EFFICIENT BAYESIAN INFERENCE OF GENERAL GAUSSIAN MODELS ON LARGE PHYLOGENETIC TREES

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Phylogenetic comparative methods correct for shared evolutionary history among a set of non-independent organisms by modeling sample traits as arising from a diffusion process along the branches of a possibly unknown history. To incorporate such uncertainty, we present a scalable Bayesian inference framework under a general Gaussian trait evolution model that exploits Hamiltonian Monte Carlo (HMC). HMC enables efficient sampling of the constrained model parameters and takes advantage of the tree structure for fast likelihood and gradient computations, yielding algorithmic complexity linear in the number of observations. This approach encompasses a wide family of stochastic processes, including the general Ornstein-Uhlenbeck (OU) process, with possible missing data and measurement errors. We implement inference tools for a biologically relevant subset of all these models into the BEAST phylogenetic software package and develop model comparison through marginal likelihood estimation. We apply our approach to study the morphological evolution in the superfamily of *Musteloidea* (including weasels and allies) as well as the heritability of HIV virulence. This second problem furnishes a new measure of evolutionary heritability that demonstrates its utility through a targeted simulation study.

1. Introduction.

1.1. Motivation. The evolutionary history of organisms shapes the distribution of their observed characteristics (Felsenstein, 1985). To account for correlation induced by this shared history, phylogenetic comparative methods (PCMs) have been developed for the analysis of quantitative traits (see e.g. Pennell and Harmon, 2013, for a review). These methods can be applied to a wide range of organisms and traits, to answer a large spectrum of biological questions on various evolutionary time frames, ranging from decades or even years in virology (Dudas et al., 2017) to millions of years in evolutionary biology (Aristide et al., 2016). Modern studies in PCMs routinely include a growing number of taxa (e.g. more than fifteen hundred in Blanquart et al. 2017), with possibly a large number of missing data for the multivariate measurements of continuous traits (e.g. almost 25% in Schnitzler et al. 2017), and with intricate phylogenetic and temporal structure. The underlying biological processes at play often have complex dynamics, and are only measured imperfectly, with a variable amount of noise.

1.2. Model. PCMs posit a continuous-valued stochastic process running on the branches of a phylogenetic tree that gives rise to trait values, possibly measured with noise, at the tree tips for observed organisms.

Keywords and phrases: Statistical Phylogenetics, Phylodynamics, Ornstein-Uhlenbeck Process, Bayesian Inference, Hamiltonian Monte Carlo, Model Selection, BEAST, Heritability, HIV, Musteloidea, Total Evidence

46 *Phylogenetic Tree.* The phylogenetic tree represents the evolutionary relationship among
 47 the organisms studied. We assume, without loss of generality, that the tree is calibrated in
 48 time, so that branch lengths represent actual time. For organisms evolving rapidly, such as
 49 viruses, the observations at the n tips of the tree are not necessarily contemporaneous (see
 50 Figure 1, left). We denote by m the total number of internal and external nodes in the tree
 51 ($m = 2n - 1$ if the tree is binary).

52 *Stochastic Process on the Tree.* We assume that a continuous trait evolves over time accord-
 53 ing to a stochastic process. When a speciation event occurs in the tree, the process is split into
 54 two conditionally independent processes with the same distribution (see Figure 1). Only the
 55 values of the process at the tips of the tree are potentially observed. This model enforces a
 56 phylogenetic correlation structure on the observations, as shown below.

FIG 1. Realization of a univariate BM process (right) with variance $\sigma^2 = 0.05$ and mean root value $\mu = 0$ on a timed phylogenetic tree (left). Observations span 3 years (2006 – 2008, tips Z^1 to Z^5). t_{12} is the time of shared evolution between tips 1 and 2. ℓ_1 is the length of the branch ending at tip 1.

57 *Brownian Motion (BM).* The simplest stochastic process (Cavalli-Sforza and Edwards,
 58 1967; Felsenstein, 1985) assumes that a multivariate trait Z_t of dimension p evolves in time
 59 t following a Brownian motion B_t with variance $\mathbf{R}$, such that $dZ_t = \mathbf{R}^{1/2}dB_t$ for all $t \geq 0$
 60 and at the tree root $Z^r \sim \mathcal{N}(\mu, \Gamma)$. Under this process, the covariance between trait k at node
 61 i and trait l at node j ($1 \leq k, l \leq p$ and $1 \leq i, j \leq m$) is the product of (i) the covariance R_{kl}
 62 between traits k and l and (ii) the shared evolutionary time t_{ij} between species i and j , i.e.
 63 the time from the root to their most recent common ancestor (see Figure 1), plus the contribu-
 64 tion of the root itself: $\text{Cov}[Z_k^i; Z_l^j] = t_{ij}R_{kl} + \Gamma_{kl}$ (see e.g. Clavel, Escarguel and Merceron,
 65 2015). A constant drift ν can be added to the mean value of the trait (Gill et al., 2016), in
 66 which case the expectation of trait k at species i at time t_i is $\mathbb{E}[Z_k^i] = t_i\nu$.

67 *Ornstein-Uhlenbeck (OU).* The OU process was proposed as a model for traits evolving
 68 under stabilizing selection (Hansen, 1997) and has become widely used across evolutionary
 69 biology (see e.g. Cooper et al., 2016, and references therein). The OU process generalizes BM
 70 by adding a deterministic call-back term to a given value β that is interpreted as the optimal
 71 value of the trait of a species in a given environment: $dZ_t = -\mathbf{A}(Z_t - \beta)dt + \mathbf{R}^{1/2}dB_t$ for
 72 all $t \geq 0$. Matrix $\mathbf{A}$ is the “selection strength” that is constrained to have positive real parts
 73 of its eigenvalues and controls the dynamics of the pull toward the optimum. The covariance
 74 between two multivariate traits at two nodes can be explicitly formulated (Bartoszek et al.,
 75 2012; Clavel, Escarguel and Merceron, 2015), and, compared to the BM where the variance
 76 increases linearly in time, is bounded by the stationary variance $\mathbf{V}$ of the process.

Observation Model and Individual Variation. The processes described above are meant to capture the evolutionary dynamics of traits across organisms. However, various other sources of variation may contribute to the observed data, such as measurement error, independent environmental variation, or intra-specific variation (see e.g. [Hadfield and Nakagawa, 2010](#), for a review). As outlined in the next section, we include these biological phenomena in our model as an extra layer, that links the realization of the process at the tips $\mathbf{Z}^i$ to the actual measurements $\mathbf{Y}^i$ through a Gaussian observation model.

1.3. *Scope of the article.* In this work, we propose a general and efficient Bayesian framework to rigorously analyze this broad class of evolutionary models.

State of the Art. Since their introduction in the seminal article of [Felsenstein \(1985\)](#), PCMs have undergone extensive development, resulting in increasingly realistic models. We limit references here to those that specifically relate to the inference problem in a general setting and refer to e.g. [Harmon \(2019\)](#) for a recent and more comprehensive overview of these models. [Clavel, Escarguel and Merceron \(2015\)](#) provide a comprehensive maximum-likelihood framework to fit a wide range of models, using explicit estimators that can in some cases be computationally prohibitive. [Pybus et al. \(2012\)](#), [Freckleton \(2012\)](#), [Mitov et al. \(2019\)](#) describe and implement likelihood computation algorithms that are linear in the number of observations in a framework similar to the one described here. This algorithm is exploited in [Mitov, Bartoszek and Stadler \(2019\)](#) to conduct maximum-likelihood inference. From a Bayesian perspective, [Pybus et al. \(2012\)](#) and [Cybis et al. \(2015\)](#) describe a standard Markov chain Monte Carlo (MCMC) inference framework for BM, while [Hassler et al. \(2020\)](#) extend it to include measurement errors with possible missing values, and [Fisher et al. \(2020\)](#) to use an efficient Hamiltonian Monte Carlo (HMC) sampler on the rates of a relaxed random walk ([Lemey et al., 2010](#)). Other more general models have been developed, including: non Gaussian models (using e.g. Levy processes, see [Landis, Schraiber and Liang 2013](#); [Duchen et al. 2017](#), or general Fokker - Plank equations, see [Boucher et al. 2018](#)); models where the trait explicitly impacts the tree (with BiSSE and related methods, see [Maddison, Midford and Otto 2007](#); [Fitzjohn, Maddison and Otto 2009](#); [Fitzjohn 2010](#); [Goldberg, Lancaster and Ree 2011](#); [Fitzjohn 2012](#)); models where species interact with each other (through mutualism or competition, see [Nuismer and Harmon 2015](#); [Manceau, Lambert and Morlon 2016](#); [Drury et al. 2016](#); [Bartoszek et al. 2017](#); [Drury et al. 2018](#); [Aristide and Morlon 2019](#), or through migration [Bartoszek 2017](#); [Duchen et al. 2020a](#)); models with varying and heritable intraspecific variance ([Kostikova et al., 2016](#)); or models in high-dimensional trait settings (using pseudo or penalized likelihood, see [Goolsby 2016](#); [Clavel, Aristide and Morlon 2019](#)). These models are however outside of the scope of the present work, that focuses on the general Gaussian model as presented in the next section (see Definition 1).

