Fast simulation of reconstructed phylogenies under global, time-dependent birth-death processes

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ABSTRACT
Motivation: Diversification rates and patterns may be inferred from reconstructed phylogenies. Both the time-dependent as well as the diversity-dependent birth-death process can produce the same observed patterns of diversity over time. To develop and test new models describing the macro-evolutionary process of diversification, generic and fast algorithms to simulate under these models are necessary. Simulations are not only important for testing and developing models but play an influential role in the assessment of model fit.

Results: In the present paper I consider as the model a global, time-dependent birth-death process where each species has the same rates but rates may vary over time. For this model I derive the likelihood of the speciation times from a reconstructed phylogenetic tree and show that each speciation event is independent and identically distributed. This fact can be used to simulate efficiently reconstructed phylogenetic trees when conditioning on the number of species, the time of the process or both. I show the usability of the simulation by approximating the posterior predictive distribution of a birth-death process with decreasing diversification rates applied on a published bird phylogeny (family Cettiidae).

Availability: The methods described in this manuscript are implemented in the R package TESS, available from the repository CRAN (http://cran.r-project.org/web/packages/TESS/). Contact: hoehna@math.su.se

1 INTRODUCTION

The birth-death process is arguably the most commonly used process to model species diversity (Nee, 2006; Ricklefs, 2007). In 1994 Nee et al. (1994) introduced the birth-death process to estimate diversification rates from reconstructed phylogenetic trees. It was then used to estimate speciation and extinction rates that are constant over time and equal for all species (Yang and Rambal, 1997). In recent years the constant, equal rate birth-death process has been extended to: time-dependent speciation and extinction rates (Rabosky, 2006; Rabosky and Lovette, 2008; Alfaro et al., 2009; Morlon et al., 2011); trait-dependent speciation and extinction rates (Maddison et al., 2007; FitzJohn et al., 2009); and diversity-dependent speciation rates (Etienne et al., 2012). The models used for parameter inference have become increasingly complex and different models — such as the time-dependent speciation and the diversity-dependent speciation models — can produce almost indistinguishably close fits to observed phylogenetic trees. Thus, studies investigating the different patterns generated by the different models need to be compared. An evident approach is to use simulations under one model and inference under any of the available models. Furthermore, simulations play an important role in investigations of which parameters can be estimated (Quental and Marshall, 2009; Liow et al., 2010).

However, simulating trees has gained less attention and remains a challenge in several situations (Hartmann et al., 2010). One might be interested in simulating trees under three different conditions when the process starts with a single species at time $t_0 = 0$:

- Simulating a tree for a given time $t$.
- Simulating a tree for a given number of species $n$.
- Simulating a tree for a given number of species $n$ that have evolved over time $t$.

Additionally, the conditions can be modified if the process starts with the most recent common ancestor (MRCA) and thus there are two species at time $t_0 = 0$ (Stadler, 2011). I consider the time-dependent speciation and extinction rates birth-death process as the model of choice for simulations in this paper. This process contains the constant rate birth-death process as a special case.

Simulations under the first condition, a given time $t$ of the process, are straightforward from a theoretical point of view. One only needs to simulate the exponentially distributed waiting times until the next event, either a speciation event or an extinction event, and pick a random species. Nevertheless, implementing this forward simulation might be challenging and inefficient in situation when the rates are not constant and both rates are high. Additionally, there is no available software for simulating reconstructed trees for a given time $t$ when the rates vary continuously. An overview of available software packages for simulating trees under a time-dependent birth-death process is given in Stadler (2011).

