BP&P

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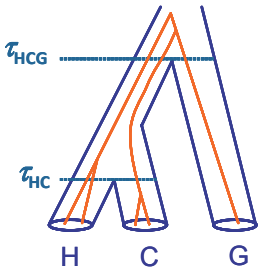

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0. Introduction

BPP is a Bayesian Markov chain Monte Carlo (MCMC) program for analyzing DNA sequence alignments from multiple loci and multiple closely-related species under the multispecies coalescent (MSC) model (Rannala and Yang, 2003; Yang, 2002). The program can be used to conduct four different analyses, specified using two variables in the control file:

A00 (`speciesdelimitation = 0, speciestree = 0`): estimation of the parameters of species divergence times and population sizes under the MSC model when the species phylogeny is given (Rannala and Yang, 2003);

A01 (`speciesdelimitation = 0, speciestree = 1`): inference of the species tree when the assignments are given by the user (Rannala and Yang, in preparation);

A10 (`speciesdelimitation = 1, speciestree = 0`): species delimitation using a user-specified guide tree (Yang and Rannala, 2010; Rannala and Yang, 2013);

A11 (`speciesdelimitation = 1, speciestree = 1`): joint species delimitation and species tree inference of unguided species delimitation (Yang and Rannala, 2014).

Underlying all those analyses is the MSC model, which specifies the probabilistic distribution of gene trees given the species tree (Rannala and Yang, 2003; see also Takahata et al., 1995; Yang, 2002). The basic parameters in the MSC model include the species divergence times (τs), measured by the expected number of mutations per site, and population size parameters $\theta = 4N\mu$, where N is the effective population size and μ is the mutation rate per site per generation so that θ is the average proportion of different sites between two sequences sampled at random from the population. For a species tree with s species, there are $s - 1$ divergence times (τs) and at most $2s - 2$ population size parameters (θs). Analysis A00 is to estimate those parameters when the species delimitation and species tree is fixed. Analyses A01, A10, and A11 compare different MSC models.

See Yang (Yang, 2014: Chapter 9) for a discussion of Bayesian inference under the MSC model. Chapters 7 and 8 of the same book describes Bayesian MCMC algorithms. Please also look at the BPP tutorial, which illustrates the four analyses (Yang, 2015).

Assumptions made in the MSC model implemented in the program include no recombination within a locus, free recombination between loci, no migration (gene flow) between species, and neutral clock-like evolution. The ideal loci are thus loosely linked short genomic segments: each segment is short (within 500 or 1000bp) so that recombination within the segment is rare while different segments should be far apart so that recombination between them is so common that the segments have more or less independent histories. It is assumed that the genealogical trees are not affected by natural selection. Protein-coding gene sequences appear to be useable in a BPP analysis since most proteins are performing similar functions in closely related species and the main effect of purifying selection on nonsynonymous mutations is a reduction of the neutral mutation. In a few analyses, exons were noticed to give consistent results with the introns or noncoding DNA (Shi and Yang, 2018). At any rate it is a good idea to separate the noncoding and coding regions of the genome into two datasets. Genes that are under species-specific directional selection may distort the shape of the genealogical tree and should be used with caution. The program currently uses the JC69 mutation model (Jukes and Cantor, 1969) to correct for multiple hits, and mutation rate is assumed to be constant over time. The use of the JC model and the clock assumption mean that the program should be limited to closely related species with sequence divergence not much higher than 10%. Work is under way to extend those models.

Note that a major 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 process may also vary along the genome, this heterogeneity is believed to be much less important. Thus multiple genes from the mitochondrial genome should be treated as one 'locus' in the MSC-based analysis. Typically the mitochondrial genome has different mutation rate from the nuclear genome, and also a different effective population size from the autosomes. While the model of locus-rate variation (option variable locusrate) and the heredity scalar (option variable heredity) are designed to deal with this, it may be prudent to analyze the loci from the nuclear genome separately from the single mitochondrial locus. Also the model assumes that the sequences are random samples from the different species. If some sequences from the same species are identical, they should all be used, and it is incorrect to use the haplotypes only, which will lead to biased parameter estimates. Similarly it is incorrect to filter loci based on bootstrap support values and use only those loci with high phylogenetic information signal.

How to cite the program. You can cite the BPP tutorial and the original papers that described the methods you used (see above). Describe the priors you used since they are necessary for reproducibility. If you conduct a joint analysis of species delimitation and species tree inference, your method description may look like the following (replace the numbers in green with those you used):

"Joint Bayesian species delimitation and species tree estimation was conducted using the program BPP (Yang, 2015). The method uses the multispecies coalescent model to compare different models of species delimitation (Yang and Rannala, 2010; Rannala and Yang, 2013) and species phylogeny (Yang and Rannala, 2014; Rannala and Yang, 2017) in a Bayesian framework, accounting for incomplete lineage sorting due to ancestral polymorphism and gene tree-species tree discordance. The population size parameters (θs) are assigned the inverse-gamma prior IG(3, 0.002), with mean 0.002/(3 – 1) = 0.001. The divergence time at the root of the species tree (τ_0) is assigned the inverse-gamma prior IG(3, 0.004), with mean 0.002, while the other divergence time parameters are specified by the uniform Dirichlet distribution (Yang and Rannala, 2010: equation 2). Each analysis is run at least twice to confirm consistency between runs."

1. Compiling and running BPP

1.1 Compiling the program

This manual applies to BPP versions 3.4 and 4.0. Both are C programs 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, this document) is included in the release. BPP 4 is an improved rewrite, available at https://github.com/bpp/bpp.

BPP is a command-line program, so the best way of running it is from the command line, rather than double-clicking on the file name from your file explorer. 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`

WINDOWS and MAC OSX executables will be provided at the download site. For LINUX or MAC OSX, you may need to compile the programs to generate the executable file `bpp`. This needs to be done only once. 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`
`md bin`	`md bin`
`cd src`	`cd src`
`gcc -o bpp -O3 bpp.c tools.c -lm`	`make`
`mv bpp ../bin`	`mv bpp ../bin`

The `-o` flag specifies the name of the resulting executable file, the `-O3` flag is for optimizing the code, and `-lm` is to link to the math library. Then move the generated executable file (bpp) into the bin/ folder.

The same source code can be compiled into a simulation program (MCcoal). See the section *The simulation program (MCcoal)* later in this document for details.

1.2 Trial run

Run the program from a command box (rather than double-clicking the executable) so that you will see the error messages. In the bpp/ folder, run the program by typing the following command:

On WINDOWS	On LINUX/UNIX/MAC OSX
`bin\bpp bpp.5s.ctl`	`bin/bpp bpp.5s.ctl`

Or move to the `examples/` folder, and run the example analysis as follows.

On WINDOWS	On LINUX/UNIX/MAC OSX
`cd examples` `..\bin\bpp ChenLi2001.bpp.ctl`	`cd examples` `../bin/bpp ChenLi2001.bpp.ctl`
`cd examples` `..\bin\bpp Li3lociHuman.bpp.ctl`	`cd examples` `../bin/bpp Li3lociHuman.bpp.ctl`

You may use the graphical interface BPPX, written by Bo Xu. This documentation assumes that you run BPP from the command line. If you have not used the command line before, here are simple tutorials that you should go through first:
http://abacus.gene.ucl.ac.uk/software/CommandLine.Windows.pdf
http://abacus.gene.ucl.ac.uk/software/CommandLine.MACosx.pdf

2. File formats

2.1 Sequence data file and individual map (Imap) file

The sequence file. The sequence alignments are in the phylip/paml format, with one alignment following the other, all in one file. The sequence name should be separated from the sequence by a line break or by at least two spaces. Have a look at the sequence files test5s.txt and ChenLi2001.txt. An alignment is called a locus. Every locus must have at least 2 sequences, different loci can have different numbers of sequences, and some species may be missing at some loci.

By default (cleandata = 0), alignment gaps and ambiguity nucleotides are used in the likelihood calculation, with gaps treated as question marks (see Yang, 2006, pp. 107-108). If cleandata = 1, all columns with gaps or ambiguity characters are removed before analysis.

The Imap file. Each sequence name has a tag after ^. For example, the sequence name GI01234567^Specimen1 has the individual ID Specimen1. An Imap file then maps the individuals (specimens) to the species/populations. For example, the following Imap file

maps Specimen1 to species A.

```
Specimen1  A
Specimen2  A
Specimen3  A
Specimen4  B
Specimen5  C
Specimen6  C
```

The MSC model implemented in BPP uses only the population ID (such as A, B, … in the example), and does not use the individual ID. Also when BPP reads the sequence names, it use the individual ID to retrieve the species ID for each sequence but then ignores the sequence name. The motivation for the use of the Imap file and this two-layer design is that one may analyze the same sequence data with different population/species assignments, which can be achieved by editing the small Imap file without editing the much bigger sequence data file.

2.2 Control file

2.2.1 The control variables

The default control file name is bpp.ctl. Lines beginning with an asterisk are comments. Most often the order of the lines is unimportant, but there are exceptions (as explained below). We use ChenLi2001.bpp.ctl in the examples/ folder to explain the variables in the control file. This is for analysis A00: parameter estimation under the MSC using multiple-loci multiple-species data on a fixed species tree (Rannala and Yang, 2003; Burgess and Yang, 2008). We will then comment on the other analyses (A01, A10, and A11). Please also read the section on the example datasets later in this document.

