

Simultaneously estimating evolutionary history and repeated traits phylogenetic signal: applications to viral and host phenotypic evolution

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Summary

1. Phylogenetic signal quantifies the degree to which resemblance in continuously valued traits reflects phylogenetic relatedness. Measures of phylogenetic signal are widely used in ecological and evolutionary research and are recently gaining traction in viral evolutionary studies. Standard estimators of phylogenetic signal frequently condition on data summary statistics of the repeated trait observations and fixed phylogenetics trees, resulting in information loss and potential bias.

2. To incorporate the observation process and phylogenetic uncertainty in a model-based approach, we develop a novel Bayesian inference method to simultaneously estimate the evolutionary history and phylogenetic signal from molecular sequence data and repeated multivariate traits. Our approach builds upon a phylogenetic diffusion framework that models continuous trait evolution as a Brownian motion process and incorporates Pagel's λ transformation parameter to estimate dependence among traits. We provide a computationally efficient inference implementation in the BEAST software package.

3. We evaluate the synthetic performance of the Bayesian estimator of phylogenetic signal against standard estimators and demonstrate the use of our coherent framework to address several virus-host evolutionary questions, including virulence heritability for HIV, antigenic evolution in influenza and HIV, and *Drosophila* sensitivity to sigma virus infection. Finally, we discuss model extensions that will make useful contributions to our flexible framework for simultaneously studying sequence and trait evolution.

Key-words: adaptation, Bayesian phylogenetics, comparative approach, virulence, virus evolution

Introduction

A central premise of comparative biology is that evolutionary history shapes the distribution of phenotypic traits in extant species. The field has long realized that shared ancestry induces statistical dependence among observed trait values and that this correlation structure needs to be taken into account in comparative analyses (Felsenstein 1985; Harvey & Purvis 1991). Recent advances in comparative analyses of continuously valued trait data focus on the tempo and mode of trait evolution, for example testing for different evolutionary rates (O'Meara *et al.* 2006), identifying rate shifts (Revell *et al.* 2012) and on correlations among traits (Revell & Collar 2009). However, the question also frequently arises to what extent

shared ancestry needs to be controlled for (Freckleton, Harvey & Pagel 2002). Different measures have been proposed to quantify and test the tendency for related species to share similar traits, which is generally referred as 'phylogenetic signal'. This quantification is particularly relevant for traits that are heavily impacted by ecological as well as evolutionary processes (Losos 2008).

Measures of phylogenetic signal are often classified into indices based on phylogenetic autocorrelation or on Brownian diffusion models of trait evolution (Münkemüller *et al.* 2012). Building on spatial autocorrelation functions, phylogenetic autocorrelation quantifies the degree of correlation across observations that the evolutionary history explains, but does not provide a generative model for how the traits arise along this history; popular examples include Moran's I (Moran 1950) and Abouheif's C_{mean} (Abouheif 1999). On the other hand, Blomberg's K (Blomberg, Garland & Ives 2003) and

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Pagel's λ (Pagel 1999) are commonly used measures that advance a Brownian diffusion process along the history as a data generative model and serve to both quantify and test for phylogenetic correlation. Under both measures, a value of 0 reflects independence across observations, whereas a value of 1 suggests that traits arise according to the generative process. Recent performance evaluations of different indices based on simulations under Brownian diffusion indicate that Pagel's λ and Abouheif's C_{mean} perform well in testing procedures and that Pagel's λ provides the most reliable quantification of phylogenetic signal (Münkemüller *et al.* 2012; Shirreff *et al.* 2013).

Phylogenetic comparative methods are permeating evolutionary biology, but they have only sporadically been adopted in virus evolutionary studies due to a strong genotypic focus and a comparatively lower availability of phenotypic trait data. There is however a growing interest in studying a variety of viral phenotypic measurements ranging from traits that remain close to the genotype, like antigenic properties, to those that may be heavily impacted by the host environment, such as virulence or infections traits (Hartfield, Murall & Alizon 2014). Because of its importance for vaccine selection, antigenic evolution has been extensively studied in human influenza viruses. Although performing assays to measure influenza antigenic properties may be relatively straightforward, they produce challenging data explaining perhaps why antigenic evolutionary studies have largely been divorced from, or at best contrasted against (Smith *et al.* 2004), sequence evolution (but see Bedford *et al.* (2014) for recent efforts to integrate both).

The human immunodeficiency virus (HIV) also evades humoral immune responses within a host, but this has far less impact at the population or epidemiological scale as compared to the continuous turnover characteristic for antigenic drift in seasonal influenza. The question still remains to what extent the virus may adapt to humoral immunity at the population level. Current studies addressing this generally do not account for phylogenetic dependence among the viruses for which antigenic neutralization is measured [e.g. Bunnik *et al.* (2010); Euler *et al.* (2011)], and it is unclear how this affects the results. The interest in HIV adaptation at the population level also extends to cell-mediated immunity (Kawashima *et al.* 2009), drug therapy (Little *et al.* 2002) and viral fitness, all of which can impact virulence and disease severity. This leads to a more general question as to what extent the HIV genotype can control for the rate of progression to AIDS. Disease progression rates vary extensively among HIV patients, but they can be predicted by the level of viraemia in early infection, referred to as set point viral load (spVL) (Mellors *et al.* 1996). To assess the heritability of spVL, many studies have focused on HIV transmission pairs, which resulted in a broad range of heritability estimates [e.g. Tang *et al.* (2004); Hollingsworth *et al.* (2010); van der Kuyl *et al.* (2010)], although this may be narrowed down by interpreting the results using a consistent measure of heritability (Fraser *et al.* 2014). Phylogenetic signal estimators have recently been proposed as an alternative approach to study spVL heritability (Alizon *et al.* 2010;

Shirreff *et al.* 2013). This represents a rare application of the comparative approach to continuously valued viral characters, but one that in essence also attempts to disentangle the impact of ecological – in this case the host environment – and evolutionary processes.

Virulence may therefore also be addressed from the host perspective, as closely related hosts may exhibit more similarity in their susceptibility to viral infection. This has been elegantly addressed for sigma viruses in fruit flies (Longdon *et al.* 2011). By experimentally testing the susceptibility of 51 host species to viral infection with three different host-specific viruses, this study showed that the host species phylogeny is a strong determinant of viral persistence and replication in novel *Drosophila* hosts. Such investigations represent interesting applications for phylogenetic signal estimators that can both test and quantify the degree to which the host phylogeny controls pathogen susceptibility.

The examples of viral phenotypic evolution discussed above are all based on traits that can be subject to considerable quantification error (inherent to the assays) or natural individual variation. To characterize the measurement error, many studies produce repeated measures, but then generally condition on the trait means and variances for each strain or taxon in the subsequent analysis. This is akin to how intraspecific variation has been traditionally treated by maximum likelihood estimation procedures in phylogenetic comparative approaches across different species, which raises the problem of error propagation [but see Lynch (1991) and Housworth, Martins & Lynch (2004) for a notable exception]. To address this, Revell & Graham Reynolds (2012) have proposed a Bayesian method to accommodate intraspecific variation through simultaneously inferring species means and trait evolutionary model parameters. While taking into account uncertainty, a joint Bayesian inference also allows for cross talk between the different model components (Revell & Graham Reynolds 2012). Importantly however, the problem of error propagation extends to all aspects of comparative phylogenetic estimation, adding further to its imprecision. For example, there is generally considerable uncertainty in the reconstructed tree, including both branch lengths and tree topology estimates, to which trait evolutionary processes are typically fitted. Although phylogenetic error can be empirically captured by considering a (posterior) distribution of trees (Barker, Meade & Pagel 2007; Longdon *et al.* 2011; de Villemereuil *et al.* 2012), all these separate efforts indicate that trait evolutionary analyses would benefit from a general and coherent statistical framework, one that solely conditions on the observed sequence and trait data, rather than on data summary statistics such as trait means and variances of repeated measures and collections of independently inferred phylogenies. This has recently become realistic by the development of an integrated Bayesian inference approach that connects sequence and trait evolutionary processes in a phylogeographic context (Lemey *et al.* 2009, 2010). Here, we build upon these Bayesian phylogenetic diffusion models and extend them to simultaneously estimate evolutionary history and trait phylogenetic signal. This framework has several additional advantages, including the general

