Detecting episodic evolution through Bayesian inference of molecular clock models

John H Tay[1], Guy Baele[2], Sebastian Duchene[1*].

[1]Peter Doherty Institute for Infection and Immunity, Department of Microbiology and Immunology, University of Melbourne, Melbourne, Australia

[2]Department of Microbiology, Immunology and Transplantation, Rega Institute, KU Leuven, Leuven, Belgium

*email: sduchene@unimelb.edu.au

Abstract (250 words max.)

Molecular evolutionary rate variation is a key aspect of the evolution of many organisms that can be modelled using molecular clock models. For example, fixed local clocks revealed the role of episodic evolution in the emergence of SARS-CoV-2 variants of concern. Like all statistical models, however, the reliability of such inferences is contingent on an assessment of statistical evidence. We present a novel Bayesian phylogenetic approach for detecting episodic evolution. It consists of computing Bayes factors, as the ratio of posterior and prior odds of evolutionary rate increases, effectively quantifying support for the effect size. We conducted an extensive simulation study to illustrate the power of this method and benchmarked it to formal model comparison of a range of molecular clock models using (log) marginal likelihood estimation, and to inference under a random local clock model. Quantifying support for the effect size has higher sensitivity than formal model testing and is straight-forward to compute, because it only needs samples from the posterior and prior distribution. However formal model testing has the advantage of accommodating a wide range molecular clock models. In contrast, the random local clock had low power for detecting episodic evolution. In an empirical analysis of a data set of SARS-CoV-2 genomes, we find 'very strong' evidence for episodic evolution. Our results provide guidelines and practical methods for Bayesian detection of episodic evolution, as well as avenues for further research into this phenomenon.

Keywords: Bayesian model selection, prior sensitivity, effect size, episodic evolution, molecular clock, virus molecular evolution, Markov chain Monte Carlo

1 Introduction

Episodic evolution occurs when evolutionary change is accelerated over short periods of time, such as through short-lived mutational bursts (Gillespie, 1984). Many microbial pathogens are known to undergo such periods

of increased molecular evolutionary rates. Notably, the large number of mutations in SARS-CoV-2 variants of concern (VOC) is explained by such dynamics (Hill et al., 2022, Lythgoe et al., 2022, Markov et al., 2023, Neher, 2022, Tay et al., 2022). Similar processes are known to occur in other viruses, such as during within-host evolution in influenza viruses (Xue et al., 2018) and in HIV lineages subject to hypermutation mediated by APOBEC3G/G (Simon et al., 2005). A major consequence of evolutionary rate acceleration is that it can result in increased transmissibility and virulence, as appears to be the case in some Ebola virus (EBOV) lineages (Mbala-Kingebeni et al., 2019) and SARS-CoV-2 VOCs, or drug resistance, which has been suggested for *Mycobacterium tuberculosis* (Cohen et al., 2015).

An effective method to detect episodic evolution using genome data is to estimate evolutionary rates for branches in phylogenetic trees using molecular clock models. Here, the 'evolutionary rate' refers to the number of substitutions per site per unit time, such that it represents the combination of the instantaneous mutational process and the rate at which mutations are fixed (i.e. substitutions). Under this framework, branches that correspond to lineages expected to have undergone episodic evolution should therefore exhibit higher evolutionary rates.

The simplest molecular clock model is the strict clock (SC), where all branches are assigned the same evolutionary rate. The most flexible approaches, involve an uncorrelated 'relaxed molecular clock', in which evolutionary rates can vary across branches in the tree according to an underlying statistical distribution. For example, evolutionary rates across branches can be independent draws from a lognormal or gamma distribution (typically abbreviated as UCLD and UCGD, for uncorrelated clock with a lognormal and gamma distribution, respectively; reviewed by Ho and Duchene 2014 and Guindon 2020). An explicit model for episodic rates is the fixed local clock (FLC) (Worobey et al., 2014, Yoder and Yang, 2000). In the FLC model a set of branches, known as the *foreground*, are allowed to have a different evolutionary rate to the *background* (Fig. 1). To describe episodic evolution in the emergence of SARS-CoV-2 VOCs, the stem branches (i.e. between the stem and crown node) leading up to VOC clades are designated as the foreground (Gräf et al., 2021, Tay et al., 2022) (see also Fig. 1C).

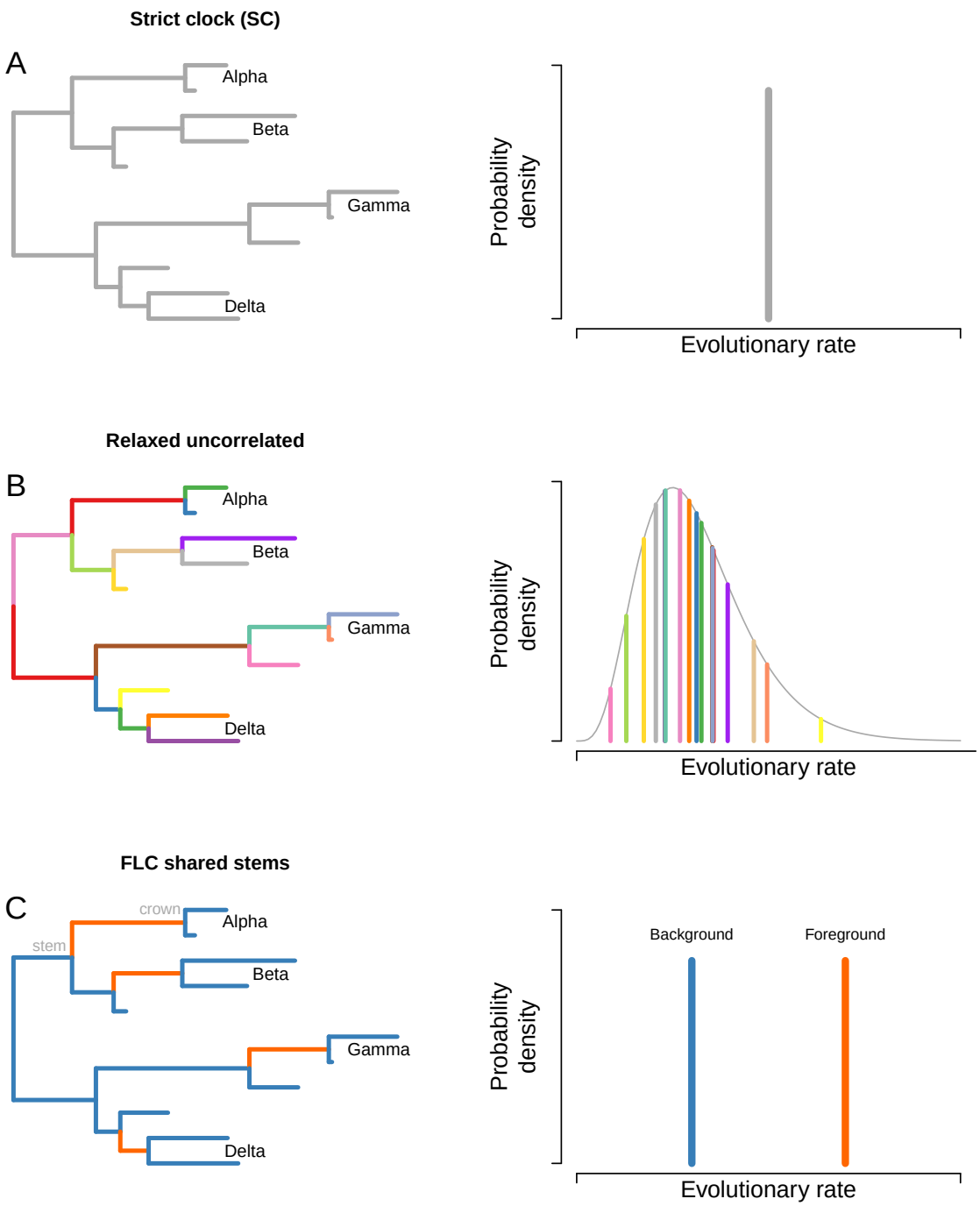

Figure 1: Examples of molecular clock models. A. is a strict clock model (SC), where all branches share a single evolutionary rate. B. is an uncorrelated relaxed clock model, in which the evolutionary rates across branches are independent draws from an underlying statistical distribution, such as a lognormal or a gamma (Γ) distribution (typically abbreviated as UCLD and UCGD, respectively). Note that branches are coloured according to those drawn from the distribution on the right. C. represents a fixed local clock model, where stem branches leading up to variants of concern (VOCs; those leading to clades labelled as Alpha, Beta, Gamma and Delta, following variant names) are designated as the 'foreground' and assigned a rate that differs from the 'background'. In C, we have labelled the stem and crown nodes of variant Alpha, where evolutionary rate changes would have occurred.

Bayesian methods for quantifying the strength of evidence for episodic evolution include formal model testing. For example, one can compare the fit of the FLC with that of the SC and UCGD by comparing their associated (log) marginal likelihoods, allowing to compute Bayes factors of one clock model over the others. Molecular clock models are treated as explicit hypothesis, with the UCGD and SC presenting a form of 'null' and the FLC the hypothesis of interest. Here, the log Bayes factor is the difference in log marginal likelihoods (i.e. the average fit) between competing models (Sinsheimer et al., 1996). Following Kass and Raftery (1995), a log Bayes factor of two competing models, M_1 and M_2, $0 < logBF_{12} \leq 1$ is considered as not worth more than 'bare mention' for evidence of M_1 over M_2. A $1 < logBF_{12} \leq 3$ is considered as 'positive evidence', $3 < logBF_{12} \leq 5$ is 'strong', and $logBF_{12} > 5$ is 'very strong'. There exist multiple methods for estimating log marginal likelihoods in Bayesian phylogenetics (Lartillot and Philippe, 2006, Oaks et al., 2019), with generalised stepping-stone sampling being the most accurate to date (Baele et al., 2016, Fourment et al., 2020).

