SOFTWARE TOOL ARTICLE

The EpiFusion Analysis Framework for joint phylodynamic and epidemiological analysis of outbreak characteristics

[version 1; peer review: 2 approved, 1 approved with reservations]

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Abstract

The fields of epidemiology and viral phylodynamics share the ultimate goal of disease control, but concepts, methodologies and data employed by each differ in ways that confer complementary strengths and different areas of weakness. We recently introduced EpiFusion, a model for joint inference of outbreak characteristics using phylogenetic and case incidence data via particle filtering and demonstrated its usage to infer the effective reproduction number of simulated and real outbreaks. Here we provide a series of vignettes demonstrating data analysis using the EpiFusion Analysis Framework, consisting of the R package EpiFusionUtilities and the Java program in which the model is implemented, including an example using a new feature incorporated since EpiFusion's last description: the option to provide a phylogenetic tree posterior as the phylogenetic data input to the program. By outlining these examples, we aim to improve the usability of our model, and promote workflow reproducibility and open research.

Keywords

research software, phylodynamics, epidemiology, joint inference, effective reproduction number, infectious disease, modelling, R, java, particle filtering

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Open Peer Review

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Introduction

Implementing mathematical models of infectious disease outbreak characteristics using computational tools is an important aspect of public health research.[1,2] Tools are often distributed as packages or libraries in popular programming languages such as R, Python or Julia, or as standalone executable software.[3–6] Distributing and documenting workflows is important for the purposes of reproducible research, and to enable appropriate implementation of epidemiological models.[7–9] Previously we outlined EpiFusion, a novel method for modelling infectious disease outbreak characteristics conditioned on case incidence and phylogenetic trees using particle filtering, and validated its usage to infer infection trajectories and the effective reproduction number R_t.[10] Here, we present the EpiFusion Analysis Framework, consisting of this EpiFusion model implemented as a Java command line tool, and the EpiFusionUtilities R package for data and output processing.

EpiFusion consists of a 'single process model, dual observation model' particle filtering structure, where particles simulate outbreak trajectories and characteristics through time (process model) and are evaluated against phylodynamic and epidemiological data at resampling steps (observation models). EpiFusion uses case incidence and phylogenetic tree(s) as its data input but can also be run with either data type alone. The force of infection over time β is fit within the particle filter, and the recovery rate, case sampling rate, and genomic sequence sampling rate (γ, ϕ and ψ respectively) are fit via Markov Chain Monte Carlo (MCMC). Further information including model theory and validation are provided in Ref. 10. The EpiFusion model is packaged as a Java[11] command line tool and takes eXtensible Markup Language (XML) files[12] as input.

In this article we provide instruction on implementing the recommended workflow ('The EpiFusion Analysis Framework') using an EpiFusion model. This includes data pre-processing, parameterisation, and eventual output parsing from within an R session, using the R package EpiFusionUtilities (https://github.com/ciarajudge/EpiFusionUtilities). We also demonstrate start-to-finish use cases for complete analysis of two outbreak datasets using EpiFusion and EpiFusionUtilities.

Methods

Operation

EpiFusion is implemented as an open-source Java software (version 8 or later) and can be used as a command line tool or from within the EpiFusionUtilities R package (https://ciarajudge.github.io/EpiFusionUtilities/). The latest stable version of the program is available for download under Releases on the project Github repository (https://github.com/ciarajudge/EpiFusion/releases). The source code for the latest development version is also available at this repository for users who wish to clone the repository and compile the program from source. EpiFusion can be called using its full file path, or from any working directory on your system by creating a symbolic link *(Appendix 1).*

EpiFusionUtilities is implemented as an open source R[13] (version 3.5.0 or later) package and is available to install from Github using the R package devtools[14]:

```r
# install from Github
devtools::install_github("https://github.com/ciarajudge/EpiFusionUtilities")
```

The key steps of the EpiFusion Framework workflow are outlined below in brief in the 'Implementation' section.

Implementation

The EpiFusion Analysis Framework consists of three main steps: (i) Data Processing and Parameterisation (ii) Running EpiFusion and (iii) Parsing and Interpreting the output (Figure 1). All three steps can be carried out using `EpiFusionUtilities`, but it is also possible to manually assemble an XML input file and run `EpiFusion` from the command line using an executable jar file.

Data processing and parameterisation

EpiFusion XML

EpiFusion uses input files written in eXtensible Markup Language (XML) to provide all data and parameterisation to the program. These files contain `Loggers`, `Data`, `Analysis`, `Model`, `Parameters` and `Priors` sections where various aspects of the model and analysis may be specified (Table 1). A full breakdown of the options available within each section is included in the Supplementary Information *(Appendix 2).*

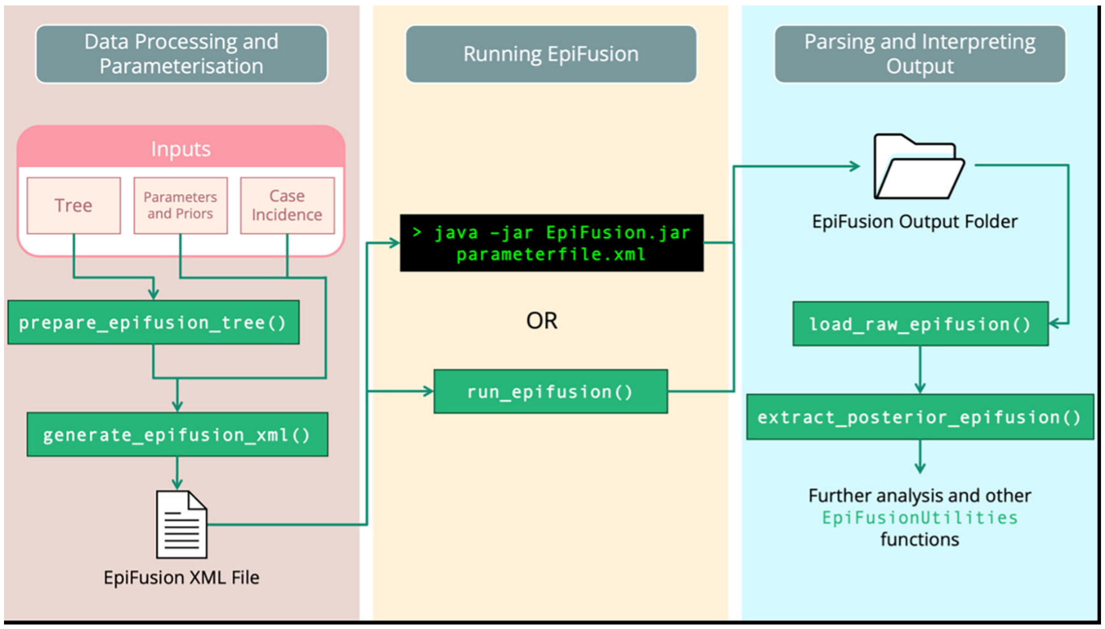

Data Processing and Parameterisation
Running EpiFusion
Parsing and Interpreting Output
Inputs
Tree
Parameters and Priors
Case Incidence
prepare_epifusion_tree()
generate_epifusion_xml()
EpiFusion XML File
> java -jar EpiFusion.jar parameterfile.xml
OR
run_epifusion()
EpiFusion Output Folder
load_raw_epifusion()
extract_posterior_epifusion()
Further analysis and other EpiFusionUtilities functions

Figure 1. Recommended EpiFusion Framework workflow, using EpiFusionUtilities functions (green) to prepare data and parse results.

Table 1. Main sections of EpiFusion XML parameter file structure.

XML section	Description
Loggers	Provides detail on the program output, including specifying the file path of the output folder that should be created, and the frequency at which the program both logs the state of the MCMC to the output files and prints to the console.
Data	Provides the case incidence data and/or a phylogenetic tree or trees. These can be supplied either directly within the XML document or by providing full file paths to files containing the data)
Analysis	Parameterises the method for fitting the infectivity parameter beta - this is the daily probability per infectious individual of infecting another individual.
Model	Allows further customisation of the EpiFusion model structure. Currently, this only includes specification of the epidemiological observation model.
Parameters	Specifies many assorted parameters for the model, for example number of MCMC steps per chain, number of MCMC chains, and number of particles in the particle filter.
Priors	Prior distribution specification for parameters to be fit via particle MCMC. A range of distribution options are available, including: Normal, Truncated Normal, Poisson, Uniform, Uniform Discrete, Beta and Fixed (if a parameter should be fixed to a specific value and not inferred via MCMC).

Assembling parameter XML files

EpiFusion XML files may either be populated manually using templates available at the `EpiFusion` Github repository under examples, or created using a number of functions in the `EpiFusionUtilities` package.

The first step of processing case incidence or tree data for EpiFusion input is to select an 'index date'. This date should equate approximately to the 'date of origin' of the outbreak under consideration, i.e. the suspected date of the first infection/importation to the geographical or demographic system under study (day '0'). The index date should fall before any birth events (internal nodes / branching events) in the tree or observed epidemiological cases. This date is provided to the processing functions to enable the case and incidence data to be rooted in numerical time units. All trajectory samples will assume that the outbreak originated with one individual becoming infected on the index date. All times in the EpiFusion input and output will be in relation to this date and measured in days.

```
index_date <- as.Date("2024-01-01")
```

If there is uncertainty in the date of origin of the outbreak we recommend setting the index date to earlier than the estimated date. The resulting trajectories will likely demonstrate high uncertainty during the earliest days of the outbreak, until the point of the time series at which some data becomes available.