Simulating phylogenies under models with a non-zero extinction rate is challenging when one is interested in simulating a tree with a fixed number $n$ of extant species (Stadler, 2011). The general sampling approach (GSA) introduced by Stadler (2011) in the program TreeSim, is the currently only available approach to simulate trees under the first and second condition. However, TreeSim only implements changes of speciation and extinction rates at specific, pre-defined times. Furthermore, when one chooses a model with high species turnover (the extinction rate being close to
Extinction

(see Figure 1.b). From the reconstructed tree with removing all extinct branches gives the reconstructed tree Neet et al. 1994, Equation 24) by

\[ P(N(T) = n | N(t) = 1) = \frac{(1 - P(N(T) > 0 | N(t) = 1) \exp(r(t,T)))^{n-1}}{P(N(T) > 0 | N(t) = 1)^2 \exp(r(t,T))} \]

which leads to the probability of obtaining a single lineage at time \( T \)

\[ P(N(T) = 1 | N(t) = 1) = P(N(T) > 0 | N(t) = 1)^2 \exp(r(t,T)) \]

Equation (3) can be derived from a geometric distribution with parameter \( P(N(T) > 0 | N(t) = 1) \exp(r(t,T)) \) multiplied by the probability of survival of the process \( P(N(T) > 0 | N(t) = 1) \). Thus, the probability of \( n \) species at time \( T \) given that we had \( 1 \) species at time \( t \) \((N(t) = 1)\) and the species survives, denoted by \( S(1, t, T) \), is

\[ P(N(T) = n | N(t) = 1, S(1, t, T)) = \frac{(1 - P(N(T) > 0 | N(t) = 1) \exp(r(t,T)))^{n-1}}{P(N(T) > 0 | N(t) = 1)^2 \exp(r(t,T))} \]

Now, following the reasoning of Thompson (1975, p. 54-58), I give the joint probability distribution of all speciation times \( \{ t \} \) of the reconstructed
tree given that we sample (or stop) the process at time $T$
\[
P_{BD}(\{t\}|N(t_0) = 1, T) = P(N(T) = 1|N(t_0) = 1) \times \prod_{i=1}^{n-1} \big(i \times \lambda(t_i) \times P(N(T) = 1|N(t_i) = 1)\big).
\]
(6)

The intuitive interpretation of the probability density is that the process starts with one species that survives until the present time ($P(N(T)=1|N(t_0)=1)$). Then, for each speciation event we multiply with the density to obtain a speciation event at time $t_i$ ($i\lambda(t_i)$) and the probability that this new species has one sampled descendant at the present ($P(N(T)=1|N(t_i)=1)$). A short proof is given in the Appendix.

Using Equation (3) and Equation (6) we can compute the probability of the conditioned process on $n$ lineages at the present time $T$ results
\[
P_{BD}(\{t\}|N(t_0) = 1, N(T) = n, T) = \frac{\prod_{i=0}^{N-1} i \times \lambda(t_i) \times P(N(t)=1|N(t_i)=1)}{1 - P(N(T)>0|N(t_0)=1) \exp\{r(t_0, T)\}}.
\]
(7)

Note, the factor $i$ in front of $\lambda(t_i)$ is simply a combinatorial constant representing the $(n-1)!$ possible orderings of the speciation events (Al豆dov and Popovic, 2005). Hence, the probability density of each single speciation event are iid by the definition of independence $P_{BD}(t_j|\{t_i\}, N(t_0)=1, N(T)=n, T) = P_{BD}(t_j|N(t_0)=1, N(T)=n)$ and have the probability density
\[
P_{BD}(t) \propto \lambda(t)P(N(T) = 1|N(t) = 1).
\]
(8)

This has previously been shown by Yang and Rannala (2007) for constant rates and been exploited by Ηohna et al. (2011) for estimations of model parameters.

I will now show how to use these probability distributions to simulate reconstructed trees under the three different conditions.

2.1 Simulating reconstructed trees for a fixed time $t$ and number of species $n$

I will start developing the algorithm for simulating reconstructed trees when conditioning on both — the time of the process $t$ and the number of sampled, extant species $n$ — although this might not be the common or natural condition for simulating reconstructed trees. It is however used by the two other simulation conditions. Random speciation events are drawn by solving the inverse cumulative distribution function of the speciation times.

