Smarter dating and faster proposals: revisiting the phylogenetic relaxed clock model

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Abstract

Author summary

Introduction

The molecular clock hypothesis states that the evolutionary rates of biological sequences are approximately constant throughout time [1]. By applying this assumption under a phylogenetic framework, evolutionary trees and divergence dates of life forms can be inferred from biological sequences, such as nucleotides and amino acids [2,3]. In Bayesian phylogenetics, these trees and their associated parameters are estimated as probabilitiy distributions [4–6]. Statistical inference is typically performed by sampling under the Metropolis-Hastings Markov chain Monte Carlo (MCMC) algorithm [7,8]. Bayesian phylogenetic clock models have been implemented in many platforms including BEAST [9], BEAST2 [10], MrBayes [11], and RevBayes [12].

The simplest phylogenetic clock model – the strick clock – makes the convenient assumption that the evolutionary rate is constant across all lineages [4,5,13]. However, molecular substitution rates are known to vary over time, over population sizes, over evolutionary pressures, and over DNA replicative machineries [14–16]. Moreover, any given dataset may be clock-like (i.e. a small variance in substitution rate across lineages) or non clock-like (a large variance). In the latter case, a strict clock is probably not suitable.

This led to the development of relaxed (uncorrelated) clock models, under which each branch in the phylogenetic tree has its own substitution rate [3]. Branch rates can be drawn from a range of probability distributions including log-normal, exponential, gamma, and inverse-gamma distributions [3,17,18]. This class of models is widely used, and has aided insight into many recent biological problems, including the 2016 Zika virus outbreak [19] and the COVID-19 pandemic [20].

Finally, although not the focus of this article, the class of correlated clock models assumes some form of auto-correlation between rates over time. The correlation itself can invoke a range of stochastic models, including compound Poisson [21] and CIR processes [17], or it can exist as a series of local relaxed clocks [22]. However, due to the correlated and discrete nature of such models, the time until MCMC convergence may be cumbersome, particularly for larger datasets [22].

With the increasing availabilty of biological sequence data, the development of efficient Bayesian phylogenetic methods is more important than ever. The performance

of MCMC is dependent not only on computational runtime but also the efficacy of an MCMC setup to achieve its convergence. A critical task therein lies the further advancement of MCMC operators. Recent developments in this area include the advancement of guided tree proposals [23–25], coupled MCMC [26, 27], adaptive multivariate transition kernels [28], and other explorative proposal kernels [29, 30]. In the case of clock models, smart tree proposals can account for correlations between substitution rates and divergence times [31]. The rate parameterisation itself can also affect the ability to "mix" during MCMC [3, 18, 31].

While a range of advanced operators and other MCMC optimisation methods have arisen over the years, there has yet to be a widescale performance benchmarking of such methods as applied to the relaxed clock model. In this article, we systematically evaluate how the relaxed clock model can benefit from i) different rate parameterisations, ii) the use of Bactrian proposal kernels [29], iii) tree operators which account for correlations between rates and times, and iv) adaptive multivariate operators [28]. Our findings suggest that...

Models

Preliminaries

Let $\mathcal{T}$ be a binary rooted time tree with N taxa (and $2N-2$ branches). Let L be the number of sites within the multiple sequence alignment D, and let L_{eff} be the effective number of sites in the alignment (i.e. the number of site patterns). The posterior density of a phylogenetic model is described by

$$p(\mathcal{T}, \vec{\mathcal{R}}\,, \sigma, \theta | D) \propto p(D|\mathcal{T}, r(\vec{\mathcal{R}}\,), \theta)\; p(\mathcal{T}|\theta)\; p(\vec{\mathcal{R}}\,|\sigma)\; p(\sigma)\; p(\theta), \tag{1}$$

for rate standard deviation σ and other model parameters θ. $\vec{\mathcal{R}}$ is a vector of abstracted substitution rates, which is transformed into real rates by $r(\vec{\mathcal{R}}\,)$. Three methods of representing rates as $\vec{\mathcal{R}}$ are presented in **Rate parameterisations**.

Under the *relaxed clock model*, each internal and leaf node is assigned a substitution rate $r_i = r(\mathcal{R}_i)$, which corresponds to its parent branch. There are a total of $|\vec{\mathcal{R}}\,| = 2N-2$ rates, which are independently distributed under the relaxed clock model prior [3].

Rate parameterisations

In Bayesian inference, the way parameters are represented in the model can affect the mixing ability of the model and the meaning of the model itself [32]. Three methods for parameterising substitution rates are described below, and are later evaluated in **Results**. Each parameterisation technique is associated with i) an abstraction of the rates $\vec{\mathcal{R}}$, ii) some function for transforming this parameter into real rates $r(\vec{\mathcal{R}}\,)$, and iii) a prior density function of the abstraction $p(\vec{\mathcal{R}}\,|\sigma)$. The three methods are summarised in **Fig 1**.

1. Real rates

The natural (and unabstracted) parameterisation of a substitution rate is a real number $\mathcal{R}_i \in \mathbb{R}, \mathcal{R}_i > 0$ which is equal to the rate itself. Thus, under the *real* parameterisation:

