gs250	GGAGCCAACAGAGTTTAACGTTCTGTT		
^kiz1375	GGAGCCAAC	kiz1375	C
^kiz2305	GGAGCCAAC	kiz2305	C
^kiz2321	GGAGCCAAC	kiz2321	C
^kiz2405	GGAGCCAAC	kiz2405	C
^bj64	GGAGCCAAC	bj64	L
^d14	GGAGCCAAC	d14	L
^wlht2	GGAGCCAAC	wlht2	L
^ypx2767	GGAGCCAAC	ypx2767	L
^ypx2732	GGAGCCAAC	ypx2732	L
^ypx3876	GGAGCCAAC		
^ypx4225	GGAGCCAAC		
^gs49	GGAGCCAAC		
^gs132	GGAGCCAAC		
^nx4	GGAGCCAAC		
^ypx3462	GGAGCCAAC		
^ypx3893	GGAGCCAAC		

Fig. 3. The sequence data file (frogs.txt) and the Imap file (frogs.Imap.txt). In the sequence file each sequence is tagged with an individual/specimen ID (such as kiz1375, for Kunming Institute of Zoology #1375). The part of the sequence name before the caret (^) is read and then ignored. In the Imap file each individual is assigned to a species/population: for example, individual kiz1375 is assigned to species C.

2017), with the species tree in the control file used as the starting species tree. The posterior distribution should be independent of the starting species tree. If the posterior results look too different between runs that started from different starting species trees, we need to rerun the program using a larger number of samples (nsample) and/or larger sampling frequency (sampfreq).

The diploid variable indicates whether the sequences from each species are phased (0) or unphased (1). If this line is missing, all sequences in the sequence data file are assumed to be phased. If the indicator is 1 for a species, all sequences from that species are assumed to be diploid unphased data. BPP does not allow some sequences from a species to be phased while others from the same species to be unphased. In an unphased sequence, a heterozygote site is represented by using the ambiguity characters Y, R, M, K, S, and W. For example, a Y in an unphased sequence means a T/C heterozygote, but in a phased sequence, it means an ambiguity state that is either T or C. BPP handles unphased sequences by analytically integrating over different phase resolutions of the heterozygote sites in the likelihood calculation, using the approach of Gronau et al. (2011). This works for a small number of sequences per locus and may not be computationally feasible when there are many sequences at each locus.

In species tree estimation (A01 analysis), one has to specify a prior distribution for the different species tree topologies and also priors for parameters (θ s and τ s) in each species tree. Two priors for species trees are available in BPP. Prior 0 (speciesmodelprior = 0) assigns equal probabilities for the labeled histories (which are rooted trees with the internal nodes ordered by relative age), while Prior 1 (speciesmodelprior = 1) assigns equal probabilities for the

rooted trees (Yang and Rannala 2014). For instance, there are 15 rooted trees in the case of four species (A, B, C, and D), with 12 unbalanced and 3 balanced trees. Each unbalanced tree, for example, (((A, B), C), D), is compatible with only one labeled history as there is only one ordering of the internal nodes. Each balanced tree, for example, ((A, B), (C, D)), is compatible with two labeled histories, depending on whether the ancestor of A and B is older or younger than the ancestor of C and D. Prior 0 assigns the probability 1/18 to each of the unbalanced trees and 2/18 to each of the balanced trees. Prior 1 assigns the probability 1/15 to each of the 15 rooted trees. We use Prior 1, which is the default.

Within each species tree model, we assign the inverse-gamma priors $\theta \sim \text{IG}(3, 0.002)$ for all θ s and $\tau \sim \text{IG}(3, 0.004)$ for the age of the root (τ_0). The inverse-gamma $\text{IG}(\alpha, \beta)$ has mean $m = \beta/(\alpha-1)$ if $\alpha > 1$ and variance $s^2 = \beta^2/[(\alpha-1)^2(\alpha-2)]$ if $\alpha > 2$. If little information is available about the parameters, you can use $\alpha = 3$ for a diffuse prior and then adjust β so that the mean looks reasonable. For example, parameter θ measures the genetic diversity (heterozygosity) in the species. This varies among species, with 0.01 (one difference per 100 bp) to be a large value while 0.001 a small one. Parameter τ_0 measures the age of the root in the rooted species tree and depends on the species included in the data set. Thus including an outgroup species will typically mean that a larger prior mean for τ_0 is appropriate.