Another Bayesian approach for hypothesis testing consists of measuring statistical support for the effect size for a parameter of interest (Kass and Raftery, 1995, Keysers et al., 2020, Morey et al., 2016, van de Schoot et al., 2021). We propose to use this approach for detecting episodic evolution by considering the FLC model, and a well-defined hypothesis: the ratio of evolutionary rates of foreground and background branches is at least 1, meaning an episodic increase in the evolutionary rate. Alternatively, the difference in foreground and background rates can be used, with a value higher than 0 suggesting episodic evolution. The Bayes factor in this situation is the ratio of the posterior and prior odds, each of which corresponds to an evolutionary hypothesis.

Finally, Bayesian model averaging is an approach where a pool of models are considered and estimates of all parameters, including evolutionary rates, are weighted averages of the models, and with the model weights corresponding to their posterior probability. Molecular clock model averaging exists for selecting uncorrelated relaxed clock models (Baele et al., 2013, Li and Drummond, 2011). The random local clock model (RLC) is also a model averaging approach, where nodes can undergo evolutionary rate changes (Drummond and Suchard, 2010). The number and location of evolutionary rate changes is inferred, without the need for their *a priori* definition, as is the case for the FLC. However, evolutionary rates cannot be shared by non-neighbouring branches, meaning that some forms of the FLC (e.g. Fig. 1 C) are not considered.

2 Results

2.1 Simulating episodic evolution

We conducted a simulation study to demonstrate the performance of Bayesian approaches for detecting episodic evolution. Our simulations utilised 100 phylogenetic trees drawn from the posterior distribution of empirical SARS-CoV-2 data by Tay et al. (2022). These phylogenies contain 179 tips that represent curated full genomes collected between December 2019 and August 2021, and include 20 samples from each of the first four VOCs (Alpha, Beta, Gamma, and Delta) and a global sample of the diversity of the virus at the time. We simulated evolutionary rates along these trees according to the FLC model using NELSI (Ho et al., 2015), where the background branches had an evolutionary rate of 7×10^{-4} substitutions per site per year, similar to previous estimates for this virus (Boni et al., 2020, Duchene et al., 2020), and the stem branches leading up to VOCs (foreground) had evolutionary rates of 1, 1.5, 2, 3, 5, or 10-fold that of the background (a 1-fold rate increase is equivalent to the SC). For each of the 100 trees, we set each of the 6 foreground rate increases and simulated a sequence alignment with 29,903 nucleotides, resulting in 600 synthetic alignments.

We fit three molecular clock models to each simulated data set under a Bayesian phylogenetic framework: the SC, the UCGD (uncorrelated relaxed-clock with an underlying gamma distribution; Drummond et al. (2006)), and an FLC configured such that the foreground branches are the stems leading up to VOCs (see Fig. 1C). For the relaxed clock model we chose the UCGD over the more popular UCLD (lognormal distribution), because previous empirical analyses favoured the UCGD for this SARS-CoV-2 data (Tay et al., 2022). We sampled the posterior distribution using Markov chain Monte Carlo (MCMC) as implemented in BEAST 1.10 (Suchard et al., 2018).

We inspected the posterior distribution of the ratio of evolutionary rates of foreground and background branches for the analyses using the FLC model. The 95% credible intervals always included the value used to generate the data, implying no obvious estimation bias in this model. An important trend in these results is that the precision of the estimates improved with increasing foreground evolutionary rates. Indeed, estimates of the foreground to background evolutionary rate ratio are much more uncertain in simulations under the SC (i.e. the simplified case of the FLC), compared to that where the foreground branches had rates were 10-fold higher than the background (SC versus FLCx10 in Fig. 2). A likely cause of this pattern is that increasing the effect size for foreground branches results in overall more informative sequence data sets.

The degree of uncertainty in estimates has important consequences for statistical support for episodic evolution. The number of data sets for which the 95% credible interval excluded a value of 1.0, meaning that

foreground and background evolutionary rates were different, increased with the degree of episodic evolution. Notably, this was the case for 18 out of 100 simulations under the FLCx1.5, and continues to increase up to 100 for those with FLCx10 (Fig. 2).

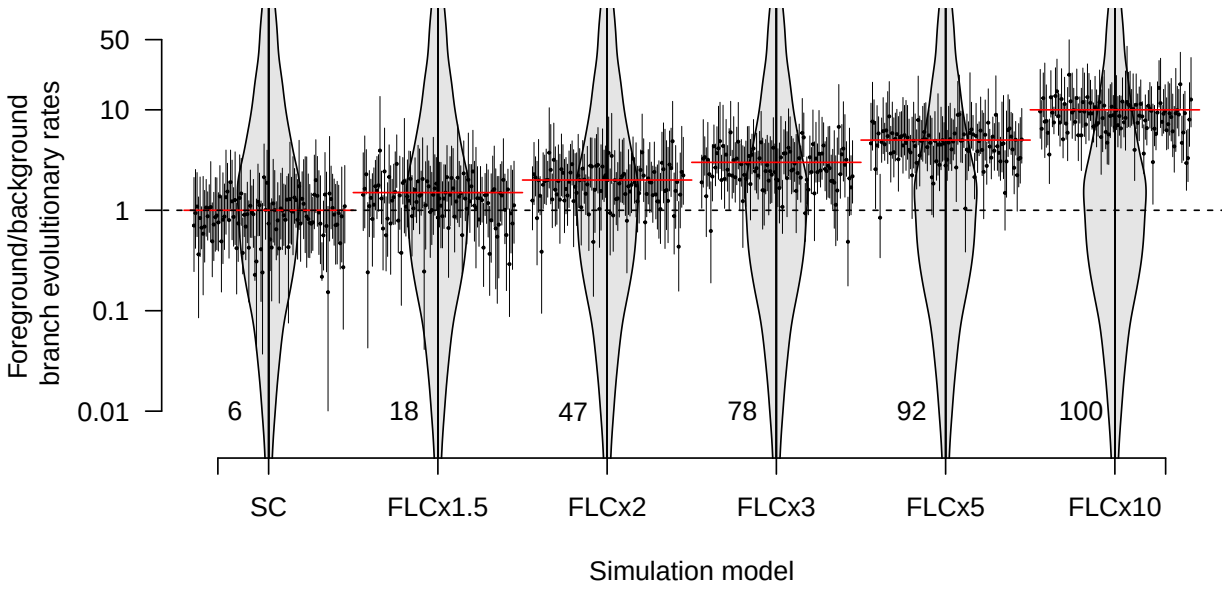

Figure 2: Posterior estimates of the foreground to background evolutionary rate ratio (foreground/background) for all simulation scenarios. SC corresponds to simulations under a strict clock and with foreground and background rates being the same. FLCx1.5 stands for simulations under a fixed local clock, where the foreground branches have an evolutionary rate that is 1.5-fold higher than that of the background, with similar nomenclature through FLCx10. Each black vertical line denotes the posterior 95% credible interval for each of the 100 simulated data sets and black dots represent the means. The dashed horizontal line represents a foreground/background evolutionary rate ratio of 1.0, such that they are equal. Red lines across each simulation model correspond to the true value used to generate the data. The grey violin plots in the background are used to show the resulting prior on the evolutionary rate ratio. Here we used a $\Gamma(\alpha = 0.5, \theta = 0.1)$ prior (where α and θ are the 'shape' and 'scale' parameters and the mean is $\alpha\theta$) for both sets of branches, which is relatively uninformative and with the foreground/background ratio centred at 1. The numbers at the bottom of each violin are the number of simulation replicates for which the 95% credible interval did not include the foreground/background evolutionary rate ratio of 1.0, which would indicate that no episodic evolution was detected.

2.2 Bayesian molecular clock model selection using log marginal likelihoods

For each simulated data set we estimated the log marginal likelihood using generalised stepping-stone sampling (Baele et al., 2016). We ranked the models and selected them according to the highest log marginal likelihood. An important consideration, however, is the variation in the log marginal likelihood. In particular, selecting a model with a log Bayes factor of 1 ('positive evidence') can be unreliable if the uncertainty in log

marginal likelihoods is more than a few log units.

To investigate uncertainty in log marginal likelihoods we randomly selected a subset of our simulations and repeated the log marginal likelihood estimations under the three molecular clock models fit to the data (SC, FLC, and UCGD). In Fig. 3 we show the resulting repeatability plot, that demonstrates the difference in log marginal likelihoods for two independent runs. We also calculated the average of the absolute difference in log marginal likelihoods between the two independent estimates for each of the three molecular clock models, $D = E(|MLE_1 - MLE_2|)$, where MLE_1 and MLE_2 are two replicates of log marginal likelihood calculations (Baele et al., 2016). The D statistic ranged from 1.70 for the FLC to 1.93 for the UCGD, such that we considered a log Bayes factor of 3 (i.e. 'strong') to select molecular clock models, because it is about one log likelihood unit higher than D and therefore likely robust to log marginal likelihood estimation uncertainty.