$$r(\vec{\mathcal{R}}\,) = \vec{\mathcal{R}}\,. \tag{2}$$

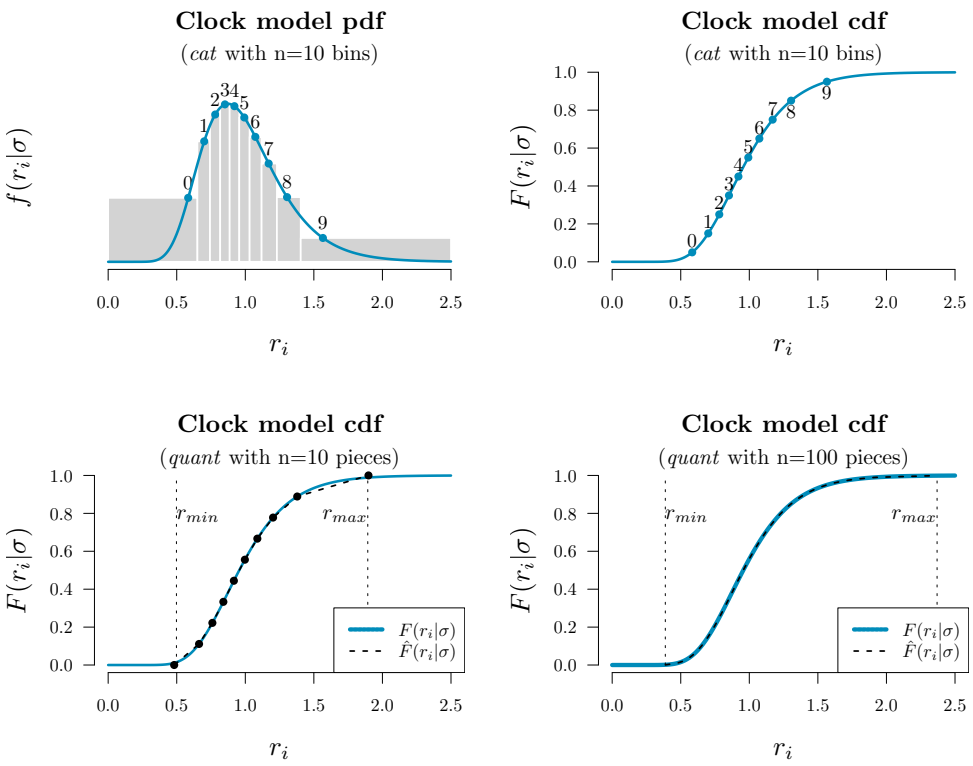

Fig 1. Methods of rate parameterisation. The *cat* and *quant* approximations are plotted on top of the true underlying rate prior distribution (*real*). In this example, rates are drawn from a LogNormal($\mu = -0.045, \sigma = 0.3$) distribution. The probability density function (pdf) and cumulative density function (cdf) of this distribution are shown.

Under the prior distribution $p(\vec{\mathcal{R}}\,|\sigma)$, rates are often log-normally or exponentially distributed with a mean of 1:

$$p(\mathcal{R}_i|\sigma) = \frac{1}{\mathcal{R}_i\sigma\sqrt{2\pi}} \exp\big(-\frac{(\ln \mathcal{R}_i - \mu)^2}{2\sigma^2}\big) \quad (\text{ LogNormal}(\mu, \sigma)\ ), \text{ or} \tag{3}$$

$$p(\mathcal{R}_i|\sigma) = p(\mathcal{R}_i) = e^{-\mathcal{R}_i} \quad (\text{ Exponential}(\lambda = 1)\ ) \tag{4}$$

where $\mu = -0.5\sigma^2$ is set such that the expected value of the log-normal distribution is 1.

Zhang and Drummond 2020 present a series of tree operators which propose internal/root node heights, and then recompute the rates of incident branches such that their genetic distances ($r_i \times \tau_i$) remain constant after the proposal [31]. By maintaining genetic distances the likelihood can also be maintained. These operators account for the correlation which exists between branch rates and branch times – a correlation which is induced by the likelihood function.

2. Categories

The category parameterisation (*cat*) is an abstraction of the *real* parameterisation. Each branch is assigned an integer from 0 to $n - 1$:

$$\vec{\mathcal{R}} \in \{0, 1, \ldots, n-1\}^{2N-2}. \tag{5}$$

The domain of $\vec{\mathcal{R}}$ is uniformly distributed:

$$p(\mathcal{R}_i|\sigma) = p(\mathcal{R}_i) = \frac{1}{n}. \tag{6}$$

Let $f(x|\sigma)$ be the probability density function (pdf) and let $F(x|\sigma) = \int_0^x f(t|\sigma)\, dt$ be the cumulative distribution function (cdf) of the prior distribution used by the underlying *real* clock model. Then, in the *cat* parameterisation, $f(x|\sigma)$ is discretised into n bins and the elements of $\vec{\mathcal{R}}$ each point to one of these bins. Each bin contains uniform probability density $1/n$. The rate of each bin is equal to the median value within the bin

$$r(\mathcal{R}_i) = F^{-1}\big(\frac{\mathcal{R}_i + 0.5}{n}\big), \tag{7}$$

where F^{-1} is the inverse cumulative distribution function (i-cdf).

The key advantage of the *cat* parameterisation is the removal of a term from the posterior density (Equation 1), or more accurately the replacement of a non-trivial $p(\vec{\mathcal{R}}\,|\sigma)$ term with that of a uniform prior. Thus, one fewer term needs to be estimated per rate.

This method was suggested in the original BEAST2 relaxed clock paper [3] and has been widely used. However, the constant distance operators since introduced by Zhang and Drummond 2020 – which are incompatible with the *cat* parameterisation – yield an increase in mixing rate under *real* by up to an order of magnitude over that of *cat*. Moreover, the implementation of the *cat* model has come under criticism [33].

3. Quantiles

Finally, rates can be parameterised as real numbers $0 < \mathcal{R}_i < 1$ which describe the rate's quantile with respect to some underlying clock model distribution. Under the *quant* parameterisation, each element in $\vec{\mathcal{R}}$ is uniformly distributed.

$$\vec{\mathcal{R}} \in \mathbb{R}^{2N-2}, 0 < \mathcal{R}_i < 1 \tag{8}$$

$$p(\mathcal{R}_i|\sigma) = p(\mathcal{R}_i) = 1 \tag{9}$$

Transforming these quantiles into rates invokes the i-cdf of the underlying *real* clock model distribution. Thus, while this approach has clear similarities with *cat*, the domain of rates here is continuous (as opposed to being confined to a discrete number of bins) and is therefore compatible with the class of operators described by Zhang and Drummond 2020 [31].