Below is a copy of the control file ChenLi2001.bpp.ctl. Note that if there are s species in the species tree, the model will involve the following parameters: $(s - 1)$ species divergence times (τs) and $(s - 1)$ ancestral θs. If a species has at least two sequences at any loci, a θ for that species will be used as well. If there is one species, the model will involve one parameter only, θ for that species. The parameters are ordered as follows: θs for the extant species, θs for the ancestral species ($s - 1$ of them), and the divergence times τs for the ancestral nodes ($s - 1$ of them).

```
          seed =  -1

       seqfile = ChenLi2001.txt
      Imapfile = ChenLi2001.Imap.txt
       outfile = out.txt
      mcmcfile = mcmc.txt

speciesdelimitation = 0        * fixed species delimitation
*speciesdelimitation = 1 0 2    * rjMCMC speciesdelimitation algorithm0(e)
*speciesdelimitation = 1 1 2 1  * rjMCMC speciesdelimitation algorithm1(a m)
 speciestree = 0        * species tree fixed
*speciestree = 1        * NNI over species/guide trees

*speciesmodelprior = 1  * 0: uniform LH; 1:uniform rooted trees; 2: uniformSLH; 3: uniformSRooted

  species&tree = 4  H  C  G  O
                    1  1  1  1
                 (((H, C), G), O);
       diploid =    1  1  1  1    * 0: phased sequences, 1: unphased diploid sequences

       usedata = 1    * 0: no data (prior); 1:seq like
         nloci = 53    * number of datasets in seqfile

     cleandata = 0    * remove sites with ambiguity data (1:yes, 0:no)?
```

```
*   thetaprior = 3 0.002 e  # inverse gamma(a, b) for theta
    thetaprior = 3 0.002   # inverse gamma(a, b) for theta
      tauprior = 3 0.03    # inverse gamma(a, b) for root tau & Dirichlet(a) for other tau's

*     locusrate = 0 2.0   # (0: No variation, 1: estimate, 2: from file) & a_Dirichlet
*      heredity = 0    # (0: No variation, 1: estimate, 2: from file) & a_gamma b_gamma
*      heredity = 1 4 4   # (0: No variation, 1: estimate, 2: from file) & a_gamma b_gamma
*      heredity = 2 heredity.txt   # (0: No variation, 1: estimate, 2: from file) & a_gamma b_gamma

* sequenceerror = #  model of sequencing errors has changed, to be described later

       finetune = 1: .01 .02 .03 .04 .05 .01 .01  # auto (0 or 1): MCMC step lengths
         print = 1 0 0 * print MCMC samples, locusrate, heredityscalars
        burnin = 2000
      sampfreq = 2
       nsample = 20000
```

seed is the random number seed. If you use a positive integer, the program will produce identical results in different runs. This is useful for debugging. If you use −1, the program will use the wall clock to generate a seed, and different runs will produce different results. It is recommended that you run the same analysis at least twice using different seeds to confirm that the results are stable across runs.

seqfile is the name of the sequence alignment file, while
Imapfile is the individual map file. The Imapfile is not needed if the data contain only one species. These two files are input.

```
speciesdelimitation = 0        * fixed species delimitation
speciestree = 0        * no change to species tree or guide trees
```

speciesdelimitation = 0 and **speciestree = 0** specify analysis A00: estimation of parameters under the MSC model. For analyses A01, A10, and A11, those two variables may be specified the value 1. See the next subsection below. If those two variables are missing or their lines are commented out, the default value of 0 is assumed. The variable `speciesmodelprior` is used in analyses A01, A10, and A11, and has no effect for analysis A00.

```
species&tree = 4  H  C  G  O
                  1  1  1  1
               (((H, C), G), O);
       diploid =  1  1  1  1    * 0: phased sequences, 1: unphased diploid sequences
```

The above block specifies 4 species in the data, which are H (human), C (chimp), G (gorilla), and O (orang). The maximum number of sequences at any locus is 1 for H, 1 for C, 1 for G, and 1 for O. These numbers serve two purposes. First, they are used to determine which θ parameters are involved in the model and should be estimated. If the number of sequences for a species is 2 or greater, θ for that species will be a parameter estimated by the program. Second, the sum of the numbers of sequences over all species specifies the maximum number of sequences at a locus. BPP always uses θ and τ (age) for every interior (ancestral) node on the species tree, and uses θ for each extant species if and only if that species has more than 2 or more sequences at some loci. In the example here, the parameters are the three ancestral θs and three node ages (τs).
If you specify 2 or more sequences for a species but there are at most 0 or 1 sequence per locus for that species in the sequence data file, θ for that species will not be identifiable or estimable. In that case, the posterior for the parameter will be the prior. Nevertheless, other results (such as the posterior distribution for other parameters or posterior probabilities for species trees and species delimitations) will still be correct. The same applies to other more complex cases of missing data and parameter unidentifiability. As an example, suppose the species tree and the numbers of sequences for two kinds of loci are as follows:

((A,B), (C,D))
locus configuration 1: 1 1 0 0
locus configuration 2: 2 0 0 1
Then θ_A, θ_{AB}, θ_{ABCD}, τ_{AB} and τ_{ABCD} are identifiable while θ_B, θ_C, θ_D, θ_{CD} and τ_{CD} are not. For example, it is impossible to estimate θ_D, θ_{CD} and τ_{CD} if no data are available from C.
As another example, suppose the species tree is
((A,B), (C,D));
locus configuration 1: 1 1 0 0
locus configuration 2: 2 0 0 10
Then θ_A, θ_D, θ_{AB}, θ_{CD}, θ_{ABCD}, τ_{AB}, τ_{CD} and τ_{ABCD} are all identifiable while θ_B and θ_C are not. We are essentially fitting a demographic model of population size change along the path D-CD-ABCD. The information in the data for θ_{CD} and τ_{CD} may be weak so those parameters may be estimated poorly.

The species tree is given in the parenthesis (Newark) format, ending with a semicolon (;). The tree is fixed if **speciesdelimitation = 0** and is used as the guide tree in the rjMCMC run for species delimitation if **speciesdelimitation** = 1 (see below).

The next line use the **diploid** variable to indicate 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. Indeed the ape sequences of Chen and Li (Chen and Li, 2001) are unphased diploid sequences. If the indicator is 1 for a species, all sequences from that species are assumed to be diploid unphased. The program does not allow some sequences from a species to be phased sequences while other sequences from the same species to be unphased. In an unphased sequence, a heterozygote site is represented using the ambiguity characters YRMKSW. N-? are allowed and are treated as ?, but other ambiguities (HBVD) are not allowed. In a phased sequence, ambiguities YRMKSWHBVD-N? are all treated as ambiguities in the usual way.

The MSC model averages over different resolutions of the heterozygote sites in the likelihood calculation, using the approach of Gronau et al. (Gronau et al., 2011). The simulation program MCcoal has been modified to simulate unphased dipoid data as well. This allows one to see the difference between the analysis of the full data and the unphased data.

usedata = 0 is for running the MCMC without sequence data to generate the prior, while **usedata = 1** is for generating the posterior.

nloci specifies the number of loci (alignments). If you have 200 loci in the data file and choose **nloci = 2**, BPP will read the first two loci only.

cleandata = 1 causes the program to remove all columns in the alignment which have gaps or ambiguity characters. **cleandata** = 0 means that those will be used in the likelihood calculation.

thetaprior = 3 0.002 specifies the inverse-gamma prior IG(α, β) for the θ parameters, with the mean to be $\beta/(\alpha - 1)$. In the example, the mean is $0.002/(3 - 1) = 0.001$ (one difference per kb).

Note that all θ parameters in the MSC model (for both modern species and extinct ancestral species) are assigned the inverse-gamma prior with the same parameters. The inverse-

gamma is a conjugate prior for θ (Hey and Nielsen, 2007), which means that both the prior and the posterior of θ will be inverse-gamma. Use of the conjugate priors allows the θ parameters to be integrated out analytically, and thus the dimension of the parameter space is reduced. This typically leads to improved mixing of the MCMC. However, with this option, the posterior of the θ parameters will not be produced. To estimate the θ parameters, add the letter e (or E) on the line, as follows

```
thetaprior = 3 0.002 e
```

Whether θs are integrated out or estimated, other results (such as the posterior of the τ parameters or the posterior probability of species trees or species-delimitation models) should be identical. A useful strategy may be to run the A01, A10, and A11 analyses without estimating the θ parameters, and after the MAP model (the best species tree or the best species delimitation model) is identified, run the A00 analysis with the model fixed to estimate all parameters.