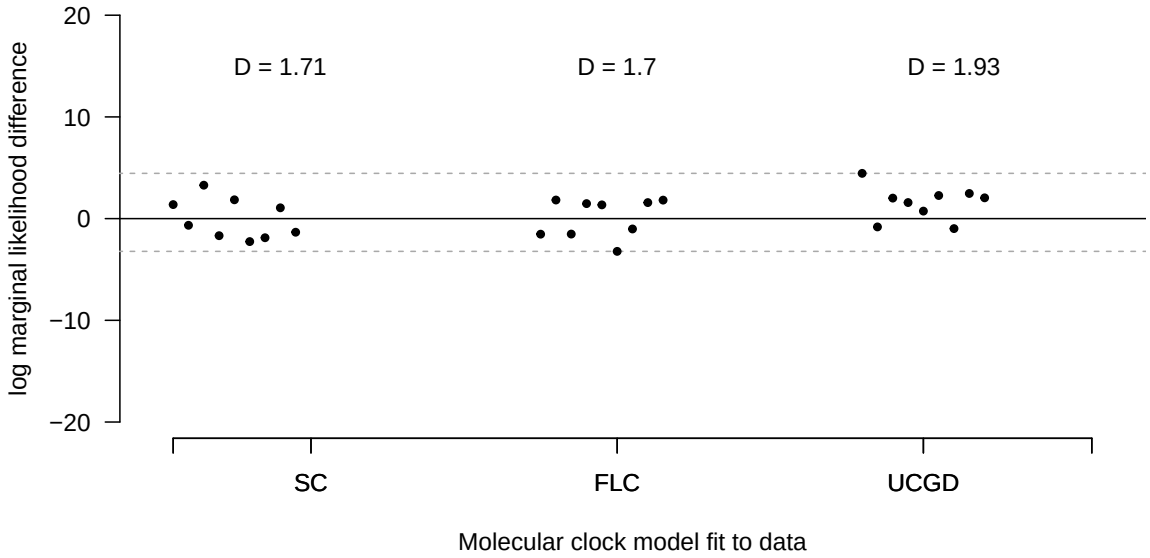

Figure 3: Repeatability plot for a subset of simulations for each of the three molecular clock models' fit to the data. We repeated the log marginal likelihood estimations for each data set and calculated their difference, which is represented by each point. The solid line denotes no difference in log marginal likelihoods and the dashed lines are for the maximum value observed. For each model we show the repeatability statistic, $D = E(|MLE_1 - MLE_2|)$, where MLE_1 and MLE_2 are two replicates of the log marginal likelihood estimates.

In our simulations under the SC we found that no data sets supported the FLC or UCGD. As expected, increasing evolutionary rates along foreground branches resulted in more data sets preferring the FLC. However, a foreground evolutionary rate of at least three-fold that of the background was necessary for most simulations to favour the FLC with 'strong' Bayes factor support ($logBF$ >3). For a five-fold evolutionary rate increase, 83 out of 100 simulations selected the FLC, with 'very strong' support. In simulations with a

ten-fold evolutionary rate increase 97 of 100 simulations displayed at least 'very strong support' for the FLC.

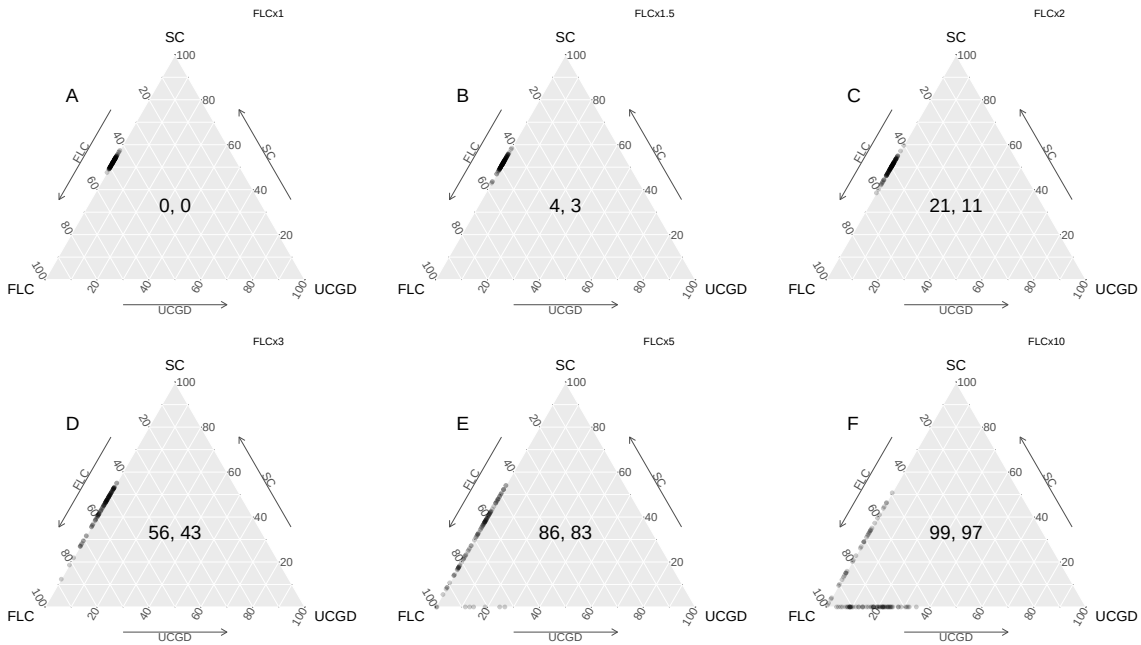

Figure 4: Ternary plots comparing the estimated log marginal likelihoods for the three molecular clock models (SC is the strict clock, FLC the fixed local clock, and UCGD the relaxed clock with underlying gamma distribution). Labels A. through F. correspond to molecular clock models used to simulate the data and match those described in Fig. 2. Each axis of the simplex represents the percentage support for a particular model, where each point indicates the level of support for each simulation. A value of 100 shows full support while 0 shows no support for a given model. The numbers in the center display the total simulations where there is 'strong' evidence that the FLC has a higher (log) marginal likelihood than the other models (log Bayes factor > 3), and those in which the log Bayes factor > 5, implying 'very strong' evidence.

2.3 Assessing statistical evidence using Bayes factors of the effect size

For our analyses under the FLC, we quantified evidence in favour of episodic evolution by comparing the posterior and prior odds, also known as a Bayes factor. We consider the hypothesis of episodic evolution as the situation where the evolutionary rate of foreground branches (r_f) is higher than that of the background branches (r_b). Because r_f and r_b are parameters of the model, we treat their ratio (or difference) as the 'effect size'. The prior probability for the hypothesis is $q(r_f > r_b)$ and its prior odds $= \frac{q(r_f > r_b)}{1-q(r_f > r_b)}$. Our prior for both r_f and r_b is a gamma distribution, $\Gamma(\alpha = 0.5, \theta = 0.1)$ (where α and θ the 'shape' and 'scale' parameters of the Γ distribution, respectively, and the mean is $\alpha\theta$=0.05), which results in $q(r_f > r_b) = 0.5$ and prior odds = 1. The posterior probability in favour of episodic evolution is $p(r_f > r_b)$, with posterior odds$=\frac{p(r_f > r_b)}{1-p(r_f > r_b)}$. To calculate the Bayes factor we take the posterior and prior odds ratio, which we present

in a logarithmic scale to facilitate comparison with other methods.

We found that the log Bayes factors of the effect size had higher sensitivity than model comparisons using log marginal likelihoods. For the data simulated under the SC, 37 out of 100 simulations had a positive log Bayes factor ($logBF > 0$, 'not worth more than a bare mention'), but only 4 displayed 'strong' ($logBF > 3$) evidence for episodic evolution. In our simulations for which the foreground branch rates were three-fold higher than that of the background (FLCx3) we found that 82 out of 100 cases had 'strong' support for episodic evolution (see Fig. 5 and Table 1). For higher evolutionary rate increases almost all simulations had 'very strong' support, at 91 for those with a five-fold rate increase and 99 for those with a ten-fold rate increase.

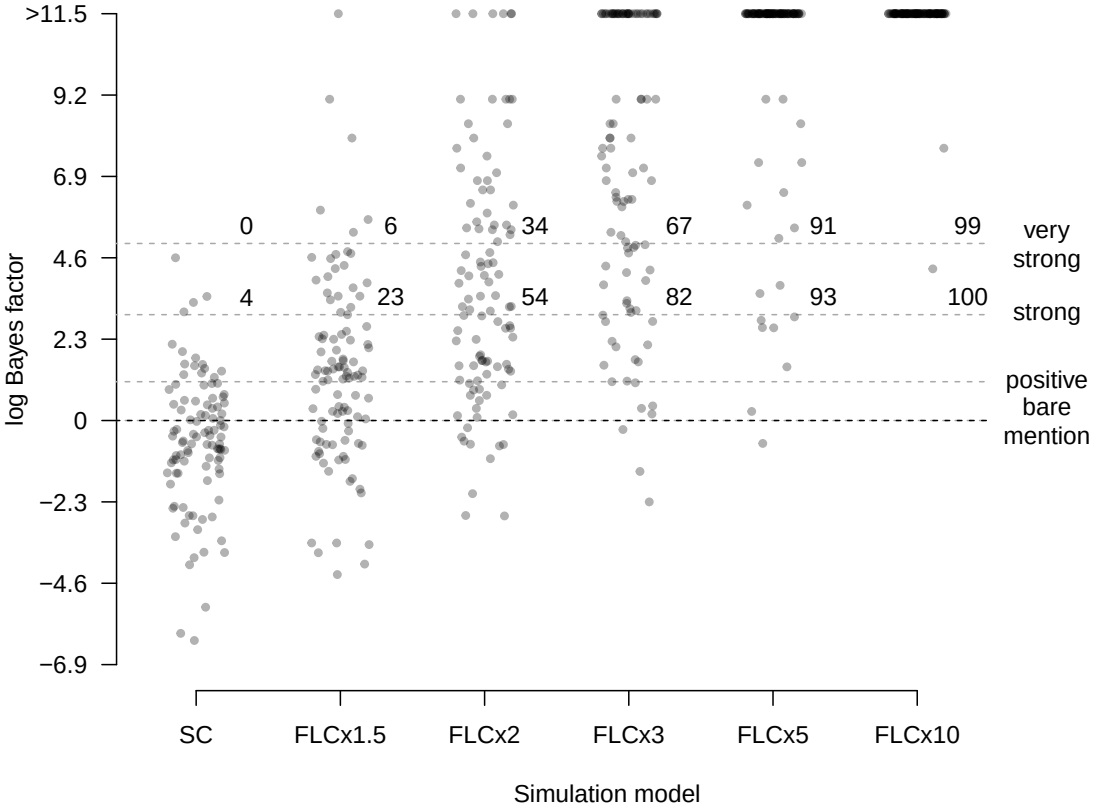

Figure 5: Statistical evidence quantified as log Bayes factors, here corresponding to the posterior and prior odds of episodic evolution. All data are analysed under the FLC molecular clock model and episodic evolution occurs when the foreground branches have higher evolutionary rates than the background branches. The models used to simulate the data are shown in the x-axis and match those described in Fig. 2. Each point represents the log Bayes factor (y axis) of a simulated data set (100 per simulation model). The dashed horizontal lines display degrees of support according to log Bayes factors (> 0 for 'not worth more than a bare mention', > 1 'positive', > 3 'strong', and > 5 'very strong'). The numbers above the dashed lines labelled 'strong' and 'very strong' correspond to the number of points above them, meaning the number of simulations with at least such support.