It is useful to plot the inverse-gamma density and calculate the 95% prior interval. The R functions for doing this are in the MCMCpack, so install the package first:

```
install.packages("invgamma");
```

Then

```
library("invgamma");
a=3; b=0.002;
curve(dinvgamma(x, a, b), from=0,
to=0.01)
qinvgamma(c(0.025, 0.975), a, b)
```

BPP collects the species trees (as well as parameters θ s and τ s) into the sample file `mcmc.txt`. At the end of the MCMC run, it summarizes the MCMC sample to produce the output file `out.txt` (fig. 4). This is self-explanatory.

Both BPP 3.4 and 4.0 implement an option of integrating out analytically the θ parameters, using conjugate inverse-gamma priors (Hey and Nielsen 2007). This reduces the state of the Markov chain, resulting in slight improvement in the mixing properties of cross-model MCMC algorithms. It is advisable to use this option in species tree estimation (the A01 analysis). The option can be turned off so that the posteriors for θ s are generated in the A00 analysis to estimate parameters on a fixed species tree, such as the maximum posterior probability (MAP) species tree.

The Example Data Set from East Asian Brown Frogs

We use as our example the five nuclear loci from East Asian brown frogs in the *Rana chensinensis* species complex (Zhou et al. 2012). There are 21–30 sequences per locus, and the

sequences are 285–489 bp long. Three morphologically recognized species exist in this group: *R. chensinensis* (clades C and L), *R. kukunoris* (K), and *R. huanrensis* (H), but *R. chensinensis* has a widespread distribution, with two populations (C and L) recognized. The sequences are unphased but were treated (incorrectly) as phased in the tutorial of Yang (2015). Here, we will use the diploid option in BPP 3.4 and 4.0 to reanalyze those data. The three input files are in the folder `frogs`. We will run each analysis twice in two folders, `frogs/r1/` and `frogs/r2/`. Start two command-line terminals. Then change directory to `r1` (or `r2`), and run the program as follows.

First, we run the A01 analysis to estimate the species tree, with the θ parameters integrated out analytically (using the control file `A01.bpp.ctl` with `thetaprior = 3 0.002` without the “e”). The MAP species tree, which is the (binary) species tree that has the maximum posterior probability (or has been visited most frequently by the MCMC algorithm), is shown in figure 5: (((LH)C)K). The MAP tree has only 32% posterior probability, indicating that the five loci have only weak phylogenetic information. The tree differs from the species tree inferred by Yang (2015, fig. 2): ((KC)(LH)), although the support is weak in both analyses. The majority-rule consensus species tree, which shows only splits found in at least half of the sampled species trees, is a star tree (fig. 4). Note that if the consensus species tree is binary, it must be the MAP tree, but otherwise the two are different.

Second, we run the A00 analysis with the species tree fixed at the MAP tree to estimate the parameters (τ s and θ s) of the MSC model (using the control file `A00.bpp.ctl` with `thetaprior = 3 0.002 e`, with the “e”). The posterior means are shown in figure 5. It is noteworthy that the diploid option affects parameter estimates. In particular, the estimates of θ are greater now, because in the analysis of Yang (2015), heterozygous nucleotides in the unphased sequences were incorrectly treated as ambiguity characters.