To prepare a tree or posterior set of trees for EpiFusion, pass an S3 phylo or multiPhylo object in R to the `prepare_epifusion_tree()` function (we recommend any standard phylogenetics R package to manipulate these objects[15,16]). This function processes a phylogenetic tree (or trees) and writes to a file, which you can specify in the arguments of the function (the default is './processedtree.tree'). It is also necessary to pass the date of the last sample in the tree(s). This function adds node and leaf labels to the tree string that correspond to their time in days after the index date.

```
prepare_epifusion_tree(tree,
                       index_date,
                       last_sequence_date,
                       "Data/Processed/processed_fixed_tree.tree")
```

To generate an EpiFusion XML file from within an R session, the `generate_epifusion_XML()` function may be used. This function populates a template XML file (included with the `EpiFusionUtilities` package) with the phylogenetic and/or case incidence data, and has default settings for parameters and priors which can also be changed by providing new values in the arguments of the function. For example below, we pass a case incidence data frame (consisting of two columns: a `Date` column named `Date`, with a numeric column `Cases` consisting of the number of cases reported on the corresponding date) and tree to the function. We also specify that we will sample from the MCMC chain every 100 steps, set our output folder path to `output_files`, and adjust the number of particles in the particle filter to 300. This creates a file in our working directory, `epifusion_input.xml`, which is ready to pass to EpiFusion.

```
logger_information <- list(fileBase = "output_files", logEvery = 100)
parameters_to_adjust <- list(numParticles = 300)

generate_epifusion_XML(tree = "Data/Processed/processed_fixed_tree.tree",
                       case_incidence = case_incidence,
                       index_date = index_date,
                       loggers = logger_information,
                       parameters = parameters_to_adjust,
                       xml_filepath = "epifusion_input.xml")
```

We include guidance on parameterisation and setting reasonable parameters and priors in the Supplementary Information alongside our more detailed description of EpiFusion XML *(Appendix 2).*

Running EpiFusion

EpiFusion can be run directly from the command line by calling an executable Java Archive (JAR) file using the following syntax. Here `EpiFusion.jar` is the file path to the executable file (i.e. in this example, the file is present in the working directory) and `epifusion_input.xml` is the file path to the parameter XML file (also present in the working directory for this example):

```
java -jar EpiFusion.jar epifusion_input.xml
```

Alternatively, it is possible to run EpiFusion from inside an R session with the EpiFusionUtilities function `run_epifusion()`. An installation of Java is still required.

```
run_epifusion("epifusion_input.xml")
```

Interpreting output

EpiFusion creates a directory within the working directory that corresponds to the file path of the `fileBase` parameter in your EpiFusion xml file. For each MCMC chain, EpiFusion will create the following output files:

- **betas**: .csv file where each row is a daily trajectory of infectivity β sampled from the MCMC
- **trajectories:** .csv file where each row is a daily trajectory sampled from the MCMC of the number of individuals infected over time
- **params:** .txt file where each column is an MCMC parameter, and each row is an MCMC sample
- **likelihoods:** .txt file of the posterior likelihoods calculated at each MCMC step
- **acceptance:** .txt file where each line logs the acceptance rate of steps between MCMC samples
- **completed:** .txt file where each line logs if the particle filter step was completed or quit due to particle depletion
- **cuminfections:** .txt file where each row is a trajectory of cumulative infections per day sampled from the MCMC
- **positivetests (only for combined or epi-only analyses):** .csv file where each row is simulated case incidence by the model which was compared to the observed case incidence. This is provided at the same resolution as the observed case incidence, i.e. there will be one column per case incidence data point.

EpiFusion will also save a copy of the parameter file used to the output folder, to record which parameters were used, and a file called 'timings.txt' with the runtime in nanoseconds.

It is possible to process this raw output manually, but `EpiFusionUtilities` provides a number of functions to do this from within R. The following functions load the raw output into an R object, plot the likelihood trace for each MCMC chain to enable inspection to decide what proportion of samples from each chain to discard as burn-in, and finally extract the posterior samples from each chain with a given proportion discarded and combine them into a single posterior while assessing convergence. To select an appropriate burn-in proportion, we recommend inspecting the likelihood and parameter trace plots to assess the point at which samples along the x-axis form a stationary distribution randomly sampled from the same region of the y-axis.[17] It is also possible to test multiple extractions `extract_posterior_epifusion()` with different burn-in proportions, and inspect the gelman-rubin statistics of the parameters, which indicate convergence if below 1.015.[18]

```
raw_output <- load_raw_epifusion("output_files/")
plot_likelihood_trace(raw_output)
full_posterior <- extract_posterior_epifusion(raw_output, 0.1)
```

Below we demonstrate the implementation of this workflow using the `EpiFusion` executable and `EpiFusionUtilities` to analyse data from a small simulated outbreak.

Use cases

Description of the simulated datasets

We demonstrate three analyses on two simulated outbreak datasets (Figures 2, 3). First we address a simple outbreak that lasts approximately three months with constant sampling effort that captures a single epidemic peak ('baseline'). In the subsection 'Full Framework Workflow' we will model this outbreak using case incidence data in conjunction with a fixed time-scaled phylogenetic tree. Next, in 'Phylogenetic Uncertainty' we will model the same outbreak whilst examining the effect of phylogenetic uncertainty by using a tree posterior generated using a BEAST analysis of genomic samples simulated from the outbreak. Finally, in 'Introducing Rate Changes', we examine an outbreak with similar transmission dynamics but where initial minimal sampling of cases and sequences is followed by a sharp increase in the sampling rate on February 5th 2024, and demonstrate how to parameterise this in an EpiFusion model. This example attempts to capture the challenges that often accompany modelling real-world outbreaks, where circumstances may evolve as an outbreak progresses (e.g. changes in case definitions affecting sampling rates, or upscaling of PCR diagnostics in response to an emerging infectious disease).

To generate the data, outbreak trajectories, and resulting weekly case incidence and a transmission tree of cases were simulated using ReMaster.[19] To give a simulated phylogenetic tree of 'sequenced samples' from the outbreak the transmission trees were downsampled, as typically only a small proportion of cases are sequenced in even heavily

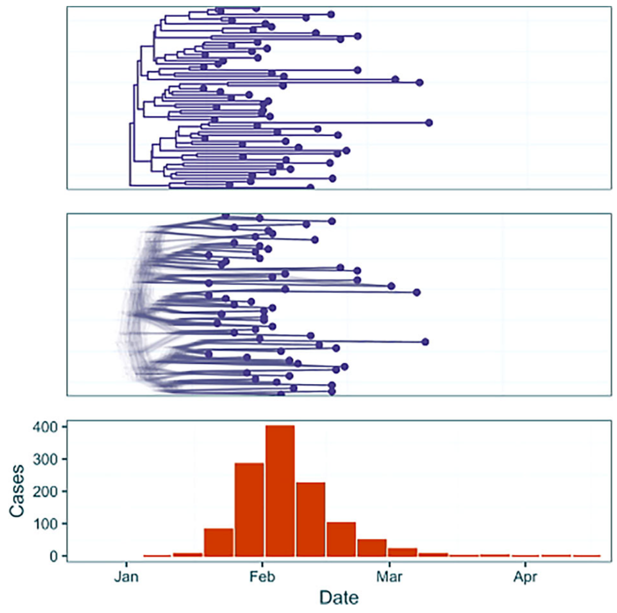


Figure 2. Data from the baseline outbreak simulated using ReMaster and BEAST 2.7.3. The dataset contains both a summary phylogenetic tree (top) and tree posterior samples (middle), and weekly case incidence (orange).

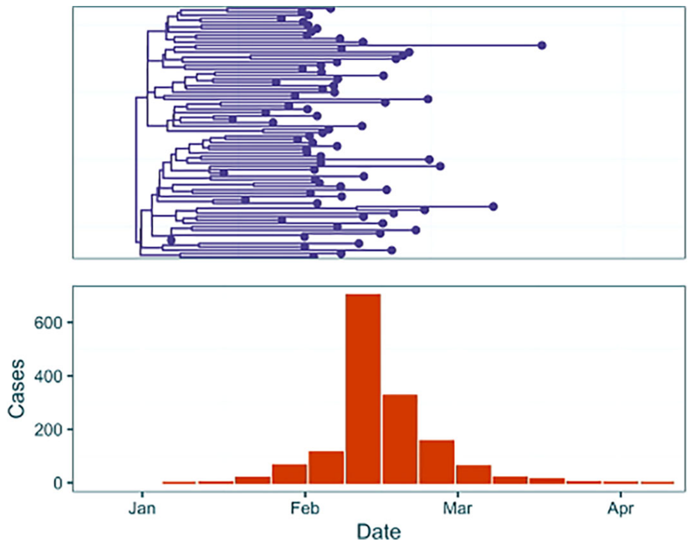


Figure 3. Data from outbreak with a step-change increase in sampling simulated using ReMaster.

sampled outbreak settings.[20,21] For the baseline outbreak genomic sequences were simulated in R from this phylogenetic tree using the function simSeq() from the R package phangorn.[16] These sequences were used to generate a tree posterior using BEAST 2.7.3[22] with a Birth Death Skyline model,[23] under a strict clock and JC69 substitution model.

The date of origin of each outbreak was arbitrarily chosen as January 1st 2024 and the case data and tree leaves were labelled accordingly. The final resulting data inputs for analysis in EpiFusion consisted of a file with a fixed time-scaled phylogenetic tree, a tree posterior file generated from sequences simulated from the outbreak, and a csv file with dated counts of weekly incidence. These raw data files are provided alongside the code below in the article repository (https://github.com/ciarajudge/EpiFusion_Vignettes). The data is also provided directly as part of the `EpiFusionUtilities` package, and can be loaded directly into R using the functions `baseline_dataset` and `sampling_dataset`.

Full framework workflow

In this example, we will use the baseline dataset to show a full workflow using the EpiFusion Framework: (i) data preparation (ii) prior and parameter specification (iii) running EpiFusion (iv) parsing and plotting output.

Data preparation

First we load and inspect the data for this example using the `EpiFusionUtilities` function `baseline_dataset()`. This function loads a data frame with weekly case incidence (formatted with two columns, Cases and Date, where Cases = the number of epidemiological cases reported on the corresponding Date), a time-scaled phylogenetic tree, and samples from a tree posterior (with 50% burn-in removed) from a BEAST analysis which we will use in a later section (Phylogenetic Uncertainty).

```
baseline_dataset()

print(baseline_caseincidence[1:5,])
##   Cases       Date
## 1     0 2024-01-08
## 2     6 2024-01-15
## 3    83 2024-01-22
## 4   285 2024-01-29
## 5   401 2024-02-05
print(baseline_tree)
##
## Phylogenetic tree with 59 tips and 58 internal nodes.
##
## Tip labels:
## sequence1|2024-01-25, sequence2|2024-01-30, sequence3|2024-02-12, sequence4|
2024-01-30, sequence5|2024-02-04, sequence6|2024-02-17, …
## Node labels:
## node_1, node_4, node_14, node_97, node_106, node_158, …
##
## Rooted; includes branch lengths.
print(baseline_treeposterior)
## 200 phylogenetic trees
```

Next we set two date objects: the ‘index date’, or the earliest date from which we will model the outbreak origin date, and the date of sampling of the last observed sequence from the dataset. Whilst for this example we know (through the simulation process) that the outbreak origin was the 1st of January 2024, it is good practice to set the index date to some time before the date that we suspect the outbreak began in the location represented by our case and phylogenetic data, to ensure the outbreak dynamics of are fully captured.

```
index_date <- as.Date("2023-12-26")
last_sequence <- as.Date("2024-03-10")
```

To prepare the tree objects for EpiFusion we can use the `prepare_epifusion_tree` function from `EpiFusionUtilities`. This function processes the tree(s) for input to EpiFusion and writes them to the provided file path. In the case where a single summary tree is provided to this function it also returns the processed tree as an R phylo object, which here we reassign to the variable `fixed_tree`.

```
fixed_tree <- prepare_epifusion_tree(baseline_tree, index_date, last_sequence,
"Data/Processed/baseline_fixed_tree.tree")
```

Definition of parameters

We will create an EpiFusion XML file using the `generate_epifusion_xml` function from `EpiFusionUtilities`. This function populates the below XML template with our data and creates a new file.