Table 1: Number of simulated data sets (out of 100) with different degrees of support for episodic evolution according to log Bayes factors of the posterior and prior odds (in logarithmic scale; see also Fig. 5). All data sets are analysed using the FLC molecular clock model, where episodic evolution occurs when the foreground branches have higher evolutionary rates than the background branches. The models used to simulate the data are shown in each row and match those described in Fig. 2.

Simulation model	$logBF > 0$	$logBF > 1$	$logBF > 3$	$logBF > 5$
SC	37	15	4	0
FLCx1.5	71	58	23	6
FLCx2	90	79	54	34
FLCx3	97	92	82	67
FLCx5	99	98	93	91
FLCx10	100	100	100	99

2.4 Prior sensitivity (when computing posterior and prior odds ratios)

An important, but often overlooked, aspect of Bayesian phylogenetic analyses is sensitivity to choice of the prior distribution (Gao et al., 2023, Moore et al., 2016). This is particularly relevant for Bayes factors using posterior and prior odds ratios. We randomly selected 25 simulated data sets and repeated the FLC analyses with a different prior on the evolutionary rate as $\Gamma(\alpha = 0.001, \theta = 1000)$ for both, foreground and background branches. Under this prior, the mean evolutionary rate is $\alpha\theta = 1.0$. Recall that our initial prior for this parameter is $\Gamma(\alpha = 0.5, \theta = 0.1)$, with mean $\alpha\theta = 0.05$. Both priors are relatively uninformative and do not have high prior probabilities for episodic evolution, at 0.38 for $\Gamma(\alpha = 0.001, \theta = 1000)$ and 0.5 for $\Gamma(\alpha = 0.5, \theta = 0.1)$. However, the commonly used $\Gamma(\alpha = 0.001, \theta = 1000)$ has more weight on low values.

The log Bayes factors using both prior parameterisations were very similar, although they tended to be slightly lower for $\Gamma(\alpha = 0.5, \theta = 0.1)$ (Fig. 6). In Fig. 7 we show the prior and posterior for two simulation replicates under the SC and the FLC where the foreground rate was 3-fold higher than the background (FLCx3). The posterior distributions are nearly identical for either prior, but there is some variation in the log Bayes factors. However, both display a similar degree of support of episodic evolution, with no support for the SC and 'very strong' support for the FLCx3.

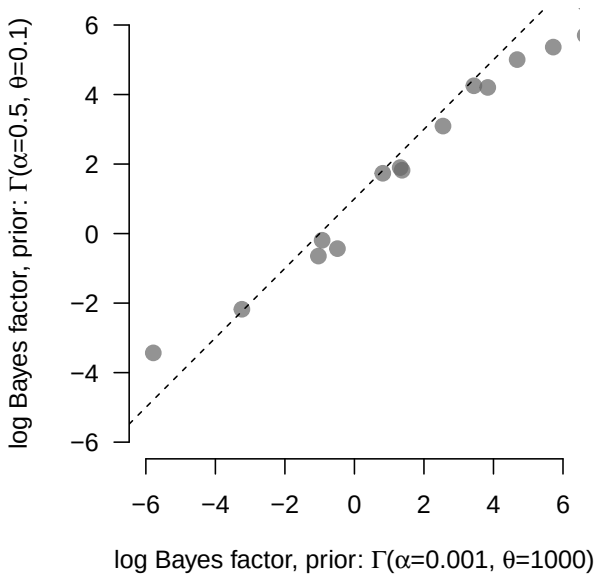

Figure 6: Comparison of log Bayes factors of posterior and prior odds of an evolutionary rate increase under the fixed local clock (FLC). The x-axis denotes values computed using a prior on the evolutionary rate of $\Gamma(\alpha = 0.001, \theta = 1000)$ and the y-axis is for those using a prior $\Gamma(\alpha = 0.5, \theta = 0.1)$. Here α is the 'shape' and θ is the 'scale' parameter. Each point is for a data set analysed under the FLC and both prior configurations. The dashed line is for y=x and thus points that fall on it are those where the log Bayes factors under both priors are identical.

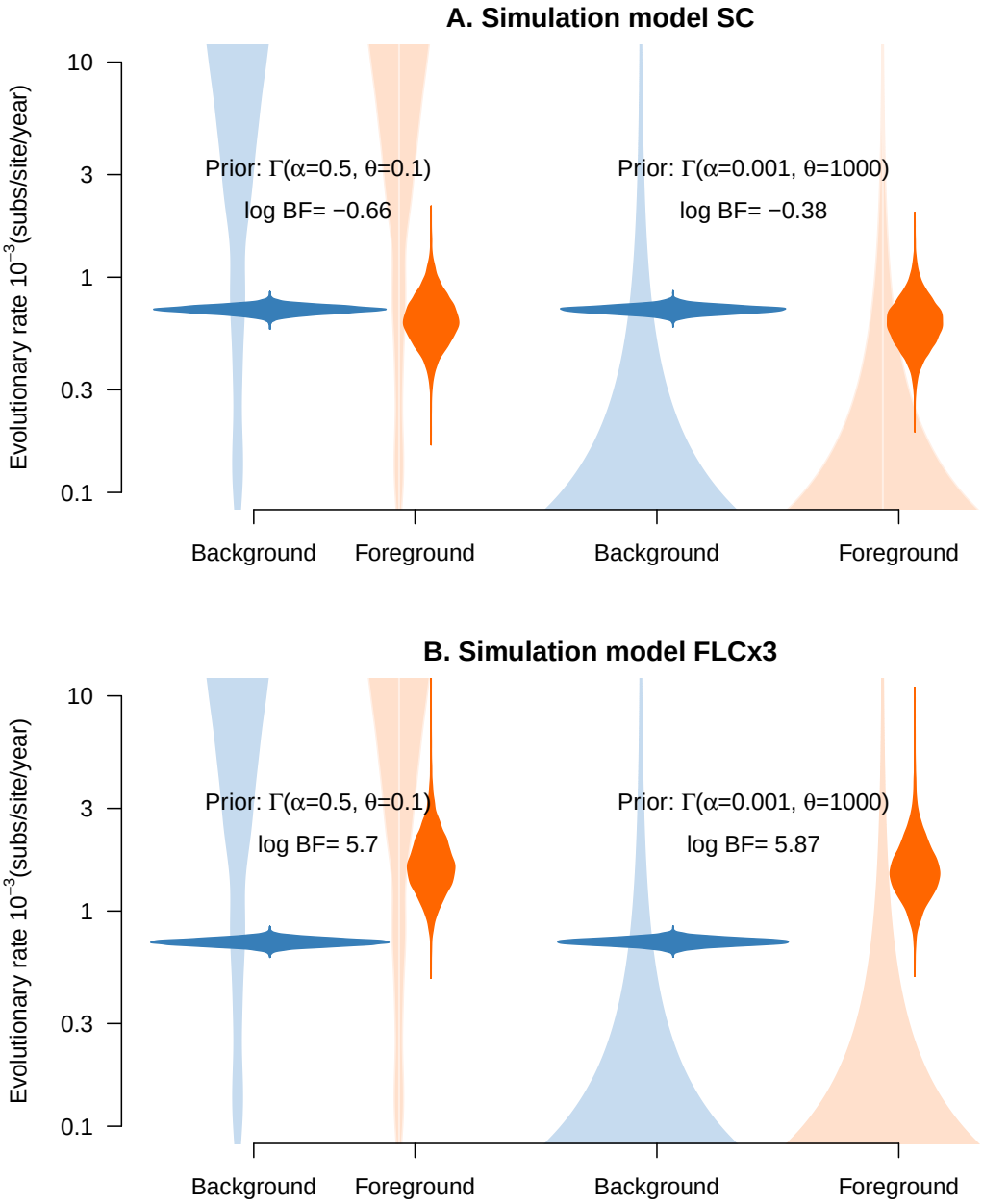

Figure 7: Prior posterior densities for background and foreground branch evolutionary rates under the fixed local clock model (FLC). The colours match those branches in Fig. 1C. The light blue densities correspond to two possible priors for the background branches, either $\Gamma(\alpha = 0.5, \theta = 0.1)$, used in most of our simulations, or $\Gamma(\alpha = 0.001, \theta = 1000)$ for assessing prior sensitivity. The light orange densities represent the prior on foreground branches, which is identical to the prior on background branches. The densities in darker shades correspond to the posterior distributions. The log Bayes factor (log BF) is shown in each case, and is calculated as the posterior and prior odds ratio of an increase in the evolutionary rate (i.e., where foreground branches have higher rates than the background). The top row (A) is for a data set simulated under a strict clock (SC) and thus expected to display no increase in evolutionary rates, and the bottom row (B) is a data set where the foreground branches had an evolutionary rate of 3-fold that of the background (FLCx3).

2.5 Bayesian model averaging with random local clocks

A different approach for detecting episodic evolution is through the use of a random local clock (RLC) model (Drummond and Suchard, 2010). In this Bayesian model averaging approach, the number and location of evolutionary rate changes is inferred, without the need for their *a priori* definition. A key consideration, however, is that evolutionary rates cannot be shared by non-neighbouring branches, meaning that the true model in our simulations is not part of those considered by the RLC. Our expectation for data simulated under the FLC is that nine clocks should be selected, corresponding to a rate change at each VOC stem and crown nodes (see Fig. 1C).

The posterior mass for our analyses under the RLC model tended to be concentrated on larger numbers of clocks with larger evolutionary rate increases, but the maximum number of clocks was consistently inferred to be below our expectation of nine. Indeed, for our simulations where the foreground branch rate was 10-fold that of the background, the RLC detected on average 3.79 clocks (Fig. 8).