From Equation (7) we have that the joint probability of all speciation times in the reconstructed tree and established that all speciation times are iid with the probability density given in Equation (8). Thus, to obtain $n-1$ speciation times (or $n-2$ in the case when starting at the MRCA), one needs to draw $n-1$ random draws from the above distribution. Such random draws can be obtained by first drawing a random number $u$ from the Uniform(0,1) distribution. Then, the speciation time $t^*$ for which the cumulative distribution function of Equation (8) equals $u$ gives the randomly drawn speciation time:
\[
u = \frac{\int_{0}^{t^*} P_{BD}(t)dt}{t} = \frac{\int_{0}^{t^*} \lambda(t)P(N(T) = 1|N(t) = 1)dt}{\int_{0}^{T} \lambda(t)P(N(T) = 1|N(t) = 1)dt}.
\]
(9)

Figure 2 shows an example of how to draw random speciation times in the sampled, reconstructed tree.

\[\text{Fig. 2. An example birth-death process with rates of: } \lambda = 1.0 \text{ if } t < 1.0 \text{ and } \lambda = 0.1 \text{ otherwise; } \mu = 0.95 \text{ if } t < 1.0 \text{ or } t > 2.0 \text{ and } \mu = 0.0 \text{ otherwise. Additionally, there is a mass-extinction event at time } t = 0.8 \text{ with a survival probability of } p = 0.2 \text{ per species. The algorithm for drawing new speciation times } t^* \text{ draws a random } u \sim \text{unif}(0,1), \text{ and searches the } t^* \text{ for which CDF}(t^*) = u.\]

The algorithm how to construct a random from the set of speciation time is given below.

2.2 Simulating reconstructed trees for a fixed time $t$

I start by simulating the number of species after the time $t$. Equation (3) gives the probability of $n$ species at time $t$. Therefore, one only needs to make a random draw $u \sim \text{unif}(0,1)$ and find the maximal $n$ for which the cumulative distribution function of Equation (3) is smaller or equal to $u$:
\[
\max_n \{ u \geq \sum_{i=1}^{n} P(N(t) = i|N(t_0) = 1) \}.
\]
(10)

Once the number of species is obtained by a random draw, the speciation times can be simulated using the above algorithm conditioning on both, the number of species and the time of the process.

2.3 Simulating reconstructed trees for a fixed number of species $n$

A tree with a fixed number of extant species can be simulated by first simulating the time of the process $T$ given that the number of species was $N(T) = n$. Let us assume that one draws a random time $T$ with some probability $P(T)$ and maximum value $max$, then simulates a tree under the birth-death process. A new time $T$ and a new tree is drawn until the tree contains $n$ species at time $T$. Thus, using Bayes Theorem, the probability of the time of the process (or the time of the origin/MRCA) is
\[
P(T|n) = \frac{\int P(N(T) = n|N(t_0) = 1) \times P(T) dt}{\int P(N(T) = n|N(t_0) = 1, t) \times P(T) dt}.
\]
(11)

If a uniform distribution as the prior distribution on the time of the process is assumed, then the probability $P(T)$ in the numerator cancels out with the probability $P(t)$ in the denominator. However, in the general form when
one wants to specify some informative prior distribution of the time of the process, then the integral needs to be computed numerically.

Only a random draw from the distribution given in Equation (11) needs to be obtained and the same algorithm as for conditioning on the number of species and the time of the process can be used.

2.4 Starting at the MRCA
In the previous sections I only considered the situations when the process starts with \( N(t_0) = 1 \). Now I will give the necessary modifications if one wants to condition on the MRCA. Let the process start with two species and both survive until the present \( S(2, t_0, t) \), then Equation (5) needs to be modified to condition on two species to start with:

\[
P(N(t) = n|N(t_0) = 2, S(2, t_0, t)) = \sum_{i=0}^{n-1} \left( P(N(t) = i|N(t_0) = 1, S(1, t_0, t)) \times P(N(t) = n - i|N(t_0) = 1, S(1, t_0, t)) \right)
\]

\[
= (n-1)(P(N(T) > 0|N(t) = 1)^2 \exp(r(T), T))^2 \\
\times (1 - P(N(T) > 0|N(t) = 1) \exp(r(T), T))^{n-2}.
\]

The rationale behind the derivation is that one could consider two independent processes, both starting with one species at time \( t_0 = 0 \), and the sum of the species of both processes equals \( n \).