It is often necessary to adjust some other parameters from their default values in this template. This can be achieved by providing additional arguments to the `generate_epifusion_xml` function, which we demonstrate below.

```
<?xml version="1.0" encoding="UTF-8"?>
<EpiFusionInputs>
  <loggers>
    <fileBase>FILESTEM</fileBase>
    <logEvery>10</logEvery>
  </loggers>
  <data>
    <incidence>
      <incidenceVals>INCIDENCE</incidenceVals>
      <incidenceTimes type="exact">INCIDENCETIMES</incidenceTimes>
   </incidence>
   <tree>
     <treePosterior></treePosterior>
   </tree>
   <epicontrib>0.5</epicontrib>
   <changetimes>0</changetimes>
  </data>
  <analysis>
    <type>looseformbeta</type>
    <startTime>null</startTime>
    <endTime>null</endTime>
    <inferTimeOfIntroduction>false</inferTimeOfIntroduction>
  </analysis>
  <model>
    <epiObservationModel>poisson</epiObservationModel>
  </model>
  <parameters>
    <epiOnly>false</epiOnly>
    <phyloOnly>false</phyloOnly>
    <numParticles>200</numParticles>
    <numSteps>2000</numSteps>
    <numThreads>8</numThreads>
    <numChains>4</numChains>
    <stepCoefficient>0.05</stepCoefficient>
    <resampleEvery>7</resampleEvery>
    <segmentedDays>true</segmentedDays>
    <samplingsAsRemovals>1</samplingsAsRemovals>
    <pairedPsi>false</pairedPsi>
  </parameters>
  <priors>
    <gamma>
      <stepchange>false</stepchange>
      <disttype>TruncatedNormal</disttype>
      <mean>0.15</mean>
      <standarddev>0.05</standarddev>
      <lowerbound>0.0</lowerbound>
   </gamma>
   <psi>
     <stepchange>false</stepchange>
     <disttype>TruncatedNormal</disttype>
     <mean>0.001</mean>
     <standarddev>0.0005</standarddev>
     <lowerbound>0.0</lowerbound>
   </psi>
   <phi>
```

```
      <stepchange>false</stepchange>
      <disttype>TruncatedNormal</disttype>
      <mean>0.02</mean>
      <standarddev>0.01</standarddev>
      <lowerbound>0.0</lowerbound>
    </phi>
    <initialBeta>
      <stepchange>false</stepchange>
      <disttype>Uniform</disttype>
      <min>0.3</min>
      <max>0.8</max>
    </initialBeta>
    <betaJitter>
      <stepchange>false</stepchange>
      <disttype>Uniform</disttype>
      <min>0.001</min>
      <max>0.05</max>
    </betaJitter>
  </priors>
</EpiFusionInputs>
```

We will generate an EpiFusion XML using the summary tree we prepared with the `prepare_epifusion_tree` function and our loaded case incidence data. First we will make lists of the various parts of the XML file we wish to override from the default. For example, the below code represents the `loggers` chunk in the default XML that details how often we sample from the MCMC (every 10 MCMC steps):

```
  <loggers>
    <fileBase>FILESTEM</fileBase>
    <logEvery>10</logEvery>
  </loggers>
```

To override this, we will make a list in R that we will later pass to the `loggers` argument of the `generate_epifusion_xml` function to specify our output folder filepath as `Results/fixed_tree` and sample from the MCMC chain every 10 steps. We will also make a `parameters` list to adjust the number of MCMC steps, thus run each chain for longer to ensure we get a satisfactory number of samples from the posterior. We will reduce the number of particles in the particle filter to 100, as this is sufficient for a short, simple analysis, and will slightly reduce runtime.

```
loggers <- list(fileBase = "Results/baseline_fixed_tree", logEvery = 10)
parameters <- list(numSteps = 10000,
                   numParticles = 100)
```

We will also slightly adjust the prior for `initialBeta`, or β_0 (infectivity at the beginning of the time series) from the default settings. As the default prior for γ is a truncated normal distribution with mean 0.15, standard deviation 0.05 and lower bound 0.0, by setting the initial β value as $0.1 < \beta < 0.5$ we indicate that the initial R_t is approximately between 0.66 and 3.33 ($R_t = \beta/\gamma$, i.e. $0.1/0.15 = 0.66$ and $0.5/0.15 = 3.33$).

```
priors <- list(initialBeta = list(stepchange = "false",
                                  disttype = "Uniform",
                                  min = 0.1,
                                  max = 0.5))
```

In this example we are happy with the other parameters in the default XML, so we can generate the XML file Data/EpiFusion_XMLs/fixed_tree_inputfile.xml with the following code:

```
generate_epifusion_XML(tree = "Data/Processed/baseline_fixed_tree.tree",
                       case_incidence = baseline_caseincidence,
                       index_date = index_date,
                       loggers = loggers,
                       priors = priors,
                       parameters = parameters,
                       xml_filepath = "Data/EpiFusion_XMLs/baseline_fixed_tree_
                       inputfile.xml")
```

Running EpiFusion

To run EpiFusion for the fixed tree example, we will use the run_epifusion function from EpiFusionUtilities to run the program within our R session:

```
run_epifusion("Data/EpiFusion_XMLs/baseline_fixed_tree_inputfile.xml")
```

On conclusion of its analysis, EpiFusion saves a timings.txt file to the output folder with the total runtime in nanoseconds, which we examine and convert to minutes below:

```
runtime <- suppressWarnings(read.table("Results/baseline_fixed_tree/timings.txt")
[1,1]) / 6e10
paste0("Runtime: ",runtime," minutes")
## [1] "Runtime: 10.5715153277833 minutes"
```

Parsing and plotting the output

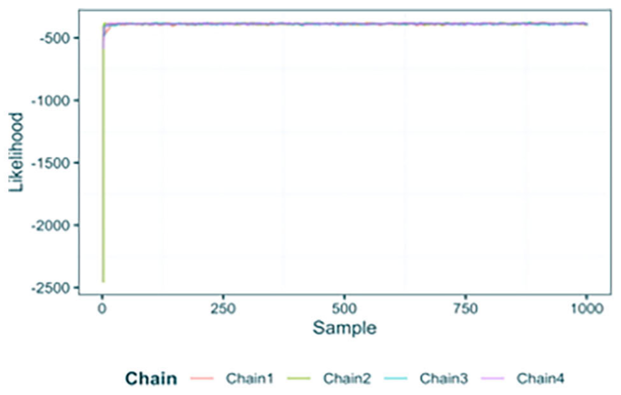
First we will use the load_raw_epifusion function to import the full raw results. This function automatically produces plots (Figure 4) of the likelihood and parameter traces using the plot_likelihood_trace and plot_parameter_trace functions. This allows us to check for convergence and help to identify what proportion of each chain to discard as burn-in.

```
raw_output_fixed <- load_raw_epifusion("Results/baseline_fixed_tree/")
```

Next we can discard the burn-in from each MCMC chain and combine all chains into a combined posterior using the extract_posterior_epifusion function which takes a raw EpiFusion object and the proportion of each chain to discard as burn-in as its arguments. By default, the function returns means and Highest Posterior Density (HPD) intervals for the trajectories and parameters fitted by EpiFusion, however by specifying include_samples = TRUE we also instruct the function to return the actual posterior samples (minus burn-in) for inspection. This greatly increases the memory used by the posterior output object in your R environment, so is recommended for initial inspection of your results but not for downstream tasks such as loading posteriors from many analyses for plotting.

```
parsed_output_fixed <- extract_posterior_epifusion(raw_output_fixed, 0.2,
include_samples = TRUE)
str(parsed_output_fixed, max.level = 2)
## List of 5
##  $ infection_trajectories : List of 4
##  ..$ mean_infection_trajectory   :  Named num [1:113] 0 1.22 1.54 1.73 1.96 …
##  .. ..- attr(*, "names")= chr [1:113] "T_0" "T_1" "T_2" "T_3" …
##  ..$ median_infection_trajectory : Named num [1:113]  0 1 1 1 2 2 2 5 6 …
##  .. ..- attr(*, "names")= chr [1:113] "T_0" "T_1" "T_2" "T_3" …
##  ..$ infection_trajectory_hpdintervals : List of 3
##  ..$ infection_trajectory_samples :'data.frame': 3208 obs. of 113 variables:
```