Arguably, a more important question than the number of clocks detected in the RLC is whether any rate changes were detected along the stems or crowns of VOC clades. To investigate this we calculated the posterior probability of rate changes along VOC stem and crown nodes, and took the average over the 100 simulation replicates. We found very low support for rate changes at these nodes (average posterior probability of a rate change ≤ 0.31). It is worth noting, however, that the average posterior probability for a rate change along any of these branches tended to be higher with larger rate changes. For example, the average posterior probability for a rate change in the crown node of Delta was 0.48 for FLCx10, but 4.1×10^{-4} for FLCx1.5 (Table 2).

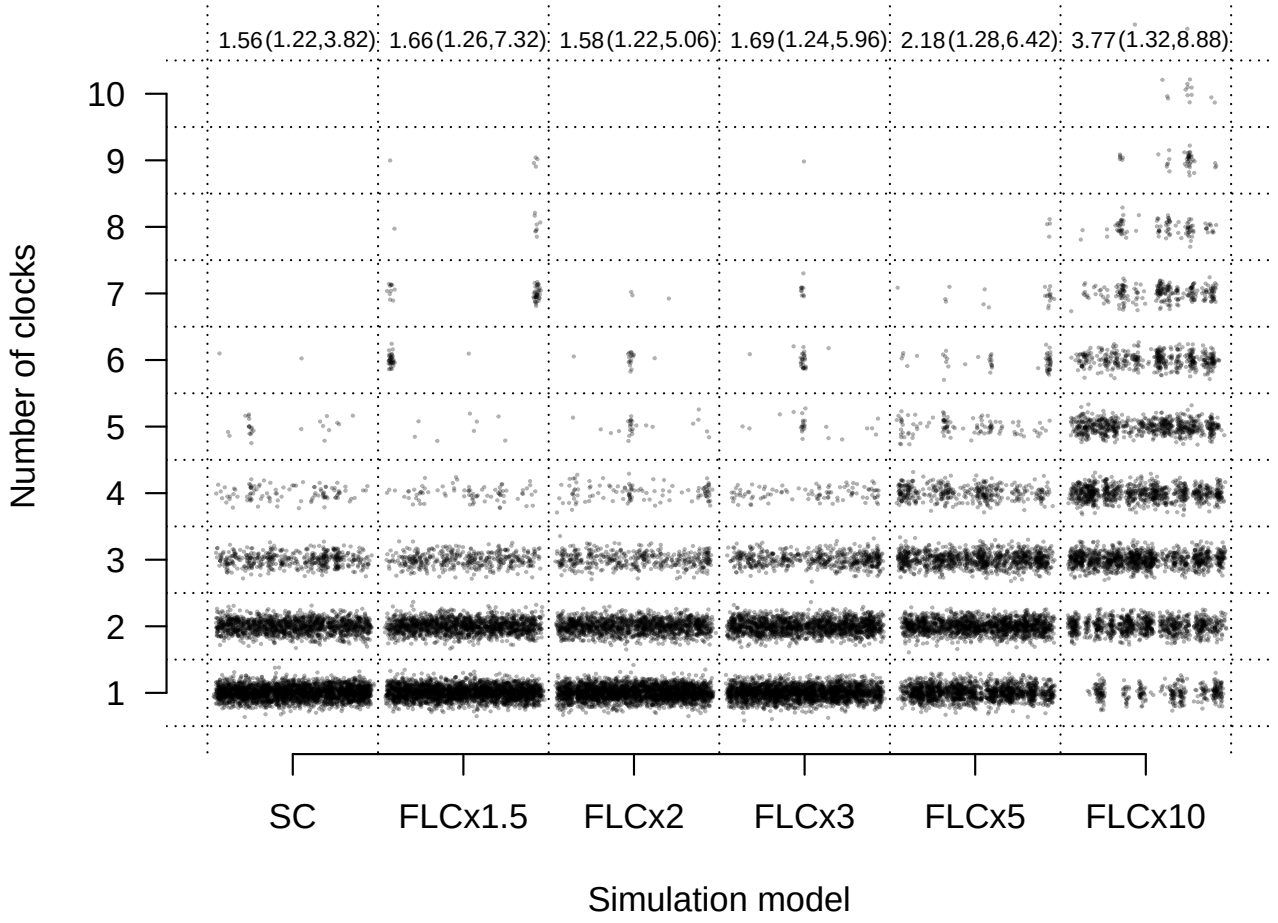

Figure 8: Posterior support for the number of local clocks inferred using Bayesian model averaging under a random local clock (RLC). Each column in the x-axis represents a simulation scenario under the fixed local clock (FLC) shown in Fig. 1. The y-axis shows the number of clocks. Each point is a sample drawn from the posterior distribution from the 100 data sets replicate data sets (arranged along each box in the x-axis). Numbers at the top correspond to the mean average number of clocks. Between parentheses we show the highest and lowest mean number of clocks, among data sets.

Table 2: Average posterior probability of evolutionary rate changes along particular branches, using the random local clock model (RLC). Numbers are computed as the posterior probability averaged over 100 simulations in each case.

Simulation model	**Alpha stem**	**Alpha crown**	**Beta stem**	**Beta crown**	**Gamma stem**	**Gamma crown**	**Delta stem**	**Delta crown**
SC	0.00	0.0043	0.00	0.0015	0.00	0.00079	0.00	0.00060
FLCx1.5	0.00	0.0076	0.00	0.011	0.00	0.00057	0.00	0.00041
FLCx2	0.00	0.025	0.00	0.0057	0.00	0.0035	0.00	0.0044
FLCx3	0.00	0.022	0.00	0.019	0.00	0.013	0.00	0.071
FLCx5	0.00	0.066	0.0060	0.081	0.00	0.11	0.00	0.22
FLCx10	0.050	0.30	0.060	0.24	0.02	0.31	0.02	0.48

2.6 Empirical analyses of SARS-CoV-2 variants of concern

We analysed an empirical SARS-CoV-2 data set to illustrate the application of the methods above. The data set was curated in a previous study by Tay et al. (2022) that displayed 'very strong' support for episodic evolution. The data set consists of 20 VOC complete genomes from each of the first four VOCs (Alpha, Beta, Gamma, and Delta) and 99 non-variant genomes, for a total of 179 whole-genome sequences.

We compared the same three models as in our simulations using generalised stepping-stone sampling (note that earlier work used path sampling and stepping-stone sampling to perform log marginal likelihood estimation). We repeated the log marginal likelihood estimation ten times to assess its variation. As expected, the FLC model had the highest support among the clock models tested. If we consider the average log marginal likelihoods over the ten replicates, the log Bayes factor of the FLC with respect to the UCGD (the second best-fitting model) is 6.35, consistently indicating 'very strong' support (see Fig. 9). These results were consistent with the log Bayes factor of the effect size, at 5.71. The ratio of the evolutionary rate of the foreground branches had a mean of 3.90 (95% credible interval: 2.21 - 8.02). We did not analyse the empirical data under the RLC because such analyses were previously conducted and the RLC did not support episodic evolution (Tay et al., 2022).

We show the inferred SARS-CoV-2 phylogeny under the FLC model in Fig. 10, with branches coloured according to the inferred evolutionary rates. Importantly, some of the branches leading up to VOCs are particularly long, such as that leading up to Gamma, with between 7 and 28 weeks and about 27 expected mutations, consistent with the large number of mutations defining VOCs and their time of emergence (Markov et al., 2023, Martin et al., 2021).

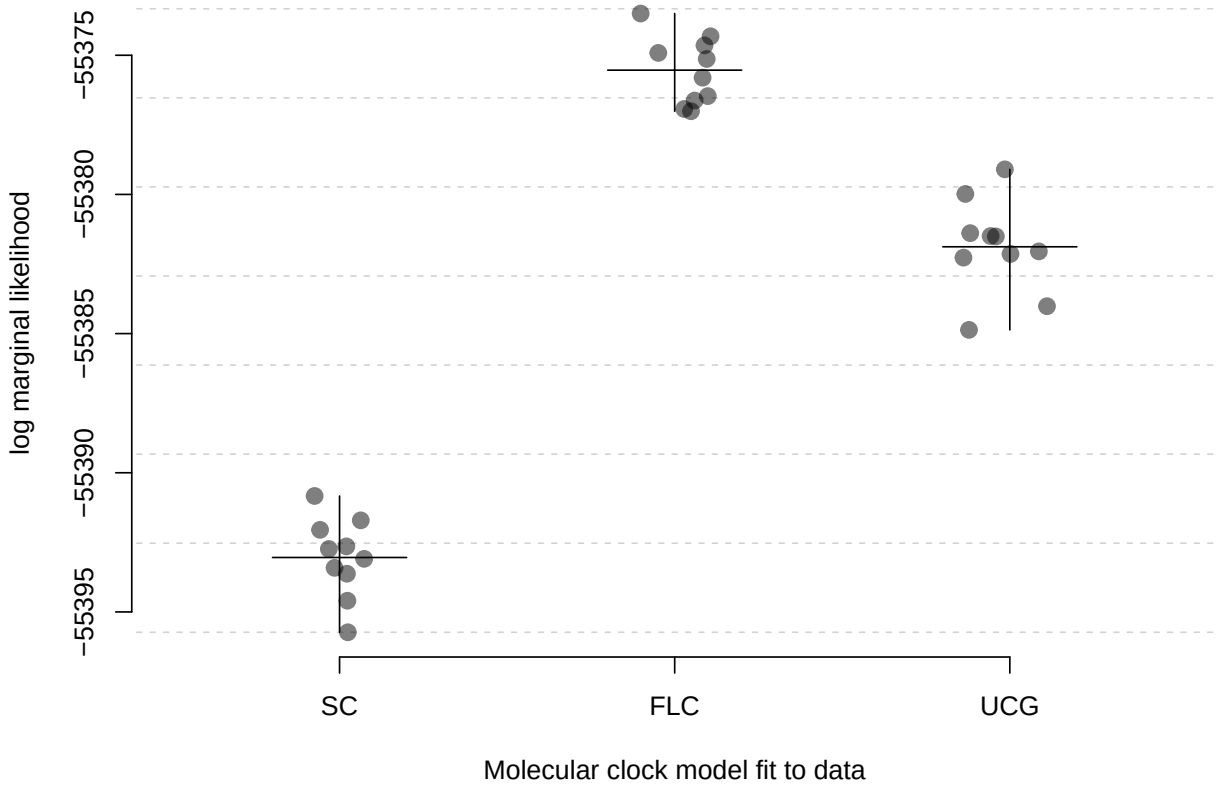

Figure 9: Comparison of the three molecular clocks for an empirical SARS-CoV-2 genome data set; strict clock (SC), fixed local clock (FLC), and uncorrelated relaxed clock with an underlying Γ distribution. The x-axis denotes the model used to analyse the data and the y-axis is the log marginal likelihood estimated using generalised stepping-stone sampling. We repeated these calculations ten times to assess their variability. Each point corresponds to a replicate and they have been jittered along the x-axis to facilitate visualisation. The horizontal solid lines represent the average log marginal likelihood across the ten simulations, and the vertical lines are for the range.