applicability to traits of any dimension, the quantification of correlations among them and the possibility for ancestral state reconstructions. Furthermore, it allows incorporating measurement uncertainty in a natural way by numerically integrating the unobserved average tip trait values based on repeated measures. We use simulations to compare estimator performance of our Bayesian implementation of Pagel's λ (λ_B) to standard phylogenetic signal estimators, and demonstrate its use through several applications to viral traits. We revisit heritability estimation for HIV virulence, contrast phylogenetic signal for antigenic evolution in influenza H3N2 and HIV-1 subtype B, and finally return to virulence, but from the perspective of *Drosophila* hosts challenged by host-specific sigma virus infection. Taken together, we demonstrate how a coherent framework can advance trait evolutionary studies and discuss extensions that may further promote its role in comparative analyses.

Methods

We begin with a brief description of Brownian diffusion along a phylogenetic tree and the construction of Pagel's λ as a measure of phylogenetic signal, parameterized within this diffusion process. Extensive derivations find themselves in Felsenstein (1985) and Pagel (1999).

PHYLOGENETIC BROWNIAN PROCESS

Let $\mathbf{Y} = \{y_{ij}\}$ be the $N \times K$ matrix of K -dimensional trait values realized at the tips of a tree \mathcal{T} for N taxa. The diffusion process posits that data \mathbf{Y} arise from conditionally independent, multivariate normally distributed displacements along each branch in \mathcal{T} . These displacements are centred around the hypothesized trait value at the parent node of the branch and have variance proportional to a $K \times K$ positive definite, symmetric matrix Σ , where the proportionality constant is the branch length. The diagonal elements of Σ describe the (relative) rates at which the different trait dimensions evolve over the tree and the off-diagonal elements reflect the covariation in trait dimensions after controlling for their shared history. Conditioning on the hypothesized ancestral trait values α at the root of \mathcal{T} , the joint distribution of $\text{vec}[\mathbf{Y}]$ falls out as

$$p(\text{vec}[\mathbf{Y}] | \Sigma, \mathbf{V}_{\mathcal{T}}, \alpha) = \frac{\exp\{-\frac{1}{2}(\text{vec}[\mathbf{Y}] - \mathbf{X}\alpha)^t(\Sigma \otimes \mathbf{V}_{\mathcal{T}})^{-1}(\text{vec}[\mathbf{Y}] - \mathbf{X}\alpha)\}}{(2\pi)^{NK/2} |\Sigma \otimes \mathbf{V}_{\mathcal{T}}|^{1/2}}, \quad \text{eqn 1}$$

where $\text{vec}[\cdot]$ is the vectorization operator that stacks the column vectors of its argument below one another, \otimes is the Kronecker product and \mathbf{X} is a $NK \times K$ design matrix in which entries in column k contain 1 for trait k or 0 otherwise, following Freckleton, Harvey & Pagel (2002). More importantly, $\mathbf{V}_{\mathcal{T}} = \{v_{ij}\}$ is an $N \times N$ variance matrix that is a deterministic function of \mathcal{T} ; we return to its definition shortly as this relates to Pagel's λ . Equation (1) relates that $\text{vec}[\mathbf{Y}]$ is multivariate normally distributed with $NK \times NK$ variance matrix $\Sigma \otimes \mathbf{V}_{\mathcal{T}}$, fallaciously suggesting computational order $\mathcal{O}(N^3K^3)$ to decompose the variance matrix and evaluate the density. We prefer to work directly with the joint density of \mathbf{Y} ,

$$p(\mathbf{Y} | \Sigma, \mathbf{V}_{\mathcal{T}}, \alpha) = \frac{\exp\{-\frac{1}{2}\text{tr}[\Sigma^{-1}(\mathbf{Y} - \mathbf{1}\alpha)^t \mathbf{V}_{\mathcal{T}}^{-1}(\mathbf{Y} - \mathbf{1}\alpha)]\}}{(2\pi)^{NK/2} |\Sigma|^{N/2} |\mathbf{V}_{\mathcal{T}}|^{K/2}}, \quad \text{eqn 2}$$

that is a matrix-normal probability density function (Dawid 1981), where $\text{tr}[\cdot]$ is the trace operator and $\mathbf{1}$ is a N -dimensional column vector of ones. Importantly, eqn (2) clarifies the smaller computational order $\mathcal{O}(N^3 + K^3)$ required to evaluate the density without specialized knowledge of Kronecker product identities. Leading software to estimate Pagel's λ for multivariate traits, such as the *caper* package (Orne *et al.* 2013) in R, relies implicitly on this representation to afford computational efficiency.

However, Pybus *et al.* (2012) show that it is possible to evaluate eqn (2) in computational order $\mathcal{O}(NK^2)$ by modelling explicitly in terms of precision Σ^{-1} and, more importantly, exploiting an original dynamic programming algorithm. We adopt this approach here since repeated evaluation of eqn (2) is the rate-limiting step in both profiling the likelihood function in a maximum likelihood framework and numerical integration in a Bayesian framework. This critical insight enables us to scale comparative methods to trees with hundreds or thousands of tips, a situation regularly encountered in viral evolution.

In brief, the dynamic programming algorithm starts with the joint density of \mathbf{Y} and the hypothesized trait values at each of the internal and root nodes in \mathcal{T} . From this joint density, we recover the marginalized density of \mathbf{Y} by integrating out the internal and root node values. We achieve this high-dimensional integration through a post-order tree traversal over the $\mathcal{O}(N)$ internal and root nodes. Each nodal visit entails a simple integration of the hypothesized value at the node to arrive at the partial density of the tip trait values descendent to the node given the unobserved value of the parent of the node or the prior distribution assumed on the root trait value. This recursive task has an analytic solution involving $\mathcal{O}(K^2)$ computational operations.

To complete specification of the Brownian diffusion process along \mathcal{T} and introduce Pagel's λ , we return to our definition of $\mathbf{V}_{\mathcal{T}}(\lambda)$ that we now explicitly parameterize in terms of λ . Let $d_{\mathcal{T}}(u, v)$ equal the sum of branch lengths along the shortest path between node u and node v in \mathcal{T} . Then, diagonal elements $v_{ii} = d_{\mathcal{T}}(v_{2N-1}, v_i)$, the time distance between the root node v_{2N-1} and tip node i . To interpret these elements, the marginal distribution of \mathbf{Y}_i given α has variance proportional to the time since the root. For ultrametric trees, these diagonal entries are all equal; for rapidly evolving pathogens, non-contemporaneous sequences are common, leading to different times. Further, the off-diagonal elements

$$v_{i\hat{i}} = \lambda \left[\frac{d_{\mathcal{T}}(v_{2N-1}, v_i) + d_{\mathcal{T}}(v_{2N-1}, v_{\hat{i}}) - d_{\mathcal{T}}(v_i, v_{\hat{i}})}{2} \right], \quad \text{eqn 3}$$

specifying the rescaled time distance between the root node and the most recent common ancestor of tip nodes i and \hat{i} . Intuitively, the covariance between two tip traits is a function of their shared evolutionary history, and the traits become conditionally independent at their most recent common ancestor node in \mathcal{T} . Scalar λ exists on the continuum between 0 and 1 (Pagel 1999), with $\lambda = 1$ returning a variance matrix $\mathbf{V}_{\mathcal{T}}$ that perfectly adheres to a Brownian process along \mathcal{T} (Felsenstein 1985). On the other hand, $\lambda = 0$ reflects the absence of any phylogenetic correlation. Intermediate values of λ indicate that the phylogeny exerts a weaker effect on the trait evolutionary process than expected from a Brownian motion model.

