# Validation of dated phylogenies in microbial population genetics

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# INTRODUCTION

Dated phylogenies, also known as tip-calibrated, time-stamped or time-calibrated phylogenies, have become a ubiquitous tool in the study of microbial population genetics (Drummond et al. 2003; Biek et al. 2015; Rieux and Balloux 2016). In a dated phylogeny, the branch lengths are measured in a unit of time, for example years or days, rather than a unit of evolution as in a standard phylogeny. Consequently, the tips of a dated phylogeny are aligned with the (typically known) dates of sampled genomes and the internal nodes are aligned with the (typically inferred) dates of common ancestors between the genomes. Many tools exist to build dated phylogenies, either from a sequence alignment using for example BEAST (Suchard et al. 2018) or BEAST2 (Bouckaert et al. 2019), or by dating the nodes of a standard phylogeny, using for example LSD (To et al. 2016), node.dating (Jones and Poon 2017), treedater (Volz and Frost 2017), BactDating (Didelot et al. 2018) and TreeTime (Sagulenko et al. 2018). The dated phylogeny is interesting in itself since it depicts the ancestral relationships of sampled genomes over time, but it is also often used as the foundation for further analysis (Didelot and Parkhill 2022), such as inference of demographics (Baele et al. 2016), phylogeography (Lemey et al. 2009) or transmission between hosts (Didelot et al. 2017).

There are many factors that can invalidate the results of a dated phylogenetics analysis. This includes in particular the confounding effect that population structure can have on dating (Duchene et al. 2015; Murray et al. 2016). This is especially true when the substructures are imbalanced (Duchêne et al. 2015), are sampled at different dates (Tong et al. 2018), have different clock rates (Wertheim et al. 2012) and when the population structure is strong (Navascués and Emerson 2009). More generally, any incorrect assumptions made by the model under which the dating analysis is performed can invalidate the results.

One approach that has been used to ensure that there are no incorrect assumptions being made in the model is to perform inference under multiple models and perform model comparison, typically by computing a Bayes Factor (Baele et al. 2012; Li and Drummond 2012; Bouckaert and Drummond 2017). However, this requires multiple runs under different models, and only provides a relative measure of model appropriateness, with no indication of how good the best model actually is in absolute terms. Another related line of research involves testing the significance of the temporal signal (Duchene et al. 2015, 2020). This can be done for example by comparing results with and without dates (Rambaut 2000) or by randomizing the leaf dates (Duchene et al. 2015).

Here we present an alternative approach, in which we seek to evaluate the correctness of an inference and detect if there are any reasons to believe that the inference is not valid. This approach is sometimes referred to as model checking, model diagnostics or model validation, and it is complementary with the model comparison methodology mentioned above. We study the distribution of residuals after fitting a model, following methodology reminiscent of regression models (Cox and Snell 1968; Dunn and Smyth 1996), but also previously applied more generally for example to epidemic models (Lau et al. 2014) or Hidden Markov Models (Zucchini and MacDonald 2009; Buckby et al. 2020).

We use simulated datasets to demonstrate that this approach can detect a wide range of problems in the inference, including the aforementioned confounding effect of population structure (Murray et al. 2016). We also demonstrate the usefulness of this approach in practice on real datasets.

