

How to validate a Bayesian model

FÁBIO K. MENDES^{1†}, REMCO BOUCKAERT^{2‡*},

LUIZ M. CARVALHO^{3†}, ALEXEI J. DRUMMOND⁴

¹Department of Biology, Washington University in St. Louis

²School of Computer Science, The University of Auckland

³Escola de Matemática Aplicada, Fundação Getúlio Vargas

⁴School of Biological Sciences, The University of Auckland

*Corresponding authors: f.mendes@auckland.ac.nz; remco@cs.auckland.ac.nz

†Authors contributed equally to this work

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Abstract

Biology has become a highly mathematical discipline in which probabilistic models play a central role, and as a result research in the biological sciences is now dependent on computational tools capable of carrying out complex analyses. These tools must not only be efficient, but also correctly implemented. Both goals are difficult to achieve for several reasons, such as the multidisciplinary nature of method development, and a still embrionic literature on good software development and statistical practices aimed at professionals from disparate fields. Here we provide guidelines for the validation of probabilistic model implementations, focusing on Bayesian approaches. This manuscript summarizes good practices for assessing the correctness of simulation and inference procedures under a model, and is available in the traditionally static print version as well as in a reproducible and executable form.

[Probabilistic models, Bayesian models, model validation, coverage]

Introduction

The last two decades have seen the biological sciences undergo a major revolution. Critical technological innovations such as the advent of massive parallel sequencing and the accompanying improvements in computational power and storage have flooded biology with unprecedented amounts of data ripe for analysis. Not only has intraspecific data from multiple individuals allowed progress in fields like medicine and epidemiology (e.g., The 1000 Genomes Project Consortium, 2015; Human Microbiome Project Consortium, 2012; Neafsey et al., 2015), population genetics (e.g., Lynch, 2007; Lack et al., 2016; de Manuel et al., 2016) and disease ecology (e.g., Rosenblum et al., 2013; Bates

et al., 2018), but now a large number of species across the tree of life have had their genomes sequenced, furthering our understanding of species relationships and diversification (e.g., Martin et al., 2013; Brawand et al., 2014; Jarvis et al., 2014; Novikova et al., 2016; Pease et al., 2016; Kawahara et al., 2019; Upham et al., 2019). However, as the old adage goes, with great power comes great responsibility: never has the data available to the average biologist been so abundant, but also never has one been so aware of both its complexity and the necessary care needed to analyze it. Almost on par with data accumulation is the rate at which new computational tools are being proposed, as evidenced by journals entirely dedicated to method advances, methodological sections in biological journals, and computational biology degrees being offered by institutions around the world.

One extreme case is the discipline of evolutionary biology (on which we focus our attention). While it could be said that many decade-old questions and hypotheses in evolutionary biology have aged well and stood up the test of time (e.g., the Red Queen hypothesis, Van Valen 1973; Lively 1987; Morran et al. 2011; Gibson and Fuentes-González 2015; the Bateson-Dobzhansky-Muller model, Dobzhansky 1936; Muller 1940; Hopkins and Rausher 2012; Roda et al. 2017), data analysis practices have changed drastically in recent years, to the point they would likely seem exotic and obscure to an evolutionary biologist active forty years ago. In particular, evolutionary biology has become highly statistical, with the development and utilization of models now being commonplace.

Models are employed in the sciences for many reasons, and fall within a biological abstraction continuum (Servedio et al., 2014), going from fully verbal, highly abstract models (e.g., Van Valen 1973), through proof-of-concept models that formalize verbal models (e.g., Maynard Smith 1978; Reinhold et al. 1999; Mendes et al. 2018), to models that interact directly with data through explicit mathematical functions (Yule, 1924; Felsenstein, 1973; Hasegawa et al., 1985; Hudson, 1990). Within the latter category, probabilistic models have seen a sharp surge in popularity within evolutionary biology, in conjunction with computational tools implementing them.

Despite the increasing pervasiveness of probabilistic models in the biological sciences, tools implementing such models show large variation not only with respect to code quality (from a software engineering perspective), but also correctness (Darriba et al., 2018). This is unsurprising given the multidisciplinary nature of model and method development, and the challenges inherent to software research funding (Siepel, 2019). The bioinformatics community is thus in dire need of resources that provide guidance for code improvement and validation.

Here, we summarize best practices in probabilistic model validation for method developers, with an emphasis on Bayesian methods. Scripts for reproducing our validation protocols and figures are available on [[Link to DeveloperManual](#)]. This repository also hosts tools for model validation within the BEAST 2 platform (Bouckaert et al., 2019).

Probabilistic models

Probabilistic models mathematically formalize natural phenomena having an element of randomness. This is done through probability distributions describing both the observed empirical data – seen as the result of one or more random instantiations of the modeled process – as well the model parameters, which abstract relevant, but usually unknown aspects of the phenomenon at hand. The historical, stochastic, and highly dimensional nature of evolutionary processes makes the utility of probabilistic models in evolutionary biology self-evident.