a



b

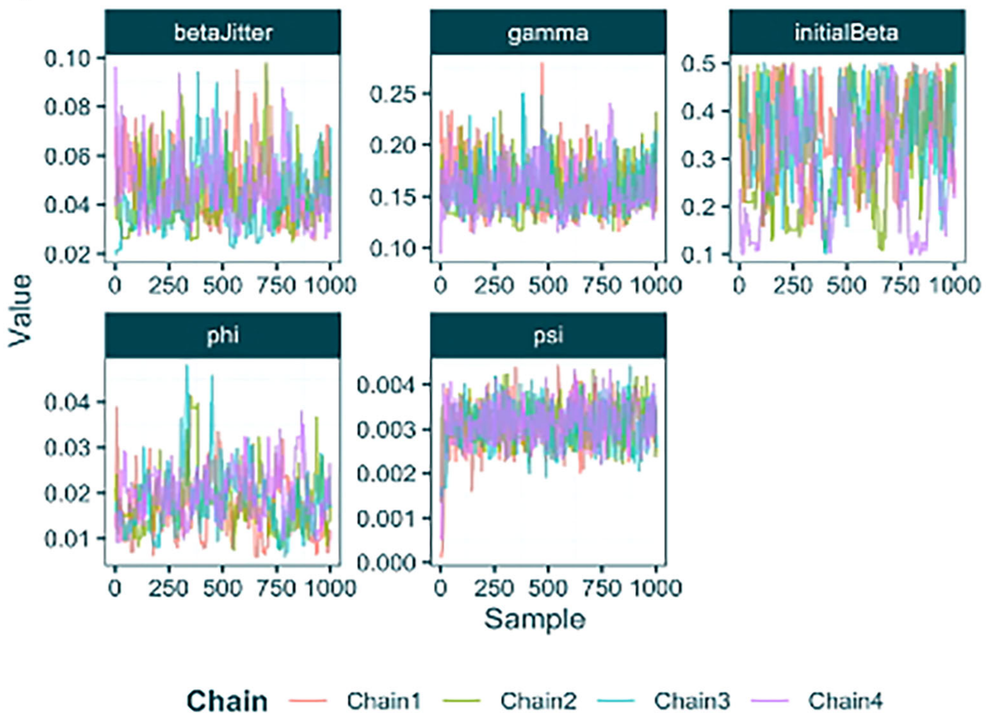


Figure 4. (a) Likelihood and trace plot from an EpiFusion analysis produced by the 'plot_likelihood_trace' function in EpiFusionUtilities. (b) Parameter trace plots from an EpiFusion analysis produced by the 'plot_parameter_trace' function in EpiFusionUtilities.

```
## $ rt_trajectories : List of 4
##  ..$ mean_rt_trajectory    :  Named num [1:113] 2.09 2.14 2.18 2.25 2.32 …
##  .. ..- attr(*, "names")= chr [1:113] "T_0" "T_1" "T_2" "T_3" …
##  ..$ median_rt_trajectory  :  Named num [1:113] 2.13 2.16 2.2 2.24 2.32 …
##  .. ..- attr(*, "names")= chr [1:113] "T_0" "T_1" "T_2" "T_3" …
##  ..$ rt_trajectory_hpdintervals : List of 3
##  ..$ rt_trajectory_samples :'data.frame': 3208 obs. of 113 variables:
## $ parameters            :List of 5
##  ..$ gamma      :List of 3
##  ..$ psi        :List of 3
##  ..$ phi        :List of 3
##  ..$ betaJitter :List of 3
##  ..$ initialBeta:List of 3
## $ fitted_epi_cases : List of 4
##  ..$ mean_fitted_epi_cases : Named num [1:15] 0.251 12.306 84.751 297.153
```

```
412.487 …
##   .. ..- attr(*, "names")= chr [1:15] "T_0" "T_1" "T_2" "T_3" …
##   ..$ median_fitted_epi_cases : Named num [1:15] 0 12 84 296 412 233 109 51 23
10 …
##   .. ..- attr(*, "names")= chr [1:15] "T_0" "T_1" "T_2" "T_3" …
##   ..$ fitted_epi_cases_hpdintervals : List of 3
##   ..$ fitted_epi_cases_samples :'data.frame': 3208 obs. of 15 variables:
## $ cumulative_infections :List of 4
##  ..$ mean_cuminfection_trajectory : Named num [1:113] 0 0.219 0.542 0.961 1.44 …
##   .. ..- attr(*, "names")= chr [1:113] "T_0" "T_1" "T_2" "T_3" …
##   ..$ median_cuminfection_trajectory   : Named num [1:113] 0 0 0 1 1 2 2 3 6 9 …
##   .. ..- attr(*, "names")= chr [1:113] "T_0" "T_1" "T_2" "T_3" …
##   ..$ cuminfection_trajectory_hpdintervals : List of 3
##  ..$ cuminfection_trajectory_samples :'data.frame': 3208 obs. of 113 variables:
```

The extracted posterior object from the `extract_posterior_epifusion` function contains mean and HPD intervals of increasing width for infection, R_t, cumulative infection and fitted epidemiological case trajectories. The `trajectory_table` function can parse these into a convenient table structured to be suitable for plotting with `ggplot2`. This table is structured with a Time column for each day in the analysis, and Mean and upper and lower HPD interval (0.95, 0.88 and 0.66) columns for each trajectory type (infection, R_t, cumulative infections).

```
traj_table <- trajectory_table(parsed_output_fixed, index_date)
colnames(traj_table)
##  [1] "Time"                           "Mean_Infected"
##  [3] "Lower95_Infected"               "Upper95_Infected"
##  [5] "Lower88_Infected"               "Upper88_Infected"
##  [7] "Lower66_Infected"               "Upper66_Infected"
##  [9] "Mean_Rt"                        "Lower95_Rt"
## [11] "Upper95_Rt"                     "Lower88_Rt"
## [13] "Upper88_Rt"                     "Lower66_Rt"
## [15] "Upper66_Rt"                     "Mean_CumulativeInfections"
## [17] "Lower95_CumulativeInfections" "Upper95_CumulativeInfections"
## [19] "Lower88_CumulativeInfections" "Upper88_CumulativeInfections"
## [21] "Lower66_CumulativeInfections" "Upper66_CumulativeInfections"

#Show the first 5 columns and 3 rows of the traj_table
knitr::kable(head(traj_table[,1:5], n = 3))
```

Time	Mean_Infected	Lower95_Infected	Upper95_Infected	Lower88_Infected
2023-12-26	0.000000	0	0	0
2023-12-27	1.218828	1	2	1
2023-12-28	1.542394	1	3	1

It is possible use this table with ggplot functions to plot and inspect the inferred trajectories. However we also provide a function, `plot_trajectories` that takes the trajectory table as input and automatically plots all three trajectory types (Figure 5).

```
plot_trajectories(traj_table)
```

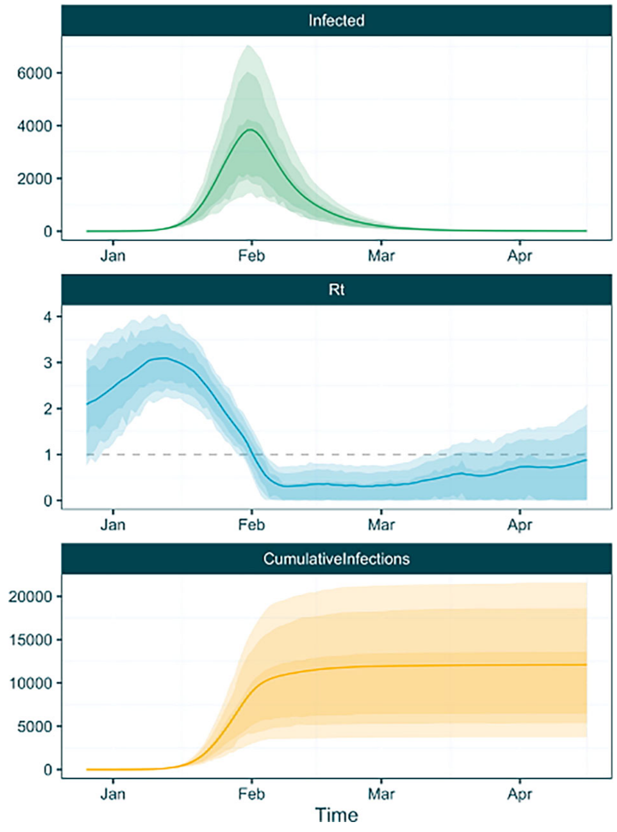


Figure 5. Infection, Rt and cumulative infection trajectories plotted by the EpiFusionUtilities function 'plot_trajectories'.

The `plot_trajectories` function also takes additional arguments to allow more customisation. For example, it is possible to provide a specific trajectory type to plot using the type argument, and specify bespoke plot colours using the `plot_colours` argument. Here we will plot only the R_t trajectories in a specified colour (pink) (Figure 6).

```
plot_trajectories(traj_table, type = "rt", plot_colours = "pink")
```

As this was a combined analysis that has used case incidence data, it is possible to examine the fit of the case incidence simulated within the model to the provided data. We already have the case incidence data loaded from the data preparation stage, so we can add the mean and HPD intervals of the fit to the existing table (Figure 7).

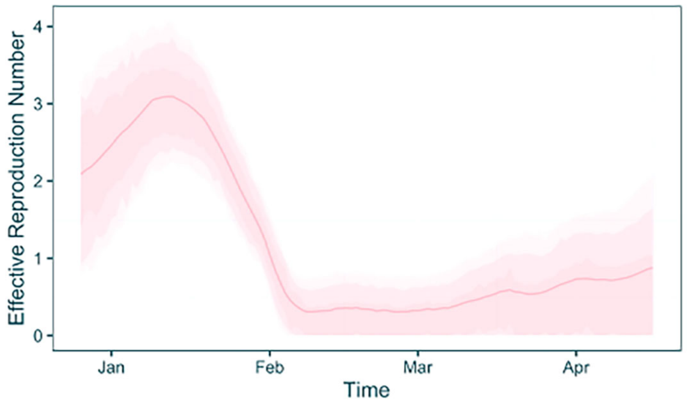


Figure 6. Inferred R(t) trajectories using a combined EpiFusion model and a fixed tree, plotted with the EpiFusionUtilities function 'plot_trajectories'.

```
epi_data_and_fit_table <- baseline_caseincidence %>%
  mutate(Stat = "Observed Cases") %>%
  full_join(data.frame(Date = baseline_caseincidence$Date,
                       Stat = "Fitted Cases",
                   Cases = parsed_output_fixed$fitted_epi_cases$
                           median_fitted_epi_cases,
                   Lower95_Cases = parsed_output_fixed$fitted_epi_cases$
fitted_epi_cases_hpdintervals$HPD0.95$Lower,
                   Upper95_Cases = parsed_output_fixed$fitted_epi_cases$
fitted_epi_cases_hpdintervals$HPD0.95$Upper
         )) %>%
  mutate(Stat = factor(Stat, levels = c("Observed Cases", "Fitted Cases")))
## Joining with ‘by = join_by(Cases, Date, Stat)’
ggplot(epi_data_and_fit_table, aes(x = Date)) +
  geom_bar(aes(y = Cases, fill = Stat), stat = "identity", position = "dodge",
col = NA, alpha = 0.7) +
  scale_fill_manual(name = "", values = c("#e95b0d", "grey")) +
  geom_errorbar(aes(ymin = Lower95_Cases, ymax = Upper95_Cases, col = Stat),
position = "dodge", show.legend = F) +
  scale_color_manual(values = c(NA, "black")) +
  lshtm_theme()
```

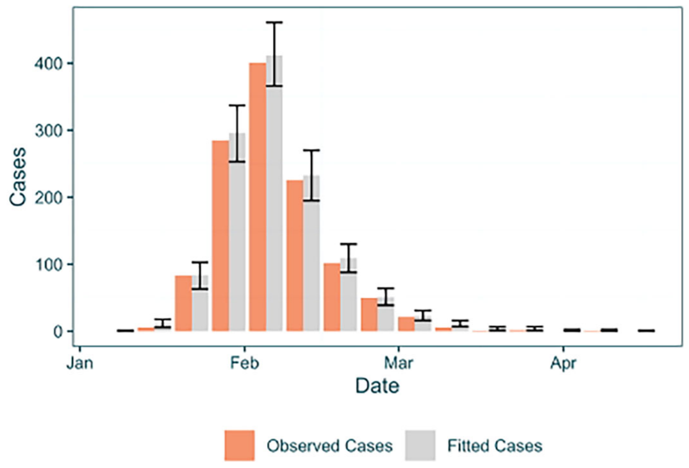


Figure 7. Fit of observed epidemiological cases to simulated cases by the EpiFusion model, plotted with ggplot2.