A potential disadvantage of the *quant* method would be the computational requirements of continuously evaluating the i-cdf, especially for trees with large N. Hence, rather than evaluating the exact i-cdf F^{-1}, an approximation $\hat{F}^{-1}$ will be used instead:

$$r(\mathcal{R}_i) = \hat{F}^{-1}(\mathcal{R}_i). \tag{10}$$

In this article we have extended *quant* through a linear piecewise approximation of the i-cdf. As the piecewise approximation is linear, evaluating the derivatives $\frac{\partial}{\partial \mathcal{R}_i}\hat{F}^{-1}(\mathcal{R}_i) = D\hat{F}^{-1}(\mathcal{R}_i)$ and $\frac{\partial}{\partial r_i}\hat{F}(r_i) = D\hat{F}(r_i)$ – which are required for computing Hastings ratios – is trivial. The approximation is comprised of n pieces (where n is fixed) and upper and lower rate boundaries $r_{\min}$ and $r_{\max}$. The approximation is displayed in **Fig 1** and further detailed in **S1 Appendix**.

Zhang and Drummond 2020 introduced several tree operators for the *real* parameterisation – including **Constant Distance**, **Simple Distance**, and **Small Pulley**. In this project, we extended these three operators so that they are compatible with the *quant* parameterisation. These are presented in **S1 Appendix**.

Bactrian proposal kernel

The step size of a proposal kernal $q(x'|x)$ should be such that the proposed state x' is sufficiently far from the current state x to explore vast areas of parameter space, but not so large that the proposal is rejected too often [34]. Yang et al. have challenged the widely used uniform proposal kernel in place of the Bactrian kernel [29, 30]. The Bactrian(m) distribution is defined as the sum of two Normal distributions:

$$\Sigma \sim \text{Bactrian}(m) \equiv \frac{1}{2}\text{Normal}(-m, 1-m^2) + \frac{1}{2}\text{Normal}(m, 1-m^2) \qquad (11)$$

where $0 \leq m < 1$ describes the modality of the Bactrian distribution. When $m = 0$, the Bactrian distribution is equivalent to a Normal$(0, 1)$ distribution. As $m \to 1$, the distribution becomes increasingly bimodal (**Fig. 2**). Yang et al. 2013 [29] suggest that Bactrian$(m = 0.95)$ yields a proposal kernel superior to the uniform kernel, by placing minimal probability on steps which are too small or too large.

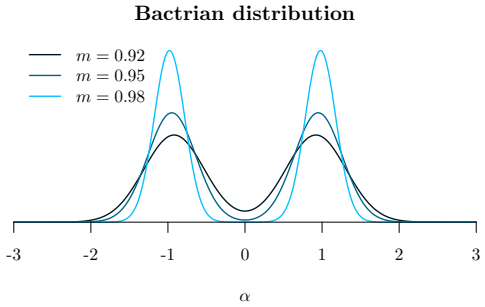

Fig 2. The Bactrian proposal kernel. Y-axis corresponds to probability density $f(\Sigma|m)$.

In this article we compare the performance of uniform and Bactrian proposal kernels in the clock model. Two Bactrian distributions are compared($m = 0.95$ and $m = 0.98$). The clock model operators which these proposal kernels apply to are described in **Table 1**.

	Operator	Proposal	Parameters
1	**Random walk operator**	$x' \leftarrow x + s\Sigma$	σ, r, q
2	**Scale operator**	$x' \leftarrow x \times e^{s\Sigma}$	σ, r
3	**Interval operator**	$y \leftarrow \frac{u-x}{x-l} \times e^{s\Sigma}$ $x' \leftarrow \frac{u+l*y}{y+1}$	q $(l = 0, u = 1)$
4	**Constant distance operators**	$x' \leftarrow x + s\Sigma$	t

Table 1. Summary of proposal kernels $q(x'|x)$ of clock model operators. In each operator, Σ is drawn from either a Bactrian(m) or Uniform distribution (distributions are normalised so that they have a mean of 0 and a variance of 1). The scale size s is tunable. The proposal kernel may apply to node heights t, clock standard deviation σ, clock rates r (*real* only), and clock rate quantiles q (*quant* only). The Scale operator acts on parameters with non-negative domains. The Interval operator proposes values which respect its domain i.e. $l < x' < u$.

Narrow Exchange Rate

The **Narrow Exchange** operator [35], widely used in BEAST [9,36] and BEAST2 [10], is similar to NNI, and works as follows (**Fig. 3**):

Step 1. Sample an internal/root node E from tree $\mathcal{T}$, where E has grandchildren.

Step 2. Identify the child of E with the greater height. Denote this child as D and its sibling as C (i.e. $t_D > t_C$).

Step 3. Randomly identify the two children of D as A and B.

Step 4. Relocate the $B - D$ branch onto the $C - E$ branch, so that B and C become siblings and their parent is D. All node heights are unchanged.

Lakner et al. 2008 [37] found that tree operators which perterb topology (such as NNI and SPR) consistently perform better than those which also change branch lengths (such as LOCAL [38] and Continuous Change [39]). If Narrow Exchange was adapted to the relaxed clock model by ensuring that genetic distances remain constant after the proposal, its performance may be improved even further. This may in turn permit proposing a new node height t_D and therefore changing branch (time) lengths.