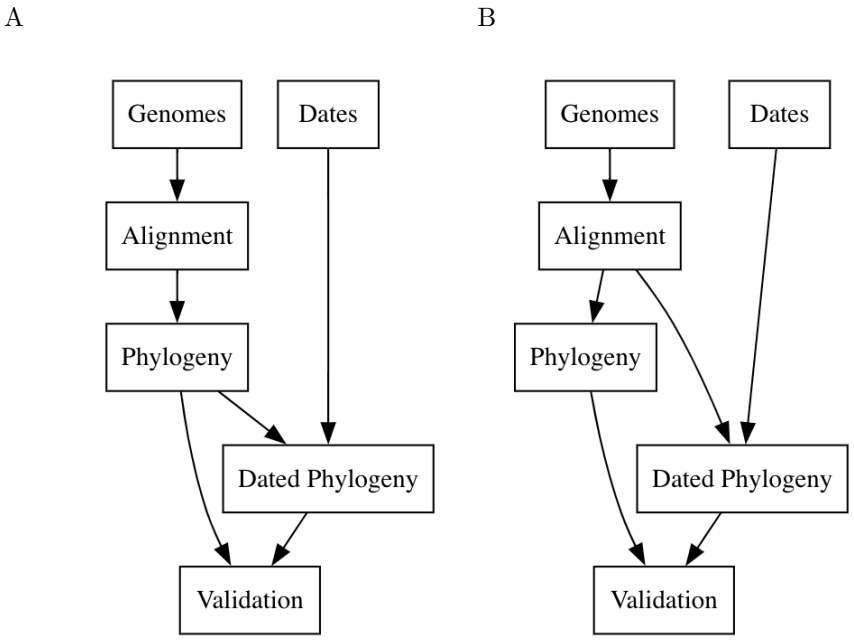


Figure 1: (A) General approach for validation of a dated phylogeny built by dating the nodes of a standard phylogeny. (B) General approach for validation of a dated phylogeny built directly from a sequence alignment.

# RESULTS

## General approach

We want to validate a dated phylogeny $\mathcal{D}$, previously constructed using any method. We propose to do so by comparing the dated phylogeny $\mathcal{D}$ with an undated phylogeny $\mathcal{L}$. If the method used to construct $\mathcal{D}$ involved dating the nodes of an undated phylogeny, for example TreeTime (Sagulenko et al. 2018) or treedater (Volz and Frost 2017), then this is readily available for validation and we therefore focus on this case in this article (Figure 1A). However, the validation methodology below can also be applied to methods that build a dated phylogeny directly from the alignment such as BEAST (Suchard et al. 2018), simply by constructing a separate undated phylogeny from the same alignment using for example PhyML (Guindon et al. 2010) or RAxML (Stamatakis 2015) (Figure 1B). For each branch in the dated phylogeny $\mathcal{D}$ we can consider its inferred length, the number of substitutions happening on that branch in the standard phylogeny $\mathcal{L}$, and the model and parameters used when building the dated phylogeny $\mathcal{D}$, in order to compute a residual for that branch (see Methods). If the inference is valid, these residuals will follow their theoretical distribution (Cox and Snell 1968; Dunn and Smyth 1996). We use this property as a way to test the validity of the dated phylogeny $\mathcal{D}$.