Outline. We complement recent advances in computing the likelihood under a general class of Gaussian models with an algorithm to analytically evaluate its gradient with respect to any of the parameters in linear time in the number of observations. This general class includes the OU process, as well as measurement error and missing data. We exploit this algorithm to develop an efficient Bayesian inference framework that relies on the use of an HMC sampler and allows for model selection through marginal likelihood estimation. We implement this framework in the BEAST phylogenetic software package ([Suchard et al., 2018](#)) for a subclass of models, namely the OU process with a diagonal selection strength matrix $\mathbf{A}$, that are of particular interest for the biological problems we study here. In Section 2, we present the general likelihood and new gradient computation algorithm (with details in Appendices A and B). In Section 3, we develop the Bayesian statistical inference framework (with details in Appendix C). Finally, in Section 4, we illustrate the method on two recently published biological datasets as well as on simulations.

126 **2. Efficient Gradient Computations.** In this section, we show how the likelihood and
 127 its gradient with respect to all the parameters in a general trait evolutionary model can be
 128 computed in linear time in the number of tips of a rooted phylogenetic tree.

129 **2.1. Statistical Model.** Conditioning on a tree $\mathcal{T}$, we define the following general Gaus-
 130 sian model of trait evolution:

131 **DEFINITION 1 (General Gaussian Model of Trait Evolution).** Let $\mathcal{T}$ be a rooted phylo-
 132 genetic tree with n tips and m internal and external nodes. At each node j , $1 \leq j \leq m$, define
 133 a latent variable $\mathbf{Z}^j$, and for each observation i , $1 \leq i \leq n$, a measure $\mathbf{Y}^i$, both of dimension
 134 p . The general Gaussian model of trait evolution on $\mathcal{T}$ is then defined in a hierarchical way
 135 as follows:

$$\begin{aligned}
 (2.1) \quad & \mathbf{Z}^r \sim \mathcal{N}(\boldsymbol{\mu}, \boldsymbol{\Gamma}) && \text{root;} \\
 (2.2) \quad & \mathbf{Z}^j \mid \mathbf{Z}^{\text{pa}(j)} \sim \mathcal{N}(\mathbf{q}_j \mathbf{Z}^{\text{pa}(j)} + \mathbf{d}_j, \boldsymbol{\Sigma}_j) && \text{propagation;} \\
 (2.3) \quad & \mathbf{Y}^i \mid \mathbf{Z}^{\text{pa}(i)} \sim \mathcal{N}(\mathbf{Z}^{\text{pa}(i)}, \mathbf{S}_i) && \text{observation;}
 \end{aligned}$$

136 where $\text{pa}(j)$ and $\text{pa}(i)$ denote, respectively, the unique parent node of node j or latent tip
 137 associated with observation i ; $\boldsymbol{\mu}$ and $\boldsymbol{\Gamma}$ are the expectation and variance of the root variable
 138 $\mathbf{Z}^r$; for any node j , $\mathbf{q}_j$, $\mathbf{d}_j$ and $\boldsymbol{\Sigma}_j$ are, respectively, the actualization, drift, and variance as-
 139 sociated with the branch going from $\text{pa}(j)$ to j ; and for any observation i , $\mathbf{S}_i$ is the variance
 140 associated with it. We further denote by $\mathbf{Y} = (\mathbf{Y}^1, \dots, \mathbf{Y}^n)^T$ and $\mathbf{Z} = (\mathbf{Z}^1, \dots, \mathbf{Z}^m)^T$ the
 141 matrices of observed and latent trait variables, and by $\mathbf{X} = (\mathbf{Y}^T, \mathbf{Z}^T)^T$ the *complete* dataset.
 142 Furthermore, we assume that $\boldsymbol{\Gamma}$, $\boldsymbol{\Sigma}_j$, and $\mathbf{S}_i$ are positive definite for any node j and observa-
 143 tion i .

144 This allows entertaining a highly generic framework that encompasses various evolution-
 145 ary scenarios, as underlined in the following three paragraphs.

146 *Stochastic Process Propagation.* Equation (2.2) describes the stochastic process that gov-
 147 erns the evolution of the latent trait. It is similar to the $\mathcal{G}_{\text{Lin}}$ model described in Mitov et al.
 148 (2019), or the generic formulation used in Bastide et al. (2018). Both the BM and OU models
 149 can be cast into this framework, by setting:

$$(2.4) \quad \mathbf{q}_j = \mathbf{I}_p, \quad \mathbf{d}_j = \ell_j \boldsymbol{\nu}, \quad \boldsymbol{\Sigma}_j = \ell_j \mathbf{R} \quad (\text{BM});$$

$$(2.5) \quad \mathbf{q}_j = e^{-\mathbf{A} \ell_j}, \quad \mathbf{d}_j = (\mathbf{I}_p - e^{-\mathbf{A} \ell_j}) \boldsymbol{\beta}, \quad \boldsymbol{\Sigma}_j = \mathbf{V} - e^{-\mathbf{A} \ell_j} \mathbf{V} e^{-\mathbf{A}^T \ell_j} \quad (\text{OU});$$

150 where, as defined in the introduction, $\boldsymbol{\nu}$ and $\mathbf{R}$ are the constant drift and variance of a simple
 151 BM, and $\mathbf{A}$, $\boldsymbol{\beta}$ and $\mathbf{V}$ are the selection strength, optimal values and stationary variances of
 152 an OU. We refer to Mitov et al. (2019) for more details concerning other models that can be
 153 described within this framework, including processes with shifts or jumps.

154 *Observation Model.* Equation (2.3) describes the observation model. The variance term $\mathbf{S}_i$
 155 can have multiple significations, from a simple measurement error, to a “meta-analysis” effect
 156 (see Hadfield and Nakagawa, 2010 for a review on observation errors), or an “intra-specific”
 157 variance (see e.g. Goolsby, Bruggeman and Ané, 2017). In the simple case where the same
 158 observation error is assumed for all measures i , $1 \leq i \leq n$, this term reduces to $\mathbf{S}_i = \mathbf{S}$. Note
 159 that, from a methodological point of view, Equation (2.3) can be considered as a particular
 160 case of Equation (2.2).

161 *Phylogenetic Factor and Repetitions.* We note that one may posit that $\mathbf{Z}^i$ is of lower di-
 162 mension $q < p$ and link to $\mathbf{Y}^i$ through a linear combination via a latent factor model (Tolkoff
 163 et al., 2018). It would also be straightforward to include several measurements associated
 164 with a single tip. For clarity, we omit these details in the main text, and refer to Appendix A
 165 for the derivations in this general framework.

166 *2.2. Likelihood Computations.* The general model of Definition 1 is Gaussian, and it is
 167 hence possible to write out the marginal distribution $p(\mathbf{Y} | \boldsymbol{\theta})$ of the measures $\mathbf{Y}$ given the
 168 parameters $\boldsymbol{\theta}$ for some specific models (see e.g. Clavel, Escarguel and Merceron, 2015 for
 169 such formulations in the multivariate OU case). However, this computation generally requires
 170 the inversion of a tree-induced variance matrix, of dimension $n \times n$. This is inefficient (worse
 171 than quadratic in n , see e.g. Raz, 2003), and is ill-suited for handling large phylogenetic trees
 172 that now frequently confront practitioners (see e.g. Jetz et al., 2012; Blanquart et al., 2017)
 173 or when the tree itself is random (Pybus et al., 2012). To alleviate this issue, it is possible to
 174 write an efficient pruning-style algorithm that is *linear* in the number n of organisms.

175 *Pruning-Style Algorithm.* Felsenstein (1973) introduced the pruning algorithm into phylo-
 176 genetics to compute the likelihood of a simple BM. It draws from classical Gaussian condi-
 177 tional propagation ideas such as the Kalman filter or other “forward-backward” algorithms
 178 (see e.g. Rabiner, 1989 for a review). Variants of this algorithm have been flowering in the
 179 literature, sometimes under different names. A non-exhaustive list of references for the BM
 180 case includes Hadfield and Nakagawa (2010), Pybus et al. (2012), Fitzjohn (2012) (Gaussian
 181 Elimination Method), Freckleton (2012), Lartillot (2014) (Phylogenetic Kalman Filter) and
 182 Cybis et al. (2015). Goolsby (2016) and Hassler et al. (2020) have recently proposed adap-
 183 tations to handle missing data. Finally, an extension of the algorithm to the general case as
 184 presented in Definition 1 (with missing data) was proposed by Bastide et al. (2018), and also
 185 more recently by Mitov et al. (2019).

186 *Implementation in BEAST.* All these methods allow computing the likelihood in linear time
 187 in n . As is frequently the case for PCMs, many have been implemented in various indepen-
 188 dent software packages, each likely with a specific use in mind (with the notable exception
 189 of the R Core Team, 2019 package PCMBase, see Mitov et al., 2019). While this explains
 190 at least partly the numerous references to the algorithm, it limits the use of several imple-
 191 mentations beyond the specific models they consider. Bayesian Evolutionary Analysis by
 192 Sampling Trees (BEAST, Suchard et al., 2018) is a widely used, well established and ver-
 193 satile phylogenetic software package. It encompasses a great variety of molecular sequence
 194 modeling tools, making it possible to conduct a coherent joint inference of both the timed
 195 phylogenetic tree and of the properties of the stochastic process, without the need to resort
 196 to a two-step analysis as is usually the case in previous methodologies (see Section 3.1 for
 197 more details). We implemented the general algorithm presented in Bastide et al. (2018) in
 198 this unified framework (with improvements, see next paragraph), allowing for its seamless
 199 integration with the realistic analyses permitted by the software.