The algorithms for simulating reconstructed phylogenies remain unchanged and only Equation (3) needs to be replaced by Equation (12).

2.5 Reconstruction of a tree from speciation times
In the previous sections I only elaborated the methods how to draw the set of speciation times. The reconstructed phylogeny can be created from the set of speciation times by first creating a set of \( n \) species. Then, repeatedly join two random species together. The time of the \( i^{th} \) speciation event is the \( i^{th} \) largest speciation time of the previously created set of speciation times. This procedure is closely related to the UPGMA method (Sokal and Michener, 1958; Felsenstein, 2004). The resulting tree is from the correct reconstructed phylogenies remain unchanged and only Equation (3) needs to be replaced by Equation (12).

2.6 Modelling mass-extinction events and random taxon sampling
The rate functions have been provided as general functions of time. Following Nee et al. (1994), mass-extinction events are modeled by

\[
r(t, s) = \int_{t}^{s} (\mu(z) - \lambda(z)) dz - \sum_{t_m \in (t,s]} \log(\rho_m)
\]

where \( t_m \) is the time and \( \rho_m \) the survival probability of the \( m^{th} \) mass-extinction event.

Then, the probability of a single species alive at time \( t \) surviving until time \( T \) \((P(N(T) > 0|N(t) = 1))\) from Equation (2) changes to

\[
P(N(T) > 0|N(t) = 1) = 1 \times \exp(-\int_{t}^{T} \mu(s) ds) - \sum_{t_m \in (t,T]} (\rho_m - 1) \exp(r(t, t_m))
\]

Note that here \( 1 - \rho_m \) represents the extinction probability at the \( m^{th} \) mass-extinction event. Furthermore, random taxon sampling is the same as a mass-extinction event at the present time Nee et al. (1994).

2.7 Implementation and numerical integration
The methods described to simulate trees are implemented in the R (R Core Team, 2012) package TESS (Tree Evolution Simulation Software). TESS can handle any type of rate functions and is designed for fast simulation of reconstructed trees. Furthermore, TESS computes the likelihood function (Equation (6)) conditioned on the time of the process, survival of the process or the time and number of taxa at present. The likelihood function can be used for Bayesian and Maximum Likelihood inference, posterior prediction and model testing (see the Supporting Information).

There are no closed form solutions known for the equations presented above. Only for the special cases when the rates are constant one can compute the probability densities analytically. Nevertheless, R provides several methods for numerical integration. TESS uses the package deSolve (Soetaert et al., 2011) to perform the numerical integration. If only a single draw or very few draws from a distribution are needed, then TESS performs a simple Monte Carlo sampling instead.

3 RESULTS
In this section I compare the performance of TESS to TreeSim and show a potential application: posterior prediction of reconstructed phylogenies.

3.1 Performance
One of the major shortcomings of the GSA as implemented in TreeSim is the very long computation time, especially in situation when \( \lambda(t) \approx \mu(t) \). Additionally, TreeSim cannot handle continuous increasing or decreasing rate functions but only constant rates in predefined intervals. Thus, I designed a challenging but hopefully interesting function of the rates. The speciation rate \( \lambda(t) = 1.0 \) for \( t \in [0.0, 0.8] \), then is increased for a time of rapid radiation as \( \lambda(t) = 2.0 \) for \( t \in [0.8, 1.0] \) and switches back to the original rate when \( t > 1.0 \). The extinction rate changes at the same time points. First, the rate is comparably high \( \mu(t) = 0.95 \) for \( t \in [0.0, 0.8] \) and then slows down to \( \mu(t) = 0.5 \) for \( t \in [0.8, 1.0] \) before switching back to the original value as well. Additionally a mass-extinction event occurs at time \( t = 0.8 \) and each species survives with probability \( p = 0.2 \). The process resembles the situation when the diversity increases only slowly but a high species turnover happens until a mass-extinction event removes most
species but also opens new niches which results into a time of rapid radiation. Furthermore, each extant species was sampled with probability \( p = 0.5 \). I ran both R packages on a MacBook Pro with a 2.3 GHz Intel Core i7 using only one core at a time to produce as fair as possible results. Simulations with the given rates where performed for \( n \in \{10, 20, \ldots, 200\} \) species sampled at the present time. For each \( n \), 100 trees were simulated and the median running time was recorded (see Figure 3). Note that the distribution of running times is very skewed for TreeSim. Therefore, the median is a more stable estimator than the mean but the median is also much lower. TESS outperforms TreeSim up to four orders of magnitude. Furthermore, TESS computation time is mostly independent of the number of species and the main challenge is to compute the integral of the probability of survival. Additionally, TESS can simulate a batch of trees faster once the integral of the survival probability is pre-computed which is shown in the simulations of 10,000 trees (TESS 10K).