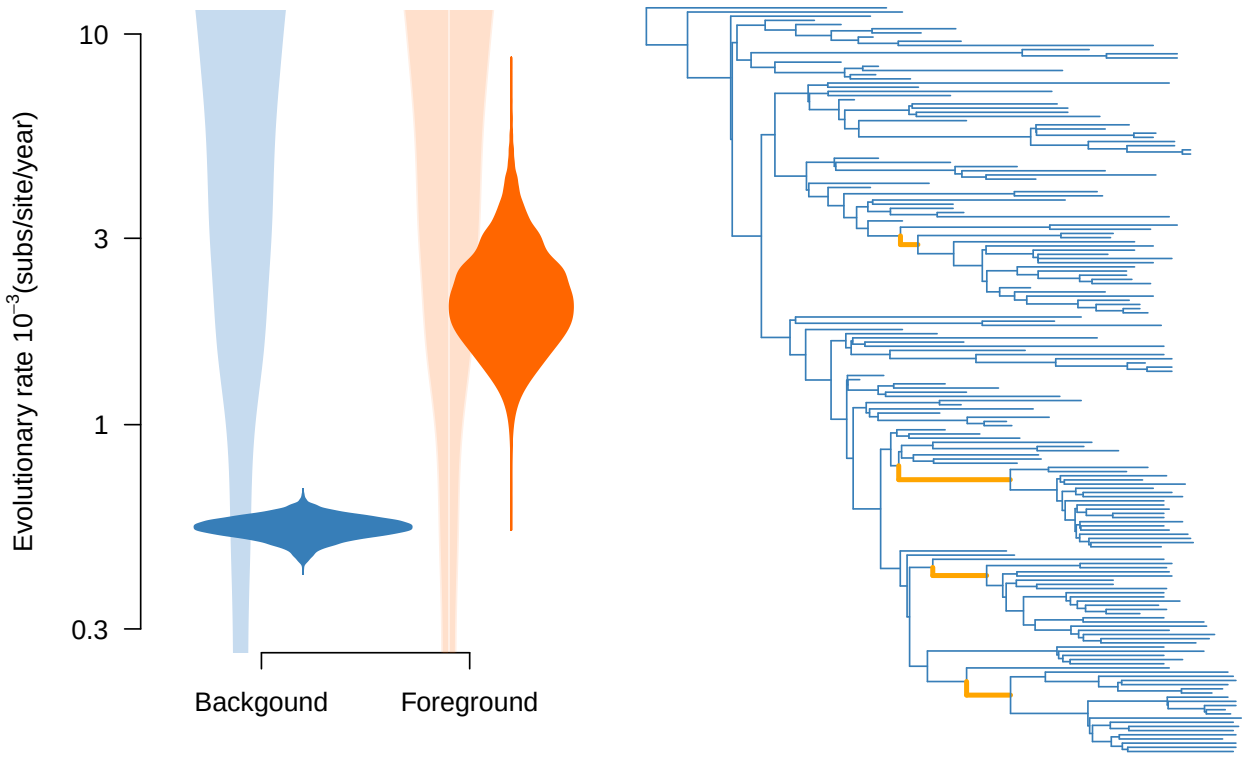

Figure 10: Empirical analysis for SARS-CoV-2 genome data set under the fixed local clock (FLC). The y-axis is the evolutionary rate and the x-axis is for the background and foreground branches, with colours matching those of the tree. The densities in light shades correspond to the prior on the evolutionary rates background and foreground branches, which is a $\Gamma(\alpha = 0.5, \theta = 0.1)$ distribution. The densities in darker shades represent the posterior. The log Bayes factor of the effect size is 5.71 ('very strong' support), and it is calculated as the log ratio of the posterior and prior odds of foreground branch rate being higher than that the background. In the phylogenetic tree, branches coloured in orange denote the stems of the first four variants of concern (VOCs): Alpha, Beta, Gamma and Delta.

3 Discussion

Detecting episodic evolution from genomic data can be challenging, particularly in situations where evolutionary rate changes are expected to occur along a small subset of branches, or even a single branch in phylogenetic trees. The main difficulty stems from the often limited amount of information available to estimate the evolutionary rate on a single branch. Our simulations are necessarily limited to one specific scenario, the emergence of SARS-CoV-2 VOCs, but they provide important guidelines for detecting episodic evolution in other organisms and outbreaks.

The signal in the data is a key consideration for the sensitivity of the approaches used here. Consider our simulations where the evolutionary rate of foreground branches was three-fold that of the background

(FLCx3). The uncertainty in the ratio of evolutionary rates for both sets of branches is sufficiently high that only 78 out of 100 simulations had 95% credible intervals that excluded a value of 1.0 (indicative of no episodic evolution; Fig. 2). Thus, only about a half of these simulations selected the FLC model with 'strong' or 'very strong' support (at 56 and 43, respectively; Fig. 4). Using log Bayes factors of the effect size yielded higher values, at 82 and 67, respectively (Fig. 5).

In contrast, our simulations with a ten-fold increase in the evolutionary rate of foreground branches with respect to the foreground yielded the lowest uncertainty in the ratio of foreground to background evolutionary rates (Fig. 2). None of the 100 simulations included a value of 1.0 for this statistic and 99 had 'strong' support in favour of the FLC over other molecular clock models (Fig. 4). Similarly, all of the simulations had 'strong' log Bayes factor support for episodic evolution according to the effect size (Fig. 5). The key factor at play here is the fact that our simulations with higher evolutionary rates for foreground branches tended to have increasing numbers of unique site patterns, meaning more information content (average of 1393 unique site patterns for SC, 1437 for FLCx5, and 1510 for FLCx10), and therefore more power to detect episodic evolution with either method. Other considerations for future studies that may impact our ability to detect episodic evolution may be the total number of sequences, the length of the genome or gene in question, and the number of foreground branches.

Our approach of assessing the effect size using posterior and prior odds to calculate the Bayes factor is practical and computationally efficient. However, the definition of the 'effect size' should be made explicit. In our study, the effect size is a convolution of two parameters, rather than a single parameter. A more tractable approach may be to quantify the effect size of an actual parameter of the model, where possible. Alternatively, in a situation similar to the one we explored here, one could consider using a hyperprior for the difference or ratio of two parameters. In any case, verifying the prior support for the hypothesis of interest is essential and straight-forward. For complex models, a simple solution is to run the analyses without data to obtain the marginal prior probability and odds of the hypothesis.

Model testing via log marginal likelihood estimation requires performing additional estimation procedures rather than simply sampling from the posterior. We found it to be more conservative than using prior and posterior odds for detecting episodic evolution, but it is important to note that model selection is much more flexible because it allows one to consider a range of sophisticated molecular clock models, including any parameterisation of the FLC. Moreover, to improve sensitivity one can employ a lower support threshold (e.g. 'positive' evidence), under the condition that variation in the marginal likelihood is lower than the level of

support required. Other approaches for model selection, including cross-validation, have recently been shown to have higher sensitivity than those based on log marginal likelihoods (Lartillot, 2023), and may mandate further research.

An alternative to model testing and using posterior and prior odds ratios is to employ an automated approach. Bayesian model averaging incorporates model uncertainty by sampling models relative to their posterior probability. The RLC model can be considered as a local molecular clock averaging method. We did not find this method to be sufficiently sensitive to detect evolutionary rate changes along VOC stem branches, but crucially, the correct model used to generate the data is not part of those that the RLC can consider. Clearly, model averaging approaches that can visit the exact model of interest, in this case the FLC, may have higher statistical power. An interesting approach that also explores a range of models, including local clocks, treats branch-specific rates as a Dirichlet process (Heath et al., 2012), implemented in the RevBayes platform (Höhna et al., 2016).

Other approaches that warrant further attention in the context of episodic evolution include those where changes in evolutionary rates are modelled as a compound Poisson process (Huelsenbeck et al., 2000). Recent developments in molecular clock models hold great promise for elucidating complex evolutionary patterns for which a hypothesis is difficult to define *a priori*, including shrinkage-based RLC models (Fisher et al., 2021), new MCMC proposals for relaxed clock models (Douglas et al., 2021), fixed local relaxed clocks (Fourment and Darling, 2018), and additive relaxed clocks (Didelot et al., 2021). Our guidelines and simulation framework will be useful to assess new methods and the evidence for episodic evolution in a range of organisms.

4 Methods

4.1 Simulations

To simulate our sequence data, we sampled 100 trees from the posterior distribution previously obtained (Tay et al., 2022) under a fixed local clock model with shared stems (labeled FLC here). Under this model, the molecular clock is parameterised with two evolutionary rates, i.e. one for the background branches and another for the foreground branches. The foreground branches are the stems leading up to VOCs and the background branches comprise all other branches, including those that form VOC clades. The premise of this model is that the evolutionary rate of the virus accelerated prior to the emergence of VOCs, but with the VOCs themselves evolving at a rate that is statistically indistinguishable from its ancestral and sister

lineages (see Fig. 1).