The central component of a probabilistic model, $\Pr(D = d|\Theta = \theta)$, allows us to describe the probability distribution over the data (d) given the model parameters (θ). This probability mass function (pmf; or its continuous counterpart, the probability density function, pdf, $f_D(d|\Theta = \theta)$) is sometimes referred to as the likelihood function. (Just for this section, we will abuse and simplify notation, and drop variable subscripts, e.g., $f_D(D = d|\Theta = \theta) \rightarrow f(d|\theta)$). As illustrated in the next sections, probabilistic models can be hierarchical, in which case there may be several likelihood functions. In a frequentist statistical framework, $f(d|\theta)$ is the sole component of a probabilistic model, and is maximized across parameter space during parameter estimation and model comparison.

In the present study we focus on Bayesian inference, however, where a probabilistic model \mathcal{M} defines a posterior probability distribution for its parameters, $f(\theta|d) = \frac{f(d|\theta)f(\theta)}{\int_{\Theta} f(d|\theta)f(\theta)d\theta}$. Here, our prior inferences or beliefs about the natural world – represented by the prior distribution $f(\theta)$ – are confronted and updated by the data through the likelihood function (or multiple likelihood functions). $f(d) = \int_{\Theta} f(d|\theta)f(\theta)d\theta$, the probability of the data, is also known as the marginal likelihood or the model evidence. Crucially, a Bayesian model includes a prior, $f(\theta)$: when models are compared, for example, $f(\theta)$ needs to be taken into account when computing the model evidence $f(d)$.

Models routinely used in evolutionary biology are often characterized by continuous parameters, and are normally complex enough to preclude analytical solutions for the posterior density $f(\theta|d)$, mainly due to the intractability of the integral appearing in the marginal likelihood. In those cases, one can make use of the fact that $f(d)$ is a constant that can be ignored (i.e., $f(\theta|d) \propto f(\theta|d)f(\theta)$), and use techniques like Markov chain Monte Carlo (MCMC) to sample the posterior distribution. This is because the MCMC algorithm that generates the Markov chain, called the Metropolis-Hastings (Metropolis et al., 1953; Hastings, 1970) algorithm, only requires the posterior to be evaluated up to a constant.

In practice, the Metropolis-Hastings algorithm samples the posterior distribution (also referred to as the “target” distribution) by means of a transition mechanism. If the proposal distribution generated by this mechanism is irreducible, positive recurrent, and aperiodic, and the resulting chain is long enough, then the sampled posterior distribution will approximate the target distribution $f(\theta|d)$ (Smith and Roberts, 1993; Tierney, 1994; Gelman et al., 2013).

We will spend time considering MCMC in particular as it is the commonly chosen technique for obtaining $f(\theta|d)$ under an implementation of model \mathcal{M} . A thorough validation effort thus entails verifying the correctness of (i) the model (i.e., $f(d|\theta)f(\theta)$), and (ii) the components involved in the MCMC transition mechanism. We note that the latter are not part of the model, however, and it is possible to sample $f(\theta|d)$ with other techniques such as importance sampling, Hamiltonian Monte Carlo (Duane et al., 1987), or even by converting the sampling problem into an optimization one (e.g., Zhang and Matsen, 2019).

Finally, we stress that we are interested in practices for verifying model *correctness*. There are other tests employed to ensure that a particular MCMC analysis is converging as anticipated. Determining that one or more independent Markov chains converged on very similar posterior distributions is not a correctness test, as those distributions might be very different from the target distribution.

Validating a Bayesian model

Validating the simulator, $S[\mathcal{M}]$

When a probabilistic model \mathcal{M} is implemented for the first time, a simulator $S[\mathcal{M}]$ must be devised and itself validated before we can validate an inferential engine $I[\mathcal{M}]$. It is $I[\mathcal{M}]$ that will be employed by users in empirical analyses.

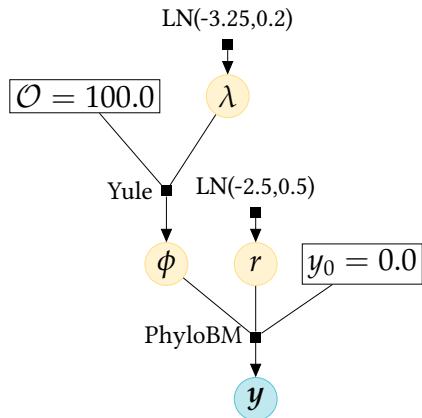


Figure 1: The graphical representation of a simple Bayesian phylogenetic model. Parameters are represented by yellow circles, observed data in blue circles, and fixed values are shown within boxes. Sampling distributions are represented by filled squares. $LN(\mu, \sigma^2)$ denote log-normal sampling distributions with mean μ and variance σ^2 in log-space. Parameter descriptions can be found in the main text.