### 3.2 Posterior Prediction

For each simulated tree I computed the posterior predictive distribution can be used to assess the fit of the model to the observed data (Gelman et al., 2003). Posterior predictive tests have the advantage to take the uncertainty in the model parameters into account, if applied an a Bayesian analysis (Bollback, 2002). If only the point estimate is used, e.g. the maximum likelihood estimate, then it is possible to compute the distribution of number of species at any time \( t \) using Equation (3) or the probability of \( n \) species in the reconstructed tree at time \( t \) given in Nee et al. (1994). Instead, when the uncertainty of the estimated parameters is of interest, an analytical solution is infeasible in most situations because commonly there does not exist a closed form solution for the posterior distribution, as for the model considered in this paper. Here I will present an example how simulating reconstructed trees can be used to approximate the posterior predictive distribution and evaluate the fit of the model. If the observed statistic fall outside the 95% confidence interval, then we can reject the model.

I used the species tree phylogeny from Aaström et al., 2011, Additional file 7 containing the bird family Cettiidae. Phillimore and Price (2008) showed that most bird phylogeny show patterns of a diversification slowdown. The rate functions with a slowdown in diversification can be modeled by the functions \( \lambda(t) = \lambda_0 + \lambda_1 \exp(-\alpha t) \) and \( \mu(t) = \mu. \) I choose an exponential prior distribution for each parameter with rate 0.1 which is comparable flat but still puts most prior probability on small parameter values and hence on more simple models, i.e. the parameter combination \( \mu = 0 \) represents the pure birth model. The likelihood is obtained from Equation (6), starting at the MRCA and conditioned on the survival of both species.

The posterior distribution was approximated using a Markov chain Monte Carlo algorithm (Metropolis et al., 1953; Hastings, 1970). The parameter values were changed using a scaling proposal and each parameter was updated once per iteration. The MCMC was run for 55000 iterations and the first 5000 iterations were considered as the burnin period. Convergence was assessed using the Geweke test of convergence to same distribution in two parts of the run (Geweke, 1991). Every 10th sample from the posterior distribution was used to simulated one tree conditioned on the time of the process (see Figure 4), number of taxa at the present time and the \( \gamma \)-statistic (Pybus and Harvey, 2000). The histograms of the predicted number of taxa and \( \gamma \)-statistic values indicate that the observed values fall well within the 95% confidence intervals. The observed lineage-through-time (LTT) plot and the posterior predictive LTT plots both show significant decrease in the rate of diversification. More generally, the observed LTT plot falls well within the ranges of posterior predicted LTT curves. This indicates that the model fits the data. However, the large variance in the prediction shows the uncertainty and possibly a different model could fit the data more closely.

The MCMC algorithm as well as a basic posterior predictive test is available in TESS. The MCMC algorithm is kept as general as possible so that any possible model might be used. See the additional file PosteriorPredictionCettidae.R that was used for this study.

### 4 DISCUSSION

In the present paper I only considered simulations of reconstructed phylogenies. Most estimated phylogenies are reconstructed phylogenies because no information about extinct species is available, such as ancient molecular sequences, and only the extant species are included. Nevertheless, phylogenetic trees including some extinct species might provide more information in estimating the time-dependent diversification rates. In these cases, either the GSA or simulating the missing speciation and extinction events following the idea of Bokma (2008) are useful.