We changed the inverse gamma priors for θ s and τ_0 to evaluate their impact on the posterior probabilities for species trees. We used the shape parameter $\alpha = 3$ in the inverse-gamma as a diffuse prior, and $\alpha = 21$ as an informative prior, and then varied the prior mean by two orders of magnitude. Note that the mean of the inverse gamma $\text{IG}(\alpha, \beta)$ is $\beta/(\alpha-1)$ and the coefficient of variation is $\sqrt{1/(\alpha-2)}$, with $\alpha > 2$. The results are summarized in table 1. As the data set with five loci is small, the prior had substantial impact on the posterior. Having very large θ s in the prior tends to reduce posterior probabilities for the MAP tree, apparently because large θ s allow the poor species trees to “explain” the data easily by attributing the conflicting gene trees to random fluctuations of the coalescent process in the ancestors. Having a very small τ_0 in the prior tends to reduce posterior probabilities for the MAP tree as well, possibly because a small τ_0 causes the different species trees to look similar, pushing all coalescent events into the root species.

```

Initial MSC density and log-likelihood of observing data:
log-P0 = 1693.495406   log-L0 = -7224.742900

-3% 0.64 0.12 0.00 0.15 0.14 0.0945 0.0021 1173.83 -4441.6634
Current Pjump:      0.64363 0.12239 0.00000 0.14533 0.13900
Current finetune:   5.00000 0.00100 0.00100 0.00100 0.30000
New finetune:       15.65957 0.00038 0.00001 0.00046 0.13064

-2% 0.65 0.20 0.00 0.21 0.47 0.0455 0.0020 1130.79 -4435.1621
Current Pjump:      0.64574 0.20115 0.00000 0.20817 0.47400
Current finetune:   15.65957 0.00038 0.00001 0.00046 0.13064
New finetune:       49.40811 0.00025 0.00000 0.00030 0.23626

-1% 0.64 0.23 0.00 0.25 0.20 0.0255 0.0021 1170.83 -4430.6203
Current Pjump:      0.64186 0.23210 0.00000 0.25233 0.19950
Current finetune:   49.40811 0.00025 0.00000 0.00030 0.23626
New finetune:       99.00000 0.00018 0.00000 0.00025 0.15026

0% 0.64 0.24 0.00 0.25 0.41 0.0195 0.0018 1158.59 -4417.9870
Current Pjump:      0.64404 0.24375 0.00000 0.24667 0.41000
Current finetune:   99.00000 0.00018 0.00000 0.00025 0.15026
New finetune:       99.00000 0.00015 0.00000 0.00020 0.22142

5% 0.64 0.26 0.00 0.27 0.23 0.0330 0.0019 1143.31 -4423.5558
10% 0.65 0.26 0.00 0.27 0.23 0.0427 0.0019 1106.92 -4422.6892
15% 0.65 0.26 0.00 0.26 0.23 0.0423 0.0019 1141.57 -4423.4965
...
95% 0.64 0.26 0.00 0.26 0.24 0.0535 0.0020 1195.79 -4427.2378
100% 0.64 0.26 0.00 0.26 0.24 0.0534 0.0020 1147.06 -4427.3735

Species in order:
1. K
2. C
3. L
4. H

(A) Best trees in the sample (15 distinct trees in all)
33268 0.33268 0.33268 ((C, (H, L)), K);
24492 0.24492 0.57759 (C, ((H, L), K));
16502 0.16502 0.74261 ((C, K), (H, L));
...

(B) Best splits in the sample of trees (10 splits in all)
74262 0.742613 0011
40556 0.405556 0111
32517 0.325167 1011
19549 0.195488 1100
...

(C) Majority-rule consensus tree
(K, C, (L, H) #0.742613);

(D) Best tree (or trees from the mastertree file) with support values
((C, (H, L) #0.742613) #0.405556, K); [P = 0.332677]

```

FIG. 4. Output from BPP for analysis A01 (species tree estimation). The progress indicator is negative during the burnin, and BPP goes through four rounds of automatic step-length adjustments, aiming to achieve a near-optimal acceptance proportion of 30% for the parameter-moves (Yang and Rodríguez 2013). Sampling (in `mcmc.txt`) starts after the burn-in is over. At the end of the MCMC run, the sample is processed to calculate the posterior probabilities of the species trees (section A), which are further summarized to calculate the posterior for splits as well as the majority-rule consensus tree.

Discussion

The Choice of Sequence Loci

The ideal data for analyses using BPP are loosely linked short genomic segments (referred to as “loci”) (Takahata et al. 1995; Burgess and Yang 2008; Shi and Yang 2018). A major biological factor that the MSC model accommodates is the genealogical heterogeneity across genome: that is, different regions

of the autosomal genome have different gene tree topologies and branch lengths (coalescent times). While the mutational rate may also vary along the genome, this variation is expected to have much less impact on the species tree inference, especially when the species are close and the sequences are similar. Thus multiple genes from the mitochondrial (or chloroplast) genome should be treated as one “locus” in the MSC-based analysis. Typically the mitochondrial genome has