Although λ operates directly on $\mathbf{V}_{\mathcal{T}}$ given the phylogeny \mathcal{T} , it is often convenient to view λ as a transformation of the phylogeny to fit the Brownian motion model to the trait data. Figure 1 shows these transformations for three different values of λ on an example phylogeny relating six taxa as well as their corresponding $\mathbf{V}_{\mathcal{T}}$. The transformation involves rescaling the internal node heights, with all internal node heights equal to the root node as the most extreme case ($\lambda = 0$, starlike tree in Fig. 1). Freckleton, Harvey & Pagel (2002) demonstrate how to construct, in a maximum likelihood (ML) setting, an estimator λ_{ML} of

λ for hypothesis testing about the strength of phylogenetic signal in fully observed multivariate traits conditional on a known phylogeny.

BAYESIAN ESTIMATOR OF REPEATED TRAITS PHYLOGENETIC SIGNAL

We develop a coherent estimator λ_B set in a Bayesian framework for simultaneously estimating the phylogeny and trait signal with potentially missing or repeated measures. Assume that at each tip i in \mathcal{T} , we observe R_i trait realizations $\mathbf{X}_i = (\mathbf{X}_{i1}, \dots, \mathbf{X}_{iR_i})$. To model these repeated measures, we build upon Guo *et al.* (2007) by asserting that tip traits \mathbf{Y}_i are unobserved and characterize a sampling distribution on \mathbf{X}_i . Specifically, we posit that each observed value \mathbf{X}_{ir} is multivariate normally distributed about \mathbf{Y}_i with variance $\Gamma_i = (R_i - 1) \times \Gamma$, where Γ is an estimable $K \times K$ variance matrix that quantifies the measurement error in the observation process across all taxa. Over all taxa and all trait observations $\mathbf{X} = (\mathbf{X}_1, \dots, \mathbf{X}_N)$, we write

$$p(\mathbf{X}|\mathbf{Y}, \Gamma) = \prod_{i=1}^N \prod_{r=1}^{R_i} \left[\frac{\exp\{-\frac{1}{2}(\mathbf{X}_{ir} - \mathbf{Y}_i)' \Gamma_i^{-1} (\mathbf{X}_{ir} - \mathbf{Y}_i)\}}{(2\pi)^{K/2} |\Gamma_i|^{1/2}} \right]. \quad \text{eqn 4}$$

Conveniently, when no repeated measures exist for tip i , $R_i = 1$ and the density function in eqn (4) enforces $\mathbf{X}_i = \mathbf{Y}_i$, returning the original model.

To simultaneously estimate the phylogeny \mathcal{T} and account for its uncertainty, we further consider aligned molecular sequences \mathbf{S} from the N taxa and model \mathbf{S} using standard Bayesian phylogenetics models parameterized in terms of other phylogenetic and demographic process parameters ϕ . Conditional on \mathcal{T} , we assume independence between \mathbf{S} and \mathbf{X} , enabling us to write down the joint density $p(\mathcal{T}, \phi, \mathbf{S})$ and view it, for the purposes of this paper, as a prior on \mathcal{T} after integrating out ϕ . We refer interested readers to, for example, Suchard, Weiss & Sinsheimer (2001) and Drummond *et al.* (2012) for detailed development of $p(\mathcal{T}, \phi, \mathbf{S})$.

Combining the Brownian diffusion process, repeated measures and phylogenetic uncertainty return the joint posterior distribution

$$p(\lambda, \Sigma, \Gamma, \mathcal{T}, \phi|\mathbf{X}, \mathbf{S}) \propto p(\mathbf{X}|\lambda, \Sigma, \Gamma, \mathcal{T}) p(\lambda) p(\Sigma) p(\Gamma) p(\mathcal{T}, \phi, \mathbf{S}) = \left(\int p(\mathbf{X}|\mathbf{Y}, \Gamma) p(\mathbf{Y}|\Sigma, \mathbf{V}_{\mathcal{T}}(\lambda), \alpha) p(\alpha) d\mathbf{Y} d\alpha \right) \times p(\lambda) p(\Sigma) p(\Gamma) p(\mathcal{T}, \phi, \mathbf{S}) \quad \text{eqn 5}$$

where $p(\lambda)$, $p(\Sigma)$, $p(\Gamma)$ and $p(\alpha)$ are prior distributions, and the integration in the second line of eqn (5) reflects a data augmentation procedure with the unobserved tip traits \mathbf{Y} and root node trait α . Lemey *et al.* (2010) and Pybus *et al.* (2012) develop priors $p(\Sigma)$ and $p(\alpha)$ that enable convenient analytic and numerical integration of the augmented