Some notes about the inverse-gamma distribution. Since BPP3.4, both the θ and τ parameters are assigned the inverse gamma priors rather than the gamma priors in version 3.3 or earlier. One difference is that the gamma is light-tailed while the inverse-gamma is heavy-tailed, so that the inverse-gamma may be less influential than the gamma if your prior mean is much too small. The inverse-gamma distribution IG(α, β) has mean $m = \beta/(\alpha - 1)$ if $\alpha > 1$ and variance $s^2 = \beta^2/[(\alpha - 1)^2(\alpha - 2)]$ if $\alpha > 2$, while the coefficient of variation is $s/m = \sqrt{1/(\alpha - 2)}$. If little information is available about the parameters, you can use $\alpha = 3$ for a diffuse prior and then adjust the β so that the mean looks reasonable. Both parameters θs and τs in the MSC model are measured by genetic distance, the expected number of mutations/substitutions per site. For example, for the human species, $\theta_H \approx 0.0006$, which means that two random sequences from the human population are different at ~0.06% of sites, less than 1 difference per kb. A sensible diffuse prior is then "`thetaprior = 3 0.002`", with mean 0.001.

tauprior = 3 0.03 specifies the inverse-gamma prior IG(α, β) for τ_0, the divergence time parameter for the root in the species tree. Other divergence times are generated from the uniform Dirichlet distribution (Yang and Rannala, 2010: equation 2). In the example, the mean is 0.03/(3 – 1) = 0.015 (which means 1.5% of sequence divergence between the root of the species tree and the present time). If the mutation rate is 10^{-9} mutations/site/year, this distance will translate to a human-orangutan divergence time of 15MY .

```
locusrate = 0                 #  (0: No variation)
locusrate = 1 2.0               # (1: estimate & a_Dirichlet)
locusrate = 2 LocusRateFileName     # (2: from file)
```

locusrate = 0 (default) means that all loci have the same mutation rate. **locusrate = 1** or **2** specifies two models for variable mutation rates among loci (Burgess and Yang, 2008). **locusrate = 1** specifies the random-rates model of Burgess & Yang (Burgess and Yang, 2008: equation 4). The average rate for all loci is fixed at 1, while the rates among loci are assumed to generated from the Dirichlet distribution $D(\alpha)$. Parameter α is inversely related to rate variation, with a large α meaning similar rates among loci. If all loci are noncoding, the rates are probably similar, so $\alpha = 10$ or 20 may be reasonable, while $\alpha = 2$ or 1 may be too small. The MCMC generates the posterior for rates at loci.

locusrate = 2 LocusRateFileName specifies the fixed-rates model of Burgess & Yang (Burgess and Yang, 2008). This is the strategy used by Yang (Yang, 2002), with the relative rates

estimated by the distance to an outgroup species. The relative locus rates are listed in the file: there should be as many numbers in the file, separately by spaces or line returns, as the number of loci or **nloci**. The program re-scales those rates so that the average among all loci is 1 and then use those relative rates as fixed constants.

Note. The model of variable rates among loci implemented here has some differences from a similar model implemented in the IMa program (Hey and Nielsen, 2004). The biggest difference appears to be the parametrization. BPP defines mutation rate on a per-nucleotide basis, so the prior specifies that the expectation of the mutation rate per site is constant among loci. IMa defines mutation rate on a per-locus basis, so its prior specifies that the expectation of the mutation rate per locus is the same among loci. If locus one has 100 sites and locus two has 1000 sites, then IM assumes that the per-site rate for locus one is 10 times for locus two, while BPP assumes the same per-site rate. Also IMa constrains the geometric mean of rates across loci to be one, while BPP constrains their arithmetic mean to be one. The IM assumptions do not appear to me to be realistic.

```
heredity = 0      # (0: No variation)
heredity = 1 4 4  # (1: estimate, & a_gamma b_gamma)
heredity = 2 heredity.txt     # (2: from file)
```

heredity = 0 is the default and means that θ is the same for all loci. **heredity = 1** or **2** specifies two models that allow θ to vary among loci, which may be useful for combined analysis of data from autosomal, mitochondrial, X and Y loci. With such mixed data, the effective population sizes are different among loci, so that a heredity multiplier (inheritance scalars, Hey and Nielsen, 2004) should be applied. Other factors such as natural selection may also cause θ to deviate from the neutral expectation. BPP implements two options for this. The first option (**heredity = 1**) is to estimate the multipliers from the data, using a gamma prior with parameters α and β specified by the user. In the example above, a gamma prior $G(4, 4)$, with mean 4/4 = 1, is specified for the multiplier for each locus. The MCMC should then generate a posterior for the multiplier for each locus. The second option (**heredity = 2**) is for the user to specify the multipliers in a file, and the multipliers will then be used as fixed constants in the MCMC run. The file simply contains as many numerical values as the number of loci, separated by spaces or line breaks.

Genome	Heredity scalar
Nuclear autosome	1
X chromosome	0.75
Y chromosome	0.25
Mitochondrial	0.25

Note. The effect of the locus-specific mutation rates and the locus-specific heredity multipliers are different. A locus rate is used to multiply all θs and τs for the locus, while a heredity multiplier is used to multiply all θ parameters for the locus but not the τs. Nevertheless, those parameters are quite likely to be strongly correlated, especially when the species tree is small.

Ziheng note on 3 March 2011.
The model of sequence errors is rewritten, so that the old option is unavailable. Use
sequenceerror = 0 # (0: default: No error)

```
finetune = 0: .01 .02 .03 .04 .05 .01 .01  # auto (0 or 1): MCMC step lengths

finetune = 1: .01 .02 .03 .04 .05 .01 .01  # auto (0 or 1): MCMC step lengths
```

This is about the step lengths used in the proposals in the MCMC algorithm. The first value, before the colon, is a switch, with 0 meaning no automatic adjustments by the program and 1 meaning automatic adjustments by the program. Following the colon are the step lengths for the proposals used in the program. If you choose to let the program adjust the step lengths, burnin has to be >200, and then the step lengths specified here will be the initial step lengths, and the program will try to adjust them using the information collected during the burnin step. This option appears to work fine. Some notes about manually adjusting those finetune step lengths are provided below in section 3.2.

```
print = 1 0 0 0 * print MCMC samples, locusrate, heredityscalars, GeneTrees
burnin = 2000
sampfreq = 2
nsample = 20000
```

`print = 0` means that no MCMC samples are written into the file, which may be useful if you need the screen output only. `print = 1` generates a file mcmc.txt containing the MCMC samples. The next two flags on the same line are for printing locus rates and locus heredity scalars if those are estimated from the data using gamma priors. I suspect there may be trouble if you have many thousands of loci. I think the option of printing and processing gene trees for loci are not working, so choose 0.

MCMC samples are taken after the burnin, and in this example, are taken every 2 iterations, with a total of 20,000 samples taken. The total number of MCMC iterations is burnin + output × nsample. The resulting file can be large. For analysis A00 (speciesdelimitation = 0, speciestree = 0), this file is readable from R or Andrew Rambaut's TRACER. For other analyses (A01, A10, and A11), the sample file is not readable by R or TRACER.

`print = -1` means that BPP will bypass the MCMC. Instead it will read the MCMC sample and summarize the results. Thus with `print = 1`, the mcmc.txt file will be output, but with `print = -1`, it will be the input. Make sure that useful files are not overwritten. The `print = -1` option is useful for combining the MCMC samples from multiple runs to produce the posterior summary. Suppose you run the same analysis 3 times in different folders. After a week you are tired so you can kill the jobs. You can then merge the mcmc.txt files from the three runs into one file. Note that if the last line of the first file is incomplete, you should delete the incomplete line, and if the first line of the second file is a header line, you should delete that header line. You then run the program to summarize the posterior using the combined sample. Do not combine the MCMC samples from different analyses (with different data files or priors).

2.2.1 The four analyses (A00, A01, A10, and A11)

Here we describe the specifics of the four different analyses.

A00 (`speciesdelimitation = 0, speciestree = 0`), for estimation of the parameters of species divergence times and population sizes (τs and θs) under the MSC model when the species phylogeny is given (Rannala and Yang, 2003), has been explained in detail above using the control file ChenLi2001.bpp.ctl as an example.

If there is only one species, the MSC model will become the single-population coalescent (Kingman, 1982). Take a look at examples/yu2001.bpp.ctl, which is for analyzing a sample of 61 human sequences from Yu et al. (Yu et al., 2001) to estimate the single parameter $\theta = 4N\mu$. There is no need for the Imap file, or the need to tag the sequence names in the

sequence file (yu2001.txt): the sequence names are read and then ignored. Multiple loci may be included in the sequence file. There is no need for a species tree, so the block for specifying species names and species tree looks like this:

```
species&tree = 1  H
                100  * max number of sequences
```

In the single-species analysis, the printout on the monitor includes the posterior mean of θ, and posterior means of μt_{MRCA} for the loci, calculated up to that point in the MCMC run. If you have many loci, only the first few μt_{MRCA} are printed on the monitor.

The mcmc sample file lists $1 + g + 1$ columns for g loci: θ, μt_{MRCA} for the g loci, and the log likelihood. The μt_{MRCA} are not parameters, but are sometimes of interest as well.

Common features of analyses A01, A10, and A11. Note that A00 is a within-model inference: there is one well-specified model (the MSC model on a fixed species tree) and the parameters in the model are all well defined. The objective of the analysis is to estimate those parameters. In contrast, A01, A10, and A11 are all trans-model inferences. They move between different models, and the main objective is to calculate the posterior probabilities for those models. Each of those models is an instance of the MSC model, but the species delimitation (the number and nature of the species) and/or the species phylogeny may differ between models. In analyses A01, A10, and A11, the inverse-gamma prior specified using **thetaprior** applies to all θ parameters in all models: in other words, there may be thousands of θ parameters across the models, and each one is assigned the same IG prior. Similarly each of those MSC models (if they specify two or more delimited species) may have a parameter τ_0 for the divergence time of the root, and all those τ_0 parameters are assigned the same inverse-gamma prior. Another difference is that the sample file mcmc.txt is readable in R or by TRACER for analysis A00, but not for A01, A10, and A11.

A01 means species tree estimation when the assignments and delimitation are fixed.

```
speciesdelimitation = 0    *
       speciestree = 1    * NNI/SPR over species trees
  speciesmodelprior = 1  * 0: uniform LH; 1:uniform rooted trees; 2: uniformSLH; 3: uniformSRooted
```

This invokes the NNI or SPR algorithm to change the species tree topology, while species delimitation is fixed (so that the number of species and the assignment of individuals to species are fixed).