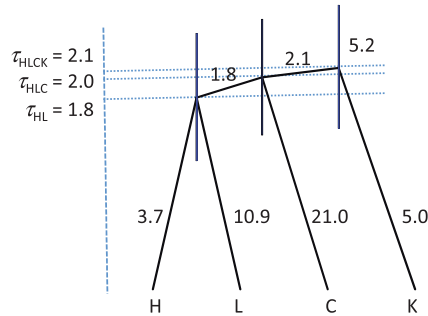


Fig. 5. The MAP species tree for four brown frog species/populations, showing the parameter estimates ($\times 10^{-3}$). The branch lengths represent the posterior means of divergence times (τ s), while the node bars show the 95% HPD intervals. Posterior means of θ s are shown along the branches. The priors used in the analysis are $\theta \sim \text{IG}(3, 0.002)$ for all populations and $\tau_{\text{KCLH}} \sim \text{IG}(3, 0.004)$ for the root age. The tree is drawn with FigTree using the BPP output FigTree.tre.

Table 1. Posterior Probability for Clades in the MAP Tree under Different Priors for the Frogs Dataset

Prior		(HL)	(HLC)	Tree	P_{jump}
$\theta \sim \text{IG}(3, 0.0002)$	$\tau_0 \sim \text{IG}(3, 0.004)$	0.69	0.35	0.28	6.6%
$\theta \sim \text{IG}(3, 0.002)$	$\tau_0 \sim \text{IG}(3, 0.004)$	0.72	0.38	0.32	5.6%
$\theta \sim \text{IG}(3, 0.02)$	$\tau_0 \sim \text{IG}(3, 0.004)$	0.45	0.30	0.19	10.0%
$\theta \sim \text{IG}(21, 0.002)$	$\tau_0 \sim \text{IG}(3, 0.004)$	0.41	0.34	0.20	12.3%
$\theta \sim \text{IG}(21, 0.02)$	$\tau_0 \sim \text{IG}(3, 0.004)$	0.47	0.36	0.24	10.0%
$\theta \sim \text{IG}(21, 0.2)$	$\tau_0 \sim \text{IG}(3, 0.004)$	0.39	0.35	0.19	14.1%
$\theta \sim \text{IG}(3, 0.002)$	$\tau_0 \sim \text{IG}(3, 0.0004)$	0.69	0.33	0.28	6.3%
$\theta \sim \text{IG}(3, 0.002)$	$\tau_0 \sim \text{IG}(3, 0.004)$	0.72	0.38	0.32	5.6%
$\theta \sim \text{IG}(3, 0.002)$	$\tau_0 \sim \text{IG}(3, 0.04)$	0.69	0.53	0.44	4.6%
$\theta \sim \text{IG}(3, 0.002)$	$\tau_0 \sim \text{IG}(21, 0.004)$	0.70	0.41	0.30	7.5%
$\theta \sim \text{IG}(3, 0.002)$	$\tau_0 \sim \text{IG}(21, 0.04)$	0.69	0.34	0.29	6.2%
$\theta \sim \text{IG}(3, 0.002)$	$\tau_0 \sim \text{IG}(21, 0.4)$	0.87	1.00	0.87	0.7%

Note.—The MAP tree is (((HL)C)K), shown in figure 5. P_{jump} is the acceptance proportion of the SPR move across species trees recorded during the MCMC run. The same analysis was run three times (with burnin = 8000, sampfreq = 2 and nsample = 10^5) to check for consistency between runs, and the combined sample was then summarized. There were mixing problems for the last analysis, with priors $\theta \sim \text{IG}(3, 0.002)$ and $\tau_0 \sim \text{IG}(21, 0.4)$, in which case we used burnin = 4×10^4 and nsample = 5×10^5 .

a different mutation rate and different effective population size from the autosomal genome. BPP has a control variable (locusrate) for rate variation among loci and another variable (heredity) for different heredity scalars for population sizes (Burgess and Yang 2008), so that in theory the program allows joint analysis of autosomal and mitochondrial loci in one data set. However, certain biological processes such as introgression and selection may affect the autosomal and mitochondrial genomes differently, and it may be useful to analyze the autosomal and mitochondrial loci as separate data sets to examine possible heterogeneity in the species tree.

The MSC model assumes that the sequences from the different species are random samples. Thus all sequences from a particular species (or a random sample of them) should be used: for example, it is not advisable to use only the distinct haplotypes because removal of the identical sequences leads to underestimation of θ s. Similarly one

should not filter loci based on bootstrap support values; using only loci with high phylogenetic information content will bias estimates.