Observed epidemiological cases are shown by the grey bars, with their corresponding fitted cases from EpiFusion shown to their right by the blue bars. The error bars for the fitted case incidence correspond to bounds of the 0.95 HPD interval.

Finally we can examine the posteriors of the MCMC parameters. The posterior extraction process uses the `R package stable`.GR to perform gelman-rubin convergence tests on each parameter, and estimate the effective sample sizes of each. If the gelman-rubin statistic is less than 1.015 this indicates MCMC convergence.[18] If the MCMC has not converged it may be necessary to run each chain for longer.

```
print(parsed_output_fixed$parameters$gamma$rhat)
## [1] 1.001274
print(parsed_output_fixed$parameters$gamma$ess)
## [1] 1052
```

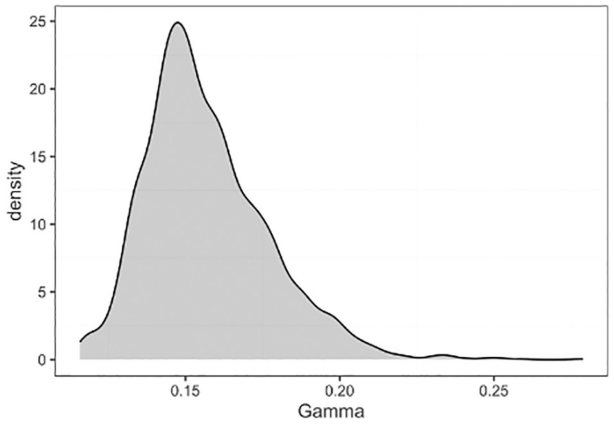


Figure 8. Posterior density of the gamma recovery/removal parameter, plotted using ggplot2.

We can also view the posterior density of a parameter by plotting the samples from the MCMC, which we can access from the posterior object due to setting `include_samples = TRUE` when we extracted the posterior earlier using `extract_epifusion_posterior` (Figure 8).

```
ggplot(data = data.frame(Gamma = parsed_output_fixed$parameters$gamma$samples),
aes(x = Gamma)) +
  geom_density(fill = "#01454f", alpha = 0.3) +
  lshtm_theme()
```

Phylogenetic uncertainty

In the previous example we modelled the baseline outbreak dataset using a fixed time-scaled phylogenetic tree as the phylodynamic data source. However, in a real outbreak setting there is often uncertainty about the evolutionary relationships and hence the tree sturcture (phylogenetic uncertainty). Bayesian tree inference approaches such as BEAST[22,24] attempt to approximate the true tree by sampling trees from a posterior set obtained through MCMC, often yielding thousands of plausible tree structures under the provided data and model. A single maximum clade credibility tree can be summarised from this 'tree posterior' and was used in EpiFusion using workflow specified above. It is valuable, however, to assess how uncertainty in the tree structure may affect epidemiological parameters inferred through EpiFusion. Currently, this can be explored by using a tree posterior as the data input into EpiFusion, and sampling a unique tree from the posterior for use with each MCMC chain. Below we demonstrate this approach by once more modelling the baseline outbreak dataset, but this time using the tree posterior as the phylodynamic data source.

Prepare data and parameters and run EpiFusion

To prepare the tree posterior (which was already loaded into our environment when we used the `baseline_dataset()` function) we once again use the `prepare_epifusion_tree` function. This function will recognise that a tree posterior has been passed, and will write the processed trees to a file without returning anything to your R session.

```
prepare_epifusion_tree(baseline_treeposterior, index_date, last_sequence, "Data/
Processed/baseline_processed_tree_posterior.tree")
```

Next we will generate the XML file for the analysis using the tree posterior. We again specify adjustments to the loggers chunk, specifying our desired output folder name and how often to sample from the MCMC and print to console. We will also increase the number of MCMC chains to 50, which, in conjunction with passing a tree posterior to EpiFusion, will instruct the model to run 50 chains, each using a different tree sampled at random from the tree posterior. This analysis will therefore take longer.

```
loggers <- list(fileBase = "Results/baseline_tree_posterior", logEvery = 5)
parameters <- list(numChains = 50)
```

Similarly to the fixed tree example, we will adjust some of the default priors. As before, we will set the initial infectivity β_0 to between 0.1 and 0.5. We will also narrow slightly narrow some priors using their inferred values as estimated from our previous example on the same dataset, in order to help the efficiency of the MCMC sampling process due to the extra number of chains we are running.

```
priors <- list(initialBeta = list(stepchange = "false",
                                   disttype = "Uniform",
                                   min = 0.1,
                                   max = 0.5),
               betaJitter = list(stepchange = "false",
                                  disttype = "Uniform",
                                  min = 0.005,
                                  max = 0.05),
               phi = list(stepchange = "false",
                                   disttype = "TruncatedNormal",
                                   lowerbound = 0.0,
                                   mean = 0.02,
                                   standarddev = 0.005))
```

Finally we generate an XML file using these parameters and priors for input into EpiFusion and run it:

```
generate_epifusion_XML(tree = "Data/Processed/baseline_processed_tree_posterior.
tree",
                      case_incidence = baseline_caseincidence,
                      index_date = index_date,
                      loggers = loggers,
                      parameters = parameters,
                      priors = priors,
                      xml_filepath = "Data/EpiFusion_XMLs/tree_posterior_
inputfile.xml")

run_epifusion("Data/EpiFusion_XMLs/tree_posterior_inputfile.xml")
```

Inspecting each chain

To examine the results of the model using the tree posterior we will again load the raw results with `load_raw_epifusion`. This time we will set `suppress_plots` to `true`.

```
raw_phylouncertainty <- load_raw_epifusion("Results/baseline_tree_posterior/",
suppress_plots = TRUE)
```

To examine the effect the inclusion of the tree posterior has on the analysis, we can use another EpiFusionUtilities function `plot_chainwise_trajectories`. This function operates similarly to the `plot_trajectories` function, but separates the trajectories by chain for inspection, while discarding a proportion of the trajectories of each chain for burn-in. This allows us to see how the sampled tree, which differs between each chain, affects the inferred trajectories (Figure 9). Here we can see that most of the chains converge on a similar set of trajectories to our fixed tree analysis, but some chains (and thus, some sampled trees) suggest other trajectory possibilities.

```
plot_chainwise_trajectories(raw_phylouncertainty, 0.2)
## [1] "WARNING: Chain 1 got stuck, with an acceptance rate of 0.0."
## [1] "WARNING: Chain 34 got stuck, with an acceptance rate of 0.0."
## [1] "WARNING: Chain 50 got stuck, with an acceptance rate of 0.0."
```

In this plot we see a further capability of the `plot_chainwise_trajectories` function. MCMC chains that have become ‘stuck’ i.e. enter a state space where they do not accept any further MCMC steps and have an acceptance rate of 0% are plotted with dotted lines, to enable users to identify and discard them when extracting the posterior using the `discard_chains` argument of the `extract_posterior_epifusion` function.

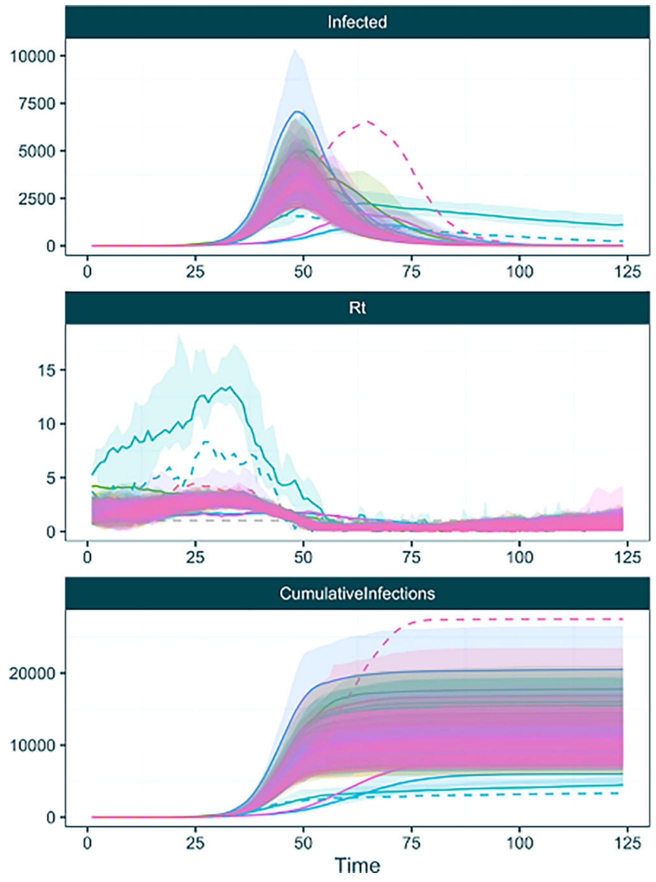


Figure 9. Inferred infection, R_t and cumulative infection trajectories plotted using the 'plot_chainwise_trajectories' function of EpiFusionUtilities.