A10, for species delimitation using a user-specified guide tree (Yang and Rannala, 2010; Rannala and Yang, 2013), is specified using

```
speciesdelimitation = 1 0 2   * speciesdelimitation algorithm0 and finetune(e)
speciesdelimitation = 1 1 2 1 * speciesdelimitation algorithm1 finetune (a m)
  speciesmodelprior = 1  * 0: uniform LH; 1:uniform rooted trees
```

The first line specifies rjMCMC algorithm 0, with $\varepsilon = 2$ in equations 3 and 4 of Yang & Rannala (Yang and Rannala, 2010). Reasonable values for ε are 1, 2, 5, etc.
The second line specifies rjMCMC algorithm 1, with $\alpha = 2$ and $m = 1$ in equations 6 and 7 of Yang & Rannala (Yang and Rannala, 2010). Reasonable values are $\alpha = 1$, 1.5, 2, etc. and $m = 0.5$, 1, 2, etc.

The two algorithms in theory should produce identical results. The variable

speciesmodelprior specifies Priors 0 and 1. Prior 0 means equal probabilities for labeled histories (which are rooted trees with internal nodes ordered by their age). This is the prior used by Yang & Rannala (Yang and Rannala, 2010: equation 2). Prior 1 means equal probabilities for rooted trees. This is now the default. The prior with the user specified probabilities for nodes described by Rannala and Yang (Rannala and Yang, 2013) is deleted in the current version. You will have to use version 2.2 for that.

A11, for joint species delimitation and species tree inference or for unguided species delimitation (Yang and Rannala, 2014), is specified as follows.

```
speciesdelimitation = 1 0 2    * rjMCMC speciesdelimitation algorithm0(e)
*speciesdelimitation = 1 1 2 1  * rjMCMC speciesdelimitation algorithm1(a m)
       speciestree = 1    * NNI over species trees
 speciesmodelprior = 1  * 0: uniform LH; 1:uniform rooted trees; 2: uniformSLH; 3: uniformSRooted
```

In this case, BPP will use the rjMCMC algorithm (either algorithm 0 or algorithm 1 of Yang and Rannala, 2010) to change the species delimitation model and the NNI/SPR move to change the species tree topology.

For A11, **speciesmodelprior** can take the four values 0, 1, 2, 3, which mean Priors 0, 1, 2, 3, respectively. As mentioned above, Prior 1 (which is the default) assigns equal probabilities to the rooted species trees, while Prior 0 means equal probabilities for the labeled histories (rooted trees with the internal nodes ordered by age). Priors 2 and 3 assign equal probabilities for the numbers of species ($1/s$ each for 1, 2, ..., s species given s populations) and then divided up the probability for any specific number of species among the compatible models (of species delimitation and species phylogeny) either uniformly [Prior 3] or in proportion to the labeled histories [Prior 2]. Priors 2 and 3 are mentioned by Yang and Rannala (Yang and Rannala, 2014) and implemented by Yang (Yang, 2015). Prior 3 may be suitable when there are many populations.

3. Screen and file outputs

3.1 A00 screen output

First we consider the simple analysis under the MSC model with the species tree fixed (A00: `speciesdelimitation` = 0, `speciestree` = 0). We use this case to explain the acceptance proportions of MCMC moves. The screen outputs for analyses A01, A10, and A11 will have differences, which will be described later.

Make the window wider, with 100 or 120 columns, say, before you run the program. (On Windows, you right-click the window title bar and choose Properties – Layout and change Window Size Width.) Pay attention to screen outputs, especially at the start of the run, to make sure that the control file and sequence data file are read correctly by the program, and that the acceptance proportions are reasonable. Use Ctrl-C to terminate the run, if needed.

Here is an outline of the steps taken by the program. The sample output is from analyzing the example dataset ChenLi2001.txt, on a fixed species tree. The differences for the species delimitation analysis are discussed later. The program first prints out the species tree, as well as a population-population table, which describes the descendant-ancestor relationship between populations and which you may ignore. It then defines the θ and τ parameters involved in the model.

The program then reads and processes the sequence data file.

It then generates the initial values for parameters θs and τs by using the gamma priors and the initial gene trees and coalescent times by sampling from the prior. The program prints out the initial θs and τs, as well as the initial log MSC gene-tree density lnpG0 (Rannala and Yang, 2003) and the log sequence likelihood lnL0 (Felsenstein, 1981), and starts the MCMC.

```
MCMC settings: 2000 burnin, sampling every 2, 20000 samples
Approximating posterior, using sequence data
(Settings: cleandata=0 print=1 saveconP=1 moveinnode=1)

Starting MCMC...

Initial parameters, np = 10.
Genetrees generated from the MSC density.
  0.00093  0.00101  0.00108  0.00100  0.00106  0.00107  0.00098  0.01461  0.00922  0.00805
lnpG0 = 2458.5040  lnL0 = -43947.4120
 -4% 0.14 0.00 0.00 0.04 0.45  0.0009 0.0010 0.0011  0.0147 0.0071 0.0058 2330.02 -43766.9113
(nsteps = 5)
Current Pjump:      0.14267  0.00245  0.00057  0.04467  0.46200
Current finetune:   0.01000  0.02000  0.03000  0.04000  0.05000
New     finetune:   0.00447  0.00015  0.00030  0.00552  0.08706

 -2% 0.15 0.84 0.42 0.02 0.20  0.0007 0.0008 0.0006  0.0141 0.0066 0.0054 2305.87 -43727.7353
(nsteps = 5)
Current Pjump:      0.15443  0.84480  0.42057  0.01533  0.19600
Current finetune:   0.00447  0.00015  0.00030  0.00552  0.08706
New     finetune:   0.00217  0.00119  0.00046  0.00026  0.05433

 -1% 0.27 0.39 0.30 0.53 0.44  0.0007 0.0008 0.0005  0.0143 0.0063 0.0049 2364.60 -43723.3313
(nsteps = 5)
Current Pjump:      0.25658  0.37186  0.28314  0.50667  0.42800
Current finetune:   0.00217  0.00119  0.00046  0.00026  0.05433
New     finetune:   0.00182  0.00155  0.00043  0.00052  0.08488

  0% 0.27 0.28 0.27 0.21 0.15  0.0006 0.0008 0.0006  0.0148 0.0062 0.0052 2374.94 -43727.0222  0:05

(nsteps = 5)
Current Pjump:      0.26527  0.28023  0.27257  0.21200  0.14800
Current finetune:   0.00182  0.00155  0.00043  0.00052  0.08488
New     finetune:   0.00158  0.00143  0.00038  0.00035  0.03944

  5% 0.29 0.30 0.30 0.35 0.51  0.0007 0.0008 0.0006  0.0151 0.0061 0.0051 2379.62 -43728.0652  0:12
 10% 0.32 0.32 0.33 0.33 0.52  0.0007 0.0008 0.0006  0.0145 0.0061 0.0052 2399.95 -43723.9478  0:17
 15% 0.31 0.31 0.32 0.34 0.52  0.0007 0.0008 0.0006  0.0146 0.0061 0.0052 2274.55 -43725.6273  0:22
 20% 0.31 0.31 0.32 0.34 0.53  0.0007 0.0008 0.0005  0.0147 0.0061 0.0052 2409.03 -43725.7590  0:28
```

Then on the same line, it prints out a percentage progress indicator (with negative values for

burnin), followed by the acceptance proportions for the MCMC moves (highlighted in red in the sample output), and by the posterior means of the parameters (in this example there are three θ parameters and three τ parameters). The last two numbers before the time used is the log MSC density (for the current gene trees) and log sequence likelihood (average from the past samples). In this example, the program uses four rounds of automatic step-size adjustments, so that the acceptance proportions are close to 0.3 or lie in the interval (0.15, 0.7). See below.

The program will then read and process the file mcmc.txt to calculate the mean, min, max, median, and percentiles, and histogram information. This may take quite some time if many samples (say, 1M) are collected in the file. The program also generates a file FigTree.tre, which can be read by Andraw Rambaut's figtree program to make a publication-quality tree, showing the HPD intervals for divergence times (τs) as node bars. See the notes at the end of the file.

The mcmc.txt sample file generated in analysis A00 can be read in R or TRACER.

3.2 Adjusting step lengths for MCMC moves (finetune)

You can use the automatic adjustment of the finetune variable (MCMC step lengths), which appears to be reliable, but make sure that the acceptance proportions are neither too small nor too large. Below are notes for manual adjustments of the step lengths. Often I use automatic adjustments to generate good step lengths and then copy them into the control file, so that the next time the automatic procedure starts with already good step lengths.

Below are some notes about adjusting the step lengths manually (`finetune = 0`).