The Limits of BPP

The question is often asked what are the limits on the size of data sets that can be analyzed by BPP? The short answer is that many modern data sets are too large to be simultaneously analyzed using BPP in a reasonable time period (hours or days). The computational demands increase with an increase in either the number of species, the number of loci, the number of sequences per locus, or the number of sites per sequence. Increasing the number of species increases both the number of possible species trees and the number of parameters on each species tree so that the parameter space becomes much larger. As in conventional phylogenetic inference the number of rooted species trees increases explosively with the number of species (s). The number of parameters on each species tree ($s-1 + 2s-1 = 3s-2$) increases linearly with s . Increasing the number of loci should also have a near linear effect on the computation for each MCMC iteration. However a compounding factor is that with more loci, the posterior for the parameters (θ s and τ s) in each species tree become highly concentrated, making it more difficult for the algorithm to move from one species tree to another; this can create mixing problems. Increasing the number of sequences adds to the size of the gene trees, as well as the number of variables (such as coalescent times) to update during the MCMC. The number of sites per sequence has the least impact in terms of computational expense.

Finally the type of analysis also matters. The A00 analysis generates the posterior distribution of the parameters (θ s and τ s) when the species tree and the MSC model is fixed. The MCMC algorithm implemented in the BPP program for this inference has been successfully applied to genomic data sets of >50,000 loci (Burgess and Yang 2008). The other three analyses (A01, A10, and A11), including analysis A01 discussed in this protocol, are transmodel inferences (in the terminology of Green 2003), in which the Markov chain moves between different models, each of which is an instance of the MSC model, although the number of species and the species phylogeny may differ among the models. The main objective of the transmodel inference is the calculation of posterior model probabilities. Poor mixing is a common problem with transmodel algorithms.

For species tree estimation (the A01 analysis), BPP has been used to analyze data sets of 919 loci (19 sequences per locus, median length 706 bp) from Philippine shrews (genus *Crocodyra*) (Giarla and Esselstyn 2015; Rannala and Yang 2017) and data sets of ~10,000 loci (17 sequences per locus, 200 or 1,000 bp) from five species of gibbons (Carbone et al. 2014; Shi and Yang 2018). However, the algorithm was noted to have mixing problems in analysis of such large data sets.

Mixing Problems and MCMC Diagnosis

Mixing problems in MCMC affect the efficiency, rather than the correctness, of the algorithm. A correct MCMC algorithm should visit the different models in proportion to their

posterior probabilities. An efficient algorithm should jump between models frequently while an inefficient (lazy) algorithm may stay in one model for a long time before it jumps, and then remains in the new model for a long time before it jumps again. Both algorithms are correct in the sense that in the long run they both visit the models in proportion to their posterior probabilities. However, the lazy algorithm may be very inefficient as it takes an extremely long chain to generate reliable results because of the infrequent model jumps. The main symptom for poor mixing of the transmodel algorithm is that the chain gets stuck in one model (or a subset of models), and multiple runs (each over a finite but large number of iterations) produce different results. Mixing problems tend to be worse and occur more frequently for larger data sets but can occur even for small data sets.

Note that many of the standard MCMC diagnosis tools may not be very useful for transmodel algorithms. From our experience, comparing the results of multiple runs using different starting species trees may be the most effective way of ensuring the reliability of the results in transmodel algorithms. There are no hard rules for deciding how large a difference between runs is too large as this depends on the computing resources and the absolute running time, but accuracy at the percentage point seems desirable. Note that the variance of the estimate of the posterior model probability based on an MCMC sample of size N is $P(1-P)/(NE)$, where P is the true posterior model probability, E is the efficiency of the MCMC sample, and NE is the effective sample size (ESS) (see, e.g., Yang 2014, p. 214). Thus to reduce the SE of the estimate by one half, one needs a 4-fold increase in the MCMC sample size.