When we extract the posterior from our raw output object (while discarding the 'stuck' chains), the chains will be combined and this uncertainty will be represented in our posterior estimates. To further understand this uncertainty we will extract the posterior sample using the `extract_posterior_epifusion` function and again create a trajectory table using the `trajectory_table` function. Using this table, and our trajectory table from the fixed tree analysis, we can use ggplot2 to plot the trajectories from both analyses to demonstrate the effect of the phylogenetic uncertainty on the estimates (Figure 10). The tree posterior approach is characterised by a widening of the HPD intervals around the mean fitted infection trajectory, due to the phylogenetic uncertainty.

```
posterior_phylouncertainty <- extract_posterior_epifusion(raw_phylouncertainty,
0.3, discard_chains = c(1, 34, 50))
phylouncertainty_trajtable <- trajectory_table(posterior_phylouncertainty,
as.Date("2023-12-15")) %>%
  mutate(Approach = "Tree Posterior")

combined_trajtable <- traj_table %>%
  mutate(Approach = "Fixed Tree") %>%
  rbind(phylouncertainty_trajtable)

ggplot(combined_trajtable, aes(x = Time, col = Approach, fill = Approach)) +
  geom_line(aes(y = Mean_Infected)) +
  geom_ribbon(aes(ymin = Lower95_Infected, ymax = Upper95_Infected), col = NA,
alpha = 0.2) +
  geom_ribbon(aes(ymin = Lower88_Infected, ymax = Upper88_Infected), col = NA,
alpha = 0.2) +
  geom_ribbon(aes(ymin = Lower66_Infected, ymax = Upper66_Infected), col = NA,
alpha = 0.2) +
  lshtm_theme() +
  labs(y = "Individuals Infected") +
  facet_wrap(~Approach, ncol = 1)
```

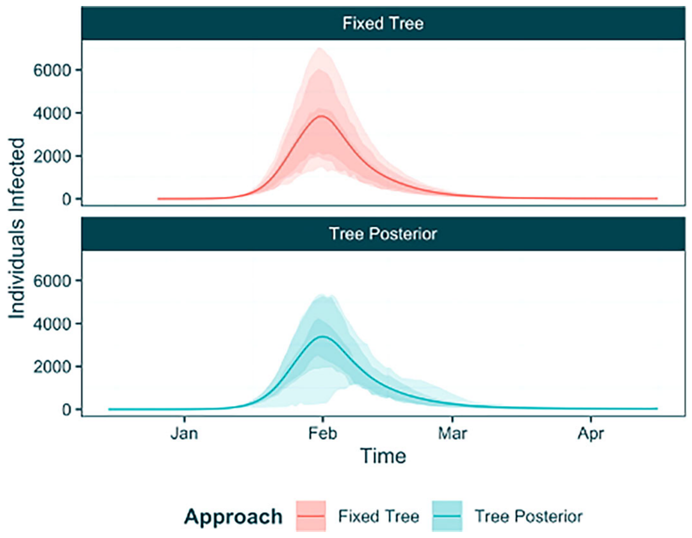


Figure 10. Inferred infection trajectories from EpiFusion analyses using a fixed tree (red) vs a tree posterior (blue).

This approach to combining chains sampled under different phylogenetic trees to form unified posteriors should be carefully employed. For the purposes of demonstration here we sample 50 trees in unique chains, however to adequately approximate the full tree posterior it is advised to conduct more samples. Further, if there is significant disparity in the inferred trajectories from different chains (i.e. under different trees), we recommend reexamining the tree posterior to check for overt phylodynamic uncertainty in your tree data and considering whether employing EpiFusion is suitable with highly uncertain phylogenies.

Introducing rate changes

While our previous examples with the baseline dataset describe an outbreak with constant sampling throughout, real life scenarios are often more complicated as the rates that govern our model, β, γ, ϕ and ψ vary over time. β is allowed to vary over time by default and is fit in the particle filter, but below we will address a common scenario where rates of sampling cases and genomes (ϕ and ψ) increase sharply at a given time point. For example, this occurred during the Brazilian Zika outbreak in 2015, where sampling sharply increased following the introduction of widespread PCR testing.[25]

Data preparation

We can load the data for this example using the EpiFusionUtilities function `sampling_dataset()`. We will use the same index date for this analysis as previously, but for this dataset the last sequence in the tree was sampled on March 17th, so we will adjust the 'last_sequence' date accordingly. As in our other examples, we prepare our tree data for EpiFusion using the `prepare_epifusion_tree` function.

```
sampling_dataset()
print(sampling_caseincidence[1:5,])
print(sampling_tree)

last_sequence <- as.Date("2024-03-17")

sampling_fixed_tree <- prepare_epifusion_tree(sampling_tree, index_date,
last_sequence, "Data/Processed/sampling_fixed_tree.tree")
```

Advanced parameterisation: Time variant prior distributions

In this example we wish to parameterise the step-increase in sampling on February 5th in our model. We will do this by setting a ‘time variant prior’ for case sampling rate phi when we generate the XML file, and using the ‘paired psi’ feature (*Supplementary Information Appendix 3*) to pair the genomic sampling rate psi to the case sampling rate.

Previously in the EpiFusion input files, the phi block in the prior section consisted of the following XML code:

```
<phi>
    <stepchange>false</stepchange>
    <disttype>TruncatedNormal</disttype>
    <mean>0.02</mean>
    <standarddev>0.01</standarddev>
    <lowerbound>0.0</lowerbound>
</phi>
```

A phi parameter with a step change is adjusted to look like this:

```
<phi>
  <stepchange>true</stepchange>
  <changetime>
    <x0>
      <disttype>FixedParameter</disttype>
      <value>35</value>
    </x0>
  </changetime>
  <distribs>
    <x0>
      <disttype>TruncatedNormal</disttype>
      <mean>0.002</mean>
      <standarddev>0.0001</standarddev>
      <lowerbound>0.0</lowerbound>
    </x0>
    <x1>
      <disttype>TruncatedNormal</disttype>
      <mean>0.025</mean>
      <standarddev>0.005</standarddev>
      <lowerbound>0.0</lowerbound>
    </x1>
  </distribs>
</phi>
```

The key differences here include the setting of the stepchange parameter to true, and the introduction of two new sub-nodes, changetimes and distribs, that contain the prior distribution details for the times of the rate changes in days from the index date (changetimes), and the rates themselves (`distribs`). For a rate with n change points, there must be $n+1$ distributions in `distribs` and n distributions in changetimes. These distributions are provided in tags with the format xn. While these adjustments can be made manually, it is also possible to parameterise this through the priors argument of `generate_epifusion_xml` using nested lists.

First we will make a list of the phi changetimes (in this example there is only one). In this scenario we ‘know’ the date of the step change in sampling - February 5th, 41 days after our index date - so we will provide it as a fixed parameter. However it is feasible to infer this change, if desired, by providing any discrete non-fixed prior distribution for this parameter.

```
phi_changetimes <- list(x0 = list(disttype = "FixedParameter",
                                  value = 41))
```

Next we will provide prior distributions for phi before and after the provided change time in the `distribs`.

```
phi_distribs <- list(x0 = list(disttype = "TruncatedNormal",
                               mean = 0.005,
                               standarddev = 0.002,
                               lowerbound = 0.0),
                     x1 = list(disttype = "TruncatedNormal",
                               mean = 0.05,
                               standarddev = 0.02,
                               lowerbound = 0.0))
```

The list structure we introduce below using the `changetimes` and `distribs` we have created mirrors the structure of the XML chunk.

```
phi_prior <- list(stepchange = "true",
             changetime = phi_changetimes,
             distribs = phi_distribs)
```

We can then feed this to the priors argument when we generate the XML file. We also will set `pairedPsi` to true in the parameters, and provide an empty `pairedPsi` in the priors. This specifies that psi is not to be fit by MCMC, and the genomic sampling rate psi is calculated as a proportion of the case sampling rate using the proportion of genomic sequences to cases in the data. Further information on this process is available in the Supplementary Information.

```
generate_epifusion_XML(tree = "Data/Processed/sampling_fixed_tree.tree",
                       case_incidence = sampling_caseincidence,
                       index_date = index_date,
                       loggers = list(fileBase = "Results/sampling_step_change",
logEvery = 5),
                       parameters = list(pairedPsi = "true",
                                         numSteps = 10000),
                       priors = list(phi = phi_prior,
                                     pairedPsi = ""),
                       xml_filepath = "Data/EpiFusion_XMLs/sampling_fixed_tree_
inputfile.xml")

run_epifusion("Data/EpiFusion_XMLs/sampling_fixed_tree_inputfile.xml")
```

Pairing `psi` with `phi` in this way is optional; here we couple the rates as we know they should change at the same time. It is also possible to parameterise these separately, e.g. an increase in sequencing without a corresponding increase in case sampling.

Parsing results

To complete our analysis we will load our results using the `load_raw_epifusion` function once more, and inspect the parameter trace. Here we will suppress the automatically created plots, and specifically plot the parameter trace of interest (the time varying parameters) using the `plot_parameter_trace` function, but changing the default type from all to timevar (Figure 11).

```
raw_sampling <- load_raw_epifusion("Results/sampling_step_change/",
suppress_plots = TRUE)
plot_parameter_trace(raw_sampling, type = "timevar")
```

Here the output from `plot_parameter_trace` looks slightly different to previous versions (e.g. Figure 4). The function automatically recognises the presence of parameters that vary over time, and plots their piecewise constant values (y-axis) across time (x-axis) in step graphs. This allows the inferred value over time to be intuitively understood

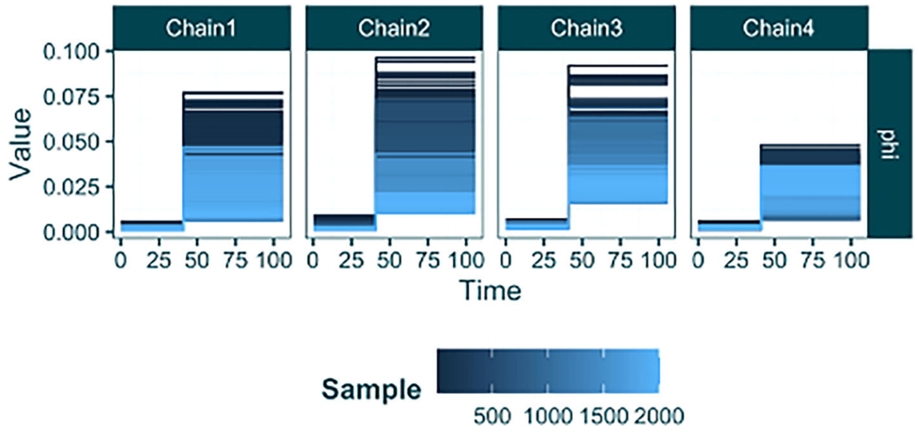


Figure 11. Parameter traces from an EpiFusion analysis with a case sampling rate (phi) step change.

from the plot. The lines are coloured by their sample index on a continuous gradient, making visible the values to which the each chain has converged (light blue). Here we see that despite each chain initiating at different values, the initial and final sampling rates across each chain converge to approximately the same values. This is shown by the light blue (later MCMC samples) lines occurring at the same y-axis value in each chain trace plot for the phi parameter.