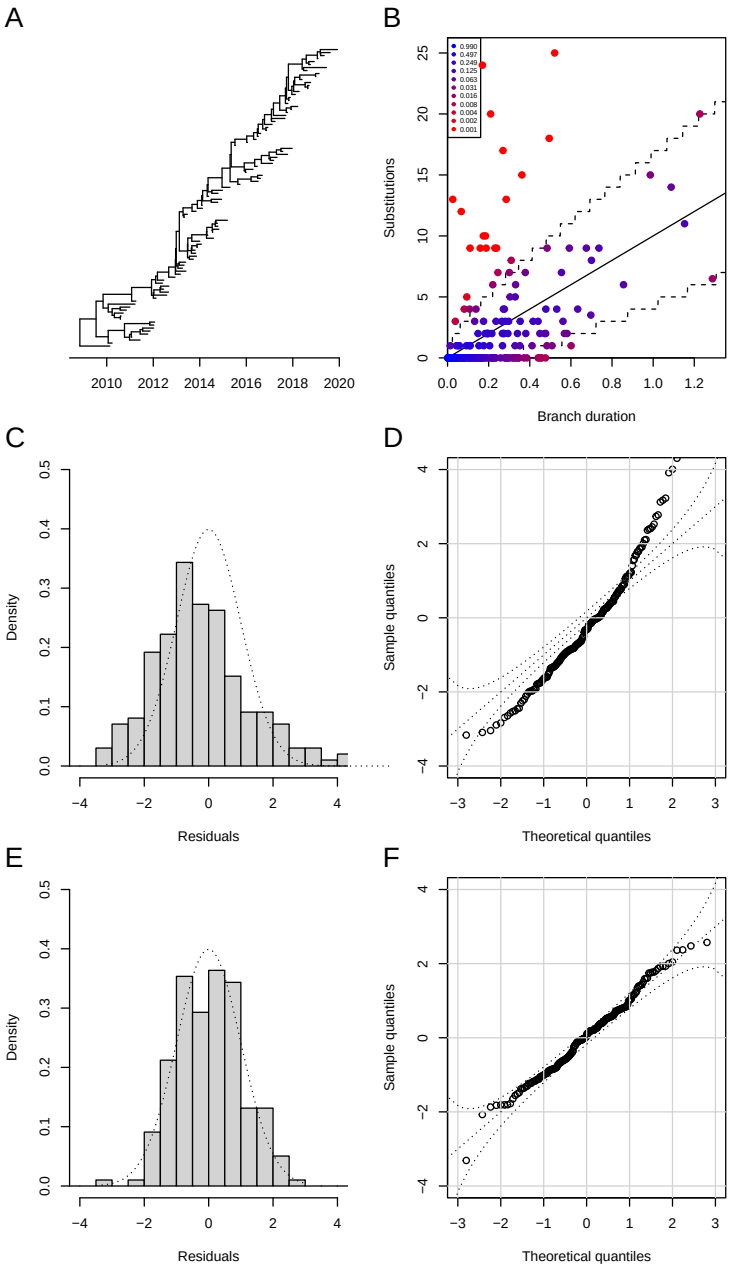


Figure 2: (A) Simulated dated phylogeny. (B) Distribution of substitutions generated by a relaxed clock model on the branches of the dated phylogeny, with their probability under a strict clock model. (C) Distribution of residuals after inference under a strict clock model. (D) QQ plot of residuals after inference under a strict clock model. (E) Distribution of residuals after inference under a relaxed clock model. (F) QQ plot of residuals after inference under a relaxed clock model.

## Motivating example

A dated phylogeny was simulated including 100 leaves uniformly distributed between 2010 and 2020, under the heterochronous coalescent model (Drummond et al. 2002) with constant population size $N_e g = 1$ year (Figure 2A). We applied the additive relaxed clock model (Didelot et al. 2021) to this dated phylogeny, with mean clock rate $\mu = 10$ substitutions per year and relaxation parameter $\omega = 5$ (Equation 3). Consequently, some branches had many more or less substitutions compared to what would be expected under a strict clock model with $\mu = 10$, and the probabilities of these branches under this model would be low (Figure 2B). Nevertheless, a root-to-tip regression seemed very satisfactory, with $R^2 = 0.94$ and $p < 10^{-4}$ for a date randomization test (Figure S1).

We applied BactDating (Didelot et al. 2018) to reconstruct the dated tree, incorrectly assuming a strict clock model (Equation 1). The clock rate was estimated to be $\mu = 10.5$ [9.4;11.6] and the root date 2008.6 [2008.1;2009.1] which is approximately correct. However, the residuals for the branches were not distributed as Normal(0,1) (Figure 2C) and a QQ plot revealed significant deviation (Figure 2D). The Anderson-Darling test rejects the hypothesis of standard normality of the residuals ($p = 3.03 \times 10^{-6}$). We repeated the same analysis incorrectly assuming a strict clock model using LSD (To et al. 2016), node.dating (Jones and Poon 2017), treedater (Volz and Frost 2017) and TreeTime (Sagulenko et al. 2018), all of which led to similar results (Figure S2).

We applied BactDating again, but this time used the correct additive relaxed clock model (Equation 3). The clock rate estimated to be $\mu = 11.3$ [8.8;14.1], the root date was 2008.9 [2007.7;2009.8] and the relaxation parameter was $\omega = 6.4$ [4.2;8.9], all of which is approximately correct. The residuals looked approximately distributed as they should be both when plotting them against their theoretical distribution (Figure 2E) and when constructing a QQ plot (Figure 2F). The Anderson-Darling test did not reject the hypothesis of standard normality of the residuals ($p = 0.658$).