Here, we present the **Narrow Exchange Rate** (NER) operator. Let r_A, r_B, r_C, and r_D be the clock rates of nodes A, B, C, and D, respectively. In addition to the modest topological change applied by Narrow Exchange, NER also proposes new clock rates $r_A{}'$, $r_B{}'$, $r_C{}'$, and $r_D{}'$. While NER does not alter t_D (i.e. $t_D{}' \leftarrow t_D$), we also consider NERw - a special case of the NER operator which embarks t_D on a random walk:

$$t_D{}' \leftarrow t_D + s\Sigma \tag{12}$$

for random walk step size $s\Sigma$ where s is a tunable scaler parameter and Σ is drawn from a uniform or **Bactrian proposal kernel**. NER (and NERw) are compatible with both the *real* and *quant* parameterisations. Analagous to the Constant Distance operator, new rates are proposed such that genetic distances between nodes A, B, C, and E are maintained. Thus, there are $\binom{4}{2} = 6$ pairwise distance constraints.

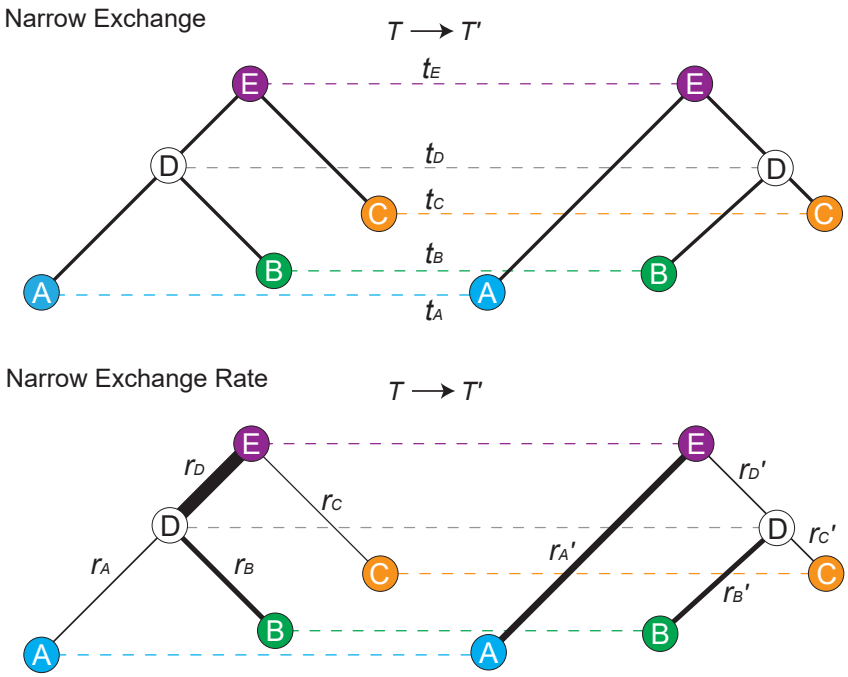

Fig 3. Depiction of Narrow Exchange and Narrow Exchange Rate operators. Proposals are denoted by $\mathcal{T} \to \mathcal{T}'$. The vertical axis corresponds to node height t. In the bottom figure, branch rates r are indicated by line thickness. In this example, the $\mathcal{D}_{AE}$ and $\mathcal{D}_{CE}$ constraints are satisfied.

$$\begin{aligned}
\mathcal{D}_{AB}: \quad & r_A(t_D - t_A) + r_B(t_D - t_B) = \\
& r_A'(t_E - t_A) + r_D'(t_E - t_D') + r_B'(t_D' - t_B) && (13)\\
\mathcal{D}_{AC}: \quad & r_A(t_D - t_A) + r_D(t_E - t_D) + r_C(t_E - t_C) = \\
& r_A'(t_E - t_A) + r_D'(t_E - t_D') + r_C'(t_D' - t_C) && (14)\\
\mathcal{D}_{AE}: \quad & r_A(t_D - t_A) + r_D(t_E - t_D) = \\
& r_A'(t_E - t_A) && (15)\\
\mathcal{D}_{BC}: \quad & r_B(t_D - t_B) + r_D(t_E - t_D) + r_C(t_E - t_D) = \\
& r_B'(t_D' - t_B) + r_C'(t_D' - t_C) && (16)\\
\mathcal{D}_{BE}: \quad & r_B(t_D - t_B) + r_D(t_E - t_D) = \\
& r_B'(t_D' - t_B) + r_D'(t_E - t_D') && (17)\\
\mathcal{D}_{CE}: \quad & r_C(t_E - t_C) = \\
& r_C'(t_D' - t_C) + r_D'(t_E - t_D') && (18)
\end{aligned}$$

Further constraints are imposed by the model itself:

$$r_i > 0 \text{ and } r_i' > 0 \text{ for } i \in \{A, B, C, D\} \quad (19)$$

$$\max\{t_B, t_C\} < t_D' < t_E. \quad (20)$$

Unfortunately, it is not possible to solve all six $\mathcal{D}_{ij}$ constraints without permitting non-positive rates or illegal trees. Therefore rather than conserving all six pairwise

distances, NER conserves a *subset* of distances. It is not immediately clear which distances should be conserved.

Automated generation of operators and constraint satisfaction

The total space of NER operators is comprised of all possible subsets of distance constraints (i.e. $\{\}, \{\mathcal{D}_{AB}\}, \{\mathcal{D}_{AC}\}, \ldots, \{\mathcal{D}_{AB}, \mathcal{D}_{AC}, \mathcal{D}_{AE}, \mathcal{D}_{BC}, \mathcal{D}_{BE}, \mathcal{D}_{CE}\}$) which are solvable. The simplest NER – the null operator denoted by NER$\{\}$ – does not satisfy any distance constraints. This is equivalent to Narrow Exchange.

As it is unclear which NER variants would perform the best, we developed an automated pipeline for generating and testing these operators.