200 *Efficiency and Numerical Robustness.* When sampling the parameters in a wide region of
 201 the space, as is typically done in a Bayesian analysis (see Section 3), numerical robustness is
 202 particularly important, as small divergences due to possibly ill-conditioned matrices can ac-
 203 cumulate over the tree traversal, and lead to diverging results. We tackled this issue using two
 204 independent developments. First, we reduced the number of operations actually performed
 205 during the traversal of the tree thanks to a careful analysis of the iteration steps, making the al-
 206 gorithm both more efficient and more robust. Second, we increased the numerical robustness
 207 by using a dedicated linear algebra library (the Efficient Java Matrix library, Abeles, 2016)
 208 to conduct the computations. Combined with the use of a Moore-Penrose pseudo inverse,

this made our handling of the singular or near-singular matrices induced by the presence of missing data (see Bastide et al., 2018, Hassler et al., 2020) more numerically stable. These developments are presented in detail in Appendix A.1.

2.3. Gradient Computation. When performing statistical inference, either in a maximum likelihood or a Bayesian framework, having access to the gradient of the likelihood at relatively cheap computational cost facilitates faster and more accurate algorithms (see Section 3). In this section, we present a novel algorithm to compute the gradient of the likelihood with respect to any parameter in the general setting presented in Definition 1. The algorithm relies on two main ingredients: (1) as in Fisher et al. (2020), we express the derivative of the likelihood as the conditional expectation of a given function of the latent traits $\mathbf{Z}^j$ at the internal nodes j , conditional on the observed traits $\mathbf{Y}$; and (2) we use a pre-order algorithm inspired from the “downward” phase in Bastide et al. (2018) to compute this expectation in a linear time in n .

Gradient as a Conditional Expectation. We rely here on Fisher’s identity (Cappé, Moulines and Rydén, 2005) that links the gradient of the log-likelihood $\log p(\mathbf{Y} | \boldsymbol{\theta})$ of the observed variables to the conditional expectation of the completed log-likelihood $\log p(\mathbf{Z}, \mathbf{Y} | \boldsymbol{\theta})$:

PROPOSITION 1 (Fisher’s Identity; Cappé, Moulines and Rydén, 2005). *Under broad assumptions, that are verified for Gaussian densities, the following identity holds (Equation 10.12 in Cappé, Moulines and Rydén, 2005):*

$$(2.6) \quad \nabla_{\boldsymbol{\theta}} [\log p(\mathbf{Y} | \boldsymbol{\theta})] = \mathbb{E} [\nabla_{\boldsymbol{\theta}} [\log p(\mathbf{X}_s, \mathbf{Y} | \boldsymbol{\theta})] | \mathbf{Y}],$$

where $\mathbf{X}_s$ represents any subset taken from the complete data.

Applying this identity, we obtain the gradient of the likelihood with respect to any parameter $\boldsymbol{\theta}_j = (\mathbf{q}_j, \mathbf{d}_j, \boldsymbol{\Sigma}_j)$ or $\boldsymbol{\theta}_i = \mathbf{S}_i$ of the model:

PROPOSITION 2 (Gradient with respect to Branch Parameters). *Under the general model of Definition 1, for any observation or node k ($1 \leq k \leq n + m$), the following identity holds:*

$$(2.7) \quad \nabla_{\boldsymbol{\theta}_k} [\log p(\mathbf{Y} | \boldsymbol{\theta}_k)] = \frac{\partial \mathbf{n}_k^T}{\partial \boldsymbol{\theta}_k} \cdot \mathbf{Q}_k(\mathbf{M}_k - \mathbf{n}_k) + \frac{\partial \text{vech}(\mathbf{Q}_k)^T}{\partial \boldsymbol{\theta}_k} \cdot \frac{1}{2} \text{vech}(\mathbf{Q}_k^{-1} - (\mathbf{M}_k - \mathbf{n}_k)(\mathbf{M}_k - \mathbf{n}_k)^T - \mathbf{V}_k)$$

with vech the symmetric vectorization operation (Magnus and Neudecker, 1986); and where $\mathbf{n}_k$, $\mathbf{M}_k$, $\mathbf{Q}_k^{-1}$ and $\mathbf{V}_k$ are parameters, representing the expectations and variances of two Gaussian densities (see Equations A.15 and A.23 in Appendix A.2), that can be computed in one pre-order traversal of the tree.

PROOF OF PROPOSITION 2. Let k be an observation or node index with associated trait variable $\mathbf{X}^k$ ($1 \leq k \leq n + m$). As in Fisher et al. (2020), we decompose the observations $\mathbf{Y}$ as $\mathbf{Y} = (\mathbf{Y}_{[k]}, \mathbf{Y}_{[k]})$, where $\mathbf{Y}_{[k]}$ denotes the observations that are “below” node k , i.e. that have k as an ancestor, and $\mathbf{Y}_{[k]}$ denotes the observations that are “above” node k , i.e. that do not have k as an ancestor. The tree conditional structure then induces the decomposition:

$$p(\mathbf{X}^k, \mathbf{Y}_{[k]}, \mathbf{Y}_{[k]} | \boldsymbol{\theta}_k) = p(\mathbf{Y}_{[k]} | \mathbf{X}^k) p(\mathbf{X}^k | \mathbf{Y}_{[k]}, \boldsymbol{\theta}_k) p(\mathbf{Y}_{[k]}),$$

where only the middle term depends on parameters θ_k associated with the branch ending at node k . Applying Proposition 1 with $\mathbf{X}_s = \mathbf{X}^k$, we get that:

$$\nabla_{\theta_k} [\log p(\mathbf{Y} | \theta_k)] = \mathbb{E} \left[\nabla_{\theta_k} \left[\log p(\mathbf{X}^k | \mathbf{Y}_{[k]}, \theta_k) \right] | \mathbf{Y} \right].$$

Using the pre-order formulas presented in Appendix A.2.1 (Equation A.15), we can see that $\mathbf{X}^k | \mathbf{Y}_{[k]}$ is normally distributed, with expectation $\mathbf{n}_k$ and precision matrix $\mathbf{Q}_k$. Applying standard derivation formulas to a log Gaussian density (see e.g. Magnus and Neudecker, 1986), we have:

$$(2.8) \quad \nabla_{\theta_k} \left[\log p(\mathbf{X}^k | \mathbf{Y}_{[k]}, \theta_k) \right] = \frac{\partial \mathbf{n}_k^T}{\partial \theta_k} \cdot \mathbf{Q}_k (\mathbf{X}^k - \mathbf{n}_k) + \frac{\partial \text{vech}(\mathbf{Q}_k)^T}{\partial \theta_k} \cdot \frac{1}{2} \text{vech} \left(\mathbf{Q}_k^{-1} - (\mathbf{X}^k - \mathbf{n}_k)(\mathbf{X}^k - \mathbf{n}_k)^T \right),$$

From Appendix A.2.2 (Equation A.23), we know that $\mathbf{X}^k | \mathbf{Y}$ is normally distributed, with expectation $\mathbf{M}_k$ and variance $\mathbf{V}_k$. Equation (2.7) is then obtained by taking the conditional expectation of the above expression (2.8). $\square$

Chain Rule. In Equation (2.7), we express the gradient of the likelihood with respect to any branch parameter using only quantities that can be computed in two traversals of the tree, one post-order and one pre-order. This provides the basis for an algorithm to compute the gradient of the likelihood with respect to any parameter with a linear complexity in n . Indeed, one only needs to apply the derivation chain rule to: (1) obtain the gradient of the branch parameters $\mathbf{n}_k$ and $\mathbf{Q}_k$ with respect to the natural parameters of the process at hand; and (2) obtain the gradient of the likelihood with respect to parameters shared between several branches. We tackle task (1) using the pre-order formulas in Appendix B. Task (2) is a straightforward application of the chain rule over all the levels of the hierarchical model:

$$(2.9) \quad \frac{\partial p(\mathbf{Y} | \theta, \mathcal{T})^T}{\partial \theta} = \sum_{k=1}^{n+m} \frac{\partial \theta_k^T}{\partial \theta} \nabla_{\theta_k} [p(\mathbf{Y} | \theta, \mathcal{T})].$$

Complexity. Appendix A implies that all the moments $\mathbf{n}_k$, $\mathbf{M}_j$, $\mathbf{Q}_k^{-1}$ and $\mathbf{V}_k$ ($1 \leq k \leq n + m$) appearing in Proposition 2 can be computed in linear time in the number of observations. Since formulas (2.7) and (2.9) only involve linear algebra operations in a space of the dimension of the parameters, the total complexity remains linear in n . In addition, we note that the sum in Equation (2.9) does not need to follow the tree order, as all the quantities are pre-computed, and hence can be parallelized easily, reducing the actual computation time.

3. Statistical Inference. In the previous section, we showed how both the likelihood and its gradient with respect to all the parameters of the models can be efficiently computed simultaneously. These quantities are the cornerstone of many statistical analyses, and allow for a wide range of analyses, from maximum likelihood to model selection (for examples in trait evolution, see e.g. Clavel, Aristide and Morlon, 2019). Here, taking advantage of the comprehensive Bayesian inference framework made available through the BEAST phylogenetics software package, we propose a new Bayesian approach that relies on the use of an efficient HMC sampler to perform both posterior inference and marginal likelihood estimation.