A simulator conventionally requires a parameter value as input (i.e., a θ value, where θ might represent more than one parameter), or a prior distribution on those values, $f_\Theta(\cdot)$. (Note that we use “.” when referring specifically to the generative function, rather than the value it takes given input.) The simulator then outputs a sample of random variable(s), which for hierarchical models will include not only an instantiation of data D , but also of a subset of the parameters in θ .

In the case of hierarchical models, it is sometimes useful to consider $S[\mathcal{M}]$ as a collection of component simulators, each characterized by a different sampling distribution. For example, $S[\mathcal{M}]$ for the whole integrative model in figure 5 can be seen as an ensemble comprised by:

1. $S[f_\Theta(\cdot)]$ (where $\Theta = \{\Lambda, R, Y_0\}$), which jointly simulates $\theta = \{\lambda, r, y_0\}$,
2. $S[f_{\Phi|\Lambda}(\cdot|\Lambda = \lambda)]$, which simulates a Yule tree ϕ given a value of λ simulated in (1),
3. $S[f_{Y|\Phi,R}(\cdot|\phi, r)]$, which simulates an array of k continuous-trait

values, \mathbf{y} , given a phylogeny ϕ with k species and an evolutionary rate r (simulated in (1) and (2), respectively).

Being able to isolate the building blocks of a hierarchical model simulator helps divide and conquer the validation task, especially when some, but not all of the sampling distributions are well-known parametric distributions, or when they result from well characterized stochastic processes (see below).

One way to validate a probabilistic model simulator is by using it to sample a large number of data sets given a set of parameters. These parameters can be seen as characterizing a “population” of the entities being modeled. For each data set, one can then construct α -confidence intervals (where $\alpha \in (0, 1)$ gives the credibility level) for certain summary statistics (e.g., mean, variance, covariance). If the simulator is behaving as expected, one should be able to verify that the (population) summary statistic is contained approximately $\alpha\%$ of the time within their α -confidence intervals. An example is the Yule model (also known as the pure-birth model; Yule 1924), a continuous-time Markov process that has been classically employed in phylogenetics to model the number of species in a clade (Yule, 1924; Aldous, 2001). Under a Yule process with a species birth rate of λ , the expected tree height, $E[t_{\text{root}}]$, for a tree with n tips is:

Box 1: Models characterized by well-known parametric distributions

One commonly used model in macroevolution for the study of continuous traits is the phylogenetic Brownian motion model (“PhyloBM” in Fig. X; Felsenstein 1973). The pdf characterizing this model’s sampling distribution is in fact the pdf of the multivariate normal (MVN) probability distribution:

$$\begin{aligned} \log f(\mathbf{y} | \mathbf{y}_0, r, T) = & -\frac{1}{2} \left[n \log(2\pi) + \log|rT| \right] \\ & -\frac{1}{2} \left[(\mathbf{y} - \mathbf{y}_0)^T (rT)^{-1} (\mathbf{y} - \mathbf{y}_0) \right], \end{aligned} \tag{1}$$

where \mathbf{y} corresponds to the observed values of a trait scored for n species, \mathbf{y}_0 is the trait value at the root of the tree, r is the instantaneous rate of change (i.e., the evolutionary rate, and sometimes represented by σ^2), and rT is the variance-covariance matrix. T is a matrix whose elements are deterministically defined by tree Φ ’s topology and branch lengths; see Fig. 1 below).

The probability density function in equation (1) describes the distribution that would result from an infinite number of BM “experiments” (each experiment being non-mean-reverting, and representing an independent evolutionary trajectory). Under this model, $\theta = \{\mathbf{y}_0, r, T\}$ and $d = \{\mathbf{y}\}$ (but note that sometimes researchers treat ϕ and consequently T as data).

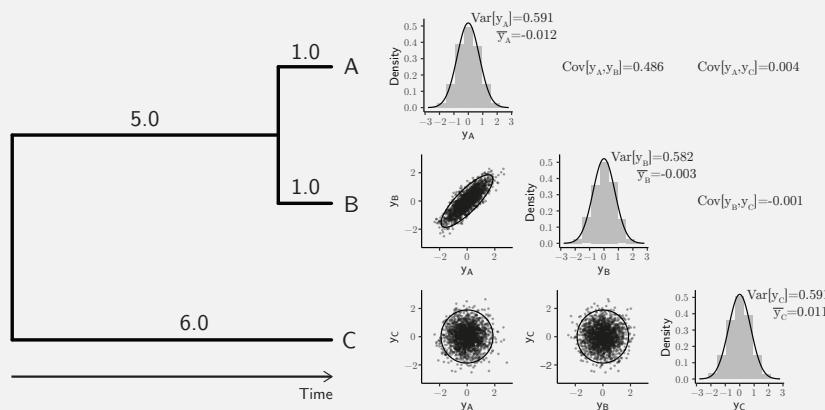


Figure 2: A sample of 1000 draws from a MVN distribution, each representing the evolutionary trajectory of one continuous trait along the species tree on the left. The root trait value, y_0 , and the evolutionary rate of the process, r , were set to 0.0 and 0.1, respectively. The panel on the right shows histograms of 1000 trait values sampled from the MVN for each species, as well as their covariation.