First note the following line in the control file ChenLi2001.bpp.ctl. Here `finetune = 0` means that MCMC step lengths will not be adjusted automatically, and they are used as given.

```
finetune = 0: .01 .02 .03 .04 .05 .01 .01  # auto (0 or 1): MCMC step lengths
```

There are seven finetune steplengths here. They are in a fixed order and always read by the program even if the concerned proposal is not used. The first five of them are ε_1, ε_2, ε_3, ε_4, ε_5, described in Rannala & Yang (Rannala and Yang, 2003). These are the step lengths used in the MCMC proposals that (1) change internal node ages in the gene tree, (2) prune and re-graft nodes in the gene tree, (3) update θs, (4) update τs using the rubber-band algorithm, and (5) implements the mixing step. The 6th and 7th are for the proposals that change the locus rates or heredity multipliers and that change the sequencing errors, respectively. If the model assumes the same rate for all loci and does not use heredity multipliers, the 6th proposal step is not used. If the model assumes no sequencing errors, the 7th step is not used. The acceptance proportions for the first five proposals are always printed out on the screen, but those for the 6th and 7th are printed out only if the concerned proposal is used in the model. In the example above, only the first five proposals are used in the algorithm and we will change the step lengths so that the acceptance proportions become close to 30%. If the acceptance proportion is too small (say, <0.10)), decrease the corresponding finetune parameter. If the acceptance proportion is too large (say, >0.80), increase the finetune parameter.

Run the program for a small number of iterations and look at the screen output for the acceptance proportions.

```
lnpG0 = 2440.0952  lnL0 = -43946.6769
  0% 0.13 0.00 0.00 0.04 0.43  0.0009 0.0009 0.0008  0.0150 0.0067 0.0057 2399.70 -43752.8348  0:05
  5% 0.14 0.00 0.00 0.04 0.44  0.0009 0.0009 0.0008  0.0148 0.0064 0.0057 2340.71 -43738.0172  0:09
 10% 0.14 0.00 0.00 0.04 0.44  0.0009 0.0009 0.0008  0.0149 0.0064 0.0056 2383.91 -43739.3830  0:14
```

Here the second acceptance proportion, at 0.00, is much too small, which means that the

corresponding step-length (0.02 in the control file) is much too large. Terminate the run (Ctrl-C) and decrease the value in the control file. Then run the program again (use the up ↑ and down ↓ arrow keys to retrieve past commands). Repeat this process a few times until every acceptance proportion is neither too small nor too large. In this example, changing 0.02 to 0.002 brings the acceptance proportion to ~24%, which is slightly too small but already good enough.

Those MCMC proposals are used in all four analyses (A00, A01, A10, A11), so that the description here applies to all of them. Note that the finetune parameters affect the efficiency of the MCMC or how fast one can obtain reliable results. In theory they do not change the results if all runs using different finetune parameters are long enough to generate reliable results.

3.4 A01: species tree estimation

Suppose we use the control file bpp.4s.ctl to run analysis A01: species tree estimation with species delimitation and assignment fixed (speciesdelimitation = 0, speciestree = 1).

The screen output will look like this:

```
  5% 0.28 0.28 0.28 0.35 0.29  0.0000  0.0033 0.0138 2403.43 -43721.0651  0:13
 10% 0.27 0.27 0.27 0.36 0.30  0.0000  0.0028 0.0142 2304.08 -43723.0804  0:21
 15% 0.26 0.27 0.26 0.36 0.30  0.0000  0.0025 0.0143 2308.11 -43723.8564  0:27
    ^^ Pjump for MCMC moves ^^  PSPR   theta  tau     lnpG    E(lnL)
```

Here the five Pjump values are the acceptance proportions for the five conventional MCMC moves, as discussed above. PSPR is the acceptance proportion for the SPR/Nodeslider moves that change the species phylogeny (Rannala and Yang, 2017). The next two numbers are posterior means of θ for the root population (-1 is printed if θs are integrated out) and τ_0 for the root age. The last two numbers are the log MSC gene-tree density (Rannala and Yang, 2003) and the average log sequence likelihood (Felsenstein, 1981).

The MCMC sample of species trees is collected in the file mcmc.txt. Below are two lines from that file. The numbers after : are the branch lengths (τs), while those after # are θs. I hope to change the output format to be more consistent with the Newark format.

```
(((H #0.000708: 0.004728, C #0.000740: 0.004728) #0.002814: 0.001190, G #0.000574: 0.005918)
#0.004132: 0.007626, O #0.002036: 0.013544) #0.003835;
(((H #0.000708: 0.004728, C #0.000740: 0.004728) #0.002814: 0.001190, G #0.000574: 0.005918)
#0.003740: 0.007351, O #0.002786: 0.013268) #0.003506;
```

The BPP summary of the sample will look like the following.

```
Read tree sample, count trees & splits
tree 20000  (((H, C), G), O);
 20001 trees read, 1 distinct trees.

Species in order:
 1. H
 2. C
 3. G
 4. O

(A) Best trees in the sample (1 distinct trees in all)
  20001  1.00000  1.00000  (O, (G, (H, C)));

(B) Best splits in the sample of trees (2 splits in all)
  20001   1.00000  1100
  20001   1.00000  1110
```

```
(C) Majority-rule consensus tree
(O, (G, (H, C) #1.000000) #1.000000);

(D) Best tree (or trees from the mastertree file) with support values
(O, (G, (H, C) #1.000000) #1.000000);  [P = 1.00000]
```

Section (A) lists the species trees in decreasing order of posterior probabilities. From this you can easily identify the 95% or 99% credibility set of species trees. Section (B) lists the splits (or bipartitions) and their posterior probabilities. The splits here take into account the location of the root, and may be different from the splits for unrooted trees. Section (C) prints the majority-rule consensus tree, with posterior probabilities for nodes.

3.3 A10: species delimitation using rjMCMC

We apply rjMCMC algorithm to the frogs data (A10: speciesdelimitation = 1, speciestree = 0).

```
cd frogs\r1
..\..\bin\bpp ..\A10.bpp.ctl
```

The screen output will look like the following. The species delimitation models that can be generated from the fixed guide tree are listed, together with their prior probabilities calculated by BPP. (As a check, if you use usedata = 0, the MCMC should be sampling from this prior distribution.) The species delimitation model is represented using four 0-1flags for the four interior nodes 6, 7, 8, 9 in the guide tree, with 0 for 'collapsed' and 1 for 'resolved'. Note that the tips in the guide tree are numbered 1, 2, …, s for s potential species, while the interior (ancestral) nodes are numbered $s + 1$, $s + 2$, …, $2s – 1$, with $s + 1$ to be the root of the guide tree. The numbering is through a tree-traversal algorithm, fixed by the program. This same order is used to specify the divergence time parameters (τs), so you can work out the order by looking at the list of nodes in the screen output (look at the "population by population table", "# species divergence times in the order:", etc.).

```
Number of species-delimitation models =  5
        delimitation model   1: 000  prior  0.20000
        delimitation model   2: 100  prior  0.20000
        delimitation model   3: 101  prior  0.20000
        delimitation model   4: 110  prior  0.20000
        delimitation model   5: 111  prior  0.20000

[Note: Ancestral nodes in order:   5 KCLH  6 KC  7 LH]

MCMC settings: 8000 burnin, sampling every 2, 100000 samples
Approximating posterior, using sequence data
(Settings: cleandata=1 print=1 saveconP=1 moveinnode=1)

Starting rjMCMC...
PrSplit = 0.500000
rj algorithm 1: new theta from G(a=2.00, m=1.00)

Starting species-delimitation model: 111

root dist = 0.00095

Initial parameters, np = 3.
Genetrees generated from the MSC density.
  0.00108  0.00074  0.00075
lnpG0 =  752.4533  lnL0 = -3402.2670
 -3% 0.70 0.13 0.00 0.20 0.36   3 0.0198 111 P[5]=0.8750  -1.0000 0.0007  787.23 -3127.1399
(nsteps = 5)
Current Pjump:      0.69618  0.13189  0.00000  0.19758  0.35650
Current finetune:   5.00000  0.00100  0.00100  0.00100  0.30000
New     finetune:  18.97702  0.00041  0.00001  0.00063  0.36913

 -2% 0.70 0.30 0.00 0.19 0.27   3 0.0209 111 P[5]=0.8575  -1.0000 0.0007  775.05 -3125.7744
(nsteps = 5)
Current Pjump:      0.69565  0.29861  0.00000  0.18742  0.27050
Current finetune:  18.97702  0.00041  0.00001  0.00063  0.36913
New     finetune:  71.87933  0.00041  0.00000  0.00037  0.32779
```

```
 -1% 0.70 0.31 0.00 0.20 0.33   3 0.0126 111 P[5]=0.8990  -1.0000 0.0007  732.14 -3126.0675
(nsteps = 5)
Current Pjump:      0.69528  0.30537  0.00000  0.20442  0.33250
Current finetune:  71.87933  0.00041  0.00000  0.00037  0.32779
New     finetune:  99.00000  0.00042  0.00000  0.00024  0.37030

  0% 0.69 0.30 0.00 0.23 0.26   3 0.0165 111 P[5]=0.9215  -1.0000 0.0007  723.36 -3125.7568  0:11

(nsteps = 5)
Current Pjump:      0.69354  0.29812  0.00000  0.23367  0.26400
Current finetune:  99.00000  0.00042  0.00000  0.00024  0.37030
New     finetune:  99.00000  0.00042  0.00000  0.00018  0.31993

  5% 0.70 0.30 0.00 0.29 0.34   2 0.0260 110 P[5]=0.8731  -1.0000 0.0007  773.34 -3125.8363  0:25
 10% 0.70 0.30 0.00 0.30 0.34   3 0.0234 111 P[5]=0.8778  -1.0000 0.0007  745.39 -3125.8263  0:39
 15% 0.70 0.30 0.00 0.30 0.34   2 0.0225 110 P[5]=0.8879  -1.0000 0.0007  762.56 -3125.9276  0:53

   ^^ Pjump for MCMC moves ^^       Prj      P[model 5]     theta    tau   lnpG     E(lnL)
```

The starting species delimitation model is generated by choosing at random one of the models defined by the guide tree or starting tree.