1. Solution finding. Using standard analytical linear-system solving libraries in MATLAB, the $2^6 = 64$ subsets of distance constraints are solved. 54 out of the 64 subsets were found to be solvable, and the unsolvables were discarded.

2. Solving Jacobian determinants. The determinant of the Jacobian matrix J is required for computng the Hastings ratio of the proposal. J is defined as

$$J = \begin{bmatrix} \frac{\partial r_A{}'}{\partial r_A} & \frac{\partial r_A{}'}{\partial r_B} & \frac{\partial r_A{}'}{\partial r_C} & \frac{\partial r_A{}'}{\partial r_D} \\ \frac{\partial r_B{}'}{\partial r_A} & \frac{\partial r_B{}'}{\partial r_B} & \frac{\partial r_B{}'}{\partial r_C} & \frac{\partial r_B{}'}{\partial r_D} \\ \frac{\partial r_C{}'}{\partial r_A} & \frac{\partial r_C{}'}{\partial r_B} & \frac{\partial r_C{}'}{\partial r_C} & \frac{\partial r_C{}'}{\partial r_D} \\ \frac{\partial r_D{}'}{\partial r_A} & \frac{\partial r_D{}'}{\partial r_B} & \frac{\partial r_D{}'}{\partial r_C} & \frac{\partial r_D{}'}{\partial r_D} \end{bmatrix}. \tag{21}$$

Computing the determinant $|J|$ invokes standard analytical differentiation and linear algebra libraries of MATLAB. 6 of the 54 operators were found to have $|J| = 0$, corresponding to irreversible proposals, and were discarded.

3. Automated generation of BEAST2 operators. Java class files are generated using string processing. Each class corresponds to a single operator, extends the class of a meta-NER-operator, and is comprised of the solutions found in **1** and the Jacobian determinant found in **2**. $|J|$ is further augmented if the *quant* parameterisation is employed.

The 48 operators generated by this pipeline are evaluated and compared in **Results**. Each operator is considered with and without a random walk on t_D and thus there are 96 total settings.

A guided adaptive leaf rate operator

A *guided* operator incorporates knowledge about neighbouring states, while an *adaptive* operator undergoes a training process to improve its efficiency over time [40]. In previous work, parsimony scores and conditional clade probabilities of neighbouring trees have been employed by guided tree operators [**?**, 24, 25] and the latter has also been explored as the basis of adaptive tree operators [24, 25]. The (adaptive) mirror kernel [30] learns a target distribution which acts as a 'mirror image' of the current point x. The adaptable variance multivariate normal (AVMVN) kernel [28, 36] learns correlations between parameters during MCMC. Baele et al. 2017 observed a large increase ($\approx 5 - 10\times$) in sampling efficiency from using the AVMVN kernel on clock rates and substitution model parameters across partitions [28].

In this article we consider application of the AVMVN kernel to the branch rates of leaf nodes. This operator is not readily applicable to internal node branch rates due to their dependencies on tree topology.

AVMVN kernel

The AVMVN kernel assumes its parameters live in $x \in \mathbb{R}^N$ and that these parameters follow a multivariate normal distribution with covariance matrix Σ_N. Hence, the kernel operates on the logarithmic or logistic transformation of the N leaf branch rates, depending on the rate parameterisation:

$$x_i = \begin{cases} \log r_i \text{ for } \textit{real} \\ \log \frac{q_i}{1-q_i} \text{ for } \textit{quant} \end{cases} \tag{22}$$

where r_i is a real rate and q_i is a rate quantile. The AVMVN probability density is defined by

$$\mathcal{AVMVN}(x) = \mathcal{MVN}\big(x, (1-\beta)\frac{\Sigma_N}{N} + \beta\frac{\mathbb{I}_N}{N}\big), \tag{23}$$

where $\mathcal{MVN}$ is the multivariate normal probability density. β ($= 0.05$) is a constant which determines the fraction of the proposal determined by the identity matrix $\mathbb{I}_N$, as opposed to the covariance matrix Σ_D which is trained during MCMC.

The AVMVN proposal kernel is computed as

$$x' \leftarrow x + \sum_{i=1}^{N}\sum_{j=i}^{N} c_{i,j} \times s\Sigma \tag{24}$$

$$\text{where } c = \texttt{cholesky}\{(1-\beta)\frac{\Sigma_N}{N} + \beta\frac{\mathbb{I}_N}{N}\}. \tag{25}$$

The $\texttt{cholesky}\{Y\}$ decomposition returns a lower diagonal matrix L, with positive real diagonal entries, such that $Y = LL'$ [41, 42]. s is a tunable step size parameter and Σ is a random variable drawn from a proposal kernel (uniform or Bactrian for instance). Our BEAST2 implementation of the AVMVN kernel is adapted from that of BEAST [36].

In Results, we evaluate this operator for its ability to estimate leaf rates. As the size of the covariance matrix Σ_N grows with the number of taxa N, AVMVN is hypothesised to work well on small trees but become less efficient with larger taxon sets.

Results

Assessment criteria and datasets

To avoid a cross-product explosion, the four targets for clock model improvement (**Rate parameterisations**, **Bactrian proposal kernel**, **Narrow Exchange Rate**, and **A guided adaptive leaf rate operator**) are evaluated sequentially, in the order presented in this paper. The setting which is considered to be the best in each step is then incorporated into the following step. This protocol and its outcomes are summarised in **Fig. 4**.

Methodologies are assessed according to the following criteria.