Summary and Perspectives

The multispecies coalescent provides a natural framework for accommodating incomplete lineage sorting and phylogenetic uncertainties at individual loci. It makes an efficient use of the information in the genomic sequence data and provides a powerful methodology for resolving challenging species phylogenies characterized by extremely short internal branches and large ancestral population sizes (Shi and Yang 2018). While easy species trees can be recovered using any methods including concatenation (although even in such cases concatenation creates biased estimates of divergence times and population sizes, Ogilvie et al. 2016), likelihood-based MSC methods have an advantage for difficult species trees generated during radiative speciation events. MSC methods are consistent (they will converge to the true species tree when the number of loci increases) and have higher efficiency (they recover the correct species tree with greater probability) than concatenation or summary methods (Ogilvie et al. 2016; Xu and Yang 2016; Shi and Yang 2018).

At the time of writing, the two versions of BPP (3.4 and 4.0) have nearly identical functionalities. Both implement the four different analyses described in Yang (2015): A00, A01, A10, and A11. Both include options for handling diploid sequences by analytically integrating out the different phase resolutions (Gronau et al. 2011), and for calculating the marginal likelihood (Bayes Factors) through thermodynamic integration

and Gaussian quadrature (Lartillot and Philippe 2006; Rannala and Yang 2017). Both include check-pointing, which may be used to restart an aborted run. BPP4.0 has a computational advantage over 3.4: depending on the data and model, the speed difference can be several-fold. Much of this improvement results from savings on repeated calculations in the computation of the gene tree density (Rannala and Yang 2003). Both versions avoid repeated calculations of conditional probabilities in the computation of the sequence likelihood, but BPP3.4 does not implement similar savings on the gene tree density. More importantly, the redesign and reimplementing of the algorithms in BPP4.0 makes it easier for parallelization. We expect future improvements will be mostly made in BPP4.0.

Currently efforts are made to improve the MCMC algorithms for better mixing efficiency and to parallelize the code to improve the computational efficiency. Work is also under way to extend the JC69 mutation/substitution model to GTR+G (Yang 1994a, 1994b) and to relax the molecular clock (Xu and Yang 2016), so that the program can be used to estimate the species tree for distantly related species. The user is advised to check the program web site for future updates.

Acknowledgments

This study is supported by a grant from the Biotechnological and Biological Sciences Research Council (BB/P006493/1) to Z.Y. We thank Patrick Goring and two anonymous reviewers for comments.

References

- Burgess R, Yang Z. 2008. Estimation of hominoid ancestral population sizes under Bayesian coalescent models incorporating mutation rate variation and sequencing errors. *Mol Biol Evol.* 25(9):1979–1994.
- Carbone L, Harris RA, Gnerre S, Veeramah KR, Lorente-Galdos B, Huddleston J, Meyer TJ, Herrero J, Roos C, Aken B, et al. 2014. Gibbon genome and the fast karyotype evolution of small apes. *Nature* 513(7517):195–201.
- Dalquen D, Zhu T, Yang Z. 2017. Maximum likelihood implementation of an isolation-with-migration model for three species. *Syst Biol.* 66(3):379–398.
- Degnan JH, Rosenberg NA. 2006. Discordance of species trees with their most likely gene trees. *PLoS Genet.* 2(5):e68.
- Degnan JH, Rosenberg NA. 2009. Gene tree discordance, phylogenetic inference and the multispecies coalescent. *Trends Ecol Evol.* 24(6):332–340.
- Edwards SV. 2009. Is a new and general theory of molecular systematics emerging? *Evolution* 63(1):1–19.
- Edwards SV, Xi Z, Janke A, Faircloth BC, McCormack JE, Glenn TC, Zhong B, Wu S, Lemmon EM, Lemmon AR, et al. 2016. Implementing and testing the multispecies coalescent model: a valuable paradigm for phylogenomics. *Mol Phylogenet Evol.* 94(Pt A):447–462.
- Giarla TC, Esselstyn JA. 2015. The challenges of resolving a rapid, recent radiation: empirical and simulated phylogenomics of Philippine shrews. *Syst Biol.* 64(5):727–740.
- Green PJ. 2003. Trans-dimensional Markov chain Monte Carlo. In: Green PJ, Hjort NL, Richardson S, editors. Highly structured stochastic systems. Oxford (UK): Oxford University Press. p. 179–196.
- Gronau I, Hubisz MJ, Gulko B, Danko CG, Siepel A. 2011. Bayesian inference of ancient human demography from individual genome sequences. *Nat Genet.* 43(10):1031–1034.