3.1. *Bayesian Phylogenetics and the Total Evidence Approach.* The likelihood and gradient algorithms presented below work conditionally on a phylogenetic tree between sampled species being known without error. However, the tree is generally a summary statistic resulting from a complex statistical analysis, and is usually inferred from molecular sequences, which are the actual observed data. Many methods in the literature follow a two-step procedure and first infer the tree from sequence data to then proceed with analyzing the continuous traits, assuming that the phylogenetic tree is known and fixed (see e.g. [Harmon, 2019](#) for a recent review of such methods). This approach suffers from two major drawbacks. First, it ignores the uncertainty in the reconstruction of the tree, which, given the difficulty of the task, can be substantial, and bias the subsequent analyses (see [Felsenstein, 2004](#) for a review, and [Bastide, 2017](#), Section 5.1, for an example in a specific case). Second, this approach does not allow for the complete use of the data available, as it ignores continuous traits for the tree reconstruction. Although, when present, sequence information tends to dominate over trait information ([Baele et al., 2017](#)), it is not always available for all sampled organisms. This is particularly true for ancient fossils, that might bear some continuous trait data, but, because of the rapid degradation of DNA molecules, can not be sequenced ([Leonardi et al., 2016](#)). We refer to Section 4.3 for an example of such a dataset, where the continuous trait constitutes the only source of information available to reconstruct the phylogenetic tree.

To overcome these limitations, we use a *total evidence approach* ([Ronquist et al., 2012](#)), that can analyze sequence and trait data jointly, using all the information available in a Bayesian analysis. Denote by $\mathbf{S}$ the sequence data (that might not be available for all the sampled species), and by ϕ all the parameters associated with the model of sequence evolution and the dating clock model, (see e.g. [Felsenstein, 2004](#) for a review of such models). The goal of Bayesian phylogenetics is then to learn about the posterior: $p(\theta, \mathcal{T}, \phi \mid \mathbf{Y}, \mathbf{S})$. One crucial assumption that we make is that, conditionally on the phylogenetic tree $\mathcal{T}$, the evolution of continuous traits and the sequences are independent, such that:

$$(3.1) \quad \begin{aligned} p(\theta, \mathcal{T}, \phi \mid \mathbf{Y}, \mathbf{S}) &\propto p(\mathbf{Y}, \mathbf{S} \mid \theta, \mathcal{T}, \phi) p(\theta, \mathcal{T}, \phi) \\ &= p(\mathbf{Y} \mid \theta, \mathcal{T}) p(\theta) \times p(\mathbf{S} \mid \mathcal{T}, \phi) p(\mathcal{T}, \phi). \end{aligned}$$

The term $p(\mathbf{S} \mid \mathcal{T}, \phi) p(\mathcal{T}, \phi)$ has been the focus of an extensive literature, and benefits from efficient methods readily available in BEAST ([Suchard et al., 2018](#)). Thanks to the tools presented in the previous section, we focus here on $p(\mathbf{Y} \mid \theta, \mathcal{T}) p(\theta)$ that deals with the study of the distribution of continuous traits among the population of species.

This conditional independence assumption, although limiting, is essential from a computational point of view. It has also proven to be useful and adequate to study a wide range of biological questions, and it is widely spread in the field of PCMs, applied to a fixed tree or in a total evidence approach (see e.g. [Felsenstein, 2004](#); [Harmon, 2019](#), for reviews). As mentioned in the Introduction (see Section 1.3), some attempts have been made to relax this assumption, with fixed trees or discrete characters (see e.g. [Fitzjohn 2012](#); [Müller, Rasmussen and Stadler 2017](#) and references therein). However, the computational burden associated to these methods currently limits their application to relatively small scale datasets.

3.2. *Hamiltonian Monte Carlo.* HMC is a powerful MCMC sampling technique, that exploits the geometrical properties of the density to be sampled through the use of Hamiltonian dynamics ([Neal, 2011](#); [Betancourt, 2017](#)). It associates to a vector of parameters of interest θ , viewed as the position of a particle in a d -dimensional space, an auxiliary independent vector $\mathbf{p}$ of “momentum”, that is typically chosen to be Gaussian: $\mathbf{p} \sim \mathcal{N}(\mathbf{0}_d, \mathbf{I}_d)$. The log joint distribution of the parameter $(\theta, \mathbf{p})$ then represents the “total energy” $H(\theta, \mathbf{p}) = U(\theta) + K(\mathbf{p})$ of the particle, with $U(\theta) = -\log p(\theta \mid \mathbf{Y}, \mathcal{T})$ the “potential energy” set to be equal to the

319 posterior density of interest, and $K(\mathbf{p}) = \mathbf{p}^T \mathbf{p} / 2$ the “kinetic energy”. The total energy is
 320 then invariant to the Hamiltonian dynamics:

$$\begin{cases} \frac{d\mathbf{p}}{dt} = -\nabla_{\boldsymbol{\theta}} U(\boldsymbol{\theta}) = \nabla_{\boldsymbol{\theta}} \log p(\mathbf{Y} \mid \boldsymbol{\theta}, \mathcal{T}) + \nabla_{\boldsymbol{\theta}} \log p(\boldsymbol{\theta}) \\ \frac{d\boldsymbol{\theta}}{dt} = +\nabla_{\mathbf{p}} K(\mathbf{p}) = \mathbf{p}. \end{cases}$$

321 The HMC sampling scheme exploits this property using proposals that approximately fol-
 322 low these dynamics, as discretized by an appropriate numerical scheme such as the leapfrog.
 323 This allows for a proposal that can have a small correlation with the current state, while still
 324 having a high probability of acceptance (Neal, 2011). Such an HMC sampler has already
 325 proven very successful in a phylogenetics context (Fisher et al., 2020; Ji et al., 2020). Our ef-
 326 ficient and general algorithm for likelihood and gradient computation, presented in Section 2,
 327 makes it now applicable to the wide variety of models covered by Definition 1.

328 **3.3. Confronting Constrained Natural Parameters.** Some of the parameters of the mod-
 329 els, such as the variance of a BM or an OU, live in constrained spaces with a non-trivial
 330 structure, that need to be sampled adequately. One standard way to deal with this structure
 331 (see e.g. Stan, 2017) is to map the constrained natural parameters $\boldsymbol{\theta}$ to a vector of indepen-
 332 dent, unconstrained parameters $\boldsymbol{\eta}$ through a smooth transformation f . The density in the new,
 333 unconstrained space is then linked to the density in the constrained space by a simple multi-
 334 plication with the determinant of the Jacobian matrix of the transformation (see e.g. Le Gall,
 335 2006), such that:

$$\nabla_{\boldsymbol{\eta}} \log p(\boldsymbol{\eta}) = \frac{\partial f^{-1}(\boldsymbol{\eta})^T}{\partial \boldsymbol{\eta}} \nabla_{\boldsymbol{\theta}} \log p(f^{-1}(\boldsymbol{\eta})) + \nabla_{\boldsymbol{\eta}} \log \left| \frac{\partial f^{-1}(\boldsymbol{\eta})^T}{\partial \boldsymbol{\eta}} \right|.$$

336 This formula allows us to easily update the constrained parameters $\boldsymbol{\theta}$ from movements in
 337 the unconstrained space of $\boldsymbol{\eta}$. We present the transformations and the associated priors used
 338 here in detail in Appendix C. In particular, we show that sampling the space of correlation
 339 matrices amounts to sampling vectors in the half-euclidean sphere, which provides an original
 340 and simple representation of the classical LKJ transformation (Lewandowski, Kurowicka and
 341 Joe, 2009).

342 **3.4. Model Selection and Marginal Likelihood Estimation.** We described above a general
 343 framework to efficiently infer the parameters of a wide class of evolutionary models. When
 344 analyzing a dataset, a question that naturally arises is the choice of the most suited model to
 345 interpret the evolutionary patterns in a specific problem.

346 **Bayesian Model Selection.** In a Bayesian setting, one natural way to compare a collec-
 347 tion of models $(\mathcal{M}_m)_{1 \leq m \leq K}$ is to compute their marginal likelihoods $p(\mathbf{Y} \mid \mathcal{M}_m)$ (see e.g.
 348 Oaks et al. 2019 for an introduction in a phylogenetic context). The marginal likelihood, that
 349 integrates all the parameters against the prior, takes the model complexity into account by
 350 design, “penalizing” complex models, that otherwise mechanically have a higher likelihood.
 351 Marginal likelihoods allow computing Bayes factors, which have a natural comparison scale
 352 (Jeffreys, 1935; Kass and Raftery, 1995). However, because of the need to integrate over the
 353 potentially very large space of parameters, this quantity is typically hard to compute, and
 354 approximations are required.