Another drawback of the GSA is that events — e.g. mass-extinction events — cannot be specified to happen at a given time before the present when one conditions on the number of species being sampled. In this manuscript I did not consider these circumstances, although the simulation procedure can be modified to accommodate events at specific times before the present without major difficulties. In this case the distribution of the time of the origin (or the MRCA) needs to be considered backwards in time instead of forwards. Finally, once the time of the process is obtained, the simulation of the single speciation events remains unchanged.

The simulation algorithm presented here in this paper relies on the factorization of the probability density function. Previously, only for the constant rate birth-death process has such a factorization been known (Yang and Rannala, 1997; Höhna et al., 2011). I derived the factorization for the time-dependent birth-death process. Unfortunately, other types of birth-death process, e.g. the diversity-dependent birth-death process (Etienne et al., 2012) or the protracted speciation process (Etienne and Rosindell, 2012), do not have a know factorization. Nevertheless, the time-dependent birth-death process might be used to approximate other birth-death processes, such as the diversity-dependent birth-death process (Rabosky and Lovette, 2008).

It is possible to simulate tree using an MCMC algorithm, once the likelihood equations are known. However, if no direct simulation technique from the desired distribution is known, then the MCMC algorithm must start from some arbitrary starting values. Furthermore, samples are random draws from the specified distribution only after the chain has reached its stationary distribution. When the chain has reached its stationarity distribution and how long it takes to do so is a challenge in itself (Gelman et al., 2003). Thus, direct sampling, as I propose here, is safer, faster and can even be used for starting values of MCMC runs.

Several of the more complicated birth-death processes, e.g. containing different rates for different species (FitzJohn et al., 2009) or heritable extinction rates (Rabosky, 2009), do not have a known likelihood function. Instead, approximate Bayesian computation (ABC) can be applied to estimate the parameters of interest (Beaumont et al, 2002) although ABC has been criticized and should be used cautiously (Robert et al., 2011). The birth-death simulations implemented in TESS rely on the likelihood function and therefore neither ABC is necessary nor are these simulations needed for parameter estimation. Nonetheless, the simulation techniques can be used to test the summary statistics used in ABC. Currently, only the number of species at the present time, the time of the origin and the \( \gamma \)-statistic have been used as summary statistics for ABC under a birth-death model (Rabosky, 2009). Using TESS it might be possible to test the performance of these summary statistics and develop new ones.
Fig. 4. Posterior predictive draws were obtained by approximating the posterior distribution with an MCMC algorithm and then simulating trees conditioned on the time of the process. For the first 100 trees the LTT curves (left plot), for each tree the number of taxa at present (middle plot), the \( \gamma \)-statistic (right plot) are plotted. The thin gray lines represent the random draws and the thicker black line the observed data. The histograms contain the 95% confidence interval (dashed lines) and the observed value marked with ‘x’. See the additional file `PosteriorPredictionCettiidae.R`.

5 CONCLUSION

Simulating reconstructed phylogenies is important for developing and testing macro-evolutionary processes but has been a challenge for non-constant birth-death models (Hartmann et al., 2010). In the present manuscript I present an algorithm that can be used to simulate reconstructed phylogenies when conditioning on the number of species being sampled, or the time of the process or both. The presented algorithms can accommodate any time-dependent rate function — continuous and discrete — and can model mass-extinction events as well as periods of rapid radiation. Compared to the currently only available software for these type of simulations, TreeSim, my implementation is four orders of magnitude faster for 200 taxa and does not need significantly more computational time to simulate larger trees. Thus, simulations under even more complex models and many taxa at present are now feasible.

I derived the probability density of the sampled, reconstructed phylogenetic tree. The derivation follows the ideas of Thompson (1975) and Nee et al. (1994). The newly derived probability density is provided in a general and new form. It shows that the speciation times are iid, which has previously only been shown for constant rates (Yang and Rannala, 1997). This enables for instance the Diversified Sampling under time-dependent speciation and extinction rates (Höhna et al., 2011).