Validating a phylogenetic BM simulator

The MVN is a well-characterized parametric distribution. When used as the sampling distribution of the phylogenetic BM process, it explicitly defines the expected trait value for each species (y_0), as well as their trait value variances and covariances. The latter comes from the variance-covariance matrix; for the tree shown in figure 1 and with $r = 0.1$, this matrix is:

$$rT = 0.1 \begin{bmatrix} 6 & 5 & 0 \\ 5 & 6 & 0 \\ 0 & 0 & 6 \end{bmatrix} \quad (2)$$

Together, variance-covariance matrix rT and $y_0 = [0.0, 0.0, 0.0]$ characterize a population of phylogenetically related species trait values whose means are 0.0, variances are 6.0, and co-variances are 5.0 (between species “A” and “B”) and 0.0 (between species “C” and either “A” or “B”).

Figure 1 shows the distributions of trait values and their variances and covariances for one sample of X independent realizations of phylogenetic BM processes. One can see that the sample’s average trait value and the variances and covariances approach their expectations. In order to be rigorous, one can follow the method described in the main text and verify that those expectations fall within their 95% confidence intervals 95% of the time, as calculated from a large number of samples (Supplementary Fig. ?? and Supplementary Table ??).

$$E[t_{\text{root}}] = \sum_{i=2}^n \frac{1}{1\lambda}. \quad (3)$$

One can then verify if $E[t_{\text{root}}]$ is 95% of the time within ± 1.96 standard errors of the average Yule-tree height (from each sampled data set). Confirming that this is the case indicates $S[f_{\Phi|\Lambda}(\cdot)]$ is correctly implemented (Fig. 3). In Box 1, we illustrate this procedure for the (parametric) sampling distribution underlying the phylogenetic Brownian motion

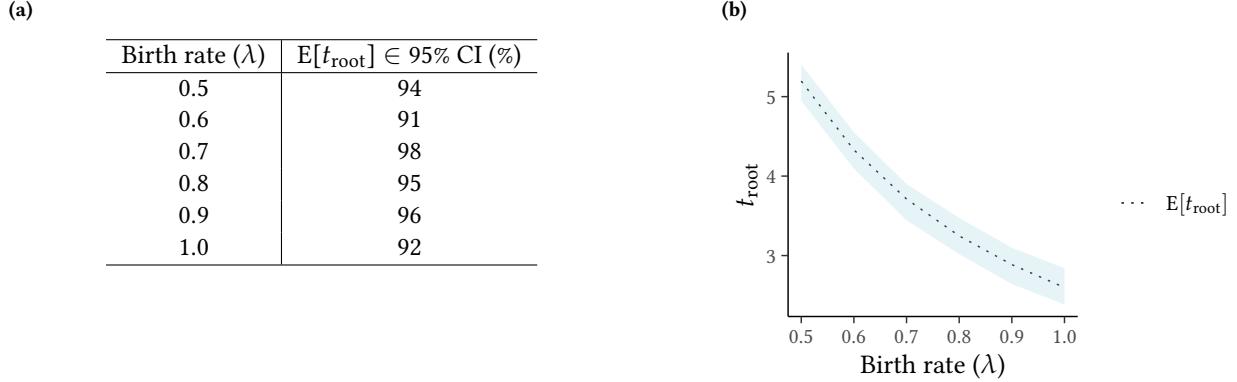


Figure 3: Validation of Yule tree simulator. (a) Number of simulated data sets (out of 100) for which the expected tree height (t_{root}) was inside the 95% CI about its sample average. Each data set consisted of 50 twenty-taxon simulated Yule trees. (b) The area shaded in light blue represents the 95% confidence interval about the average tree height, obtained from the 5,000 Yule trees simulated in (a). Simulations were carried out with the TreeSim R package (Stadler, 2011).

model (“PhyloBM”; Felsenstein, 1973). Protocols for validating $I[\mathcal{M}]$ (see below) will also automatically validate $S[\mathcal{M}]$.

We note that we have so far used $S[\mathcal{M}]$ to represent a *direct* simulator under model \mathcal{M} (Table 1), meaning each and every sample generated by $S[\mathcal{M}]$ is independent. This is contrast with other simulation strategies, such as conducting MCMC under model \mathcal{M} with no data, given specific parameter (θ) values. This latter approach may be the only option if $S[\mathcal{M}]$ has not been yet implemented, and it is predicated upon the existence of correct implementations of both an inferential engine $I[\mathcal{M}]'$ and of proposal functions. We distinguish $I[\mathcal{M}]'$ from $I[\mathcal{M}]$ because simulations are being carried out precisely to validate $I[\mathcal{M}]$. Unless MCMC simulations are done with $I[\mathcal{M}]'$ – an independent implementation of $I[\mathcal{M}]$ – they can introduce circularity to the validation task.