355 *Generalized Stepping-Stone Sampling (GSS).* In a phylogenetic context, the GSS approach
 356 (Fan et al., 2011) has been successfully used to approximate marginal likelihoods (Baele,
 357 Lemey and Suchard, 2016; Fourment et al., 2019). For a given model $\mathcal{M}$, it relies on the
 358 construction and sampling of a path between the unnormalized posterior and a “working”
 359 prior distribution $p_0(\boldsymbol{\theta} \mid \mathcal{M})$:

$$(3.2) \quad q_\beta(\boldsymbol{\theta}) = [p(\mathbf{Y} \mid \boldsymbol{\theta}, \mathcal{T}, \mathcal{M}) p(\boldsymbol{\theta} \mid \mathcal{M})]^\beta [p_0(\boldsymbol{\theta} \mid \mathcal{M})]^{1-\beta}.$$

360 When $\beta = 1$, the path likelihood is proportional to the classical posterior sampled in a stan-
 361 dard MCMC analysis, while when $\beta = 0$, it reduces to the working prior $p_0(\boldsymbol{\theta} \mid \mathcal{M})$. This
 362 working prior is chosen to match the empirical moments from a sample of the posterior distri-
 363 bution, ensuring a less vague distribution that is closer to the posterior, hence inducing a more
 364 accurate approximation for a reduced computational effort than the standard stepping-stone
 365 sampling procedure (Xie et al., 2011; Fan et al., 2011). As in Baele, Lemey and Suchard
 366 (2016), we adopt a kernel density estimator (KDE) for each parameter, using a normal ker-
 367 nel, that is log-transformed for positive parameters (see e.g. Jones, Nguyen and McLachlan,
 368 2018).

369 *Sampling the path with HMC.* The GSS estimation implies sampling from the path likeli-
 370 hood $q_\beta(\boldsymbol{\theta})$ for a sequence of β . Xie et al. (2011) and Baele, Lemey and Suchard (2016) show
 371 that choosing the path parameter as evenly spaced quantiles of a Beta distribution with shape
 372 0.3 and scale 1.0, which allows for sampling more intensely regions where β is small, and
 373 hence where the path likelihood is changing the most rapidly, yields the best performance.
 374 This sampling is usually done through a standard MCMC procedure. Here, we use the effi-
 375 cient HMC approach presented in Section 3.2, which implies taking the gradient of the log
 376 path likelihood (3.2):

$$\nabla_{\boldsymbol{\theta}} \log q_\beta(\boldsymbol{\theta}) = \beta \nabla_{\boldsymbol{\theta}} \log [p(\mathbf{Y} \mid \boldsymbol{\theta}, \mathcal{T}, \mathcal{M}) p(\boldsymbol{\theta} \mid \mathcal{M})] + (1 - \beta) \nabla_{\boldsymbol{\theta}} \log p_0(\boldsymbol{\theta} \mid \mathcal{M}).$$

377 This gradient involves a term proportional to the posterior that we already dealt with in the
 378 HMC inference, and the working distribution, that, as a product of independent KDE estima-
 379 tions, is straightforward to compute. This makes it possible to use the efficient HMC sampling
 380 scheme in the GSS marginal likelihood estimation framework already implemented and well
 381 established in BEAST (Baele, Lemey and Suchard, 2016; Fourment et al., 2019).

382 4. Applications and Simulations.

383 4.1. *Assumptions and Practical Implementation.* We showcase the usefulness of our in-
 384 ference framework using two recently published datasets, one in ecology, and one in virol-
 385 ogy. This led us to implement a subset from all the models made accessible by the method.
 386 Specifically, we limit the evolutionary model to the BM and the OU with diagonal strength
 387 of selection $\mathbf{A}$, and a shared residual variance for all the measures, possibly scaled by the tip
 388 heights ($\mathbf{S}_i = \mathbf{S}$ or $\mathbf{S}_i = t_i \mathbf{S}$ for any observation i , $1 \leq i \leq n$).

389 4.2. *Phylogenetic Heritability.* The concept of phylogenetic heritability has been defined
 390 in the field of PCMs to study the relative importance of the evolution and observation models
 391 in the total measured variance at the tip of the tree (Lynch, 1991; Housworth, Martins and
 392 Lynch, 2004). It is linked to the notion of phylogenetic signal (Pagel, 1999), and its use has
 393 recently received considerable attention in studying infection traits in the field of virology
 394 (Alizon et al., 2010; Leventhal and Bonhoeffer, 2016; Mitov and Stadler, 2018). We introduce
 395 here a general definition of the phylogenetic heritability that extends this notion to our general

framework. It relies on the expectation of the population variances computed at the latent tip level (for the $\mathbf{Z}^{\text{tip}} = (\mathbf{Z}^j)_{1 \leq j \leq n}$), and at the observation level (for the $\mathbf{Y} = (\mathbf{Y}^i)_{1 \leq i \leq n}$):

$$(4.1) \quad \mathbf{V}(\mathbf{Z}^{\text{tip}}) = \mathbb{E} \left[\frac{1}{n} [\mathbf{Z}^{\text{tip}} - \mathbb{E}_{\theta}[\mathbf{Z}^{\text{tip}}]]^T [\mathbf{Z}^{\text{tip}} - \mathbb{E}_{\theta}[\mathbf{Z}^{\text{tip}}]] \right] = \frac{1}{n} \sum_{j=1}^n \mathbb{V}\text{ar}_{\theta}[\mathbf{Z}^j],$$

$$(4.2) \quad \mathbf{V}(\mathbf{Y}) = \mathbb{E} \left[\frac{1}{n} [\mathbf{Y} - \mathbb{E}_{\theta}[\mathbf{Y}]]^T [\mathbf{Y} - \mathbb{E}_{\theta}[\mathbf{Y}]] \right] = \frac{1}{n} \sum_{i=1}^n \mathbb{V}\text{ar}_{\theta}[\mathbf{Y}^i],$$

where the expectation and variance are taken following the process of evolution and observation defined in Definition 1 with parameters θ . These quantities have closed-form expressions for all the models considered here, see e.g. Clavel, Escarguel and Merceron (2015) for the general OU case. The “heritability matrix” $\mathbf{H}$ can then be defined as:

$$(4.3) \quad H_{kl} = \frac{V_{kl}(\mathbf{Z}^{\text{tip}})}{\sqrt{V_{kk}(\mathbf{Y})V_{ll}(\mathbf{Y})}}.$$

In the case of a standard univariate trait on an ultrametric tree with only one observation per tip, this formula coincides with the classical definition found in the literature (see e.g. Mitov and Stadler, 2018).

Population versus Empirical Variance. In equation (4.3), we use the population variance, instead of the empirical one used for instance in Blanquart et al. (2017), Hassler et al. (2020). We argue in Appendix D that, when the process is not a simple BM, the population variance is more appropriate, as the empirical variance might be impaired by confounding inter-group effects if the tips are expected to have different means under the trait evolution model, which is for instance the case for an OU model on a non-ultrametric tree.

4.3. *Morphological Evolution in the Musteloidea Superfamily.* We illustrate the total evidence approach to study the evolution of some morphological features in the *Musteloidea* superfamily (including weasels and allies).

4.3.1. *Dataset and Analyses.*

Dataset. We reanalyze the dataset published by Schnitzler et al. (2017), containing 81 taxa, including 4 fossils, and 2 outgroup species. Aligned sequence data for all 77 extant taxa are available (containing 22 nuclear and 5 mitochondrial genes). Three morphological traits are measured on 65 species, including fossils, with missing data for some taxa (see Figure 2). They are carnivorian ecometric traits, defined as meaningful ratios of osteological measurements, and denoted by R1-3. Note that our Bayesian framework can readily handle this heterogeneous dataset, and jointly analyse sequence and continuous traits with missing data on both.

Questions. We aim to address the following two questions. First, does total evidence (see Section 3.1) allow for better placement of the fossils? Given that we can take trait data into account while performing phylogenetic inference, we might expect that this extra information leads to better fossil placement estimates compared with Schnitzler et al. 2017, who only considered sequence data and use monophyly constraints. Second, which model of trait evolution is most suited to explain the observed trait distribution, and does this conclusion change when we include or set aside fossil data, as suggested by Schnitzler et al. (2017)?

FIG 2. Maximum clade credibility tree with continuous traits plotted at the tips. The tree is reconstructed from both the genetic data and the trait data, using an OU model with a diagonal selection strength. The time scale for the tree is in million of years, with 0 indicating the present. Clades highlighted in blue are constrained to be monophyletic. Grey bars at nodes and fossils show the dating uncertainty. Node colors indicate the posterior support for each clade. Fossils are dotted, and highlighted in red.

430 *Sequence Evolution and Dating.* We use the same sequence evolution model as Schnitzler
431 et al. (2017) for all 27 partitions of the dataset, with estimated base frequencies and site rate
432 heterogeneity modeled using a discretized gamma distribution with 6 rate categories; an un-
433 correlated relaxed clock with an underlying gamma distribution; and an exponential growth
434 coalescent tree prior (Hasegawa, Kishino and Yano, 1985; Tavaré, 1986; Yang, 1994; Drum-
435 mond et al., 2006). Following Schnitzler et al. (2017), we constrain 5 clades to be mono-
436 phyletic, and each fossil is *a priori* assigned to one of these clades, except for *Trochiarion*
437 *albanense*, which remains unconstrained (see Figure 2). We assume a normal prior on the
438 time of the most recent common ancestor for each of those clades, with means as in Schnit-
439 zler et al. (2017), and standard deviation 1. We assume a uniform prior on fossil dates, with
440 maximum ranges taken from Law, Slater and Mehta (2018) (Table S5), except for *Teruelictis*
441 *riparius*, for which dates were extracted from the Paleobiology Database, relying on Salesa

et al. (2013). Note that these assumptions differ slightly from Schnitzler et al. (2017), who provide insufficient information to reproduce their exact pipeline.