R is widely used for inferring diversification patterns (Harmon et al., 2008). It provides a good framework to implement new methods that can be shared and used freely. The methods of this paper are implemented in the R package TESS. TESS constructs trees in the phylo format of APE (Paradis et al., 2008). Thus, the trees simulated in TESS can be directly analyzed in APE and other tools using the phylo format. The implementation is kept very general so that any time-dependent rate function can be used. Additionally, an implementation of the likelihood computation is provided that can be used either in a maximum likelihood or in a Bayesian inference, as shown in the posterior prediction example.

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**APPENDIX**

I give here a short version of the proof of the joint probability density of all speciation times of a reconstructed tree. First, let me restate the probability of obtaining exactly 1 species at time \( t \) with \( T > t \).

At any given time \( s, t < s < T \) the number of species could be between 1 and infinity, however, all but one species must go extinct until time \( T \) (Thompson, 1975):

\[
P(N(T) = 1 | N(s) = 1) = \sum_{k=1}^{\infty} k \times P(N(s) = k | N(t) = 1) \times P(N(T) = 1 | N(s) = 1)
\]

\[
\times (P(N(T) = 0 | N(s) = 1))^{k-1} \quad (14)
\]

The factor \( k \) arises because any of the \( k \) currently alive species could survive until the present and we need to consider each possibility.

Let us transform the reconstructed tree from Figure 1 into a set of branches \( b = \{\{s_0, t_1\}, \{t_1, t_2\}, \{t_1, t_3\}, \{t_2, t_4\}, \{t_3, T\}, \{t_4, T\}, \{t_4, T\}\} \). There are two types of branches, those that end at a speciation event and those that end at the sampling time \( T \). The probability density of a branch that ends at the present time is simply the probability of starting with one species and observing exactly one species

\[
P(\{t_i, T\} = P(N(T) = 1 | N(t_i) = 1) = 1). \quad (15)
\]

The probability of any other branch starting at time \( t_i \) and ending at a speciation event at time \( t_i \) is given by the probability that there were \( k \) species at time \( t_j \), one of these speciated with probability \( k\lambda(t_j) \), this species survived but all other \( k - 1 \) species went extinct before the present time:

\[
P(\{t_i, t_j\}) = \sum_{k=1}^{\infty} k \times \lambda(t_j) \times P(N(t_j) = k | N(t_j) = 1) \times (P(N(T) = 0 | N(t_j) = 1))^{k-1} \quad (16)
\]

If we multiply the equation by \( P(N(T) = 1 | N(t_j) = 1) / P(N(T) = 1 | N(t_j) = 1) \) we can see that we get exactly Equation (14)

\[
P(\{t_i, t_j\}) = 1 / P(N(T) = 1 | N(t_j) = 1) \sum_{k=1}^{\infty} k \times \lambda(t_j) \times P(N(t_j) = k | N(t_j) = 1) \times (P(N(T) = 0 | N(t_j) = 1))^{k-1} \times (P(N(T) = 1 | N(t_j) = 1)) = \lambda(t_j) \times P(N(T) = 1 | N(t_j) = 1) / P(N(T) = 1 | N(t_j) = 1) \quad (17)
\]

Finally, by multiplying the probability densities of all branches together we obtain the probability density of the reconstructed tree

\[
P(b) = \prod_{\forall b, t_j \neq T} \lambda(t_j) \times P(N(T) = 1 | N(t_j) = 1) / P(N(T) = 1 | N(t_j) = 1) \times \prod_{\forall b, t_j \neq T} P(N(T) = 1 | N(t_j) = 1) = P(N(T) = 1 | N(t_0) = 1) \prod_{i=1}^{n-1} \lambda(t_i) P(N(T) = 1 | N(t_i) = 1) \quad (18)
\]

The probability density of all branches \( P(b) \) is equivalent to the probability density of all branching times (Equation (6)).