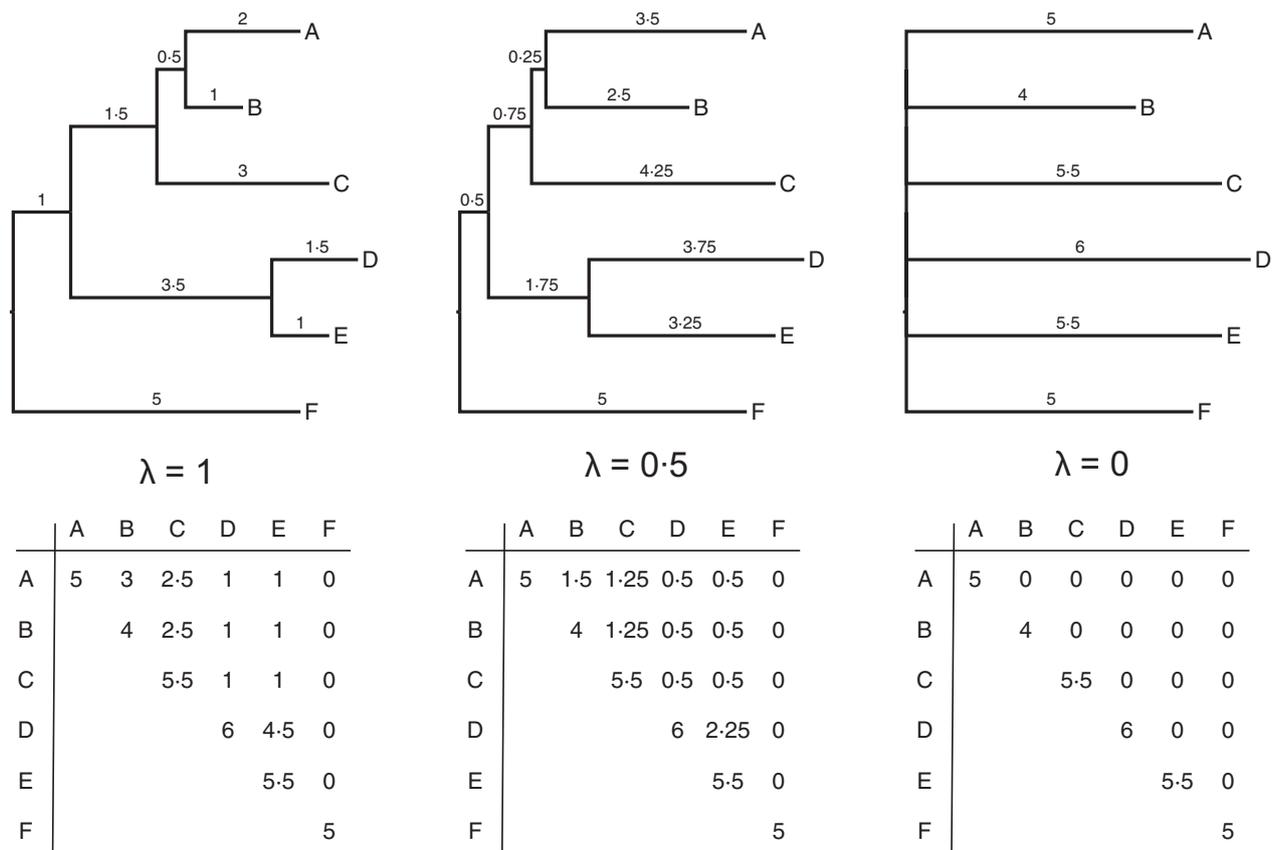


Fig. 1. Phylogenies and corresponding variance-covariance matrices for three different λ values. The tree on the left ($\lambda = 1$, no transformation) provides an example of a phylogeny estimated or hypothesized for six taxa. The numbers above the branches represent the time elapsed on the branches. The corresponding variance-covariance matrix is shown beneath the tree. Under a Brownian motion model of trait evolution, the expected covariances (off diagonals) between each pair of taxa are proportional to shared ancestry for the taxa. The expected variances (diagonal elements) for the tip traits are proportional to the summed branch lengths between the root and each tip. We multiply the off-diagonal elements by two different λ values ($\lambda = 0.5$ and 0 , respectively) in the matrices to the right and show the corresponding tree transformation. The tree for $\lambda = 0$ collapses into a star-like tree and therefore represents phylogenetic independence.

data, in the latter case via Markov chain Monte Carlo (MCMC). To construct $p\Gamma$, we structure $\Gamma^{-1} = \text{diag}(\gamma_1, \dots, \gamma_K)$ as a diagonal matrix, where *a priori* we assume γ_j is gamma-distributed with expectation 1 and a large variance 1000 for $j = 1, \dots, K$. We explore several choices for $p(\lambda)$ in the Results section; these involve the family of beta distributions that constrain $0 \leq \lambda \leq 1$ for consistency with Freckleton, Harvey & Pagel (2002).

We define our Bayesian estimator λ_B as the marginal posterior mean $E(\lambda|\mathbf{X}, \mathbf{S})$ and report uncertainty in this estimate via the 95% highest posterior density (HPD) interval of $p(\lambda|\mathbf{X}, \mathbf{S})$. We estimate these quantities by extending MCMC methods implemented in the Bayesian Evolutionary Analysis by Sampling Trees (BEAST) software package. Specifically, we use a random-scan Metropolis-with-Gibbs approach that employs standard transition kernels to integrate over the parameter spaces of \mathcal{T} and ϕ . To sample realizations of Σ and Γ , we consider Gibbs samplers, and we develop random-walk Metropolis–Hastings transition kernels on λ and missing entries in \mathbf{X} ; this latter procedure assumes a missing completely at random structure (Heitjan & Basu 1996), such that the probability that any particular datum is missing is independent of both the observed trait values and other model parameters. Integrating out the missing entries enables us to continue to draw inference in the presence of partially observed tip trait values, without needing to trim out trait dimensions that are not completely observed across all taxa or taxa that are missing traits. Finally, to compare different restricted models, for example $\lambda = 0$ (no phylogenetic signal) versus λ is random and estimates phylogenetic signal, we use Bayes factors derived from stepping stone estimation of the marginal likelihood for each competing model (Baele *et al.* 2012). We accompany our BEAST implementation with graphical user interface support in BEAUTi for setting up phylogenetic signal analyses with multivariate traits.

BENCHMARK

We extend a previous simulation study that was modelled after the heritability of set point viral load (spVL) in HIV infection history (Alizon *et al.* 2010). Briefly, this procedure involves simulating a birth–death infection process in which each branching event represents a new infection event. Starting from an initial value drawn from an empirical distribution of traits (spVL), traits diverge at transmission events. At each branching event, one daughter branch inherits the trait on the parental branch, y_P , whereas the trait on the other daughter branch evolves into y_D , following

$$y_D = h^2 y_P + M(1 - h^2), \quad \text{eqn 6}$$

where h^2 represents the heritability of the trait and M is a random variable drawn from an empirical $\log(\text{spVL})$ trait distribution (taken from one of data sets, we analyse in this study). Following the original procedure (Alizon *et al.* 2010), this process is simulated over 13 generations with a death probability of 1 out of 3 of the transmission probability; each time, a subtree of 128 taxa with associated tip trait values is drawn randomly from the process reflecting incomplete sampling. Whereas the original simulation evolved 20 replicates for four different heritability values ($h^2 = 0.3, 0.5, 0.7$ & 0.9), we also include the same amount of replicates for $h^2 = 0.1$ to ensure symmetric simulation scenarios and because existing methods have difficulties detecting low degrees of heritability (Shirreff *et al.* 2013).

We also perform simulations according to a more recent procedure proposed by Shirreff *et al.* (2013). Here, traits are simulated along an empirical phylogeny starting from the mean $\log(\text{spVL})$ at the root. Each node (and not just a single daughter branch) inherits a $\log(\text{spVL})$ according to the following process with stationary variance:

$$y_D = h^2 y_P + M\sqrt{1 - (h^2)^2}, \quad \text{eqn 7}$$

where M is now drawn from a normal distribution with the mean and variance of the population $\log(\text{spVL})$. We iterate through a wider range of heritability values (from 0.05 to 0.95 with a step size 0.05) and simulate 100 replicates for each heritability level. We use both sets of simulations to compare our Bayesian estimate of λ to a maximum likelihood estimate of lambda $[\lambda_{\text{ML}}$, for which various implementations exist, for example Revell (2012); Harmon *et al.* (2008); Orme *et al.* (2013); Paradis, Claude & Strimmer (2004); Lavin *et al.* (2008); Pinheiro *et al.* (2013)] and Blomberg's K , both obtained by the phytools R package (Revell 2012). In order to estimate confidence intervals for λ_{ML} , we extend the phytools estimation procedure to obtain the λ values for 1.92 \log likelihood units on either side of the maximum likelihood estimate and construct confidence intervals.

VIRAL EXAMPLES

We analyse four different data sets that examine the phenotypic evolution in infectious diseases; these phenotypic traits include both virus-specific characteristics and host susceptibility to viral infection. We first focus on the Swiss HIV Cohort Study (SHCS) data set (Swiss HIV Cohort Study *et al.* 2010), previously used to investigate the heritability of spVL (Alizon *et al.* 2010). This study selected HIV-1 subtype B-infected participants who had a genotypic drug resistance test and at least three HIV RNA measurements, but remained treatment-naïve at entry. In addition to spVL from cohort participants, the study also measured the declining slope of CD4+ T-cell (dsCD4) counts; this quantity also predicts virulence to some extent (Mellors *et al.* 2007). As a 'control trait' in our analyses, we followed Alizon *et al.* (2010) and considered the estimated probability that a treatment-naïve virus is resistant to zidovudine (prAZT) from the *pol* sequence using the geno2-pheno system (Beerenwinkel *et al.* 2003). We examine spVL, dsCD4 and prAZT both independently and jointly as a multivariate trait.