Total Evidence Phylogenetic Inference. We conduct phylogenetic inference using 3 different data integration scenarios: no model of trait evolution (i.e. the continuous traits are not used); a BM model; and an OU with diagonal selection strength. The latter two combine both sequence and trait evolution. We run each analysis for 100 million iterations, sample every 1000 steps, and discard the first 10% as burn-in. We specified vague priors on the parameters of the continuous processes while respecting biological constraints, as described in Appendix C. The maximum clade credibility (MCC) tree is used to represent the evolutionary history.

Fossil Placement Analysis. We assess the uncertainty of fossil placement using a method introduced by Klopstein and Spasojevic (2019). Given a sample of trees from the posterior, as the backbone tree is well resolved (see below), the method amounts to computing, for each branch of the MCC tree, the frequency a given fossil attaches to that branch. We measure frequency vector concentration using entropy; a fossil that is well resolved will be distributed over a small number of branches with high frequency, and hence has a low entropy.

Model Comparison. As in Schnitzler et al. (2017), we also conduct several model comparisons, conditioning on a tree fixed to the MCC tree from one of the previous analyses, with or without fossil species. On each tree, we test several hypotheses about the nature of trait evolution, by comparing (log) marginal likelihood estimates for the various models. Models tested in this section are the BM and OU models, but also the “trend” model, that is a BM with an added homogeneous deterministic drift (Hansen and Martins, 1996; Gill et al., 2016). See Supplementary Figure S4 for the list of all hypotheses tested. We run each analysis for 100 000 iterations, sample every 10 steps, and discard the first 10% as burn-in. 50 steps of 1000 iterations each are explored for the GSS estimation of the (log) marginal likelihood of each model.

Analysis and Representation of the Results. We use BEAST, TreeAnnotator and Tracer to conduct the analyses (Suchard et al., 2018; Rambaut et al., 2018). Trees are imported into R and plotted using treeio (Wang et al., 2019), tidytree and ggtree (Yu et al., 2017, 2018).

4.3.2. Results.

Phylogenetic Inference. Consistent with Schnitzler et al. (2017), we estimate a well-resolved backbone tree, with uncertainty mostly at the genus level, particularly in the *Mephitidae* and *Procyonidae* families (see Figure 2). Including trait information does not dramatically change the inferred relationships between extant species, confirming that molecular data are generally more informative than trait data (Baele et al., 2017).

Fossil Placement. Taking into account trait information reduces entropy scores for each of the fossils, with the OU model having the lowest entropy for 3/4 of the fossils (see Table 1 and Supplementary Figure S3). Continuous trait measurements bear different amounts of information for each fossil, which leads to different entropy score behavior. The fossil *Pannonictis* is evenly distributed over all the branches of the clade (*Mustelinae*) where it is assigned. It has a high entropy that does not decrease much when traits are taken into account. This result is not surprising given that the traits vary little among all the species of this clade (see Figure 2), and hence yield little information with respect to fossil placement. Fossils *Sivaonyx beyi* and *Teruelictis riparius* are both assigned to the same clade (*Lutrinae*). Figure 2 illustrates that their R1 trait is relatively lower compared to other members of the clade. Taking this trait into account is thus informative, and entropy decreases, with the fossil estimated to lie at the root of the tree (see Supplementary Figure S3). Finally, the species

Trocharion albanense is not assigned to any clade. Taking traits into account concentrates this fossil as a sister lineage either to *Ailurus fulgens*, or to the whole *Mephitidae* clade, which, in the assumed time range, have the most similar traits.

	<i>Pannonictis</i>	<i>Sivaonyx beyi</i>	<i>Teruelictis riparius</i>	<i>Trocharion albanense</i>
No Traits	2.84	1.80	1.24	2.28
BM	2.81	1.22	1.22	2.01
OU	2.92	1.20	1.10	1.86

TABLE 1

Entropy of the fossil position for each fossil and inference method (over 1000 trees from the posterior). Entropy should decrease if the fossils are better resolved. Maximal entropy (no information) is 5.02. OU models appear to reduce entropy for three fossils out of four.

Model Comparisons. We find that the favored model, for all tested trees, with or without fossils, is a simple BM for the first trait (R1), and an OU with diagonal selection strength but full correlation for the two other traits (R2 and R3), with R1 evolving independently from R2 and R3 (see Supplementary Figure S4). The parameter estimates are consistent with those from Schnitzler et al. (2017) (see Supplementary Figure S5). Schnitzler et al. (2017) fitted the three traits independently and used a simple penalized likelihood approach (using the Akaike Information Criterion, Akaike 1974) to demonstrate that a “trend” model is favored to the simple BM model for R1 when fossils are included. In contrast, our method is robust to the addition of these fossils that, given the missing data, amounts to the addition of two data points in the analysis (see Figure 2). The selected model has a log Bayes factor of at least 1.5 compared to the second best fitting model in all the scenarios, providing “substantial evidence” (Kass and Raftery, 1995) against the simple BM model.

4.4. Virulence Heritability in Human Immunodeficiency Viruses (HIV). New challenges for PCMs have recently emerged in infectious disease research, more specifically on the extent to which virulence is a heritable trait in HIV. Here, we employ our new modeling framework to perform a fine-grained analysis to gain insight into this problem.

4.4.1. Dataset and Analyses.

Dataset. We revisit the most comprehensive dataset on HIV-1 heritability published in Blanquart et al. (2017) and further analysed in Hassler et al. (2020). We focus on subtype B and the measurements available for male subjects who have sex with men (MSM), which comprises a dataset of 1171 viral samples. Two traits associated with HIV virulence (Alizon et al., 2010; Blanquart et al., 2017) are measured for each sample: (i) the “gold standard viral load” (GSVL) that is a standardized measure of the viral load, taken on a single sample between 6 and 24 months after infection and before initiation of antiretroviral therapy; and (ii) the CD4 cell count slope decline (see Figure 3). A dated maximum likelihood phylogeny for this dataset has recently been presented by Hassler et al. (2020). Following a similar methodology, we use it as a fixed tree in our analyses, which focus on continuous trait model selection and heritability estimations.

Questions. Disease progression varies greatly among patients. Similar to other rapidly evolving human pathogens, it is challenging to determine to what extent this variance is due to the host or virulence of the viral genotype. The pioneering application of PCMs by Alizon et al. (2010) to estimate the heritability of HIV virulence using set-point viral load

FIG 3. HIV-1 dataset from [Blanquart et al. \(2017\)](#) and [Hassler et al. \(2020\)](#). A maximum-likelihood tree estimate depicts the phylogenetic relationships for all HIV-1 subtype B viruses from MSM patients. The time scale is in calendar years (0 being the present, putting the root at around 1960). The two traits are the GSVL and the CD4 count slope decrease.

(spVL), has stimulated the generation of comprehensive data sets ([Blanquart et al., 2017](#)), but also led to a discussion concerning the underlying models ([Mitov and Stadler, 2018](#); [Bertels et al., 2018](#)). Depending on the method and datasets used, the heritability of the spVL has been quite controversial ([Leventhal and Bonhoeffer, 2016](#)), with estimates ranging from about 50% ([Alizon et al., 2010](#)), to around 30% ([Vrancken et al., 2015](#)) and to as low as about 6% ([Hodcroft et al., 2014](#)). We explore here the fit of several models of trait evolution and individual variation (Equations 2.2 and 2.3) to study their impact on heritability estimation and other parameters of interest. As the virus-host interactions are a major source of trait variation, we expect the individual variation layer to be particularly important in these models.

Trait Evolution Models. We use three different evolution models for the two traits on the tree: a multivariate BM, a multivariate OU (with diagonal selection strength), and a mixed multivariate “OU-BM” model, that has an OU model on the GSVL, and a BM model on the CD4 slope, the two still being correlated. This last model illustrates the flexibility of our framework in model specification. It is motivated by the results presented in [Blanquart et al. \(2017\)](#), and by the data distribution (see Figure 3), with the CD4 slope being much more spread out than the GSVL (with respective quartile coefficients of dispersion of -0.56 and 0.09).

Individual Variation Models. One major driver of diversity for the two traits is the interaction of the virus with its host, that is independent from the viral phylogeny. This individual variation is captured through our observation model layer. We take this variation to be either identically distributed, or scaled by the tip heights, with or without trait correlation. Scaling the independent noise by the tip heights is an empirical model inspired from Pagel’s λ model ([Pagel, 1999](#)) and is well suited for an environmental contribution that increases linearly with sampling time ([Leventhal and Bonhoeffer, 2016](#)).

Model Comparison and Model Fit. As in the previous example using a fixed tree, and using the same computational tools, priors (see Appendix C), and chain settings, we estimate (log) marginal likelihoods for all the models under study.

FIG 4. Log Marginal Likelihood Estimation (log MLE) for three different trait evolution models (BM on both traits, OU on GSVL and BM on CD4, and OU on both traits) and four observation models, either independent or correlated, and scaled by tip heights or not. The best fitting model is the OU with independent scaled errors, with a log Bayes factor of 0.77 compared to the second best model, the more complex OU with correlated scaled errors.

4.4.2. Results.

Model Selection. The model favored according to the log marginal likelihood estimation is the OU model on both traits, with an independent, scaled observation matrix (see Figure 4). Compared to the OU-BM model with the same error structure, it has a log Bayes factor support of 3.14, indicating a strong support for the more complex OU model (Kass and Raftery, 1995). In general, the scaled error models appear to be much better supported than the non-scaled ones.