Table 1: A non-exhaustive list of direct simulation software used for various models in evolutionary biology.

Software package	Model type	Platform	Reference
Seq-Gen	Molecular sequence evolution models	Standalone	Rambaut and Grass, 1997
ms	Coalescent model	Standalone	Hudson, 2002
msprime	Coalescent model	Python	Kelleher et al., 2016
SLiM	Population genetic models	Standalone	Haller and Messer, 2019
TreeSim	Birth-death models	R	Stadler, 2011
mvMORPH	Continuous trait evolution models	R	Clavel et al., 2015
diversitree	Several birth-death models	R	Revell, 2012
MASTER	Several birth-death models	BEAST 2	Vaughan and Drummond, 2013
LPhy	Several evolutionary models	Standalone	Drummond et al., 2022

Validating the inferential engine, $I[\mathcal{M}]$

The more complex the natural phenomenon under study, the more difficult it will be to strike a good balance between model practicality and realism.

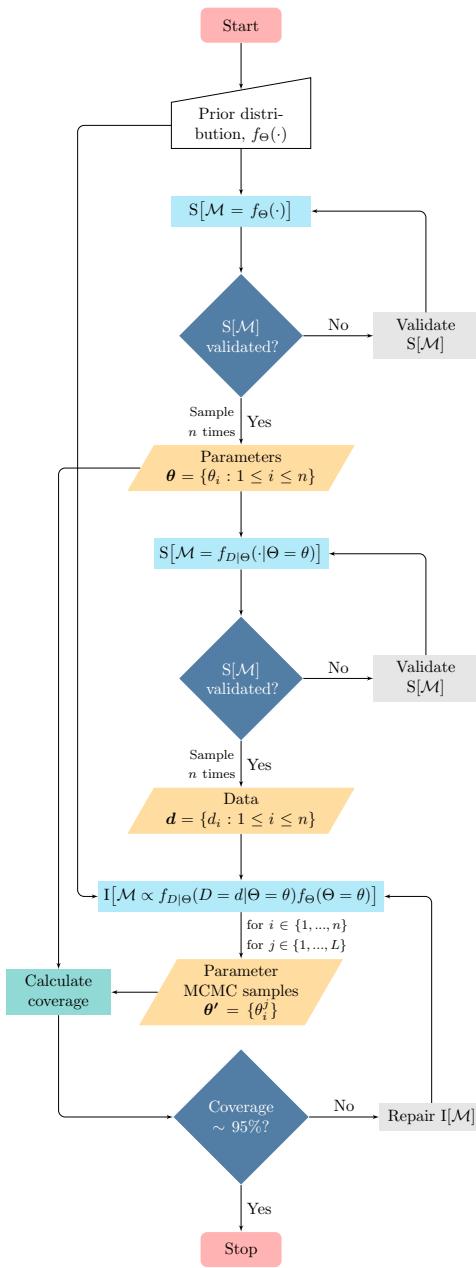


Figure 4: Flowchart of the coverage-validation of a Bayesian model.

cess (see previous section).

The popular aphorism rings true: “all models are wrong but some are useful” (Box, 1979). Very simple models are easier to implement in efficient inference tools, but will commonly make assumptions likely to be broken by the data (e.g., Sullivan and Swofford, 1997; Mendes and Hahn, 2018; Mendes et al., 2019). Conversely, complex models will fit the data better (e.g., Ogilvie et al., 2022), but may become unwieldy with increasing levels of realism.

A large number of parameters can cause overfitting and unidentifiability, and highly complex models might lead to prohibitively slow inferential analyses (e.g., Lartillot and Poujol, 2011). Deciding on the utility of a model for real-world problems is a daunting task (Brown and Thomson, 2018; Shepherd and Klaere, 2018), and is a challenge we do not address in the present contribution. Such model appraisals are normally carried out after a model is published, often in multiple contribution bouts, and are critical for a model’s longevity. Analyses of model fit against data are normally accompanied by discussions on assumption validity, and more rarely by benchmarking and scrutinization of model behavior and implementation (e.g., Maddison et al., 2007; Rabosky and Goldberg, 2015; Rabosky et al., 2013; Moore et al., 2016; Stadler, 2010; Luo et al., 2020).