After the chain has started, the five ratios after the % sign are the acceptance proportions for the conventional MCMC moves discussed above.

Next there are three numbers related to the rjMCMC move, highlighted in red above. The rest of the line shows the posterior mean for θ for the root and posterior mean for τ_0 (which exist in all species-tree or species delimitation models), the current log MSC density and average log sequence likelihood

The three numbers related to the rjMCMC move, "`2 0.0225 110`" in the example, mean that the current model is 110 (using the flags of fig. 1 in Yang and Rannala, 2010), and the rjMCMC move has the acceptance proportion 0.0225. [What does the number 2 mean?] In general the larger this proportion, the more efficient the rjMCMC algorithm is. However there is no optimal acceptance proportion for the rjMCMC move, and a value close to 0 may not necessarily mean a problem. If one model has posterior probability close to 1, the acceptance proportion should be near 0 as well. Thus both poor mixing of the rjMCMC algorithm and extreme posterior model probabilities can cause the acceptance proportion for the rjMCMC to be close to 0. It has been noted that if the rjMCMC algorithm is suffering from poor mixing, different starting species trees often lead to different results.

Next the posterior probability for the best species-tree model (the most frequently visited tree model up to now) is printed. In the example, "`P[5]=0.8879`" means tree model 5 (111) is the most favoured model, with the posterior at 0.8879.

After the MCMC is finished, the program will summarize the sample. The output looks like the following. The seven delimitation models are listed again, together with their posterior and prior probabilities. The "Guide tree with posterior probability for presence of nodes" can be copied into TreeView.

```
Summarizing the species-delimitation sample in file mcmc.txt

Number of species-delimitation models =  5
      model    prior  posterior
    1  000   0.20000   0.00000
    2  100   0.20000   0.00000
    3  101   0.20000   0.00038
    4  110   0.20000   0.10019
    5  111   0.20000   0.89943

[Note: Ancestral nodes in order:   5 KCLH  6 KC  7 LH]

Guide tree with posterior probability for presence of nodes
((K, C) #0.999620, (L, H) #0.899810) #1.000000;
```

The MCMC sample file mcmc.txt. As the number of parameters changes when the

rjMCMC moves between models, the mcmc sample file may not be very useful, so you can ignore it. Right now the header line is generated using the starting species tree and should be ignored. After the header line, each line of output has the following numbers, separated by TABs: iteration number, the number of parameters, the tree, the sampled parameter values, and lnL.

```
Gen     np      tree    tau_5KCLH       tau_6KC tau_7LH lnL
2       3       111     0.00079675      0.0007372       0.00065989      -3122.248
4       3       111     0.00074995      0.00067776      0.00059457      -3122.427
...
786     3       111     0.00037556      0.00010047      2.5634e-05      -3129.184
788     3       111     0.00065693      7.9848e-05      3.6489e-05      -3116.988
790     2       110     0.00044831      5.7933e-05      -3125.574
792     2       110     0.00060611      0.00029978      -3122.610
794     2       110     0.00060611      0.00048884      -3122.912
796     2       110     0.00041675      0.00027494      -3130.812
798     2       110     0.00034214      0.0002618       -3142.615
800     3       111     0.00044038      0.00033698      9.8583e-05      -3129.386
802     3       111     0.00060202      0.00047807      7.7594e-05      -3121.725
```

If you know the unix command grep, you can retrieve the lines for the same tree model to summarize the posterior for parameters in that model.

```
grep "3       111" mcmc.txt > result.Tree111.txt
```

In theory this should give you the same posterior as if you run analysis A00 with the species tree fixed at tree 111. In practice I think it is simpler that you edit the Imap file and the control file to run analysis A00.

Notes about running the rjMCMC algorithms.

- The rjMCMC algorithms for species delimitation allow the chain to move from one model to another but can have mixing problems. Make sure you get very similar results from multiple runs using Algorithm0 and Algorithm1 and you get the same results whatever the starting species tree is.
  The starting tree is chosen by the program at random and will vary among runs. Make sure that some of your runs are started with the one-species model, some from the fully resolved tree, and some from other trees in between. If you get consistent results among runs with different starting trees and using the two algorithms, you are unlikely to have a convergence or mixing problem.
  If you have a computer with multicore, you can run those different combinations or replicates in different folders at the same time.
- In medium-sized or large datasets with multiple loci, we have come across cases where the chain is stuck at the one-species model, or have difficulty moving into the one-species model. The problem is less common when there are only 1 or 2 loci. If you have a similar problem, you may start the analysis with 1 locus, then 2 loci, etc. to observe how the results change (you can do this by changing nloci in the control file as there is no need to change the sequence file).
- The program used the burnin to adjust the step lengths for the proposals in the MCMC algorithm. If the rjMCMC stays in species model 0, which does not have any τ parameter, no information is collected during the burnin about the proposals to change τ and the automatically adjusted step length for τ can be very poor.
- You probably need to evaluate the impact of the priors on θs and τ. See the note about the gamma distribution later in this document.

3.5 A11: joint species delimitation and species tree estimation

Again, we use the frogs example (A11.bpp.ctl) to illustrate joint species delimitation and species tree estimation (A11: speciesdelimitation = 1, speciestree = 1).

The screen output looks like this:

```
Initial parameters, np = 3.
Genetrees generated from the MSC density.

lnpG0 =  711.7015  lnL0 = -3350.8540
 -3% 0.70 0.13 0.00 0.22 0.35   4  3 0.0039 0.1455 P(4)=0.9965  -1.0000 0.0007  775.17 -3126.4790
 -2% 0.70 0.31 0.00 0.21 0.28   4  3 0.0100 0.1260 P(4)=0.9925  -1.0000 0.0008  745.60 -3125.8524
 -1% 0.70 0.30 0.00 0.22 0.35   4  3 0.0039 0.1000 P(4)=0.9980  -1.0000 0.0007  750.41 -3126.2339
  0% 0.70 0.30 0.00 0.23 0.25   4  3 0.0077 0.0920 P(4)=0.9845  -1.0000 0.0009  775.04 -3126.2217  0:13
  5% 0.70 0.30 0.00 0.24 0.34   4  3 0.0231 0.0967 P(4)=0.9368  -1.0000 0.0007  750.67 -3125.9098  0:30
 10% 0.70 0.30 0.00 0.24 0.34   4  3 0.0189 0.0954 P(4)=0.9474  -1.0000 0.0007  732.19 -3125.9363  0:46
     ^^ Pjump for MCMC moves ^^     S  p   Prj   PSPR  P(S=4)       theta   tau0   lnpG    E(lnL)
```

Here S is the number of species in the current model, and p is the number of parameters in the current model. Prj is acceptance proportion for the rjMCMC moves, while PNNI is acceptance proportion for the NNI or SPR moves. P(4)=0.9474 means that based on the states sampled, the number of species 4 has the highest posterior probability (compared with 1 species, 2 species, etc.) and the probability is 0.9474.
The next two numbers are posterior means of θ for the root population (-1 is printed if θs are integrated out) and τ_0 for the root age. These are followed by the log MSC gene-tree density and the average log likelihood.

The MCMC sample file mcmc.txt has lines like the following.

```
((K: 0.000359, C: 0.000359): 0.000271, (H: 0.000034, L: 0.000034): 0.000596); 4
((K: 0.000368, C: 0.000368): 0.000500, (H: 0.000000, L: 0.000000): 0.000868); 3
((K: 0.000378, C: 0.000378): 0.000505, (H: 0.000000, L: 0.000000): 0.000883); 3
```

The numbers after : are branch lengths (τs). If θs are estimated, they will be shown after the symbol #. Zero-length branches represent collapsed nodes, meaning that the descendent populations at that node belong to the same species. In the first sample above, there are four distinct species: A, B, C, and D, while in the second, there are three: A, BD, and C.

The summary of the sample by BPP has four sections, as follows.

```
Summarizing the species-tree sample in file mcmc.txt
read tree 100000  ((K, C), (H, L));
(A) List of best models (count postP #species SpeciesTree)
 16131  0.16131  0.16131   4 (K C L H)   ((L, H), (K, C));     0011 1100
 12736  0.12736  0.28867   4 (K C L H)   (K, (C, (L, H)));     0011 0111
  9552  0.09552  0.38419   4 (K C L H)   (H, (L, (K, C)));     1100 1110
  8009  0.08009  0.46428   4 (K C L H)   (L, (H, (K, C)));     1100 1101
  7901  0.07901  0.54329   4 (K C L H)   (C, (K, (L, H)));     0011 1011
  6638  0.06638  0.60967   4 (K C L H)   (K, (L, (C, H)));     0101 0111
…

(B)  5 species delimitations & their posterior probabilities
  95530   0.95530   4 (K C L H)
   3638   0.03638   3 (K C LH)
    533   0.00533   3 (KH C L)
    291   0.00291   3 (K CH L)
      8   0.00008   3 (KC L H)

(C)  8 delimited species & their posterior probabilities
  99701   0.99701  C
  99459   0.99459  K
  96362   0.96362  L
  95538   0.95538  H
   3638   0.03638  LH
    533   0.00533  KH
    291   0.00291  CH
```

```
       8    0.00008  KC

(D) Posterior probability for # of species
P[ 1] =    0.0000  prior[ 1] =  0.23810
P[ 2] =    0.0000  prior[ 2] =  0.23810
P[ 3] =    0.0447  prior[ 3] =  0.28571
P[ 4] =    0.9553  prior[ 4] =  0.23810
```

Section (A) lists the best models in the decreasing order of the posterior probabilities. Here a model is a full MSC model that species both the species delimitation and species phylogeny. From this section, one can easily construct the 95% or 99% credibility sets of models. This section is further summarized to produce sections B, C, and D. Section (B) gives the posterior probabilities for the top few delimitations. Section (C) lists the delimited species and their posterior probabilities, and section (D) lists the posterior probability for the number of species, together with the prior probabilities calculated by BPP.