## Confounding effect of population structure

## Benchmarking

## Real data examples

# DISCUSSION

TODO

# MATERIALS AND METHODS

## Molecular clock models

The molecular clock model determines the distribution of number of substitutions $l_i$ on a branch of the dated tree with duration $d_i$. We consider four types of molecular clock models, for each combination of discrete vs continuous and strict vs relaxed. In the discrete strict clock model (Zuckerkandl and Pauling 1962) with rate $\mu$, substitutions occur on the branches as a Poisson process with rate $\mu$ and therefore:

$$l_i \sim \text{Poisson}(d_i\mu) \tag{1}$$

A continuous version of the strict clock model can be formed based on a Gamma process (Didelot et al. 2021):

$$l_i \sim \text{Gamma}(d_i\mu, 1) \tag{2}$$

Strict clock models are based on the assumptions that the substitution rate is constant throughout the branches of the tree, but this is not always true in which case a relaxed clock model can be used which allows the rate to vary (Drummond et al. 2006). In particular here we use the additive relaxed clock model (Didelot et al. 2021), in which $\mu$ is the mean clock rate and $\omega$ determines how much this rate varies on the branches. The discrete version of this model is given by:

$$l_i \sim \text{NegativeBinomial}\left(\frac{d_i\mu}{\omega}, \frac{1}{1+\omega}\right) \tag{3}$$

whereas the continuous additive relaxed clock model is defined as:

$$l_i \sim \text{Gamma}\left(\frac{d_i\mu}{1+\omega}, 1+\omega\right) \tag{4}$$

Note that throughout this article Gamma distributions are parametrised by shape and scale and Negative Binomials by number of successes and probability of success. In the four models we have that the mean of $l_i$ is equal to $d_i\mu$. The variance of $l_i$ is equal to its mean in the two strict clock models, and equal to its mean times $(1+\omega)$ in the two relaxed clock models.

## Sampling a dated phylogeny given its estimate

We want to validate a dated phylogeny $\mathcal{D}$ by comparison with an undated phylogeny $\mathcal{L}$. If the dated phylogeny $\mathcal{D}$ was sampled from its posterior distribution for example using BactDating (Didelot et al. 2018), then residuals can be calculated directly as described in the next subsection. [1] If on the

[1] May be better to consider more than one sample and generate a distribution of p-values. But then could not show single residual value for each branch anymore.

other hand $\mathcal{D}$ is the result of a maximum likelihood estimation, or a summary tree from the posterior distribution (Heled and Bouckaert 2013), then we need to generate a sample from the posterior before residuals can be computed. [2] [3] Let $\hat{d}_i$ be a branch length in $\mathcal{D}$, on which there are $l_i$ substitutions in the undated phylogeny $\mathcal{L}$. If $\hat{d}_i$ is a maximum likelihood estimate of $d_i$ then $\hat{d}_i = l_i/\mu$. Consider the discrete strict clock model (Equation 1) and that the prior of $d_i$ is:

$$d_i \sim \text{Gamma}(k, \theta) \tag{5}$$

We can "guess" the parameters of this prior using the mean $m$ and variance $v$ of the $\hat{d}_i$ for all $i$ and applying $k = m^2\mu/(v\mu - m)$ and $\theta = v/m - 1/\mu$. [4] The posterior of $d_i$ is then:

$$d_i \sim \text{Gamma}\left(k + \hat{d}_i\mu, \frac{\theta}{1 + \theta\mu}\right) \tag{6}$$

We simulate from this distribution to get $d_i$. [5] [6] [7] [8] [9]

## Computation of residuals

[10]

Let $d_i$ be the duration of a given branch in $\mathcal{D}$ and $l_i$ be the number of substitutions on the corresponding branch of $\mathcal{L}$, that is the branch that separates the leaves in the same way. There is a unique corresponding branch in $\mathcal{L}$ for all branches in $\mathcal{D}$ except for the two branches $a$ and $b$ connected to the root of $\mathcal{D}$ for which there is only a single corresponding branch $x$. We therefore split the substitutions on $x$ proportionally between the two branches $a$ and $b$ by defining:

$$l_a = \frac{l_x d_a}{d_a + d_b} \text{ and } l_b = \frac{l_x d_b}{d_a + d_b} \tag{7}$$

The distribution of $l_i$ given $d_i$ is given by the molecular clock model. Let us for now consider that the distribution is continuous (as in Equations 2 and 4) and we will return later to the discrete case (as in Equations 1 and 3). Instead of a specific model, we consider the general case where $F_i(l_i)$ is the cumulative distribution function of $l_i$ given $d_i$. Let $u_i$ denote the uniform residual for the observation $l_i$, defined as:

$$u_i = F_i(l_i) = p(L_i \leq l_i | d_i) \tag{8}$$

[2] Need to explain why, cf script resampling.R.