When a new model \mathcal{M} is initially proposed, however, authors must ensure that their methods can at the very least robustly recover generating parameters. In this section, we discuss a few techniques that can be employed to assess the correctness of a parameter-estimation routine. These techniques assume that one can simulate from a probabilistic data-generating pro-

Coverage-validation of a Bayesian model

Our discussion on how to ensure a Bayesian model is well-calibrated and thus correct will mostly follow the ideas in Cook et al. (2006) and Talts et al. (2018). The basic idea is presented in the flowchart in figure 4, and consists of three stages: simulation, inference, and coverage calculation. Once we have a validated simulator for model \mathcal{M} , we start by sampling n parameter sets from $\theta = \{\theta_i : 1 \leq j \leq n\}$ from its prior, $f_\Theta(\cdot)$, i.e.:

$$\theta_i \sim f_\Theta(\cdot).$$

For each parameter set θ_i , we then sample a data set d_i from $f_{D|\Theta}(\cdot | \Theta = \theta_i)$:

$$d_i \sim f_{D|\Theta}(\cdot | \Theta = \theta_i),$$

These two steps conclude the “simulation” stage of this validation protocol. With $\mathbf{d} = \{d_i : 1 \leq i \leq n\}$, we use the inferential machinery $I[\mathcal{M}]$ under evaluation to compute $f_{\Theta|D}(\Theta = \theta_i | D = d_i)$ for each d_i . Recall that we assume the posterior distribution defined by $f_{\Theta|D}(\Theta = \theta | D = d)$ over Θ will be approximated with MCMC, an algorithm that generates a large sample of size L of parameter values from that posterior distribution, $\theta' = \{\theta_i^j : 1 \leq i \leq n, 1 \leq j \leq L\}$. At this point, we have concluded the inference stage of this validation pipeline.

The third stage and final stage consists of investigating coverage properties of uncertainty intervals. The critical expectation here is that if the inferential engine is correct, we will be able to obtain interval estimates with precise coverage properties. More concretely, let us first define the highest posterior density (HPD) interval. For a credibility level $\alpha \in (0, 1)$, we define $I_\alpha(d) := (a(d, \alpha), b(d, \alpha))$ such that:

$$\frac{1}{f_D(D = d)} \int_{a(y, \alpha)}^{b(y, \alpha)} f_{D|\Theta}(D = d | \Theta = \theta) f_\Theta(\Theta = \theta) d\Theta = \alpha,$$

where $f_D(D = d)$ is a constant that can be ignored. By defining $\text{Cred}(I_\alpha(d)) = \alpha$,

$$\inf_{b(d, \alpha) - a(d, \alpha)} \{I_\alpha(d) : \text{Cred}(I_\alpha(d)) = \alpha\}$$

yields the shortest interval with the required credibility. Note that we approximate a particular $I_\alpha(d_i)$ from the i -th L samples obtained with MCMC, in θ' .

Now taking a set of parameter values θ_i sampled from $f_\Theta(\cdot)$ it can be shown that $\Pr(\theta_i \in I_\alpha(d)) = \alpha$, i.e., that $100 \times \alpha\%$ HPDs have nominal coverage under the true generative model (a proof is provided in the supplementary material). More formally, the coverage of n intervals obtained as above will be distributed as binomial random variable with n trials and success probability α . When $n = 100$ and $\alpha = 0.95$, the confidence interval for the number of simulations containing the correct data-generating parameter is between 90 and 99 (Table 2). If we ascertain that

k	$\Pr(x = k)$
90	0.0167
91	0.0349
92	0.0649
93	0.1060
94	0.1500
95	0.1800
96	0.1781
97	0.1396
98	0.0812
99	0.0312
100	0.0059

Table 2: Under a correctly implemented model, coverage x (the number of true simulated values that fall within their corresponding 95%-HPDs) is binomially distributed with n trials ($n = 100$ in this case), and probability of success $p = 0.95$.

$I[\mathcal{M}]$ of a Bayesian model produces coverage lying within the expected bounds, we say the model has passed the coverage validation, and is well-calibrated and correct.

We provide examples of coverage validation attempts in figure 5, which shows coverage graphical summaries for data simulated under the model represented in figure 1. This model is deliberately simple for the sake of brevity and clarity in the discussion below. The parameters in this model are the phylogenetic tree Φ , the species birth rate λ , and the continuous-trait evolutionary rate r (we assume the continuous trait value at the root, y_0 , is known and set it to **0.0** for all simulated data sets). When the model is correctly specified between simulation and inference (“Scenario 1”, the leftmost top and bottom panels, Fig. 5), coverage is close to 95% and adequate for both λ and r , which indicates that $I[\mathcal{M}]$ – as implemented in BEAST 2, the software we used – is well-calibrated and correct.

In “Scenario 2” of figure 5 (middle top and bottom panels), however, we misspecify the model during inference, setting the prior distribution on λ to be a log-normal with a mean of -2.0 (rather than -3.25, as specified in the simulation procedure; Fig. 1). In contrast with scenario 1, coverage is 0.0 for λ and 70% for r , both much lower and outside the expected coverage bounds (Table 2). These numbers indicate that one or more of the parts comprising model \mathcal{M} used in $I[\mathcal{M}]$ differs from their counterparts in $S[\mathcal{M}]$. This result was expected because we purposefully made the models in simulation and inference differ; we know $I[\mathcal{M}]$ is correct because of the results from scenario 1. Of course, in a real-world validation experiment the model should be correctly specified, and such a result would suggest a problem with the inferential machinery (provided the the simulator had been previously validated).