4. Differences from version 3.3

i. Inverse-gamma priors on parameters. The main difference between versions 3.4 and the old version 3.3 is the change of the priors for parameters (θs and τs) from the gamma to the inverse gamma. The main reason for doing this is that one can integrate out θs analytically so that they won't be part of the state of the Markov chain. To a small extent, this reduction of the state space helps with mixing between trees and delimitation models.

ii. Diploid option for unphased sequences.
iii. Check-pointing

5. BPP4-specific features: threading and checkpointing

BPP4 can make use of available Single-Instruction-Multiple-Data (SIMD) instruction sets (also called vector instructions) available on modern processors. Currently only Intel and AMD processors, and BPP has been adapted to take advantage of the Streaming SIMD Extensions (SSE) and Advanced Vector Extensions 1 and 2 (AVX and AVX-2). BPP automatically detects and uses the best (in terms of execution speed) available instruction set on the target processor, however, one can manually specify and force an instruction set in the control file using the *arch* tag. For instance, *arch=SSE* forces BPP to use the SSE instruction set even if AVX - which has twice the register size of SSE and thus in theory yields twice the speedup - is present on the system. To completely disable vector instruction, one can use the *arch=CPU* option. The available values for the *arch* tag are 'CPU', 'SSE', 'AVX' and 'AVX2'.

BPP4 is a multi-threaded program and can be make use of multiple cores/threads on the target architecture. BPP automatically detects the number of cores on the target system and by default uses all available threads. It is possible to manually set the number of threads by providing the option 'threads = X' in the control file, where X is the requested number. To disable parallel execution use the option 'threads = 1'. [Znote: I think this is not working yet?]

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Appendix A. Marginal likelihood calculation using BFdriver 4.0

Ziheng Yang, 1 December 2016

The C program BFdriver generates control files and job subscription scripts for running MCMC (using BPP or MCMCtree) to calculate the marginal likelihood (or the Bayes factor), as described in Rannala and Yang (2016, Syst Biol).

The program takes a control file you provide (such as bpp.ctl) and generates K = 16 control files with different beta values, which are used to run bpp to sample from the different power posterior distributions. The program also generates job submission scripts and submit the jobs using qsub. All generated control files and output files are in the same current directory. The frogs dataset in the bpp release (Yang 2015 Curr Zool) is used as the example.

You need the following: a linux system with SUN grid engine managing job submission (including commonds such as qsub, qstat, qdel, etc.), and a C compiler. If you don't have this job submission system, you can use BFdriver to generate the control files and run the MCMC jobs from the command line.

(a) Compiling and running BFdriver

```
cc -o BFdriver -O3 BFdriver.c tools.c -lm
BFdriver <controlfilename> <npoints> <scriptname.sh>
BFdriver A00.ctl 16 tmp.sh
```

You may need to edit the following two lines inside BFdriver.c, and if you do, remember to compile the program after editing. Here bpp is assumed to be on your search path. You can use a full path for the executable prorgam, such as /home/gooduser/bin/bpp3.3. Also the second line is for submitting the jobs using qsub. Here the limits are set to 4G of RAM and 360 hours of running time. Check those values if necessary (and recompile).

```
fprintf(fcommand, "     echo \"bpp %s.b$I.ctl > log.b$I.txt\" > %s\n", ctlf, scriptf);
fprintf(fcommand, "     qsub -S /bin/bash -l h_vmem=4G -l tmem=4G -l h_rt=360:0:0 -cwd %s\n", scriptf);
```

(b) Running the program

Create a folder inside frogs/, say bf1:

```
mkdir frogs/bf1
cd frogs/bf1
```

Prepare a control file (A00.ctl, say) for the A00 analysis in the folder. Check that it works. This should have a fixed species tree, which is ((K, C), (L, H)). Species delimitation and species tree estimation should be turned off. The control file specifies the priors for theta, tau, and also specifies burnin, nsample, sampfreq etc. Run bpp to confirm that the control file works. Then run BDdriver as follows:

```
BFdriver A00.ctl 16 tree1.sh
```

Here A00.ctl is the control file we have prepared. K = 16 is the number of points in the Gauss-Legendre qradrature algorithm for numerical integration. You can use 8 for testing, and 16 or 32 for real calculation. tree1.sh is the temporary script file for job submission using qsub. The BFdriver command does a few things. First it reads the control file specified (A00.ctl) and creates 16 control files with names like A00.b01.ctl, ..., A00.b16.ctl. Each of those control files has the same content as A00.ctl except that one extra line is

inserted at the beginning, like the following

```
BayesFactorBeta = 0.122298 *  w=0.124629.ctl
```

This specifies the beta value when the control file is used to run bpp.

Second BFdriver creates a file named betaweights.txt, which lists the beta values and Gauss-Legendre weights. I have copied those values into an excel file in frogs/BFdriver.frogs.xls.

Third BFdriver creates a file named commands, which has the bash shell scripts for submitting the 16 jobs using qsub. You use the following to submit the jobs.

```
source commands
```

You can look at the content of tree1.sh to see the script for the last job:

```
more tree1.sh
```

which should have the content like the following:

```
bpp bpp.b16.ctl > log.b16.txt
```

You can use qstat to check the status of the 16 jobs you have submitted. When the jobs are running, they generate output files in the current folder, such as mcmc.b01.txt, out.b01.txt, and log.b01.txt (which logs the screen output). After all jobs are finished, you can use grep to extract the line with "BFbeta" from screen log (this command is at the bottom of the file commands).

```
grep BFbeta log.b*.txt
```

Then copy the ElnfX values into the excel file, and estimate the logarithm of the marginal likelihood by summing (weights * ElnfX / 2) over the 16 points.

This gives the log marginal likelihood to be –3185.93.

(c) Exercise & results

Duplicate the calculation for the alternative species tree (tree2) in the folder /bf2. Change the species tree topology to (((L, H), C), K), and use tree2.sh as the temporary job script file. Everything else should be the same as described above.

My runs gave the log marginal likelihood for tree 2 to be –3186.14. The ratio of the posterior probabilities for the two trees is then estimated to be $P_1/P_2 = \exp\{-3185.93 - (-3186.14)\} = \exp\{0.21\} = 1.24$. With BPP4.0 and the inverse gamma priors on θs and τs, the MCMC runs (A01) gave the posterior probabilities for trees 1 and 2 as 0.167 and 0.137, with the ratio 1.22. The MCMC runs and the marginal likelihood calculations seem to agree with each other. (Note that with BPP3 and the gamma priors on θs and τs, the posteriors are 0.16 and 0.13 with the ratio 1.2 (Yang 2015 fig. 4).

(d) Common errors and problems

Check the bpp control file by running bpp at the command line before submitting the jobs. Make sure that the bpp program is on your path or use a full path. You may have to edit the source file BFdriver.c and recompile.

Appendix B. The simulation program MCcoal

The program `MCcoal` can be used to simulate gene trees and sequence alignments at multiple loci under the multispecies coalescent model using a fixed species tree (Rannala and Yang, 2003). While the inference program `bpp` implements the JC model only, the simulation program allows GTR+G (and its special cases) and allows among-loci heterogeneity in the process of sequence evolution. The different loci can have different exchangeability parameters (a, b, c, d, e, f) and base compositions in the GTR model, different overall evolutionary rates, different extent of among-site rate variation (reflected in the alpha parameter for the gamma model), and different among-branch rate drifts (violation of the clock). `MCcoal` may also simulate under a continuous migration model, with a user-specified migration rate matrix between species/populations.

Look at the README.txt file for compiling the program. To run the program, type one of the following. The default control file name is `MCcoal.ctl`. Always look at the screen output to confirm that the program reads the control file correctly.

```
MCcoal
MCcoal MCcoalMigration.ctl
```

B1 Control-file options for simulating without migration

Here is a sample control file.

```
         seed = 12345
      seqfile = MySeq.txt 0 * comment out this line if you don't want seqs
     treefile = MyTree.tre  * comment out this line if you don't want trees
     Imapfile = MyImap.txt
   *   concatfile = concatenatedfile.txt  * comment out this line if you don't want
concatenated alignment
    modelparafile = modelparas.txt   * comment out this line if you don't want seqs

  species&tree = 4  A  B  C  D
                    3  2  1  1
  ((A #0.01, B #0.01) :0.01 #.01, (C, D) :0.011 #0.01) : 0.012 #0.01;
       diploid =    1  1  1  0      * 0: phased sequences, 1: diploid sequences

   loci&length = 100 1000 * number of loci & number of sites at each locus
alpha_locusrate = 50.0     * alpha for gamma for locus rate

   model = 7       * model: 0:JC69, 7:REV (GTR)
  Qrates = 0  10 5 5 5 5 10    *  1: fixed; 0: dirichlet, for TC TA TG CA CG AG
basefreqs = 0  10  10  10  10    * 0: random, Dirichlet(aT, aC, aA, aG), for base frequencies
 alpha_siterate = 0  100  20  5       * G(a, b) for alpha for sites & K for discrete gamma
    clock = 2 20   * 1: global clock; 2: independent gamma rates; 3:autocorrelated gamma rates
```

seed. Use −1 to simulate different datasets every time the program is run. If you use a positive integer the data will stay exactly the same, which is a bad idea.

seqfile, treefile, Imapfile, concatfile, modelparafile. These are names of files to be generated. If you want to simulate gene trees and not sequence files, you can comment out the line for the seqfile. concatfile will have the sequence alignment concatenated across loci. The sequence files can become very large. modelparafile records the parameter values for the GTR model, as well as the locus rate and the rates for nodes on the species tree. There is one line of output per locus.