[3] Will assume below that the input dated phylogeny is maximum likelihood estimate, not a summary of the posterior sample, in which case it would be something like a maximum a-posteriori estimate and may need adjusting.

[4] Would need justification. This is based on the idea that $\hat{d}_i$ is the MLE $s_i/\mu$ and applying the laws of total expectation and total variance to get that $\hat{d}_i$ has expectation $k\theta$ and variance $k\theta/\mu + k\theta^2$.

[5] This procedure is assuming that the $d_i$ are iid which is not true.

[6] In practice this procedure works well for iid case (cf script resampling.R) but not well for non-iid case. Reducing the variance in the posterior helps (cf line 50 of resid.R) but is a temporary ad hoc fix.

[7] Need to do other models than strict clock model. Conjugacy of priors and posteriors is not as readily available as for Poisson case. May need a Monte-Carlo method (short run of MH algorithm?) to get sample.

[8] Maybe a better way to generate a sampled $\mathcal{D}$ is to run BactDating constrained somehow to the input dated tree.

[9] Ok to be a bit approximate if we show good sensitivity and specificity on simulated datasets, but would still be good to be as close to correct as possible.

[10] Notations are not consistent. $\mathcal{D}$ sometimes refer to input dated phylogeny and sometimes to sampled dated phylogeny.

If the inference is valid, then the uniform residual $u_i$ should be distributed as Uniform(0,1), because for any random variable $X$ with cumulative distribution function $F$ we have that $U = F(X)$ is Uniform(0,1). However, it is difficult to assess how close to zero or one a value needs to be in order to be an outlier. We therefore define the normal residuals $n_i$, analogous to the residuals commonly used in regression models (Cox and Snell 1968; Dunn and Smyth 1996). The normal residuals are obtained by transforming the uniform residuals with the inverse of the cumulative distribution function $\Phi$ of a Normal(0,1) random variable:

$$n_i = \Phi^{-1}(u_i) \tag{9}$$

If the inference is valid, then the normal residual $n_i$ should be distributed as Normal(0,1) which is more convenient to work with than the Uniform(0,1) for uniform residuals. The uniform and normal residuals above can be computed directly when the clock model is continuous (Equations 2 and 4) but when the clock model is discrete (Equations 1 and 3) we need to make the following adjustment (Dunn and Smyth 1996; Brockwell 2007; Lau et al. 2014):

$$u_i \sim \text{Unif}(F_i(l_i), F_i(l_i + 1)) \tag{10}$$

## Analysis of residuals

After computation of the uniform residuals $u_i$ and normal residuals $n_i$, we use several methods to assess the validity of the dated phylogeny inference. The uniform residuals $u_i$ can be plotted as a histogram to compare their distribution with the theoretical Uniform(0,1) distribution, but as previously noted this can be difficult to interpret. We therefore prefer to use the normal residuals $n_i$ which can be plotted as a histogram to compare their distribution with the theoretical Normal(0,1). A quantile-quantile plot (QQ plot) can be used to compare the distribution of the residuals to their theoretical distribution. Anderson-Darling test (Lewis 1961) in DescTools R package implements simple hypothesis testing (unlike nortest package which is composite test). Use this to test that normal residuals are standard normal. Note this is exactly equivalent to testing the uniform residuals against Uniform(0,1). Anderson-Darling simple hypothesis testing was used in (Lau et al. 2014) via implementation in package ADGofTest, returns same results as DescTools. Both use the same C code from (Marsaglia and Marsaglia 2004). [11] [12] [13]

## Data simulation

Some simulations using DetectImports (Didelot et al. 2023b).