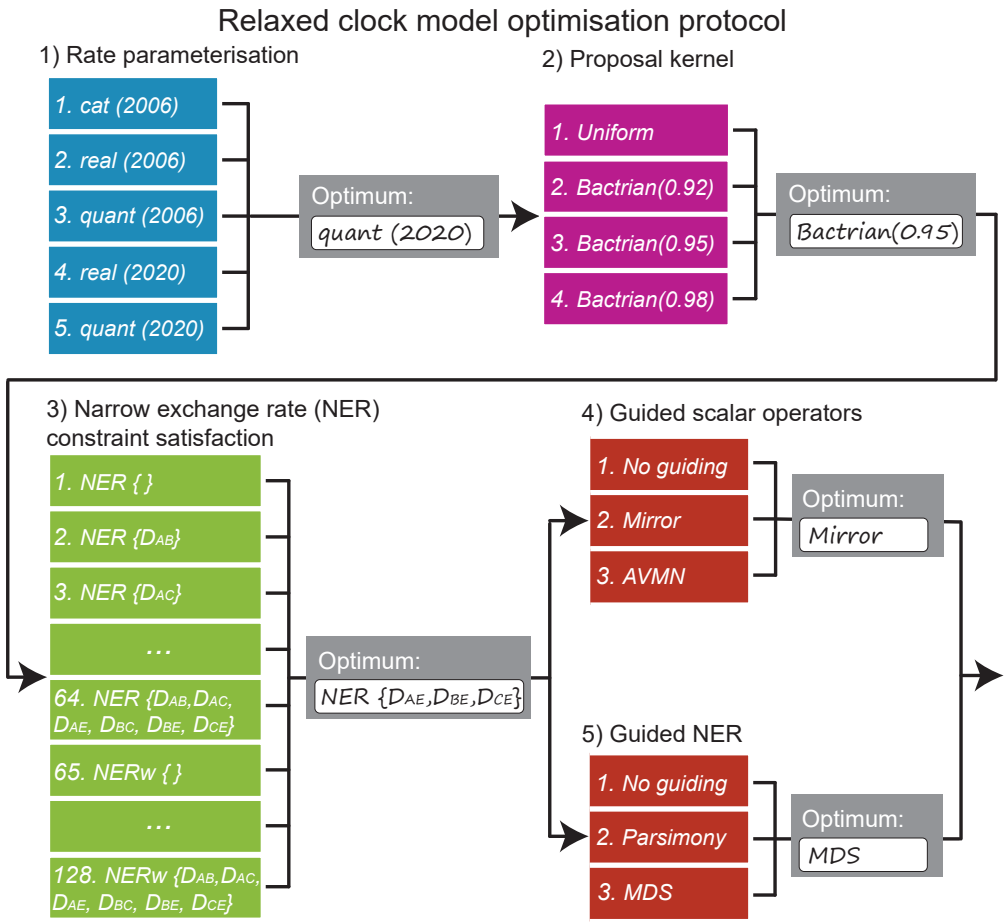

Fig 4. Protocol for optimising methodology settings. The three areas (detailed in **Models**) are optimised sequentially, where the best setting from each step is used when optimising the following step.

1. Validation. This is assessed by measuring the coverage of all estimated parameters in a well-calibrated simulation study, using 100 simulated datasets (with $N = 100$ taxa and $L = 5000$ nucleotide alignments). These are presented in **S2 Appendix**.

2. Time to convergence. Two independent MCMC chains are run and the time is measured until: a) the absolute difference in clade posterior probability between the two chains is less than 0.05 for all clades, b) the Rubin-Gelman statistic $\hat{R}$ [43] of every estimated parameter is less than 1.05, and c) the effective sample size [44] of every estimated parameter is greater than 200 in each chain.

3. Mixing of parameters. Key parameters are evaluated for the number of effective samples generated per hour (ESS/hr).

For the latter two criteria, methodologies are benchmarked using both simulated and empirical datasets – the latter were compiled [45] and partitioned [46] by Lanfear et al. as 'benchmark alignments' (**Table 2**). Each setting is run 10× per dataset and the average statistics across the $10 \times 2 = 20$ chains are reported. .

Methodologies are benchmarked using the Intel(R) Xeon(R) Gold 6138 CPU (2.00 GHz), with two processing units per pair of MCMC chains.

	N	P	L (kb)	L_{eff} (kb)	**Description**
1	38	8	9.1	6.45	Seed plants (Ran 2018 [47])
2	44	7	5.9	1.8	Squirrel Fishes (Dornburg 2012 [48])
3	44	3	1.9	0.8	Bark beetles (Cognato 2001 [49])
4	51	6	5.4	1.8	Southern beeches (Sauquet 2011 [50])
5	61	8	6.9	4.3	Bony fishes (Broughton 2013 [51])
6	70	3	2.2	0.9	Caterpillars (Kawahara 2013 [52])
7	78	8	3.4	3.1	Animals (Cannon 2016 [53])
8	80	1	10.0	4.2	*Simulated data*
9	94	4	2.2	1	Bees (Rightmyer 2013 [54])
10	106	1	0.8	0.5	Songbirds (Moyle 2016 [55])

Table 2. Datasets used during benchmarking, sorted in increasing order of taxa count N. Number of partitions P, total alignment length L, and number of patterns L_{eff} are also specified.

Comparison of rate parameterisations

We compared the three rate parameterisations described in **Rate parameterisations**. All three settings use the standard BEAST2 clock model operators from Drummond et al. 2006 [3]. *real* and *quant* additionally use the constant-distance tree operators [31]. To determine whether the difference in performance between *real*/*quant* versus *cat* is because of the constant-distance tree operators or the parameterisation itself, we also included benchmarked two additional settings: *real 2006* and *quant 2006*, which do not used the constant-distance operators. These five settings are validated in **S2 Appendix**.