- Hey J, Nielsen R. 2007. Integration within the Felsenstein equation for improved Markov chain Monte Carlo methods in population genetics. *Proc Natl Acad Sci U S A*. 104(8):2785–2790.
- Jukes TH, Cantor CR. 1969. Evolution of protein molecules. In: Munro HN, editor. Mammalian protein metabolism. New York: Academic Press. p. 21–123.
- Kubatko LS, Degnan JH. 2007. Inconsistency of phylogenetic estimates from concatenated data under coalescence. *Syst Biol*. 56(1):17–24.
- Lartillot N, Philippe H. 2006. Computing Bayes factors using thermodynamic integration. *Syst Biol*. 55(2):195–207.
- Maddison WP. 1997. Gene trees in species trees. *Syst Biol*. 46(3):523–536.
- Mallo D, Posada D. 2016. Multilocus inference of species trees and DNA barcoding. *Philos Trans R Soc Lond B Biol Sci*. 371(1702):20150335.
- Nascimento FF, dos Reis M, Yang Z. 2017. A biologist's guide to Bayesian phylogenetic analysis. *Nat Ecol Evol*. 1(10):1446–1454.
- Nichols R. 2001. Gene trees and species trees are not the same. *Trends Ecol Evol*. 16(7):358–364.
- Ogilvie HA, Heled J, Xie D, Drummond AJ. 2016. Computational performance and statistical accuracy of *BEAST and comparisons with other methods. *Syst Biol*. 65(3):381–396.
- Rannala B. 2015. The art and science of species delimitation. *Curr Zool*. 61(5):846–853.
- Rannala B, Yang Z. 2003. Bayes estimation of species divergence times and ancestral population sizes using DNA sequences from multiple loci. *Genetics* 164(4):1645–1656.
- Rannala B, Yang Z. 2013. Improved reversible jump algorithms for Bayesian species delimitation. *Genetics* 194(1):245–253.
- Rannala B, Yang Z. 2017. Efficient Bayesian species tree inference under the multispecies coalescent. *Syst Biol*. 66(5):823–842.
- Reich D, Green RE, Kircher M, Krause J, Patterson N, Durand EY, Viola B, Briggs AW, Stenzel U, Johnson PL, et al. 2010. Genetic history of an archaic hominin group from Denisova Cave in Siberia. *Nature* 468(7327):1053–1060.
- Shi CM, Yang Z. 2018. Coalescent-based analyses of genomic sequence data provide a robust resolution of phylogenetic relationships among major groups of gibbons. *Mol Biol Evol*. 35(1):159–179.
- Takahata N, Satta Y, Klein J. 1995. Divergence time and population size in the lineage leading to modern humans. *Theor Popul Biol*. 48(2):198–221.
- Xu B, Yang Z. 2016. Challenges in species tree estimation under the multispecies coalescent model. *Genetics* 204(4):1353–1368.
- Yang Z. 1994a. Maximum likelihood phylogenetic estimation from DNA sequences with variable rates over sites: approximate methods. *J Mol Evol*. 39(3):306–314.
- Yang Z. 1994b. Estimating the pattern of nucleotide substitution. *J Mol Evol*. 39(1):105–111.
- Yang Z. 2002. Likelihood and Bayes estimation of ancestral population sizes in Hominoids using data from multiple loci. *Genetics* 162(4):1811–1823.
- Yang Z. 2014. Molecular evolution: a statistical approach. Oxford (England): Oxford University Press.
- Yang Z. 2015. The BPP program for species tree estimation and species delimitation. *Curr Zool*. 61(5):854–865.
- Yang Z, Rannala B. 2010. Bayesian species delimitation using multilocus sequence data. *Proc Natl Acad Sci U S A*. 107(20):9264–9269.
- Yang Z, Rannala B. 2014. Unguided species delimitation using DNA sequence data from multiple loci. *Mol Biol Evol*. 31(12):3125–3135.
- Yang Z, Rodríguez CE. 2013. Searching for efficient Markov chain Monte Carlo proposal kernels. *Proc Natl Acad Sci U S A*. 110(48):19307–19312.
- Zhou WW, Wen Y, Fu J, Xu YB, Jin JQ, Ding L, Min MS, Che J, Zhang YP. 2012. Speciation in the *Rana chensinensis* species complex and its relationship to the uplift of the Qinghai-Tibetan Plateau. *Mol Ecol*. 21(4):960–973.