[11] Other option: Shapiro-Wilk test for normality. Usually found to be most powerful test of normality. But performs composite testing only (ie tests if Normal, not if standard Normal)

[12] Other option: Kolmogorov-Smirnov test, specific against standard Normal but not very powerful. Note this is exactly equivalent to testing the uniform residuals against Uniform(0,1).

[13] Need to check there is not too much loss of power compared to Shapiro-Wilk or even composite Anderson-Darling which can apparently be more powerful, cf https://en.wikipedia.org/wiki/Anderson-Darling_test quote : Stephens[1] notes that the test becomes better when the parameters are computed from the data, even if they are known.

Some simulations using Master (Vaughan and Drummond 2013) to simulate under the structured coalescent model (Nordborg 1997).

Some simulations using mlesky (Didelot et al. 2023a) to simulate each population with non-constant population size. The size of the $j$-th population follows a previously studied model of clonal expansion (Helekal et al. 2021):

$$N_j(t) = \frac{M_j(t-s_j)^2}{h_j^2 + (t-s_j)^2}[t \geq s_j] \tag{11}$$

Note that the square brackets are Iverson brackets. Each population starts at time $s_j$ with size $N(s_j) = 0$ and grows logistically up to its maximum $N_j(\infty) = M_j$, with $h_j$ being the time taken to reach half of this since $N_j(s_j + h_j) = M_j/2$.

## Real data

TODO

## Implementation

We implemented the analytical methods described in this paper in a new R package entitled *ValidateDating* which is available at `https://github.com/xavierdidelot/ValidateDating` for R version 3.5 or later. All code and data needed to replicate the results are included in the "run" directory of the *ValidateDating* repository.

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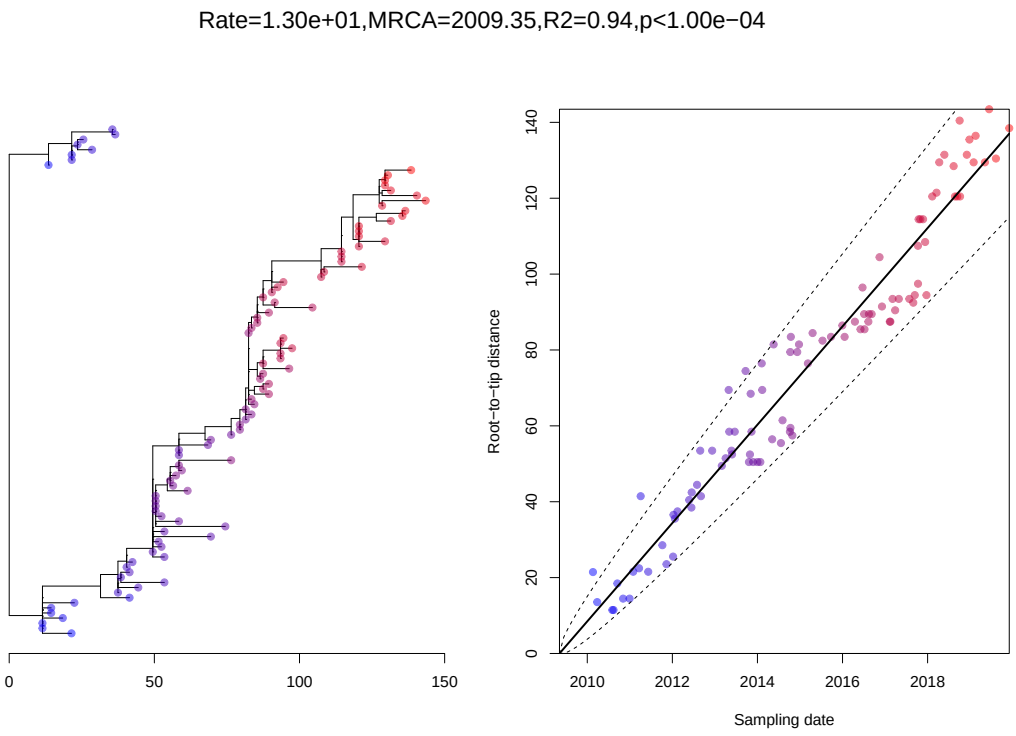


Figure S1: Root-to-tip regression analysis for the motivating example.