The sequence data for the SHCS consist of population sequences of the HIV-1 polymerase (*pol*) gene for each patient, and we used alignments in which amino acid positions that are strongly associated with antiretroviral drug resistance were removed. Having access to multiple viral load measures per patient after acute infection, but prior to start of antiretroviral therapy, the first CDC C event, or the time when the CD4 count first drops below 200 cells, Alizon *et al.* (2010) considered two different spVL criteria to distinguish different patients subsets. The 'strict' criterion only considers cases where all the viral load measurements fluctuate within a 1-log band around the patient-specific mean, whereas the 'liberal' definition applies to all cases where at least three consecutive viral loads measurements are available that fluctuate within a 1-log band of their mean. We also follow Alizon *et al.* (2010) in studying the transmission group of men who have sex with men (MSM) separately from other transmission groups that are heterosexuals and injection drug users. Because the densely sampled MSM sequences tend to cluster in phylogenetic trees (Kouyos *et al.* 2010), it is suggested that they may yield more accurate phylogenies. Furthermore, focusing on the MSM transmission group may remove some confounding factors, such as patient gender, transmission group or age, on infection trait values (Alizon *et al.* 2010). The distinction between a strict and liberal spVL definition and between MSM and the general population results in four data sets listed in Table 2. For the SHCS analysis, our full probabilistic model included a general time-reversible substitution model with discretized gamma-distributed rate variation among sites, an uncorrelated log-normal relaxed molecular clock model and a flexible Gaussian Markov random field model of population size change

through time as a tree prior (Minin, Bloomquist & Suchard 2008). For the isolates with unknown sampling times, we integrate out their dates by assuming a uniform prior distribution over a plausible time interval (Shapiro *et al.* 2011). Because the SHCS provides a large number of taxa ($n = 661$ for all risk groups and a liberal viral load criterion), we perform the different trait analyses on the full data set using an empirical tree distribution inferred separately from the nucleotide data [cfr. Lemey *et al.* (2014)]. Finally, we estimate marginal likelihoods on a fixed tree topology for the different SHCS data sets because marginal likelihood estimation, for example via stepping stone sampling, requires a series of MCMC simulations for different power posteriors; this is generally much more computationally demanding than a standard MCMC exploration of the posterior (Baele *et al.* 2012).

Our second data set explores antigenic evolution in human influenza H3N2 and consists of 1441 hemagglutinin (HA) sequences with known date of sampling and with associated antigenic measurements previously obtained by Russell *et al.* (2008). These sequences were sampled globally from 2002 to 2007 and represent a subset of strains from a larger antigenic sampling (13 000 isolates) for which HA sequence was available (Russell *et al.* 2008). Antigenicity was measured using hemagglutination inhibition (HI) assays and mapped into a two-dimensional space using multidimensional scaling (MDS) (Smith *et al.* 2004). Here, we consider the first two principal antigenic coordinates resulting from a previous MDS fit (Russell *et al.* 2008) as traits in our phylogenetic diffusion model. Given the size of the data set, we approximate phylogenetic uncertainty by integrating over a set of trees previously reconstructed as part of a phylogeographic study (Lemey *et al.* 2014).

For the third data set, we return to HIV and study antigenic evolution in the context of enhanced resistance to the broadly neutralizing antibodies (nAb) PG9, PG16 and VRC01 over the course of the HIV-1 epidemic (Euler *et al.* 2011). The sequence data set encompasses clonally sequenced viral variants from contemporary and historic seroconverters (seroconversion between 2003 and 2006 and between 1985 and 1989, respectively). To focus on the population-level evolution of neutralization resistance and avoid the impact of within-host evolutionary dynamics, we randomly choose one sequence per patient for our analyses and examine the 50% inhibitory concentration (IC_{50}) assay values for the three different antibodies. This assay measures per cent neutralization by determining the reduction in p24 production in the presence of neutralizing agent compared to the levels of p24 in the cultures with virus only; a detailed description can be found in Euler *et al.* (2011). To constrain the trait values (concentrations) to be strictly positive values under the diffusion process, we model the log-transform of the IC_{50} values observed at the tips of the tree. When the observed log IC_{50} value falls outside the tested antibody concentration range, we integrate out the concentration over a plausible IC_{50} interval. For those IC_{50} s lower than the lowest antibody concentration, we set up a uniform prior ranging from the lowest tested $\ln(nAb)$ concentration to the 32x diluted lowest tested concentration. The values at the opposite end of the spectrum, where 50% neutralization is not reached at the highest nAb concentration, are integrated out over an appropriately scaled exponential distribution. Other evolutionary models and tree priors follow the same specifications as for the SHCS analyses.

Finally, we return to infection traits and study the heritability of host susceptibility to viral infection. To this purpose, we focus on a data set that has been used to investigate the ability of three host-specific *Drosophila* sigma viruses to persist and replicate in 51 different species of *Drosophilidae* (Longdon *et al.* 2011). In this experimental study, fly species are injected with host-specific sigma virus from *D. affinis* (DAffSV), *D. melanogaster* (DMelSV) and *D. obscura* (DObsSV), and a change in viral titre is measured between day 0 and day 15 post-infection using quantitative reverse-transcription PCR.

The copy number of viral genomic RNA is expressed relative to the endogenous control housekeeping gene RpL32 (Rp49) based on species-specific primers for this gene. The authors aim at performing three replicate measures for each virus per fly species (3 replicates each of the day 0 and day 15 treatments). We incorporate these repeated measures in our analyses. We refer to Longdon *et al.* (2011) for further details on the experimental procedure. We use the sequence data for the COI, COII, 28S rDNA, Adh, SOD, Amyrel and RpL32 genes to jointly reconstruct the *Drosophila* host phylogeny with the diffusion process and follow the evolutionary model and analysis settings from Longdon *et al.* (2011).

Results

PERFORMANCE

We conduct a simulation study to compare the relative performance of the λ_B estimator to two standard indices of phylogenetic signal. We extend the simulation study by Alizon *et al.* (2010) aimed at evaluating the performance of Blomberg's K (Blomberg, Garland & Ives 2003) and Pagel's λ (Pagel 1999) in capturing the heritability of viral trait evolution. Briefly, the original simulation procedure considers a birth–death infection process with incomplete sampling and evolves a trait along the resulting transmission tree using different degrees of heritability (0.3, 0.5, 0.7 and 0.9). Here, we extend the study to a heritability value of 0.1 to explore a symmetric range of heritability values around 0.5. A comparison of the phylogenetic signal estimates for replicate data generated under different heritability values (Fig. 2) suggests that λ_B captures the underlying heritability with less bias and lower variance compared to Blomberg's K and λ_{ML} . This is confirmed by the quantitative bias and mean-squared error (MSE), which quantifies the amount by which the estimator differs from the true value, estimates as tabulated across all heritability scenarios (Table 1). Because of the somewhat distinct behaviour for small and large heritability values (Fig. 2), we also summarize the bias for heritability values smaller and larger than 0.5 (Table 1). This

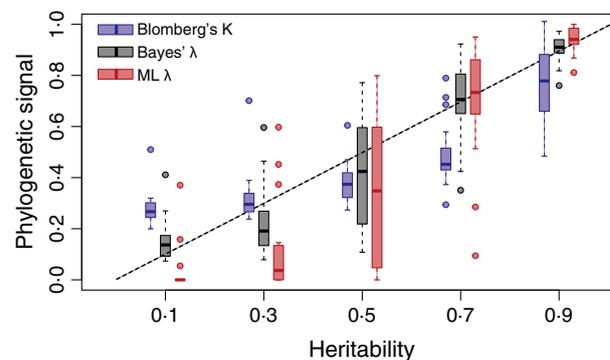


Fig. 2. Estimator performance of Blomberg's K , Pagel's λ_{ML} and λ_B on simulated data. Twenty phylogenies are simulated to model the evolution of an infection trait with known heritability ($h^2 = 0.1, 0.3, 0.5, 0.7$ & 0.9). Phylogenetic signal is then estimated on each tree using only 128 leaves to account for incomplete sampling. The box plots show the median values, the three quartiles and the outliers for Blomberg's K (blue), Pagel's λ_B (red) and λ_B (grey).