Heritability. Under the best fitted model, the heritability is estimated to be, respectively, 0.3 (95% highest posterior density interval: [0.16, 0.45]) for the GSVL, and 0.36 (95% HPDI [0.15, 0.65]) for the CD4 slope. This is in line with the selection strength estimates, with a phylogenetic half-life (in percentage of the tree height) of 0.27 (95% HPDI [0.1, 0.61]) for the GSVL, and 0.12 (95% HPDI [0.05, 0.26]) for the CD4 slope. The CD4 slope has a higher selection strength, so that the phylogenetic model allows for more individual variation (see e.g. Bastide et al., 2018), and hence the heritability, which is the relative importance of this phylogenetic model in the total variation, is mechanically higher. See Appendix E.2 for a complete presentation of the results.

Discussion. The results presented above are surprising on two accounts. First, the heritability results under the best supported OU model are different from the ones reported in Blanquart et al. (2017), who find a heritability that is larger for the GSVL than for the CD4, with estimates of 0.31 (95% HPDI [0.15, 0.43]) for the GSVL, and 0.1 (95% HPDI [0.01, 0.27]) for the CD4 slope. We note however that the selection strength parameter in Blanquart et al. (2017) is poorly estimated, with a wide confidence interval that abuts against the lower and upper limits that were arbitrarily imposed: the half-life is estimated to 0.08 (95% HPDI [0.07, 0.29]) for the GSVL, and 7.3 (95% HPDI [0.0693, 6.3×10^5]) for the CD4 slope. In contrast, the priors we set, that reflect biologically reasonable assumptions, might help us regularize the estimation of this notoriously hard to infer parameter (see e.g. Uyeda and Harmon, 2014, Bastide, Mariadassou and Robin, 2017).

Second, the log MLE favors a model with an OU on the CD4 slope, while Blanquart et al. (2017) favor a BM on this trait, which seems more reasonable from a biological point of view. It is interesting to note that when we use the less supported OU-BM model, we find estimates of the heritability to be more in line with the literature, while still on the upper range: 0.35 (95% HPDI [0.22, 0.51]) for the GSVL, and 0.21 (95% HPDI [0.12, 0.32]) for the CD4 slope. This might raise some concerns on the ability of the MLE model selection procedure to select for the best suited model in this setting. We address both concerns with a simulation study in the next section.

4.5. *Exploration of Model Selection in a Heritability Estimation Context.* In the previous section, we estimated the heritability to be lower than 50%. This means that the phylogenetic model (2.2) accounts for at most half of the total variation observed in the dataset, while individual variation (2.3) takes up the remaining part. In the absence of strong phylogenetic signal in the trait, it might be challenging to uncover the true underlying trait evolution model. Using a simulation scheme that is inspired by the empirical dataset, we explore the limits of the MLE model selection procedure in this setting.

4.5.1. *Setting.*

Base Evolutionary Scenario. We used the same fixed HIV tree as in the previous section, normalized so that it had a maximum root to tip height of one. We then simulated a bivariate trait according to a multivariate correlated OU-BM model. By analogy, the two traits are named GSVL and CD4, and the parameters of the process were taken to be similar to the ones inferred in the previous section. For the “GSVL” trait, we took a half-life of 0.3% of the tree height, a stationary variance $\sigma^2/(2\alpha)$ of 0.1, and an optimal value (equal to the root conditional value) of 1. For the “CD4” trait, we took a selection strength of 0 (BM), a variance of 0.01, and a root conditional value of -0.1 . The correlation between the two traits was set to -0.9 .

Independent Variations. On top of this evolutionary model, we added independent individual variations at each tip. Under the base scenario, the variance of this extra noise was taken to be equal to the variance of the process (0.1 on the GSVL and 0.01 on the CD4). This reflects a heritability of about 50% (computed to be of 49.4% for the GSVL and 45.9% for the CD4). We then multiplied this noise variance by a factor f varying between 0.1 and 3, leading to a maximal heritability of 0.91 and 0.89; and a minimal heritability of 0.25 and 0.22 for both traits, respectively. The estimates for the empirical data set imply a scenario where this factor f is high.

Simulation and Inference. We simulated the OU-BM process using the R package PhylogeneticEM (Bastide, Mariadassou and Robin, 2017; Bastide et al., 2018). Each scenario was repeated 50 times. On each of these datasets, we performed an analysis similar to the previous section, fitting three evolution models (the BM, the true OU-BM and the OU), with independent identically distributed individual variations at each tip.

Questions. We analysed the results to address the two following questions: (i) does the MLE model selection procedure recover the true generative model? and (ii) to what extent is heritability correctly estimated?

4.5.2. *Results.*

Model Selection. The proportion of each model being selected over the 50 repetitions is presented in Table 2. When the individual variation variance is low or equal to the evolutionary variance, the correct OU-BM model is selected in all or most of the cases. When this noise increases however, the proportion drops considerably, with the correct model being selected less than 75% of the cases when $f = 3$.

Estimation of the Heritability. In Figure 5, we show the normalized estimated heritability when we use the correct OU-BM model. When the noise level factor f increases, the variance of the estimates over the 50 repetitions increases substantially, with higher levels of noise leading to an over-estimation of the heritability for both traits. Note that the estimate is more variable for the trait under the OU model (GSVL). This is consistent with the fact that it relies on the estimate of the strength of selection, which is difficult to infer (see Supplementary Figure S7). The empirical coverage level of the 95% HPD interval remains however relatively high, never dropping below 80% (see Supplementary Figure S8).

Noise Level	0.1	0.25	0.5	0.75	1	2	3
BM	0.00	0.00	0.00	0.02	0.02	0.00	0.04
OU-BM	0.96	0.98	1.00	0.88	0.94	0.78	0.68
OU	0.04	0.02	0.00	0.10	0.04	0.22	0.28

TABLE 2

Proportion of times each model is selected over the 50 replicates. When the independent noise is small compared to the phylogenetic signal, the right model (OU-BM, bold) is almost always selected. When the independent noise become overwhelming, the phylogenetic signal is lost, and the model selection is less efficient. The HIV example explored in Section 4.4 falls under the latter category, with a high noise (or low heritability), which might explain the somewhat unexpected results provided by the model selection in the HIV example.

FIG 5. Estimation of the heritability when inferred with the correct (OU-BM) model for various levels of noise. The values are normalized by the true value used in the simulation, so they should converge to 0 (horizontal line). The distribution of the normalized individual estimates over the 50 repetitions are summarized by a box-plot within a violin plot. When the noise level increases, the estimates are more variable, and the heritability tends to be over-estimated.

Selection Strength. When the OU model is incorrectly selected over the OU-BM (which can happen almost one fourth of the time under high levels of noise), then the selection strength on the CD4 trait, that is simulated without selection, is estimated to be generally higher than the selection strength of the GSVL (see Supplementary Figure S7).

Discussion and Caveat. When the noise level is high, we observe three main artifacts in the estimation: (i) the OU model is often wrongly selected over the OU-BM; (ii) the heritability tends to be over-estimated; (iii) when the OU model is selected, the selection strength for the selection-free trait is estimated to be larger than for the trait under selection. These three artifacts are precisely the points that raised questions from a biological point of view in the previous section. This simulation study therefore illustrates that complex models of trait evolution such as the OU, that have recently been advocated in the context of heritability studies (Mitov and Stadler, 2018; Bertels et al., 2018), should be treated with caution when applied to a dataset that is burdened with high levels of noise. In our case, we simulated the data according to the exact same model used for the statistical inference (an OU-BM with residual variance). The performance of the model could be further affected by other sources of variation in a real-world example that are not accounted for in our framework. Examples of such possible mechanisms are discussed below (see Section 5.4).

4.6. Computational Efficiency of the HMC sampler. In all of the applications above, we used the standard HMC sampler described in Section 3.2, that relies on the efficient gradient computation algorithm of Section 2.3. In the case of a simple BM, Hassler et al. (2020) developed a Gibbs sampler on the variance parameter. This approach uses a simplified version of the efficient likelihood computation algorithm described in Section 2.2 to analytically

integrate missing values. It was shown to be much more efficient than a previous approach based on a numerical integration of these missing values (Cybis et al., 2015), with a minimum 25-fold speed-up in all of the configuration tested (Hassler et al., 2020).

When applying an OU process, such a Gibbs sampler cannot be used anymore, as the joint distribution of the trait values at the tips of the tree cannot be expressed as a simple Kronecker product (see e.g. Clavel, Escarguel and Merceron, 2015, for analytical expressions). Using the transformations described in Appendix C, it is however straightforward to derive an MCMC sampler based on a classical random walk (RW) Metropolis-Hasting algorithm on the space of constrained parameters. Such a sampler still relies on the efficient likelihood computation algorithm of Section 2.2. Contrary to the Gibbs sampler, that can only be used with a set of restricted priors, it has the same flexibility as the HMC sampler and can hence be targeted toward the exact same posterior distribution, allowing for fair comparisons with the HMC.