The parsing and plotting process for the rest of the results from this analysis follows the same steps as the other vignettes included in this article.

Conclusions and Discussion

The EpiFusion Analysis Framework is a novel workflow for implementing the EpiFusion joint epidemiological and phylodynamic inference model using the Java implementation of the model and the R package EpiFusionUtilities. This workflow is generalisable, utilising common R objects for data formatting (`data.frame` and `phylo` or `multiPhylo` objects) and parameterisation (list objects). We detail the full analysis workflow here, including a new feature introduced since EpiFusion's first description: introduction of the ability to explore the effect phylogenetic uncertainty by providing a tree posterior as data.

Use of this approach is recommended for outbreaks where a time-scaled phylogenetic tree and case incidence data is available, and the desired result is continuous (to a daily resolution) models of pertinent outbreak trajectories such as R_t and infections over time. The data sampled should arise from the same 'outbreak system'; specifically the genomic sequences used to build the tree and the case data should be sampled from the same approximate geographic location, time period, and population. If a time-scaled phylogenetic tree is not already assembled, this may be generated from established phylodynamic tree estimation approaches such as BEAST[22] or Nextstrain.[26] The program runs efficiently for even very large trees, but run-time increases with the length of the time series under investigation,[10] accordingly we currently do not recommend EpiFusion for analyses of time periods of longer than five years. In these instances, other programs such as EpiNow2[27] (case incidence data only) or TimTam[28,29] (both genomic and case incidence data), written in R and Java (BEAST Framework) respectively, may be more appropriate.

The documentation of reproducible analysis workflows, particularly for new tools, is essential for open research.[7–9] Providing efficient pipelines with corresponding instructions enables researchers to build on previous work to address empirical research in an efficient way, which can be of great importance during outbreak settings which are often time sensitive.[30] In the three vignettes described we provide examples of standard EpiFusion parameterisation, however there are many advanced options available to users to customise their analysis. These include capabilities for composite (non-parametric) prior distributions (*Supplementary Information Appendix 4)*, multiple epidemiological observation model options (*Supplementary Information Appendix 2, 'Model'*), multiple options for fitting β_t (*Supplementary Information Appendix 5*), and buffer zones for rate step-changes (*Supplementary Information Appendix 6*).

Exploration of the effect of phylogenetic uncertainty is now incorporated in the program through allowing the use of a time-rooted phylogenetic tree posterior (from a software such as BEAST) to be used as data within the model. This new feature is an implementation rather than theoretical advancement: For each unique MCMC chain, a new tree is randomly sampled from this posterior and used as the tree data in the model. The resulting posteriors can subsequently be examined and combined by the user with post-hoc EpiFusionUtilities functions. Incorporating phylogenetic uncertainty predictably led to increased uncertainty in the model estimates in the combined posterior. We encourage care in implementing this approach, and thoroughly examining the effect of using different trees on the model estimates using the `plot_parameter_trace` and `plot_chainwise_trajectories` functions. Further, for efficiency in our example shown above, we sample 50 trees, whereas it may be necessary to increase this number to adequately approximate the tree posterior. Going forward we aim to investigate other approaches for incorporating phylogenetic uncertainty in the model in a more comprehensive manner.

To demonstrate the advanced parameterisation options of the framework, we addressed an outbreak with a step-increase in case and genomic sequence sampling rates. While this example featured a single change in the modelled rates, this infrastructure is very flexible and can be used to add significant complexity to the model according to the user requirements.

The current framework is robust, but has some limitations. Traditionally R packages such as `EpiFusionUtilities` are distributed using the R package ecosystem CRAN. However, CRAN does not accept packages which contain binary executable code,[31] so it would not have been possible to distribute an `EpiFusion` release (an executable jar file) with the package and enable running the model from within R. A likely future step in the software development process may be to fully integrate the model into the EpiFusionUtilities R package using R/Rcpp, to allow more universal usage for all users including those without a extensive phylodynamic experience and would enable the package to be hosted on CRAN.

The requirement to provide a user defined index date, or earliest possible date of outbreak origin, is a practical implementation compromise that may result in incorrect conclusions if the index date is not set early enough (i.e., if the index date is accidentally set after the true date of outbreak origin). Currently this can be overcome by setting the index date to a longer time period before the suspected origin of the outbreak, however the resulting estimates during the earlier periods of the modelled time series should be treated carefully and will typically display high levels of uncertainty. Future distributions of this framework will aim to allow inference without this truncation to a specified index date, or allow the index date to be inferred within the model.

In conclusion, this article aims to outline a reproducible framework for utilising our novel joint inference model using a functional R package and a binary executable file. We show how different parameterisations and options for the analysis can be implemented, including how to introduce phylogenetic uncertainty through the provided tree data, and time-variant prior distributions. We hope that clearly outlining a use case of the framework will facilitate its implementation by researchers to investigate hypotheses of public health importance in the future.

Ethics and consent

Ethical approval and consent were not required.

Data availability statement and software availability

For the purposes of open research, we make available all code and data used in this manuscript on GitHub.

Source code

The source code for EpiFusion is written in Java and available on GitHub at the following repository: https://github.com/ciarajudge/EpiFusion. The source code is made available under the GNU General Public License (v3) (https://www.gnu.org/licenses/gpl-3.0.en.html). The source code at the time of this publication is archived on Zenodo: https://doi.org/10.5281/zenodo.14973874[32]

The source code for EpiFusionUtilities is written in R and is available on GitHub at the following repository: https://github.com/ciarajudge/EpiFusionUtilities. The source code is made available under the GNU General Public License (v3) (https://www.gnu.org/licenses/gpl-3.0.en.html). The source code at the time of this publication is archived on Zenodo: https://doi.org/10.5281/zenodo.14973878[33]

Underlying data and analysis code

The simulated datasets used in this manuscript have been described in Section 4 and are provided in the Open Science Framework repository 'EpiFusion Analysis Framework Software Article': https://doi.org/10.17605/OSF.IO/7W43Y.[34]

The project contains the following underlying data:

- baseline_caseincidence.RDS: weekly case incidence from the baseline simulated dataset (R data.frame)

- baseline_treeposterior.RDS: posterior of time-scaled phylogenetic trees from the baseline simulated outbreak dataset (R S3 multiPhylo object)

- baseline_tree.RDS: time-scaled phylogenetic tree from the baseline simulated outbreak dataset (R S3 phylo object)

- sampling_caseindicence.RDS: weekly case incidence from the step-change in sampling simulated dataset (R data.frame)

- sampling_tree.RDS: time-scaled phylogenetic tree from the step-change in sampling simulated outbreak dataset (R S3 phylo object)

Data are made available under the terms of the Creative Commons Attribution 4.0 International Licence (CC-BY 4.0) (https://creativecommons.org/licenses/by/4.0/).

The code used to produce all analyses and plots in this manuscript is available on GitHub at the following repository: https://github.com/ciarajudge/EpiFusion_Vignettes. This repository is archived on Zenodo: https://doi.org/10.5281/zenodo.14973889.[35]

Data and code are made available under the terms of the Creative Commons Attribution 4.0 International Licence (CC-BY 4.0) (https://creativecommons.org/licenses/by/4.0/).

Extended data

The extended data in this manuscript (additional figures, tables and appendices) is available as a Supplementary Information file which is provided in the Open Science Framework repository 'EpiFusion Analysis Framework Software Article': https://doi.org/10.17605/OSF.IO/7W43Y.[34]

The project contains the following extended data:

- supplementary_information.pdf: PDF file containing Appendices 1-6 with supplementary information.

Acknowledgements

The authors would like to thank Timothy Vaughan, Sam Abbott, Tanja Stadler, Louis Du Plessis and Timothy Russell for their role in the development of the model method, David Hodgson for his guidance on R package development and implementation, and Clara Brigitta for testing the package and vignettes.

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Open Peer Review

Current Peer Review Status: ✓ ? ✓

Version 1

Reviewer Report 03 June 2025

https://doi.org/10.5256/f1000research.178965.r376911

✓ **Leo A Featherstone**
The University of Melbourne, Melbourne, Victoria, Australia

Overview
Judge et al. present the EpiFusionUtilities R package. It provides an R interface for the EpiFusion program implemented in java, but with the key extension to accommodating phylogenetic uncertainty. This is a valuable contribution both in providing a means to address phylogenetic uncertainty and by improving accesibility through an R interface. The examples provided are comprehensive and explained with good clarity.

Some points for clarification

P6: "*It is also possible to test multiple extractions extract_posterior_epifusion() with different burn-in proportions, and inspect the gelman-rubin statistics of the parameters, which indicate convergence if below 1.015*"

It would be helpful to outline how the Gelman-Rubin statistic relates to effective sample size (ESS), which will be more familiar to BEAST users who appear to be part of the intended user base here. Could users equivalently read output into the `coda` R package to calculate ESS for MCMC traces?

P16: "*...It is valuable, however, to assess how uncertainty in the tree structure may affect epidemiological parameters inferred through EpiFusion. Currently, this can be explored by using a tree posterior as the data input into EpiFusion, and sampling a unique tree from the posterior for use with each MCMC chain...*"

Does the input strictly have to be a posterior distribution of trees? I gather the program is useful for comparing the effects of any set of candidate trees in a multiPhylo object, which I think is a really useful feature!