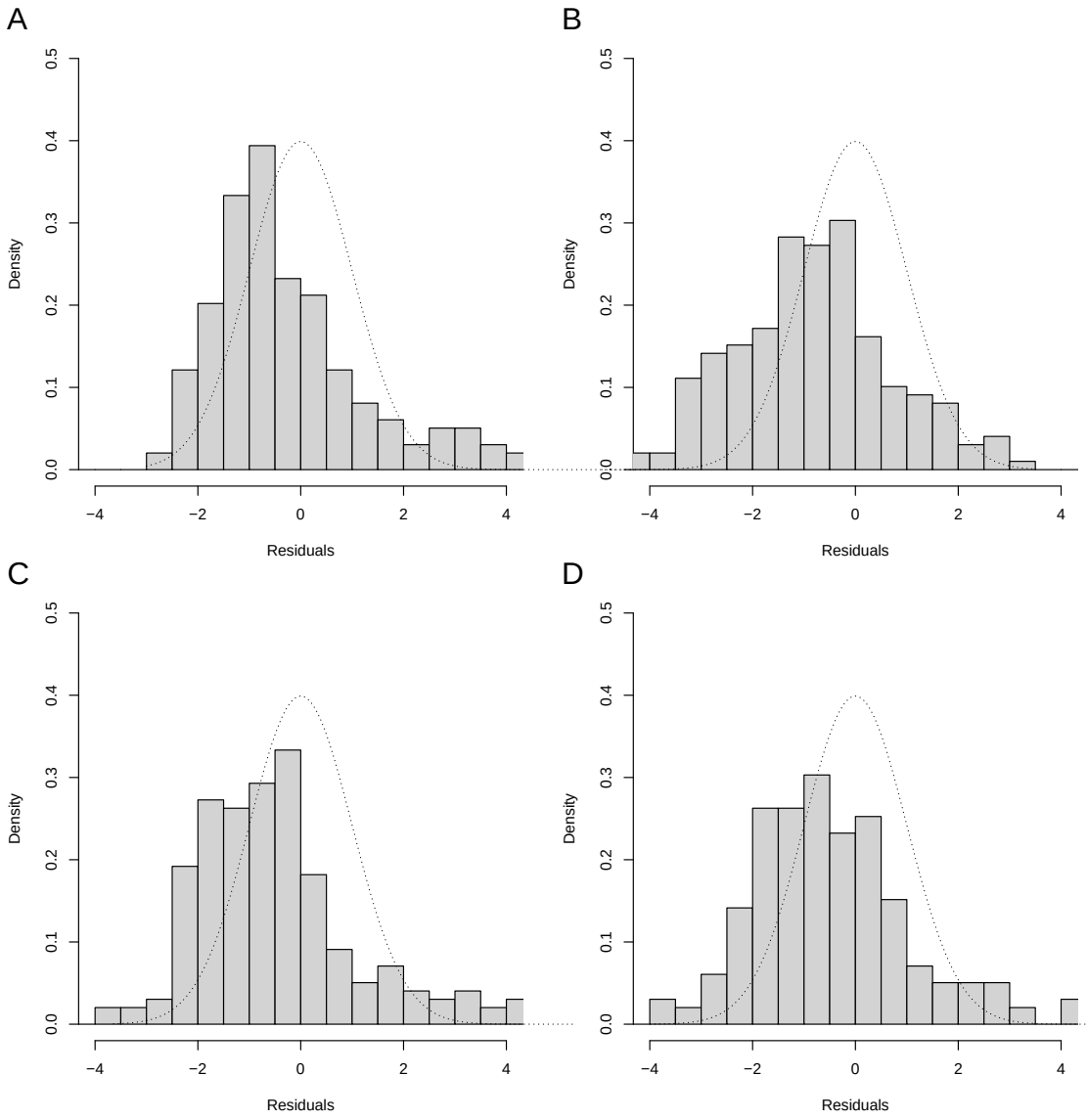


Figure S2: Residuals after application on the motivating example of a strict clock model using LSD (A), node.dater (B), treedater (C) and TreeTime (D).