Comparison of Bactrian and uniform proposal kernels on the clock model

Comparison of NER variants

The **Narrow Exchange Rate** (NER) operators are evaluated. This protocol selects the best among 48 NER (no random walk) and 48 NERw (Bactrian(0.95) random walk) operators, and has two phases. First, the best of the 96 is selected by comparing operator acceptance rates on simulated data. Second, the selected operator is benchmarked with respect to convergence time and sampling rate on real data (**Table 2**). The analyses in this section invoke the *quant* parameterisation and Bactrian(0.95) proposal kernels on clock model parameters.

Initial screening by acceptance rate

We selected the best operator variant by performing MCMC on 300 simulated datasets, where each MCMC employed all 96 NER/NERw variants. Simulated datasets have $N = 30$ taxa and an alignment with $L \sim \text{Uniform}(10^2, 10^4)$ sites. The acceptance rate of each operator is compared to that of the null operator NER{} (i.e. Narrow Exchange).

Fig. 6 shows that NER variants which satisfy the genetic distances between nodes B and A (i.e. $\mathcal{D}_{AB}$) or between B and C (i.e. $\mathcal{D}_{BC}$) usually perform worse than the standard Narrow Exchange operator, where B is the node being interchanged from the A branch to the C branch (**Fig. 3**). This is an intuitive result. If the posterior

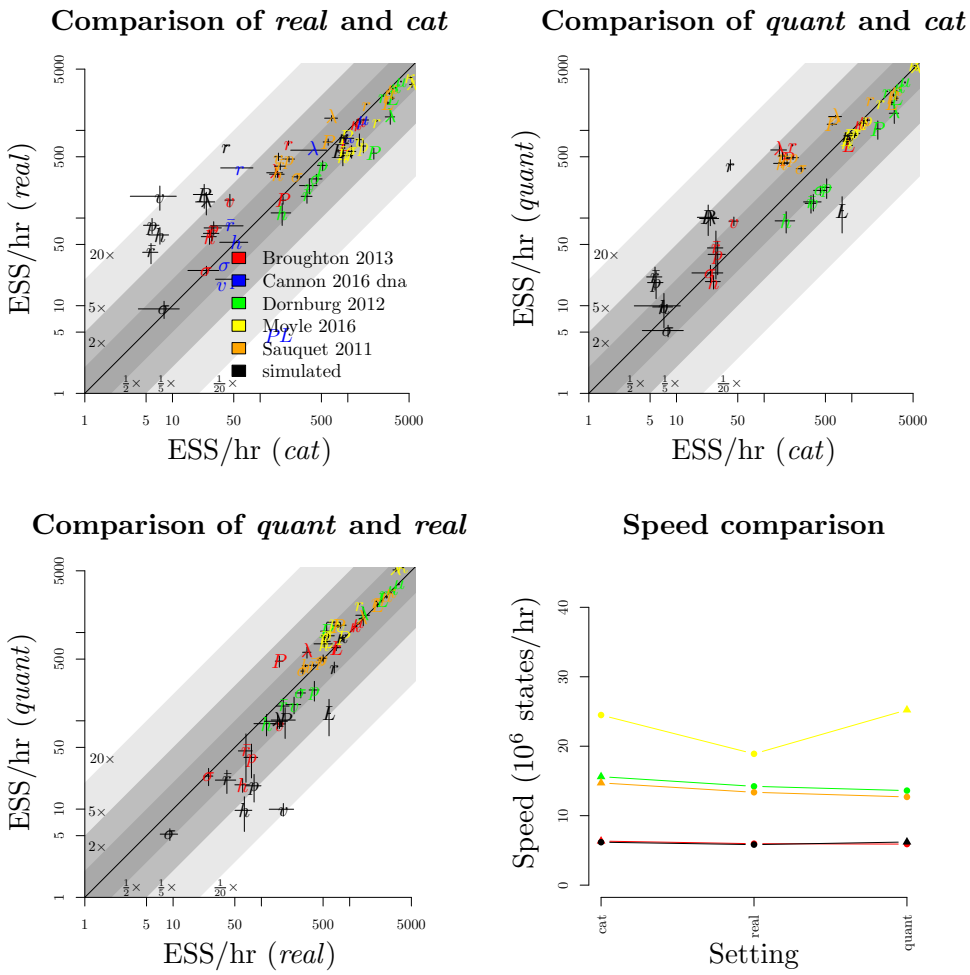

Fig 5. Rate parameterisation performance evaluation. Comparison of ESS/hr (averaged across five independent MC3 analyses) with respect to relevant terms – P: posterior density; L: likelihood, p: prior density, r: clock rate ESS averaged across all leaves, $\hat{r}$: branch rate mean, v: branch rate variance, σ: clock standard deviation, κ: HKY model transition-transversion ratio, λ: Yule model birth rate. h: tree height. Datasets are displayed in **Table 2**.

distribution is relatively flat, and the data presents high uncertainty in the positioning of B, with respect to A and C, then the topological rearrangement performed by Narrow Exchange will be favoured. However, this uncertainty in the *topology* is likely coupled with uncertainty in the *distance* between B and A or between B and C. Thus, in this case, respecting the $\mathcal{D}_{AB}$ and $\mathcal{D}_{BC}$ constraints (by proposing branch rates) makes too many unnecessary changes to the state and the operator performs worse.