[This paragraph here might or not change if we decide on a different scenario 3; this depends on Luiz’s results]: Lastly, in “Scenario 3” of figure 5 (rightmost top and bottom panels), we again correctly specified the model, but carried out substantial rejection sampling. Approximately 90% of all simulated trees were rejected based on their taxon count; trees were rejected if they had fewer than 100 or more than 200 taxa. As with scenario 1, coverage fell within the expected ranges for a correct model implementation. This result shows that, although tree size is a random

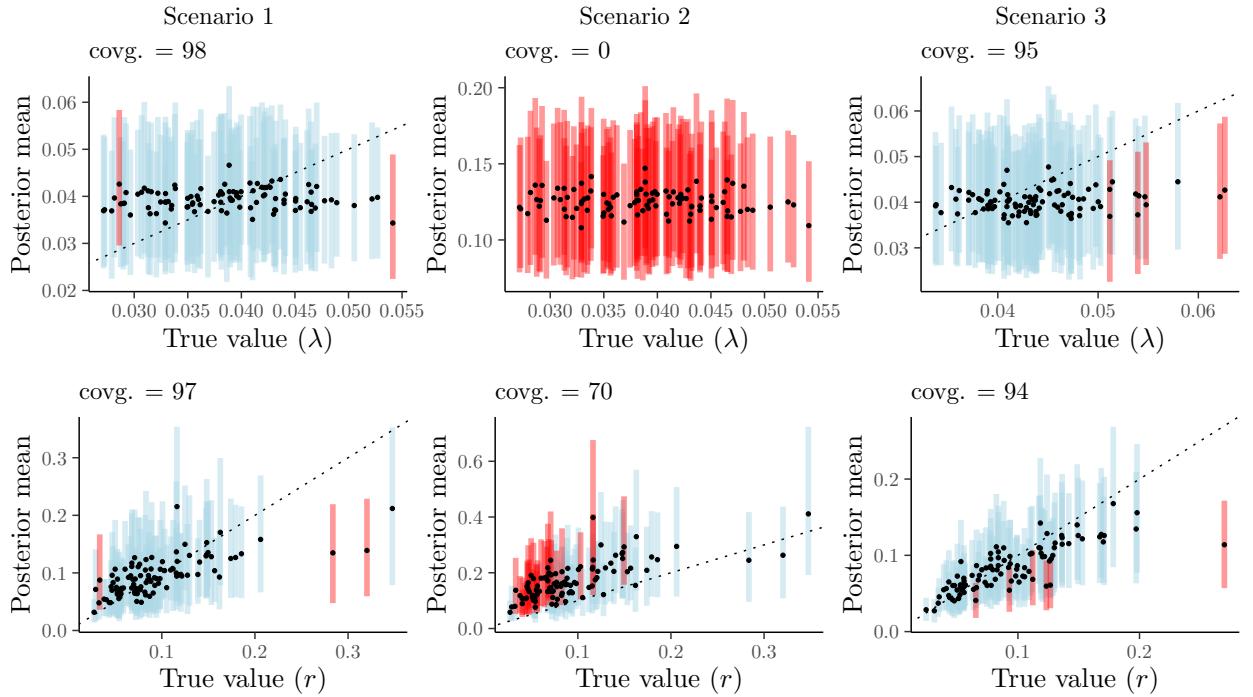


Figure 5: Coverage-validation analyses of the Bayesian hierarchical model in Fig. 1. Panels show the true (i.e., simulated) parameter values plotted against their mean posteriors (the dashed line gives $f(x) = x$). Dots and lines (100 per panel) represent true values and their 95%-HPDs, respectively. Simulations for which 95%-HPDs contained the true value are highlighted in blue, otherwise are presented in red. In “Scenario 1”, the model was correctly specified, and we simulated trees with 3 to 300 taxa using rejection sampling (approximately one in ten trees were rejected). In “Scenario 2”, the model was incorrectly specified in inference (see main text), and we used the same data sets simulated in “Scenario 1”. In “Scenario 3”, the model was correctly specified, but rejection sampling was more intense (we rejected a large number of trees, approximately 90%, keeping those having between 100 to 200 tips).

variable being filtered in simulation, it does not affect coverage because it is not a free parameter during inference (being instead fixed to the truth).

The three scenarios we explored above illustrate how coverage-validation results can be interpreted in terms of model implementation correctness. However, we can further capitalize on this validation setup and gauge how accurate our inferential tool can be for different parameters. I (FKM) will discuss the following passages with Luiz, as the term ‘identifiability’ may be improved on. The more identifiable a parameter is, the higher should the correlation between its posterior mean and its generating “true” value be. In all our scenarios the species birth-rate λ was not easily identifiable given the sizes of the phylogenetic trees. In a separate analysis, we analyzed the same model save for one difference: phylogenetic trees could only have up to fifteen terminal taxa. The results are shown in figure 5c. Here, it can be seen that the inference machinery is less accurate and that the λ parameter is less identifiable. We note that unidentifiability should not be taken as a sign that a model is incorrect – in figure 5c, coverage is still appropriate.