Table 1. Comparison of bias, mean-squared error (MSE) and coverage for different phylogenetic signal estimators across a range of heritability values

Estimator	Bias			MSE	Coverage
	Total	$h^2 < 0.5$	$h^2 > 0.5$		
Blomberg's K	0.031	-0.101	0.120	0.048	NA
Pagel's λ_{ML} , MLE	0.073	0.130	-0.024	0.045	0.830
λ_B , $\beta(1.0,1.0)$	0.018	0.009	-0.004	0.020	0.820

confirms the finding that existing methods have difficulties in estimating relatively low heritability values [$h^2 < 0.4$, Shirreff *et al.* (2013)]; Blomberg's K and λ_{ML} result in over- and underestimation, respectively (Table 5). λ_B follows the sigmoidal pattern for λ_{ML} to a lesser extent and is characterized by smaller biases for both small and large heritability values.

We also report estimator coverage for λ_B and λ_{ML} ; coverage reflects the probability that the true value from which the data derive falls within the model estimated nominal confidence interval. The uncertainty for the λ_B estimator is quantified by a 95% Bayesian high posterior density (HPD) interval, the size of which - unlike a frequentist confidence interval - does not necessarily have to correspond to the nominal coverage. To obtain a 95% confidence interval (CI) for λ_{ML} , we construct a likelihood ratio test and find its points of rejection using a numerical optimizer in R. The need to independently implement a confidence interval constructor reflects the fact that λ_{ML} is frequently reported as a point estimate without quantifying its uncertainty. We note that Blomberg's K phylogenetic signal statistic is computed as a ratio of two MSE ratios, comparing observed against expected ratios under Brownian

motion, and is therefore less amenable to constructing confidence intervals (Blomberg, Garland & Ives 2003).

We continue to explore the effect of prior specification on λ_B estimates and compare the $\beta(1,1)$ prior to a U-shaped prior [$\beta(0.5,0.5)$] and several bell-shaped priors. Figure 3 shows that bias and MSE can be further minimized while raising coverage close to nominal values for a $\beta(2,2)$ prior. This prior may help to linearize the general sigmoidal relationship between λ and the known heritability spectrum as well as reduce the generally large variance of λ estimates for intermediate heritability values. We therefore adhere to this prior specification in further data analyses, but accompany the λ estimates with a posterior divergence measure to quantify the potential prior influence. Specifically, we computed the Kullback–Leibler (KL) divergence between the prior and posterior using the FNN package in R (Boltz, Debreuve & Barlaud 2007; Beygelzimer *et al.* 2013).

We find largely similar performance differences among the three estimators, and for the different beta priors on λ_B , in a simulation analysis following a procedure similar to that of Shirreff *et al.* (2013) (Supporting Information).

HIV-1 INFECTION TRAITS

We estimate phylogenetic signal for spVL and dsCD4, either as separate traits or in combination as a bivariate trait, in the different SHCS data sets and include prAZT as a control (Table 2). Because the latter is measured directly from the genotype, it is expected to be strongly heritable. We confirm this by estimates of λ_B that are consistently close to maximum phylogenetic signal for the different SHCS data sets (Table 2). We also compare model fit of the standard diffusion model with estimable λ_B to a model that represents no phylogenetic

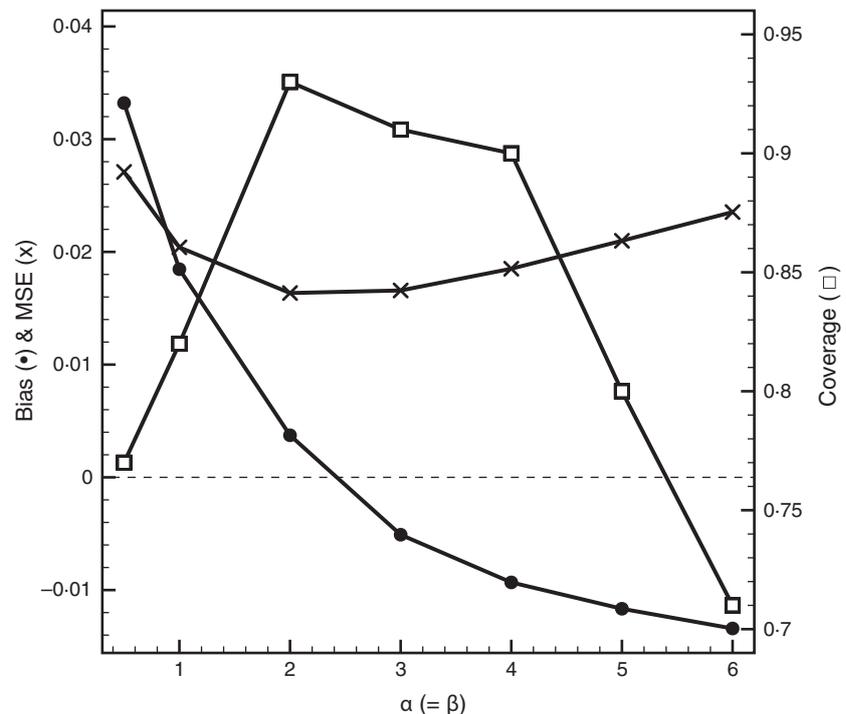


Fig. 3. Performance of the Bayesian phylogenetic signal estimation under various priors on λ_B . Different $\beta(\alpha,\beta)$ priors are explored for $\alpha = \beta$. We plot bias (filled black circles) and MSE (crosses) according to the primary axis and coverage (open squares) according to the secondary axis. The dotted horizontal line represents zero bias and MSE.

signal ($\lambda_B = 0$) and a model with perfect Brownian phylogenetic signal ($\lambda_B = 1$) using Bayes factors in Table 3. This demonstrates that the support for a nonzero phylogenetic signal for prAZT increases as the data set size increases while, in line with the very high λ_B estimates for prAZT, the support for a perfect Brownian phylogenetic signal increases. For all prAZT λ_B estimates, there is also a relatively high KL divergence between the prior and posterior distribution, suggesting that the phylogenetic signal estimates are well informed by the trait data.

In accordance with Alizon *et al.* (2010), we find a relatively high phylogenetic signal for spVL (posterior mean = 0.501, 95% HPD [0.165–0.857]), but only in the MSM subset with the strict spVL criterion (Table 2). In contrast to the prAZT, the spVL phylogenetic signal decreases as the data set increases in size and also the support for a nonzero signal is low, and there is even support in favour of the absence of spVL phylogenetic signal in the MSM liberal data set. The KL divergence indicates that the posterior divergence from the prior is limited, implying that the data do not contribute strongly to the phylogenetic signal estimate in the MSM strict data set. The fact that the posterior stays close to prior distribution for λ_B is also illustrated in Fig. S1 under both a $\beta(2,2)$ and $\beta(1,1)$ prior. Phylogenetic signal estimates for dsCD4 are generally lower, with a similar decrease for larger data sets (and increase in KL), and no data set supports a nonzero signal. The same is true for spVL and dsCD4 as a bivariate trait; in this case, the two dimensions show a negative correlation (correlation coefficient posterior mean = -0.22 , Table 2).