By default `MCcoal` prints out the sequence alignment at each locus, but if you use file format 1 (`seqfile = MySeq.txt 1`), the program will print out site pattern counts instead. The file may then be smaller. This format is readable by `bpp` and `3s`, but not by other programs.

diploid. This specifies a switch for each species, with 1 meaning diploid sequences and 0 phased haploid sequences. With the current specification, that the program will simulate 6, 4, 2, 1 sequences for A B C D, and then combine every two sequences from A B C into one diploid sequence with heterozygote sites (represented using YRMKSW). If you use the same random number seed, the diploid data generated using the sampling configuration (3 2 1 1) with diploid = 1 1 1 0 should match the haploid sequences generated using the sampling configuration (6 4 2 1) with diploid = 0 0 0 0.

species&tree. This block specifies the number and names of species, the species tree, and the parameters under the multispecies coalescent model, including the species divergence times (τ) and population size parameters ($\theta = 4N\mu$). For the example above, 100 loci, each of 1000 sites, will be simulated, on the species tree ((A, B), (C, D)), with 3, 2, 1, 1 sequences for A, B, C, D, so that there are 7 sequences at each locus. The divergence time parameters (τs) are after ':' in the tree, while the population size parameters (θs) are after '#'. Thus we have 0.01 for all θs, and $\tau_{ABCD} = 0.012$, $\tau_{AB} = 0.01$, and $\tau_{CD} = 0.011$. We need θ_A and θ_B because 2 or more sequences are sampled from species A and B. Parameters θ_C and θ_D are unnecessary in this case as only one sequence is sampled from C and D: if you specify them, they will be ignored by the program. Note that both θ and τ are measured by the expected number of mutations per site, so that $\theta = 0.01$ means that two sequences sampled from the population are 1% different. For reference, the estimate for the human species is $\theta \approx 0.0006$.

model. This can take two possible values: 0 for JC69 and 7 for GTR. The next few lines are relevant for the GTR model.

Qrates specifies the exchangeability parameters in the GTR model (a, b, c, d, e, f for TC, TA, TG, CA, CG, and AG, in Yang 1994 JME 39:105-111). There are two options, either to have those rates fixed for all loci or to have them sampled at random. The first has the following format (note that the first 0-1 number is a switch):

```
Qrates = 1   2 1 1 1 1 2    *  1: fixed; for TC TA TG CA CG AG
```

The input is one integer value (0 or 1) which acts as a switch followed by six real numbers. The line above fixes the rates at $a = 2$, $b = c = d = e = 1$ and $f = 2$, so that the transition rate is twice as high as the transversion rate, and the model corresponds to K80 or HKY. Note that only the relative rates matter, because the rate matrix (Q) is scaled so that the average rate (over the base frequencies) is 1 and branch lengths are measured in the expected number of mutations/substitutions per site. Nevertheless the program expects six rate parameters in the input here.

```
Qrates = 0   10 5 5 5 5 10    *  0: dirichlet, for TC TA TG CA CG AG
```

The second option, above, is to sample a b c d e from the Dirichlet distribution for every locus. The above specifies Dir(10, 5, 5, 5, 5, 10), with parameters $\alpha_a = 10$, $\alpha_b = \alpha_c = \alpha_d = \alpha_e = 10$, and $\alpha_f = 20$. The rates generated from the Dirichlet sum to 1, and they are rescaled. Note that larger values for those parameters mean less variance, while the mean for a, say, is given by α_a/α with $\alpha = \alpha_a + \alpha_b + \alpha_c + \alpha_d + \alpha_e + \alpha_f$. The specification here gives on average a transition/transversion rate ratio of 2 (if the base frequencies are fixed at ¼ each), but it varies among loci.

basefreqs. The base frequency parameters (π_T, π_C, π_A, π_G) can also be fixed or sampled from the Dirichlet distribution, like the Qrates. The two options are as follows.

```
basefreqs = 1 0.15 0.35 0.15 0.35    * 1: fixed; Base frequencies are for TCAG.
basefreqs = 0   10   10   10   10     * 0: random, Dirichlet(aT, aC, aA, aG), for base frequencies
```

alpha_siterate. The gamma shape parameter (α) for rate variation among sites of the same locus can be fixed or sampled from a gamma distribution. The possible options are as follows. When alpha is fixed, the same value is used for all loci. Otherwise different αs are used for different loci. Note that given alpha, the rates for sites at the same locus have a

G(α, α) distribution with mean 1 (Yang, 1993, 1994). The fourth option below will allow the program to sample α from G(100, 20), with mean 5, for each locus and then use G(α, α) to describe the among-site rate variation for the locus.

```
alpha_siterate = 1 0           * 1: alpha fixed at 0(inf), one rate for all sites at the locus
alpha_siterate = 1  5.6 0      * 1: alpha fixed at 5.6, K = 0(inf) for continuous gamma
alpha_siterate = 1  5.6 5      * 1: alpha fixed at 5.6, K = 5 categories for discrete gamma
alpha_siterate = 0  100  20  5 * 0: alpha sampled from G(100, 20), with mean 5, K = 5 for discrete gamma
```

alpha_locusrate = 50. This is about the overall rate for the whole locus. alpha_locusrate = 0 (default) means that all loci have the same overall rate. If a positive real value is given, the overall rate for the locus is sampled from a (continuous) gamma distribution, G(50, 50) with mean 1 in the example.

clock. This is used to specify the global molecular clock or the relaxed-clock models. There are three options: 1 means the global molecular clock (the default), 2 means independent gamma rates for species-tree nodes, and 3 means autocorrelated gamma rates for species-tree nodes. The options are as follows.

```
clock = 1 * 1: global clock; 2: independent gamma rates; 3:autocorrelated gamma rates
clock = 2 20 * 2: independent gamma rates from G(20, 20) with mean 1
clock = 3 20 * 3: autocorrelated gamma rates, from G(20, 20/ancestral_rate)
```

clock 1 (global clock) is assumed if the clock variable is missing in the control file. In the case of clock 2, the rate for every species on the species tree is generated as an independent gamma variable, from G(20, 20). In the case of clock 3, the rate for the root of the species tree is generated as a gamma variable from G(20, 20). Then a recursive algorithm is used to generate the rates for the descendent nodes on the species tree given the rate for the mother species: G(20, 20/mother_rate), with the mean to be the rate of the mother node. Note that the commonly used autocorrelated rates model in relaxed clock dating programs assume the geometric Brownian motion, in which the variance of the new rate is proportional to the time of separation from the ancestral node. The autocorrelated gamma model is different, with the variance given as $20/(20/\text{mother\_rate})^2 = \text{mother\_rate}^2/20$, with a larger variance if the mother rate is high. I am not happy with either this autocorrelated gamma model or the geometric Brownian motion model, and may change this option later.

After the rates for nodes (populations) on the species tree are generated for the locus, they are used to calculate the gene-tree branch lengths. Gene-tree branches residing in the same species/populations have the same rate (the rate of that species at the locus), while those residing in different species may have different rates. A branch on a gene tree may have segments residing in different species/populations, and the length of the branch (measured by the expected number of mutations/substitutions per site) is calculated by adding up those segments.

B2 Simulating with migration

```
migration = 7   * number of pops (order fixed by program)

          A      B      C      D     ABCD     AB     CD
A         0    1.1    1.2    1.3      0      0     -1
B       0.1      0    1.4    1.5      0      0     -1
C       0.2    0.4      0    1.6      0    1.7      0
D       0.3    0.5    0.6      0      0    1.8      0
ABCD      0      0      0      0      0      0      0
AB        0      0    0.7    0.8      0      0    1.9
CD       -1     -1      0      0      0    0.9      0
```

The control file for simulating migration as well as coalescence includes a block as above (see the file MCcoalMigration.ctl). The line migration = 7, where 7 is the number of populations on

the species tree, tells the program to simulate migrations. This number is fixed by the species tree and is used here for error checking. This is then followed by a migration matrix, of size 7×7. The names of the populations are read and ignored by the program, and the order of the populations is fixed. You should run the program without the migration matrix first and then use the screen output to use the correct order of populations to specify the migration matrix.

In the migration matrix, values 0 and −1 mean that migration is either impossible (for example, if the two populations did not live at the same time) or not allowed (if the two populations were contemporary but no migration between them is assumed to occur). Use 0 for both cases here. Positive values are scaled migration rates, with the element on the ith row jth column to be $M_{ij} = N_j m_{ij}$, where m_{ij} is the migration rate per generation in population j from population i, or the proportion of individuals in population j that are immigrants from population i, and where μ is the mutation rate per site per generation. In the example above, $M_{A \to B} = 1.1$, which means that on average 1.1 individuals are immigrants from population A to population B.

Note that the θ parameter for a modern species has to be specified in the tree file even if only one sequence is sampled from that species but migrations into that species are allowed by the migration matrix.