P22 *Figure 11*:
I'm concerned that any patterns in colour could be obscured by overlapping of states. Would you consider faceting by sub-groupings of samples or perhaps implementing some version of a

parallel coordinates plot to better tease apart states? Here's an example: (https://beastiary.wytamma.com/plots/parallel-coordinates/) from the beastiary [1] program.

P22: "*The program runs efficiently for even very large trees, but run-time increases with the length of the time series under investigation,10 accordingly we currently do not recommend EpiFusion for analyses of time periods of longer than five years*"

It sounds like the number of data points in the time series is the issue rather than the total timespan. If so, can you provide an upper limit on the workable number of rows in the time series? E.g. I assume 5 years of monthly or weekly time series data would be better than daily?

P23: "*Currently this can be overcome by setting the index date to a longer time period before the suspected origin of the outbreak, however the resulting estimates during the earlier periods of the modelled time series should be treated carefully and will typically display high levels of uncertainty. Future distributions of this framework will aim to allow inference without this truncation to a specified index date, or allow the index date to be inferred within the model.*"

Please elaborate on how overly conservative index dates affect inference. Is it correct to intuit that having very old index date would deflate estimates of transmission rate? How would this interact with the 5-year time limit you proposed above?

P17: Typo: "*We will also narrow slightly narrow some priors*"

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Is the rationale for developing the new software tool clearly explained?
Yes

Is the description of the software tool technically sound?
Yes

Are sufficient details of the code, methods and analysis (if applicable) provided to allow replication of the software development and its use by others?
Yes

Is sufficient information provided to allow interpretation of the expected output datasets and any results generated using the tool?
Yes

Are the conclusions about the tool and its performance adequately supported by the findings presented in the article?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Phylodynamics; Phylogenetics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 15 May 2025

https://doi.org/10.5256/f1000research.178965.r376910

?

Claire Guinat
Interactions Hôtes-Agents Pathogènes (IHAP), Université de Toulouse, INRAE, Toulouse, France

Overall comment
This documentation is very relevant to facilitate the correct use of the EpiFusion tool and is essential for open research. However, the text and code should be harmonized/complemented by what is also presented in the online tutorial on the GitHub page. The first part, which I think aims to be more generic, is a bit confusing; it becomes clearer when we reach the applications. I ran the different parts of the code, which run smoothly. However, here are a few points that might improve the manuscript:

- I also went to https://github.com/ciarajudge/EpiFusion/tree/main/examples
  => What are the links between the three tutorials provided on GitHub and this paper? The connection is unclear. It would be helpful to integrate all sources of information or clarify their respective purposes to avoid fragmentation.
- Table 1 – About Data
  => Is the tree time-calibrated? Please clarify whether this refers to an MCC tree or a posterior sample of time-scalibrated trees.
- About Parameters
  => The term “parameters” usually refers to model parameters. Here, it appears to refer to inference parameters or hyperparameters. Please clarify this distinction.
- About Model
  => What are the available model options? The XML shows a Poisson model, but further explanation is needed on what each model represents (e.g., incidence process), and how to choose one over another.
- Add library(EpiFusionUtilities) after devtools::install_github(...)
  => This addition would improve clarity and ensure reproducibility for users unfamiliar with the package.
- Index_case <- as.Date(“2024-01-01”)
  => If this date cannot be estimated and must be fixed, what are the implications? Could you clarify how users should handle uncertainty, perhaps by suggesting a sensitivity analysis? Also, is this date just an example or related to real data?
- “If there is uncertainty in the date of origin of the outbreak we recommend setting the index date to earlier than the estimated date.”

=> Please clarify what is meant by "estimated date"—is it based on genetic data, case reports, or another source?
- “To prepare a tree or posterior set of trees for EpiFusion”
  => The documentation should clarify whether trees must be time-calibrated. This is crucial for users preparing their data.
- prepare_epifusion_tree(...)
  => Are users expected to use the tree provided in the GitHub tutorial? Providing a simple MCC tree as an example would be helpful for learning purposes.
- “This function populates a template XML...”
  => It would help to list default parameter values and priors explicitly here. Also, clarify terminology: "priors" and "parameters" typically refer to biological model settings, whereas here they seem to relate to the inference framework (MCMC, particle filtering, etc.).
- CSV case format for incidence data
  => Please give an explicit description of the required format (e.g., columns, headers). You could refer to the full workflow tutorial but be explicit here too.
- generate_epifusion_XML(...)
  => The full workflow tutorial uses a different example and mentions priors, but it's unclear which parameters are being referred to. I recommend listing all adjustable priors (e.g., ψ, ϕ, betajitter) and defining them clearly.
- run_epifusion("epifusion_input.xml")
  => Could you include an estimate of run time or a way to monitor progress, similar to BEAST logs? This would help users plan their analyses.
- Output files (betas.csv, params.txt, acceptance.txt)
  => Clarify that params_chain0.csv is the output referenced as params.txt. Are these MCMC parameters or model parameters? Define what the “acceptance” file contains and how to interpret acceptance rates.
- “It is possible to process this raw output manually...”
  => Before visualizing outputs, users should be advised to assess convergence (e.g., trace plots, Gelman-Rubin statistics). Mention this step earlier in the process.
- Rt estimates
  => Where are the Rt estimates? Can users specify whether Rt is estimated from tree only, case data only, or combined? Clarifying this would be very useful.
- Use cases section
  => The term “Use cases” might be misleading, since “case” is already used for case incidence data. Perhaps “Applications” would be a clearer section title.
- “Finally, in ‘Introducing Rate Changes’”
  => Consider rewording as “Introducing sampling rate changes” to be more specific.
- Structured populations
  => Is it possible to use the method for structured populations and estimate different Rt values per subpopulation? Clarify whether this is supported or planned.
- “To generate the data...” using ReMaster.19
  => Do we assume that all cases are reported? Clarify whether the incidence file reflects total or sampled incidence, since under-reporting is common in real-world outbreaks.
- “...csv file with dated counts of weekly incidence”
  => Does incidence have to be weekly? Could it be daily or in another time unit? Specify how to align tree and case data temporal scales.
- Methods vs. Use Cases
  => The “Use Cases” section is much clearer than the “Methods” section. Consider stating

explicitly that the methods part is a generic overview and that detailed application examples follow.

- include_samples = TRUE
  => It is unclear what changes when this is set to TRUE. Does it apply burn-in and return a trimmed posterior sample? Please clarify the effect of this option.
- parsed_output_fixed <- extract_posterior_epifusion(...)
  => If users want to compute their own summary statistics (e.g., medians), should they use the raw output after burn-in? Clarify the recommended procedure.
- “Finally we can examine the posteriors of the MCMC parameters...”
  => Introduce convergence checks (Gelman-Rubin, ESS) earlier in the workflow. Also, define acronyms like ESS and rhat, and explain what these values represent and how to interpret them.
- print(parsed_output_fixed$parameters$gamma$rhat) output
  => It’s not clear to beginners what this output means. Relate it clearly to the earlier explanation of convergence diagnostics.
- “However, in a real outbreak setting there is often uncertainty...”
  => Typo: "sturcture" should be "structure".
- “...sampled at random from the tree posterior...”
  => Please clarify: if multiple trees are sampled, does this represent a sensitivity analysis across tree uncertainty? The phrasing earlier in the document suggests that only one tree is used per run.
- “...run 50 chains, each using a different tree sampled...”
  => This is now clearer, thanks. It might help to emphasize that this is the way to explore tree uncertainty.
- Side question
  => I wonder why the authors of the original EpiFusion paper are not included in this manuscript? Their inclusion would make sense if the method is being described or re-implemented.

Is the rationale for developing the new software tool clearly explained?

Yes

Is the description of the software tool technically sound?

Yes

Are sufficient details of the code, methods and analysis (if applicable) provided to allow replication of the software development and its use by others?

Partly

Is sufficient information provided to allow interpretation of the expected output datasets and any results generated using the tool?

Partly

Are the conclusions about the tool and its performance adequately supported by the findings presented in the article?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: epidemiology, infectious diseases, transmission dynamics, phylodynamics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 07 May 2025

https://doi.org/10.5256/f1000research.178965.r374093

✓

Vijaykrishna Dhanasekaran
The University of Hong Kong, Hong Kong, Hong Kong
Ruopeng Xie
The University of Hong Kong, Hong Kong, Hong Kong

This manuscript provides a valuable practical extension of the EpiFusion framework, offering clear guidance for implementing joint phylodynamic-epidemiological analyses via the EpiFusionUtilities R package. The inclusion of vignettes and real-world outbreak case studies significantly enhances reproducibility and accessibility, bridging the gap between theoretical models and applied public health research. Below are suggestions to further strengthen the work:

1. The emphasis on reproducibility is a major strength. To ensure seamless replication across systems, the authors could:
· Encapsulate dependencies by providing Docker containers or platform-agnostic virtual environments (e.g., via renv).
· Document software versions explicitly, including a sessionInfo() output, R/Java version requirements, and a requirements.txt file (or equivalent) to help users mirror the analysis environment.

2. While the focus on EpiFusionUtilities is appropriate, the manuscript would benefit from a comparative discussion of how EpiFusion complements or differs from established tools (e.g., BEAST, PhyDyn, Outbreaker2). A concise table or paragraph highlighting unique features (e.g., joint modeling of genomic and epidemiological data) and limitations (e.g., computational scalability) would help users assess its suitability for their needs.

3. The manuscript includes analyses of two outbreak datasets. Briefly justify their selection and relevance to contemporary public health challenges to strengthen the narrative. A more explicit discussion of the framework's assumptions and limitations would improve its practical utility:
· The model assumes a single infected individual (single index) initiates the outbreak. Discuss its sensitivity to this assumption and whether the model can accommodate multiple introductions.

· While the manuscript notes that phylogenetic uncertainty affects parameter estimation, it should also address how variation in tree-building methods (e.g., ML vs. Bayesian approaches) might propagate into epidemiological inferences.
· Guidance on computational performance (e.g., runtime benchmarks for larger datasets or complex models) would help users gauge feasibility for their own work.

Is the rationale for developing the new software tool clearly explained?
Yes

Is the description of the software tool technically sound?
Yes

Are sufficient details of the code, methods and analysis (if applicable) provided to allow replication of the software development and its use by others?
Yes

Is sufficient information provided to allow interpretation of the expected output datasets and any results generated using the tool?
Yes

Are the conclusions about the tool and its performance adequately supported by the findings presented in the article?
Yes

Competing Interests: No competing interests were disclosed.

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.