Phylogenetic signal in the MSM strict data set does not find strong support using BFs or KL information gain.

This may be due to the typical starlike nature of HIV-1 subtype phylogenies that is very similar to the null model for phylogenetic signal estimation. The λ_B estimate may therefore be mostly informed by clusters of closely related viruses from epidemiologically linked patients. The maximum clade credibility (MCC) tree with ancestral spVL trait estimates for the MSM strict data set in Fig. 4 illustrates this point. In the tree, we indicate a number of clusters with closely related viruses and similar spVL values. Because these clusters have relative recent MRCAs, constituting the density at small node heights in the bimodal node height density plot (Fig. 4), they may offer the strongest resistance against transforming the phylogeny into a fully starlike tree. In fact, it has been suggested that restricting the data set to MSM patients, which are more densely sampled in the SHCS, yields more accurate phylogenies because of better resolution of transmission chains between patients and that this may explain a higher spVL heritability in the MSM strict data set (Alizon *et al.* 2010). We therefore examine whether a differential proportion of such transmission clusters in the different SHCS data sets may be responsible for the differences in λ_B estimates, by splitting up the data set into one that contains only taxa that share relatively recent nodes with other taxa (< 15 years) and one that exclusively has taxa related by deeper branching patterns (internal node > 15 years), and performing separate analyses on each.

Estimates for the prAZT control trait indicate that taxa sharing more recent common ancestry are indeed most informative about phylogenetic signal. Both the λ_B estimates and the associated KL divergences are high for taxa

Table 2. Phylogenetic signal estimates for the SHCS data sets

Data set	<i>n</i>	prAZT		log(spVL)		dsCD4		dsCD4,log(spVL)		
		λ_B	KL	λ_B	KL	λ_B	KL	λ_B	KL	<i>r</i>
MSM strict	134	0.964 (0.911,0.999)	3.421	0.501 (0.165,0.857)	0.036	0.315 (0.024,0.628)	0.398	0.368 (0.056,0.727)	0.215	-0.195 ($-0.377,-0.050$)
all strict	230	0.986 (0.965,1.000)	3.725	0.303 (0.038,0.596)	0.599	0.303 (0.022,0.632)	0.513	0.206 (0.026,0.431)	1.380	-0.221 ($-0.344,-0.097$)
MSM liberal	404	0.997 (0.992,1.000)	3.858	0.228 (0.037,0.352)	1.397	0.181 (0.012,0.366)	1.631	0.113 (0.015,0.256)	2.398	-0.235 ($-0.323,-0.144$)
all liberal	661	0.992 (0.974,1.000)	3.758	0.158 (0.050,0.285)	2.328	0.093 (0.038,0.194)	2.747	0.119 (0.023,0.212)	2.665	-0.221 ($-0.288,-0.148$)

Table 3. Comparing different phylogenetic signal models for the SHCS data sets using Bayes factors. We report Bayes factors for comparing a model in which parameter λ_B is estimated (*or free*) versus a model in which it is fixed to 0 (no phylogenetic signal), BF_{f_0} , and or comparing a model in which the Pagel's λ_B is estimated versus a model in which it is fixed to 1 (phylogenetic signal expectation under Brownian motion), BF_{f_1}

Data set	prAZT		log(spVL)		dsCD4		dsCD4 & log(spVL)	
	BF_{f_0}	BF_{f_1}	BF_{f_0}	BF_{f_1}	BF_{f_0}	BF_{f_1}	BF_{f_0}	BF_{f_1}
MSM strict	1.637	1.998	1.363	4.536	-1.696	7.359	-0.870	10.249
All strict	14.068	-3.790	0.601	32.837	-1.452	8.037	-0.661	68.446
MSM liberal	53.682	-9.713	-0.921	77.527	-3.111	65.486	-2.931	130.696
All liberal	120.742	-10.861	0.234	168.002	-3.816	458.896	-0.702	404.368

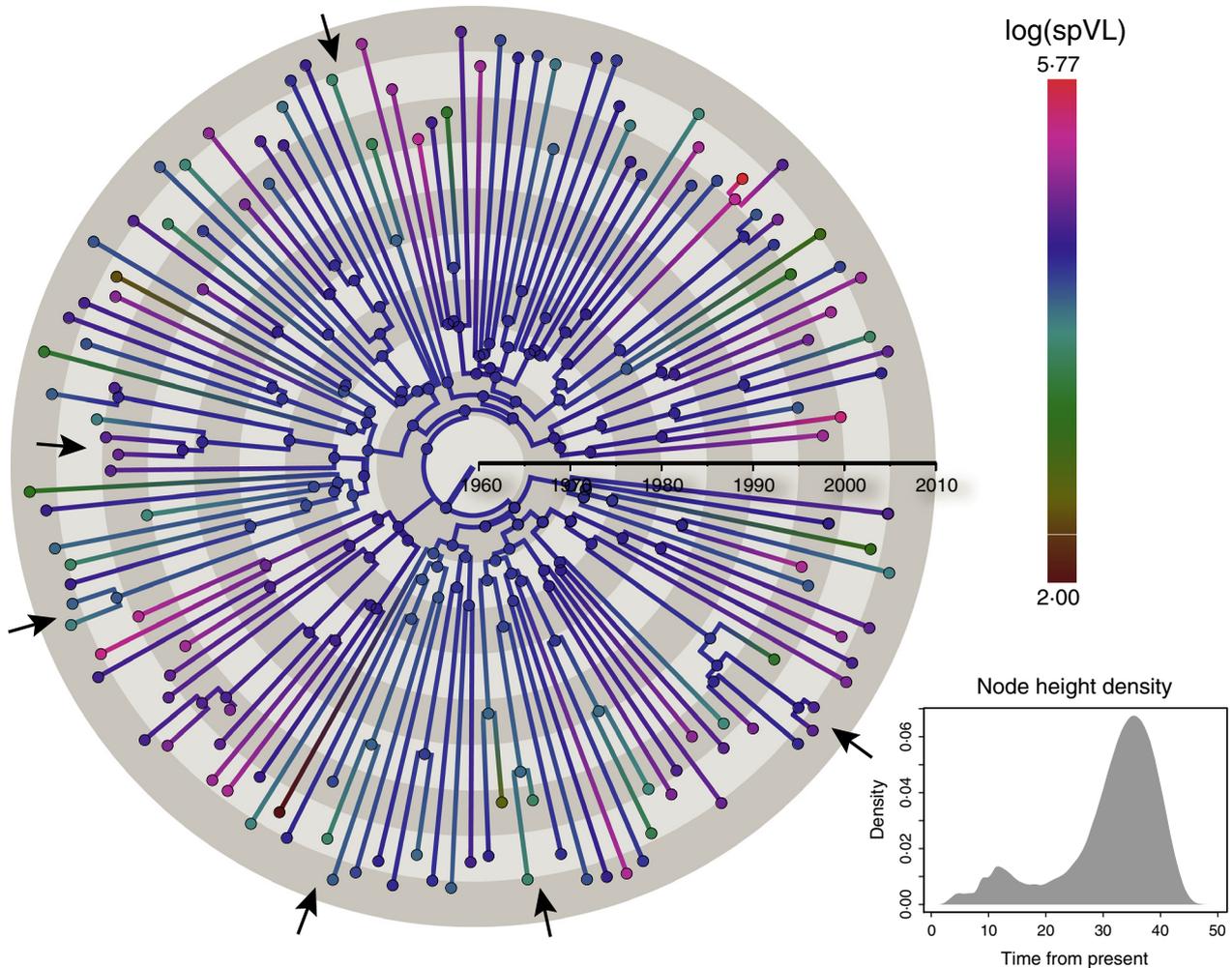


Fig. 4. Maximum clade credibility tree for the MSM strict analysis. We colour the nodes in the tree according to the observed (external nodes) or estimated (internal nodes) log spVL trait and the branches with a gradient between the relevant nodes. The arrows point at example clusters of closely related taxa with roughly similar trait values. The density plot in the lower right corner summarizes the marginal node height density from the full posterior distribution.

that descend from relatively recent nodes (< 15 years in Table 4), whereas the λ_B estimates are much closer to their prior expectation of 0.5 for the remaining taxa yielding considerably lower KL divergences. For spVL, however, the KL divergences remain low for the small data sets composed of taxa descending from more recent nodes, and focusing also on transmission clusters in the other data sets does not lead to noticeably higher phylogenetic signal estimates; this suggests that a higher phylogenetic accuracy for transmission clusters does not explain the higher phylogenetic signal.