The background branches were always assigned an evolutionary rate of 7×10^{-4} substitutions per site per year, similar to previous estimates (Boni et al., 2020, Duchene et al., 2020). For the foreground branches, we assigned evolutionary rates that were 1, 1.5, 2, 3, 5, or 10-fold that of the background branches using the R package NELSI (Ho et al., 2015). Note that a rate increase of 1-fold corresponds to a strict molecular clock model (SC). For each evolutionary rate increase in VOC stem branches, we simulated the evolution of a multiple sequence alignment according to a GTR+Γ_4 substitution model with 29,903 nucleotides to match the genome length of SARS-CoV-2 using Seq-Gen (Rambaut and Grass, 1997).

4.2 Phylogenetic analyses and log marginal likelihood calculations

We analysed each of the 600 simulated data sets using BEAST 1.10 (Suchard et al., 2018), under four molecular clock models: the SC, which has a single parameter, i.e. the evolutionary rate; the UCGD that requires two parameters, i.e. the mean and shape of the gamma distribution, but note that the true number of parameters includes the evolutionary rates across all branches; the FLC that has two parameters for the evolutionary rates: one on the background and one on the foreground branches; and the RLC, where the number and location of local clocks is treated as a random variable (Drummond and Suchard, 2010) and the local clock rates are estimated by multiplying a baseline evolutionary rate. Another popular clock model is the relaxed uncorrelated with an underlying lognormal distribution, UCLD, (Drummond et al., 2006). In earlier analyses of SARS-CoV-2 VOCs we found the UCGD had higher support than the UCLD for these data, and thus chose the UCGD here. We assigned a $\Gamma(\alpha = 0.5, \theta = 0.1)$ prior distribution for the evolutionary rates in all models (local clock rates in the FLC, the mean for the UCGD, and the baseline rate of the RLC) and an exponential distribution for the shape parameter of the underlying gamma distribution in the UCGD model (Table 3).

Table 3: Molecular clock models, parameters and priors used in our Bayesian phylogenetic inference and log marginal likelihood estimation. SC: strict molecular clock, UCGD: uncorrelated relaxed clock with an underlying gamma (Γ) distribution; FLC: fixed local clock with two evolutionary rates (VOC stem rates as foreground and the rest as background). In the Γ distribution the α corresponds to the 'shape' parameter and θ is the 'scale' and the mean value is their product, $\alpha\theta$.

Model	**Priors (of the molecular clock model)**
SC	evolutionary rate $\sim \Gamma(\alpha = 0.5, \theta = 0.1)$
UCGD	mean $\sim \Gamma(\alpha = 0.5, \theta = 0.1)$, shape $\sim$ Exp(0.33)
FLC	background and foreground rate $\sim \Gamma(\alpha = 0.5, \theta = 0.1)$ or $\Gamma(\alpha = 0.001, \theta = 1000)$

One consideration of the FLC model here is that foreground branches can become unidentifiable if the topology is co-estimated. To address this problem, we enforced monophyly for each of the four VOCs, with the rest of the tree topology being estimated in the analysis. We also enforced monophyly in the SC, UCGD, and RLC models to ensure that tree space was comparable among the molecular clock models being compared.

For our inference under the different molecular clock models, we specified the GTR+Γ_4 substitution model and an exponential growth coalescent tree prior to match the simulation conditions. Because we were interested in estimating log marginal likelihoods, we ensured that priors on all parameters were proper probability distributions (i.e. they integrate to one) (Table 4) (Baele et al., 2013).

Table 4: Priors on substitution model and tree prior. The α parameter in the Dirichlet distribution is known as the 'concentration' and it determines the degree to which the substitution rates in the GTR matrix can differ.

Parameter	**Prior distribution)**
GTR substitution rates	Dirichlet($\alpha = 1$)
Shape of Γ distribution for rate heterogeneity	Exp(mean=0.5)
Effective population size	Exp(mean=100)
Exponential growth rate,	Laplace(mean=0, scale=10)

To sample the posterior distribution, we employed Bayesian inference through Markov chain Monte Carlo (MCMC) as implemented in BEAST 1.10 (Suchard et al., 2018), running the analyses for a total of 10^7 iterations, sampling every 10^3 iterations. We assessed convergence and sufficient sampling from the stationary distribution by verifying that the effective sample size (ESS) was at least 200 for all parameters in the model using Beastiary (Wirth and Duchene, 2022b). In the empirical data analyses we doubled the chain length and reduced sampling frequency accordingly.

We estimated log marginal likelihoods using generalised stepping-stone sampling (Fan et al., 2011). This estimator requires specifying working distributions for all parameters, including the tree (Baele et al., 2016). As working priors for the continuous parameters we used the default in BEAST 1.10 (generally a kernel density estimate of the parameter posterior distribution that is transformed accordingly). For the tree we used the product of exponential distributions determined by intercoalescent times, which is the most general working distribution for the coalescent (Baele et al., 2016).

Importantly, the working distribution requires the same monophyletic constraints on VOC clades, as described above. We specified 100 path steps between the unnormalised posterior and the working distribution,

according to equally spaced quantiles from a $\beta(0.3, 1.0)$ distribution. Each path step had a chain length of 10^6 steps. To assess variation of the log marginal likelihood estimates, we took a subset of simulations and repeated the log marginal likelihood calculations to calculate a repeatability statistic (Baele et al., 2016).

4.3 Posterior and prior odds and log Bayes factors

To obtain the Bayes factor of the effect size, we calculated the posterior and prior odds of episodic evolution. The posterior probability is the proportion of samples from the MCMC for which the evolutionary rate of the foreground branches is higher than that of the background. The posterior odds can then be obtained as explained in the section 'Assessing statistical evidence using Bayes factors of the effect size'. We can use the same approach for the prior probability and prior odds by running the analyses without sequence data. Formally, the probability that one random variable is larger than another is a convolution of probability distributions, with a difficult analytic solution. A more practical approach is to simulate from the corresponding statistical distributions, here Γ, using scientific software, such as R (e.g. Fig. 11).

```
prior_foreground <- rgamma(10000, shape = 0.5, scale = 0.1)
prior_background <- rgamma(10000, shape = 0.5, scale = 0.1)
prior_probability <- mean(prior_foreground > prior_background)
prior_odds <- prior_probability / (1 - prior_probability)
```

Figure 11: Example of R code to calculate the prior probability and prior odds, where the prior on the evolutionary rates of foreground and background branches is $\Gamma(\alpha = 0.5, \theta = 0.1)$.

4.4 Empirical data of SARS-CoV-2 variants of concern

Our empirical data analyses and simulation settings are based on a data set constructed in a previous study (Tay et al., 2022). These data consist of 99 non-VOC SARS-CoV-2 complete genomes downloaded through Nextstrain (Hadfield et al., 2018) from the GISAID database (Elbe and Buckland-Merrett, 2017, Shu and McCauley, 2017). VOC genomes were included by randomly selecting 20 sequences for the first four reported VOCs (Alpha, Beta, Gamma and Delta), such that the complete alignment comprised 179 whole-genome sequences. To obtain the data, we used the R package GISAIDR (Wirth and Duchene, 2022a). The sequences were aligned using MAFFT v7.49 (Katoh and Standley, 2013) and sites likely to contain sequencing errors were excluded according to those reported previously (De Maio et al., 2020).

5 Supplementary Material

Supplementary data are available online.

6 Acknowledgements

JHT and SD were supported by the Australian Research Council (FT220100629) and the Australian National Health and Medical Research Council (grant number 2017284). GB acknowledges support from the Internal Funds KU Leuven under grant agreement C14/18/094 and from the Research Foundation – Flanders ('Fonds voor Wetenschappelijk Onderzoek – Vlaanderen', G0E1420N and G098321N). The authors acknowledge efforts by originating and submitting laboratories for the sequence data in GISAID EpiCoV on which our empirical analyses are based. This research was undertaken using the LIEF HPC-GPGPU Facility hosted at the University of Melbourne. This Facility was established with the assistance of LIEF Grant LE170100200.

7 Data availability

The data underlying this article are available in GISAID at gisaid.org, and all accession numbers are provided in Supplementary Material online.

References

G. Baele, W. L. S. Li, A. J. Drummond, M. A. Suchard, and P. Lemey. Accurate model selection of relaxed molecular clocks in Bayesian phylogenetics. *Molecular Biology and Evolution*, 30(2):239–243, 10 2013. ISSN 0737-4038. doi: 10.1093/molbev/mss243. URL `https://doi.org/10.1093/molbev/mss243`.

G. Baele, P. Lemey, and M. A. Suchard. Genealogical working distributions for Bayesian model testing with phylogenetic uncertainty. *Systematic Biology*, 65(2):250–264, 2016.

M. F. Boni, P. Lemey, X. Jiang, T. T.-Y. Lam, B. W. Perry, T. A. Castoe, A. Rambaut, and D. L. Robertson. Evolutionary origins of the SARS-CoV-2 sarbecovirus lineage responsible for the COVID-19 pandemic. *Nature Microbiology*, 5(11):1408–1417, 2020.

K. A. Cohen, T. Abeel, A. Manson McGuire, C. A. Desjardins, V. Munsamy, T. P. Shea, B. J. Walker, N. Bantubani, D. V. Almeida, L. Alvarado, et al. Evolution of extensively drug-resistant tuberculosis over

four decades: whole genome sequencing and dating analysis of Mycobacterium tuberculosis isolates from KwaZulu-Natal. *PLoS Medicine*, 12(9):e1001880, 2015.

N. De Maio, C. Walker, R. Borges, L. Weilguny, G. Slodkowicz, and N. Goldman. Issues with SARS-CoV-2 sequencing data, 2020. URL `https://virological.org/t/issues-with-sars-cov-2-sequencing-data/473`.

X. Didelot, I. Siveroni, and E. M. Volz. Additive uncorrelated relaxed clock models for the dating of genomic epidemiology phylogenies. *Molecular Biology and Evolution*, 38(1):307–317, 2021.