Given the literature (Neal, 2011; Ji et al., 2020; Fisher et al., 2020), we expect HMC to be more efficient than a simple RW, with better scalability in the number of possibly correlated parameters. To explore computational gains in this context, we reran analyses on the two datasets explored in the previous section. We selected the models with, respectively, the least number of parameters (the BM with no residual error) and the highest number of parameters (the diagonal OU with correlated residual errors). We ran each analysis 10 times and compared the average effective sample size (ESS) per minute for each parameter. We found that HMC delivered appreciable speed-up, with a median 4.37-fold increase in ESS per minute over all the parameters and configuration tested. The speed-up was particularly relevant for the most complex model on the large HIV tree, with an approximate 5 to 10-fold increase in ESS per minute for the variance and selection strength parameters, that are known to be correlated and hence particularly difficult to estimate with a classical RW sampler. We refer to Appendix E.4 for the detailed set up and results of this analysis.

We note that, as both the HMC and RW samplers use the same likelihood computation algorithm (although the RW does not make use of the gradient), the speed-ups reported here are mostly due to the sampling technique, and not the algorithms described in this work. In particular, we here used a classical HMC, with a fixed number of steps and step sizes in the numerical approximation scheme. Further speed-ups may be obtained by using more refined versions of the HMC sampler, for instance using the No-U-Turn Sampler (Hoffman and Gelman, 2014) for optimal exploration of the space, or through the preconditioning of the posterior using an adequate mass matrix (Girolami and Calderhead, 2011; Neal, 2011; Ji et al., 2020). These improvements could be the focus of future work.

5. Discussion.

5.1. Fossil Placement Using Continuous Traits. The subject of combining morphological traits with molecular data, which are typically not available for fossils, has received considerable attention over the last few years. Several studies focusing on discrete morphological characters showed that combining both sources of information could improve fossil placement (Wiens, 2009; Wiens et al., 2010), either using a fixed tree through maximum likelihood (Berger and Stamatakis, 2010), or with a total evidence approach (Ronquist et al., 2012; Gavryushkina et al., 2017). A recent trend appears to favor continuous morphological characters over discretized ones, but these may be more challenging to use in an inference framework (Parins-Fukuchi, 2018a). This motivated the development of several methods for fossil placement (Revell et al., 2015; Parins-Fukuchi, 2018b) or divergence time estimation (Álvarez-Carretero et al., 2019) on known phylogenetic trees, using quantitative traits. Given the potentially highly informative value of continuous morphological characters, some attempts have also been made to infer phylogenies without any molecular data, although with

limited success (Varón-González, Whelan and Klingenberg, 2020). This is consistent with previous findings that sequence information generally tends to dominate over trait information in a joint inference framework (Baele et al., 2017).

In Section 4.3, we showed how the total evidence framework could be used to inform fossil placement. Overall, we observed limited accuracy gains when using continuous trait information (see Section 4.3.2). However, in this example, only three continuous characters were available, offering limited information. It would be interesting to test this framework on more extensive morphometric datasets that include several dozens of traits (Álvarez-Carretero et al., 2019), and for which the associated signal might be stronger. Combined with phylogenetic factor analysis (Tolkoff et al., 2018), it might provide a good alternative to fixed tree approaches previously mentioned.

5.2. Heritability of Virulence Estimation. In line with previous work (Leventhal and Bonhoeffer, 2016; Mitov and Stadler, 2018), we found in Sections 4.4 and 4.5 that the value and quality of the heritability estimate was strongly dependent on our ability to, first, select for the right model of evolution and, second, to infer the parameters of this model with sufficient precision. As shown in our simulation study, both of these tasks are however challenging when the level of individual variation is high, i.e. the level of heritability is low. This result may not be surprising from a statistical point of view. Indeed, when the level of individual variation is high, then the evolutionary model on the phylogeny only explains a small proportion of the observed tip variation, and it hence becomes difficult to discriminate the signature of one particular model over another in the observed data.

These results call for extreme caution when interpreting not only the heritability estimates obtained in this PCM framework, but also all the other parameters inferred from the model, such as the correlation between the traits (see Appendix E.2). The quality of the estimates, in particular for the OU model, can depend on many factors, including the shape and size of the phylogenetic tree (Cooper et al., 2016). In line with previous recommendations (see e.g. Pennell et al., 2015; Cooper et al., 2016), we found here that it could be useful to complement the empirical analysis of a dataset with tailored simulation studies, designed to explore the potential limits or blind spots of a given configuration.

In the HIV example presented here, the main conclusion that emerges, irrespective of the exact estimate values and favored models, is that the heritability of these virulence traits appears to be limited. Overall, this indicates that host factors constitute an important contribution to the virulence of HIV infections, and that mechanisms underlying complex host-pathogen interactions remain to be explored in more details (Bartha et al., 2017).

5.3. Comparison with Maximum Likelihood Approaches. Numerous maximum likelihood tools exist to fit and compare complex models of evolution on a fixed tree. Such tools are widely used, usually fast, and have proven useful in many situations. For instance, both case studies cited in this article (Schnitzler et al., 2017; Blanquart et al., 2017) used a maximum likelihood framework for their analyses. In addition, the R (R Core Team, 2019) PCMs ecosystem is well developed, so that there exist many specialized packages that cover a wide range of models with various sets of assumptions (see Section 1.3).

Although usually more computationally demanding, Bayesian methods complement maximum likelihood approaches in a number of ways. In particular, the priors' regularizing effect ensures that the parameters estimates remain biologically reasonable (see Appendix C). This feature proved useful in both our applications, where, in contrast, maximum likelihood methods had difficulties in estimating some of the parameters, with estimates lying on arbitrarily fixed upper or lower bounds, yielding poor biological interpretation.

The Bayesian framework also allows for the computation of the marginal likelihood of a model, that provides a theoretically consistent way to perform model selection. Some penalised likelihood methods, such as the BIC criterion, can be seen as approximations of this gold standard, although rather coarse (Lebarbier and Mary-Huard, 2006). The GSS estimation used here, although computationally intensive, has been shown on the other hand to be one of the most precise methods in a phylogenetic context (Fourment et al., 2019).

Finally, one further strength of our framework is that it is integrated, and allows for the use of all of the extensive BEAST modeling features. Many R packages, on the other hand, although very useful in some situations, have often been developed independently, and their strengths cannot be combined into one global analysis. This is particularly true when one wishes to use a total evidence approach. Most maximum likelihood methods assume a fixed tree, while our Bayesian framework can combine state-of-the-art phylogenetic reconstruction methods with the complex and realistic continuous trait models described here.

5.4. Modeling Assumptions. As detailed in the introduction (see Section 1.3), the framework described here relies on several simplifying assumptions, that are common to most standard PCMs. Relaxing those assumptions usually comes at the cost of a substantial increase in computational complexity, and requires the development of specific algorithms, that are not covered here.

Several simulation studies have been designed to challenge those standard assumptions. They usually rely on a complex trait simulator, that is tailored to a given biological system, and aimed at producing realistic datasets. For instance, in a macro-evolutionary context, Duchen et al. (2020b) study the impact of asymmetrical inheritance on the ability of classical models to describe the produced patterns of trait distribution among species, and show that they can be flawed in some cases. Similarly, in the field of virology, several epidemic models have already been proposed to test the accuracy of virulence heritability estimations (Leventhal and Bonhoeffer, 2016; Mitov and Stadler, 2018).

5.5. Concluding Remarks. Motivated by the need to accommodate OU processes, we have presented an efficient inference procedure for a broad class of trait evolution models in a Bayesian inference framework. Using two empirical examples and a simulation study, we have demonstrated its applicability to answer a large spectrum of biological questions, in fields ranging from paleontology to virology.

At the core of the inference procedure, the likelihood and now gradient computation algorithms are linear in the number of observations, making them efficient on large trees, and applicable to a broad class of Gaussian evolutionary processes, including but not limited to the popular OU model. This algorithm however has worse than quadratic complexity in the number of latent traits propagated on the tree. On the other hand, using techniques such as phylogenetic factor analysis (Tolkoff et al., 2018), this latent dimension can be reduced to a manageable size. In this work, we made use of efficient gradient computation algorithm in a Bayesian context. Note that this gradient could be more broadly exploited in other settings, such as in a maximum likelihood inference.

All the formulas are written here for the general model of Definition 1. Specific formulas are however only implemented for a sub-set of the possible models, namely OU models with diagonal selection strength, with a constant or time-scaled noise, and with only one observation for each tip. Extensions to more general models will be required in order to study specific datasets and answer relevant biological questions. Although the main core mechanism remains unchanged, some derivations may still be needed to propagate the gradient in such complex models. For instance, dealing with the OU with a general selection strength implies taking the derivative of a matrix exponential with respect to a matrix, which is a notoriously difficult problem, and may require some approximations (Al-Mohy and Higham,

2010). However, in the quest for ever more complex models, particular care should be taken concerning practical and theoretical identifiability issues, as illustrated by our simulation study.

6. Data and Scripts. All the scripts and data used in this manuscript are publicly available as a GitHub repository: https://github.com/pbastide/HMC_OU.

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SUPPLEMENTARY MATERIAL

Post and Pre-Order Algorithms. Formal and detailed description of the post and pre-order algorithms used to compute the likelihood and its gradient.

Gradients and Chain Rules Formulas. Formal derivation of the gradient formulas with respect to natural parameters.

Constrained Parameters. Description of the smooth transformations used to map the constrained parameters to an unconstrained space.

The Heritability Statistics. Case study of the new population variance phylogenetic heritability on a toy example.

Supplementary Figures. Supplementary figures for biological applications and simulations.

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