Simulation-based calibration (SBC) [@Luiz: Or any other header that makes sense, to help organize the inference validation section*]*

Talts et al. (2018) show that one can devise other tests that might be more powerful to detect problems than just looking at the coverage of Bayesian HPD intervals. In particular, they show (Theorem 1 therein) that if the inference machinery $I[\mathcal{M}]$ works as intended, the distribution of the rank $r^{(i)} r^j$ of the j -th sample from an MCMC chain ($\theta^j = \{\theta^1, \theta^2, \dots, \theta^L\}$) in $\theta_s^{(i)}$ will follow a uniform distribution on $[1, L + 1]$. In other words, if one were to sort all L samples from the output of an MCMC experiment by a given parameter value, the first (smallest) 10% of all samples should account for approximately 10% of the total posterior density; the next 10% of (larger) samples should account, again, for approximately 10% of the total posterior density, and so on. [

@Luiz: this was my shot at verbalizing the mathematical description. Is this correct?

Adherence to this distribution can be investigated by constructing histograms (Talts et al., 2018) as well as by looking at the empirical cumulative distribution function (ECDF) and their confidence bands (Säilynoja et al., 2021).

We illustrate the SBC procedure on the model represented in figure 1... [

@Remco or @Luiz: to be done

Items to discuss/consider after example:

- Would it help to have a flowchart for the SBC procedure?
- What happens when the distribution of ranks is not uniform, and is, say, shifted towards low or high ranks?

How can this help us fix or understand a model better? (see second point below);
- Show how a misspecified model can cause this test to fail (if we take the Yule + phyloBM model as an example, as I suggest, there are only a few parameters to be misspecified, so maybe it’s easy to get this test to fail and know exactly why. One way to do that with that model, I’d suspect, is to throw away all trees with fewer than,

say, 15 leaves – I would imagine that would distort our λ rank distribution, even if the model still pass the well-calibrated test);

- What are the downsides of SBC relative to just verifying a model is well-calibrated? You'd have to simulate 1,000 data sets instead of 100 (so you have, say, 100 per 10% bin). Very computationally intensive!
- What are we going to recommend to the reader? That they really always go all the way to SBC? That's asking for too much. We have to argue that the first type of test is enough for correctness.

Box 1: Validating a phylogenetic model with respect to its phylogenetic tree parameter

Given the centrality of the phylogenetic tree (Φ) in comparative analyses, we must pay close attention to how we investigate this parameter when validating a phylogenetic model. Analyzing phylogenetic trees is made challenging by tree space being a complex mix of a discrete and continuous component, due to trees being comprised by both a topology and set of node times (Semple and Steel, 2003; Gavryushkin and Drummond, 2016). Additionally, there is no canonical total-ordering structure for trees, which complicates a validation procedure such as SBC.

To get around this difficulty, we propose computing one or more phylogenetic metrics, or functionals, for which total-ordering holds and thus for which ranks can be obtained. [@Luiz: my suggestion is to focus on RF here] One such metric is the well-known Robinson-Foulds distance (RF; Robinson and Foulds, 1981), which measures [@Luiz: brief description of RF] relative to another phylogenetic tree. In order to compute a relational metric like the RF distance during validation, we must have a reference phylogeny Φ_0 to which we can compare our focal generating phylogeny Φ and its posterior MCMC samples. The simulation-based calibration protocol remains the same, with an additional step in which we generate Φ_0 (see Algorithm ?? in the supplementary material). Figure X [@Luiz: see my description of new Fig. X below] shows validation results for the RF metric for trees simulated under the model illustrated in Fig 1. Figure Xa and Xb show the coverage of 95%-HPD intervals, and the rank distribution of the RF metric, respectively. The coverage of the RF statistic is very close to 95, and the rank distribution is approximately uniform on $(1, L + 1)$; together, both panels indicate this model is correct. We consider other phylogenetic tree metrics that could be used as an alternative to RF in the supplementary material (Supplementary Figs. ?? and ??).

[@Luiz: include Fig Xa and FigXb here; this figure would consist of just the RF panels; all other metrics have would be placed in the supp. material]

Concluding remarks

As more data is generated and made publicly available, the more will researchers in the life sciences require computational methods with which to analyze it. If such methods are not correctly implemented, conclusions drawn from the data will be of reduced or void of any significance. Here we discussed guidelines for the validation of computational methods implementing Bayesian probabilistic models. This manuscript is also followed

by a supplementary text further illustrating these guidelines that is linked with a live document available on <https://github.com/rbouckaert/DeveloperManual>. We hope our guidelines can help raise the standards for software package releases required by users, developers and reviewers alike, and consequently lead to computational tools that are more efficient, better documented, and most importantly, correctly implemented.

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