J. Douglas, R. Zhang, and R. Bouckaert. Adaptive dating and fast proposals: Revisiting the phylogenetic relaxed clock model. *PLoS Computational Biology*, 17(2):e1008322, 2021.

A. J. Drummond and M. A. Suchard. Bayesian random local clocks, or one rate to rule them all. *BMC Biology*, 8(1):1–12, 2010.

A. J. Drummond, S. Y. W. Ho, M. J. Phillips, and A. Rambaut. Relaxed phylogenetics and dating with confidence. *PLoS Biology*, 4(5):e88, 2006.

S. Duchene, L. Featherstone, M. Haritopoulou-Sinanidou, A. Rambaut, P. Lemey, and G. Baele. Temporal signal and the phylodynamic threshold of SARS-CoV-2. *Virus Evolution*, 6(2):veaa061, 2020.

S. Elbe and G. Buckland-Merrett. Data, disease and diplomacy: GISAID's innovative contribution to global health. *Global Challenges*, 1(1):33–46, 2017.

Y. Fan, R. Wu, M.-H. Chen, L. Kuo, and P. O. Lewis. Choosing among partition models in Bayesian phylogenetics. *Molecular Biology and Evolution*, 28(1):523–532, 2011.

A. A. Fisher, X. Ji, A. Nishimura, P. Lemey, and M. A. Suchard. Shrinkage-based random local clocks with scalable inference. *arXiv preprint arXiv:2105.07119*, 2021.

M. Fourment and A. E. Darling. Local and relaxed clocks: the best of both worlds. *PeerJ*, 6:e5140, 2018.

M. Fourment, A. F. Magee, C. Whidden, A. Bilge, F. A. Matsen IV, and V. N. Minin. 19 dubious ways to compute the marginal likelihood of a phylogenetic tree topology. *Systematic Biology*, 69(2):209–220, 2020.

J. Gao, M. R. May, B. Rannala, and B. R. Moore. PrioriTree: a utility for improving phylodynamic analyses in BEAST. *Bioinformatics*, 39(1):btac849, 2023.

J. H. Gillespie. The molecular clock may be an episodic clock. *Proceedings of the National Academy of Sciences*, 81(24):8009–8013, 1984.

T. Gräf, G. Bello, T. M. M. Venas, E. C. Pereira, A. C. D. Paixão, L. R. Appolinario, R. S. Lopes, A. C. D. F. Mendonça, A. S. B. da Rocha, F. C. Motta, et al. Identification of a novel SARS-CoV-2 P.1 sub-lineage in Brazil provides new insights about the mechanisms of emergence of variants of concern. *Virus Evolution*, 7(2):veab091, 2021.

S. Guindon. Rates and rocks: strengths and weaknesses of molecular dating methods. *Frontiers in Genetics*, 11:526, 2020.

J. Hadfield, C. Megill, S. M. Bell, J. Huddleston, B. Potter, C. Callender, P. Sagulenko, T. Bedford, and R. A. Neher. Nextstrain: real-time tracking of pathogen evolution. *Bioinformatics*, 34(23):4121–4123, 2018.

T. A. Heath, M. T. Holder, and J. P. Huelsenbeck. A Dirichlet process prior for estimating lineage-specific substitution rates. *Molecular Biology and Evolution*, 29(3):939–955, 2012.

V. Hill, L. Du Plessis, T. P. Peacock, D. Aggarwal, R. Colquhoun, A. M. Carabelli, N. Ellaby, E. Gallagher, N. Groves, B. Jackson, et al. The origins and molecular evolution of SARS-CoV-2 lineage B.1.1.7 in the UK. *Virus Evolution*, 8(2):veac080, 2022.

S. Y. Ho and S. Duchêne. Molecular-clock methods for estimating evolutionary rates and timescales. *Molecular Ecology*, 23(24):5947–5965, 2014.

S. Y. Ho, S. Duchêne, and D. Duchêne. Simulating and detecting autocorrelation of molecular evolutionary rates among lineages. *Molecular Ecology Resources*, 15(4):688–696, 2015.

S. Höhna, M. J. Landis, T. A. Heath, B. Boussau, N. Lartillot, B. R. Moore, J. P. Huelsenbeck, and F. Ronquist. RevBayes: Bayesian phylogenetic inference using graphical models and an interactive model-specification language. *Systematic Biology*, 65(4):726–736, 2016.

J. P. Huelsenbeck, B. Larget, and D. Swofford. A compound Poisson process for relaxing the molecular clock. *Genetics*, 154(4):1879–1892, 2000.

R. E. Kass and A. E. Raftery. Bayes factors. *Journal of the American Statistical Association*, 90(430): 773–795, 1995.

K. Katoh and D. M. Standley. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution*, 30(4):772–780, 2013.

C. Keysers, V. Gazzola, and E.-J. Wagenmakers. Using Bayes factor hypothesis testing in neuroscience to establish evidence of absence. *Nature Neuroscience*, 23(7):788–799, 2020.

N. Lartillot. Identifying the best approximating model in bayesian phylogenetics: Bayes factors, cross-validation or waic? *Systematic Biology*, page syad004, 2023.

N. Lartillot and H. Philippe. Computing bayes factors using thermodynamic integration. *Systematic Biology*, 55(2):195–207, 2006.

W. L. S. Li and A. J. Drummond. Model averaging and Bayes factor calculation of relaxed molecular clocks in Bayesian phylogenetics. *Molecular Biology and Evolution*, 29(2):751–761, 09 2011. ISSN 0737-4038. doi: 10.1093/molbev/msr232. URL `https://doi.org/10.1093/molbev/msr232`.

K. A. Lythgoe, T. Golubchik, M. Hall, T. House, G. MacIntyre-Cockett, H. Fryer, L. Thomson, A. Nurtay, D. Buck, A. Green, et al. Lineage replacement and evolution captured by the United Kingdom Covid Infection Survey. *MedRxiv*, 2022.

P. V. Markov, M. Ghafari, M. Beer, K. Lythgoe, P. Simmonds, N. I. Stilianakis, and A. Katzourakis. The evolution of SARS-CoV-2. *Nature Reviews Microbiology*, pages 1–19, 2023.

D. P. Martin, S. Weaver, H. Tegally, J. E. San, S. D. Shank, E. Wilkinson, A. G. Lucaci, J. Giandhari, S. Naidoo, Y. Pillay, et al. The emergence and ongoing convergent evolution of the SARS-CoV-2 N501Y lineages. *Cell*, 184(20):5189–5200, 2021.

P. Mbala-Kingebeni, A. Aziza, N. Di Paola, M. R. Wiley, S. Makiala-Mandanda, K. Caviness, C. B. Pratt, J. T. Ladner, J. R. Kugelman, K. Prieto, et al. Medical countermeasures during the 2018 Ebola virus disease outbreak in the North Kivu and Ituri provinces of the Democratic Republic of the Congo: a rapid genomic assessment. *The Lancet Infectious Diseases*, 19(6):648–657, 2019.

B. R. Moore, S. Höhna, M. R. May, B. Rannala, and J. P. Huelsenbeck. Critically evaluating the theory and performance of Bayesian analysis of macroevolutionary mixtures. *Proceedings of the National Academy of Sciences*, 113(34):9569–9574, 2016.

R. D. Morey, J.-W. Romeijn, and J. N. Rouder. The philosophy of Bayes factors and the quantification of statistical evidence. *Journal of Mathematical Psychology*, 72:6–18, 2016.

R. A. Neher. Contributions of adaptation and purifying selection to SARS-CoV-2 evolution. *Virus Evolution*, 8(2):veac113, 2022.

J. Oaks, K. F. Cobb, V. N. Minin, and A. D. Leaché. Marginal likelihoods in phylogenetics: a review of methods and applications. *Systematic Biology*, 68(5):681–697, 2019.

A. Rambaut and N. C. Grass. Seq-Gen: an application for the Monte Carlo simulation of DNA sequence evolution along phylogenetic trees. *Bioinformatics*, 13(3):235–238, 1997.

Y. Shu and J. McCauley. GISAID: Global initiative on sharing all influenza data–from vision to reality. *Eurosurveillance*, 22(13):30494, 2017.

V. Simon, V. Zennou, D. Murray, Y. Huang, D. D. Ho, and P. D. Bieniasz. Natural variation in Vif: differential impact on APOBEC3G/3F and a potential role in HIV-1 diversification. *PLoS Pathogens*, 1 (1):e6, 2005.

J. S. Sinsheimer, J. A. Lake, and R. J. Little. Bayesian hypothesis testing of four-taxon topologies using molecular sequence data. *Biometrics*, pages 193–210, 1996.

M. A. Suchard, P. Lemey, G. Baele, D. L. Ayres, A. J. Drummond, and A. Rambaut. Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. *Virus Evolution*, 4(1):vey016, 2018.

J. H. Tay, A. F. Porter, W. Wirth, and S. Duchene. The emergence of SARS-CoV-2 variants of concern is driven by acceleration of the substitution rate. *Molecular Biology and Evolution*, 39(2):msac013, 2022.

R. van de Schoot, S. Depaoli, R. King, B. Kramer, K. Märtens, M. G. Tadesse, M. Vannucci, A. Gelman, D. Veen, J. Willemsen, et al. Bayesian statistics and modelling. *Nature Reviews Methods Primers*, 1(1):1, 2021.

W. Wirth and S. Duchene. GISAIDR, 2022a. URL `https://doi.org/10.5281/zenodo.6474693`.

W. Wirth and S. Duchene. Real-time and remote MCMC trace inspection with Beastiary. *Molecular Biology and Evolution*, 39(5):msac095, 2022b.

M. Worobey, G.-Z. Han, and A. Rambaut. A synchronized global sweep of the internal genes of modern avian influenza virus. *Nature*, 508(7495):254–257, 2014.

K. S. Xue, L. H. Moncla, T. Bedford, and J. D. Bloom. Within-host evolution of human influenza virus. *Trends in Microbiology*, 26(9):781–793, 2018.

A. D. Yoder and Z. Yang. Estimation of primate speciation dates using local molecular clocks. *Molecular Biology and Evolution*, 17(7):1081–1090, 2000.