Fig. 6 also reveals a cluster of NER variants which – under the conditions of the simulation – performed better than the null operator NER{} around 25% of the time and performed worse around 10% of the time. One such operator is NER$\{\mathcal{D}_{AE}, \mathcal{D}_{BE}, \mathcal{D}_{CE}\}$. This variant conserves the genetic distance between all child nodes A, B, and C, and the grandparent node E. This is performed by proposing rates for r_A, r_B, and r_C while obeying the distance constraints imposed by the operator. Exploring this operator further, we can see that NER$\{\mathcal{D}_{AE}, \mathcal{D}_{BE}, \mathcal{D}_{CE}\}$ is at its best

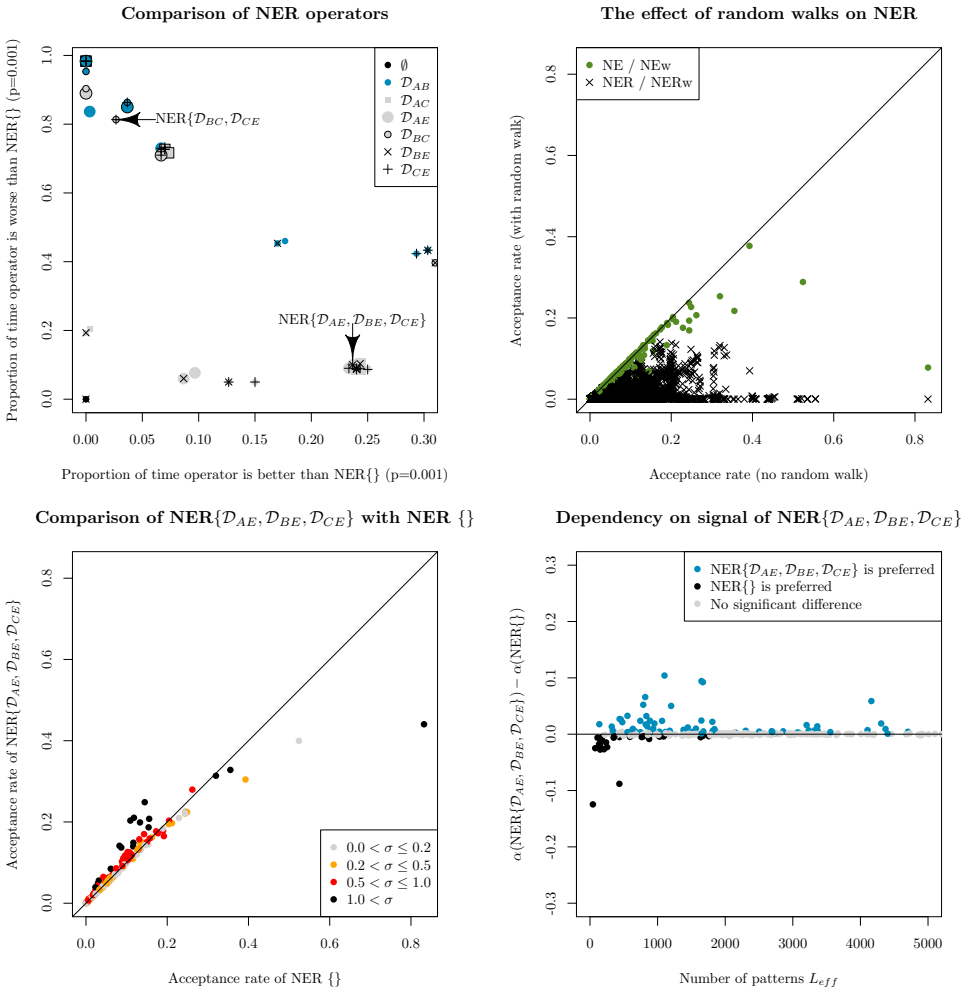

Fig 6. Screening of NER and NERw variants by acceptance rate. Top left: comparison of NER variants with the null operator NER{} (i.e. Narrow Exchange). Each of the 48 operators are represented by a single point, uniquely encoded by the point stylings. The number of times each operator is proposed and accepted is compared with that of NER{}, and one-sided z-tests are performed to assess the statistical significance between the two acceptance rates ($p = 0.001$). This process is repeated for each of 300 simulated datasets. The axes of each plot are the proportion of these 300 simulations for which there is evidence that the operator is better than NER{} (x-axis) or worse than NER{} (y-axis). Top right: comparison of NER and NERw acceptance rates. Each point is one NER/NERw variant from a single simulation. Bottom: relationship between the acceptance rates α of NER$\{\mathcal{D}_{AE}, \mathcal{D}_{BE}, \mathcal{D}_{CE}\}$ and NER{} with the clock model standard deviation σ and the number of patterns L_{eff}. Each point is a single simulation.

when there is a large variance in branch rate i.e. when clock standard deviation σ is high ($\sigma \gtrsim 0.5$ for $N = 30$), corresponding to data which is not clock-like. On the other hand, NER{} is much preferred when the operator's acceptance rate is high ($\gtrsim 0.15$) – corresponding with datasets with a small number of site patterns ($L_{eff} \lesssim 500$ for $N = 30$) and thus poor signal. Overall, NER$\{\mathcal{D}_{AE}, \mathcal{D}_{BE}, \mathcal{D}_{CE}\}$ outperforms the standard Narrow Exchange operator when the data is not clock-like and contains

enough signal.

Finally, **Fig. 6** shows that by applying a (Bactrian) random walk to t_D – the height of internal node D – the acceptance rate of NER plummets dramatically. This effect is most dominant for the NER variants which satisfy distance constraints (i.e. the operators which are not NER{}). This result is unfortunate however not unexpected, and is consistent with Lakner et al. 2008 [37], who observed that tree operators perform best when they change either topology, or branch lengths, but not both.

Although there are several operators tying for first place, we selected the NER$\{\mathcal{D}_{AE}, \mathcal{D}_{BE}, \mathcal{D}_{CE}\}$ operator to proceed to the next round of optimisation.

Benchmarking convergence time

Evaluating the AVMVN leaf rate operator

Clock model averaging

Discussion

Conclusion

Supporting information

S1 Appendix. Rate quantiles. The linear piecewise approximation used in the *quant* parameterisation is described. Constant distance tree operators [31] are extended to the *quant* parameterisation.

S2 Appendix. Well-calibated simulation studies. Methodologies are validated using well-calibated simulation studies.

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