HUMAN INFLUENZA H3N2 ANTIGENIC EVOLUTION

We study antigenic drift in human influenza H3N2 based on a large sequence data set sampled between 2002 and 2007 with matching HI assay data (Russell *et al.* 2008). HI assays measure the cross-reactivity of viruses against reference antisera and the resulting table with measurements across a multitude of viruses is frequently mapped in two-dimensional space

based on MDS approaches (Smith *et al.* 2004). Here, we treat the two principal coordinates of a previous MDS analysis on the HI data as traits evolving along the influenza genealogy and estimate their phylogenetic signal (Table 5). We find relatively high signal for the first coordinate, but approximately half of that signal for the second coordinate. These differences are also reflected in the reconstructed trait patterns along the evolutionary history (Fig. 5). Despite the much lower phylogenetic signal for the second coordinate, it still receives support for a nonzero estimate (Table 5). The large amount of influenza data included in the analysis may contribute to the more precise λ_B estimates compared to the smaller SHCS HIV data sets, and probably to a large extent also to the different order of magnitude in BF support. Both the magnitude of the phylogenetic signal for the two coordinates as a bivariate trait and its support appear to be dominated by the signal for the first coordinate. As expected from coordinates resulting from an MDS analysis, we find also little correlation between them when accounting for the ancestral history ($r = -0.098$ [$-0.158, -0.051$]).

Table 4. Phylogenetic signal estimates for the SHCS data sets

Data set	<i>n</i>		PrAZT				log(spVL)			
			< 15		> 15		< 15		> 15	
	< 15	> 15	λ_B	KL	λ_B	KL	λ_B	KL	λ_B	KL
MSM strict	39	95	0.926 (0.798,1.000)	2.604	0.553 (0.136,0.957)	0.031	0.537 (0.168,0.881)	0.049	0.569 (0.186,0.941)	0.058
All strict	99	131	0.996 (0.903,1.000)	3.351	0.631 (0.199,0.992)	0.185	0.37 (0.098,0.683)	0.330	0.509 (0.111,0.882)	0.011
MSM liberal	276	128	0.996 (0.989,1.000)	3.858	0.462 (0.071,0.856)	0.017	0.243 (0.065,0.421)	1.376	0.464 (0.102,0.841)	0.029
All liberal	396	265	0.997 (0.994,1.000)	3.856	0.363 (0.043,0.783)	0.193	0.184 (0.063,0.319)	2.036	0.341 (0.043,0.658)	0.338

Table 5. Phylogenetic signal estimates for human influenza H3N2 antigenic evolution

Trait	λ_B	KL	BF_{f_0}	BF_{f_1}
PC 1	0.821 (0.761,0.879)	2.611	1327.565	290.183
PC 2	0.420 (0.258,0.563)	0.921	31.773	406.558
PC1,PC2	0.731 (0.668,0.787)	2.246	1350.579	675.260

HIV-1 RESISTANCE TO BROADLY NEUTRALIZING ANTIBODIES

By comparing viruses isolated from individuals who seroconverted in recent years to viruses from individuals who seroconverted early in the epidemic, Bunnik *et al.* (2010) showed a decreased sensitivity of HIV-1 to polyclonal and monoclonal antibodies. Euler *et al.* (2011) extend this by examining whether the circulating HIV-1 population has also evolved towards resistance against neutralizing activity of the recently identified broadly neutralizing monoclonal antibodies (MAbs) PG9, PG16 and VRC01 (Walker *et al.* 2009; Zhou *et al.* 2010). Here, we adopt a phylogenetic perspective on such studies that traditionally ignore shared ancestry and ask whether these viral evolutionary patterns translate into a noticeable phylogenetic signal for resistance to neutralization. Table 6 lists the λ_B estimates for the IC_{50} measurements, both as univariate and combined multivariate traits. The mean posterior phylogenetic signals suggest intermediate heritability of resistance to neutralization for the three MAbs, but the broad credible intervals and low KL divergences indicate that these estimates are poorly informed by the data. For the PG9 IC_{50} in particular, a nonzero estimate cannot be supported. The lower mean trivariate trait signal is accompanied by a somewhat higher KL and an even stronger support against a nonzero estimate. As expected from the fact that PG9 and PG16 both mainly bind to a quaternary epitope on the second variable loop in the viral envelope trimer (Walker *et al.* 2009), their $\log(IC_{50})$ values are highly correlated (0.95 [0.831,0.963]). VRC01 on the other hand is directed against the CD4-binding site (Zhou *et al.* 2010), and its IC_{50} values are only moderately correlated with those for PG9 (0.362 [0.015,0.649]) and PG16 (0.462 [0.146,0.730]). We illustrate the evolutionary pattern for resistance against PG16 and VRC01 in Fig. 6

and also summarize the mean $\log(IC_{50})$ across all lineages at different points through time below the trees. The latter indicates an overall rise in resistance through time, in particular within the first 5 years since the early samples in the mid-to-late 1980s. The early time points generally have lower resistance, and although they may indeed have led to less descendants, it is also cautious to bear in mind potential sampling artefacts.

DROSOPHILA SUSCEPTIBILITY TO SIGMA VIRUS INFECTION

In our final example, we investigate the ability of three host-specific sigma viruses (family *Rhabdoviridae*) to persist and replicate in different species of *Drosophilidae*. This has been previously studied by infecting 51 *Drosophila* species with three host-specific viruses and measuring virus titres at fixed time points (Longdon *et al.* 2011). The authors use a phylogenetic mixed model to demonstrate that host relatedness strongly determines the viral persistence and replication in new hosts. Here we revisit this problem by diffusing the virulence measure, \log_2 viral load [$\log_2(VL)$], over the host phylogeny and measuring its phylogenetic signal while accommodating the multiple measurements through numerical integration of the unobserved average trait values at the tips. Wing size (a proxy for body size) is included as a control as it is expected to be a heritable trait for *Drosophila* species.

Our analysis indicates that the capacity to infect and replicate in different hosts for three different host-specific sigma viruses (DAffSV, DMelSV and DObsSV) shows relatively high phylogenetic signal, matching that of wing size in the *Drosophila* species (Table 7). These estimates are associated with relatively high KL divergences and strong support in favour of a nonzero estimate, but also strong support against a perfect Brownian trait evolutionary process. When combined into a trivariate trait, the virulence evolutionary patterns showed similar, moderate-to-high positive correlations (0.760 [0.545–0.936], 0.675[0.408,0.954] and 0.661 [0.436,0.856] between DAffSV and DMelSV, between DAffSV and DObsSV, and between DMelSV and DObsSV, respectively). In Fig. 7, we illustrate the trait evolutionary patterns for susceptibility to infection with two viruses from relatively closely related hosts.

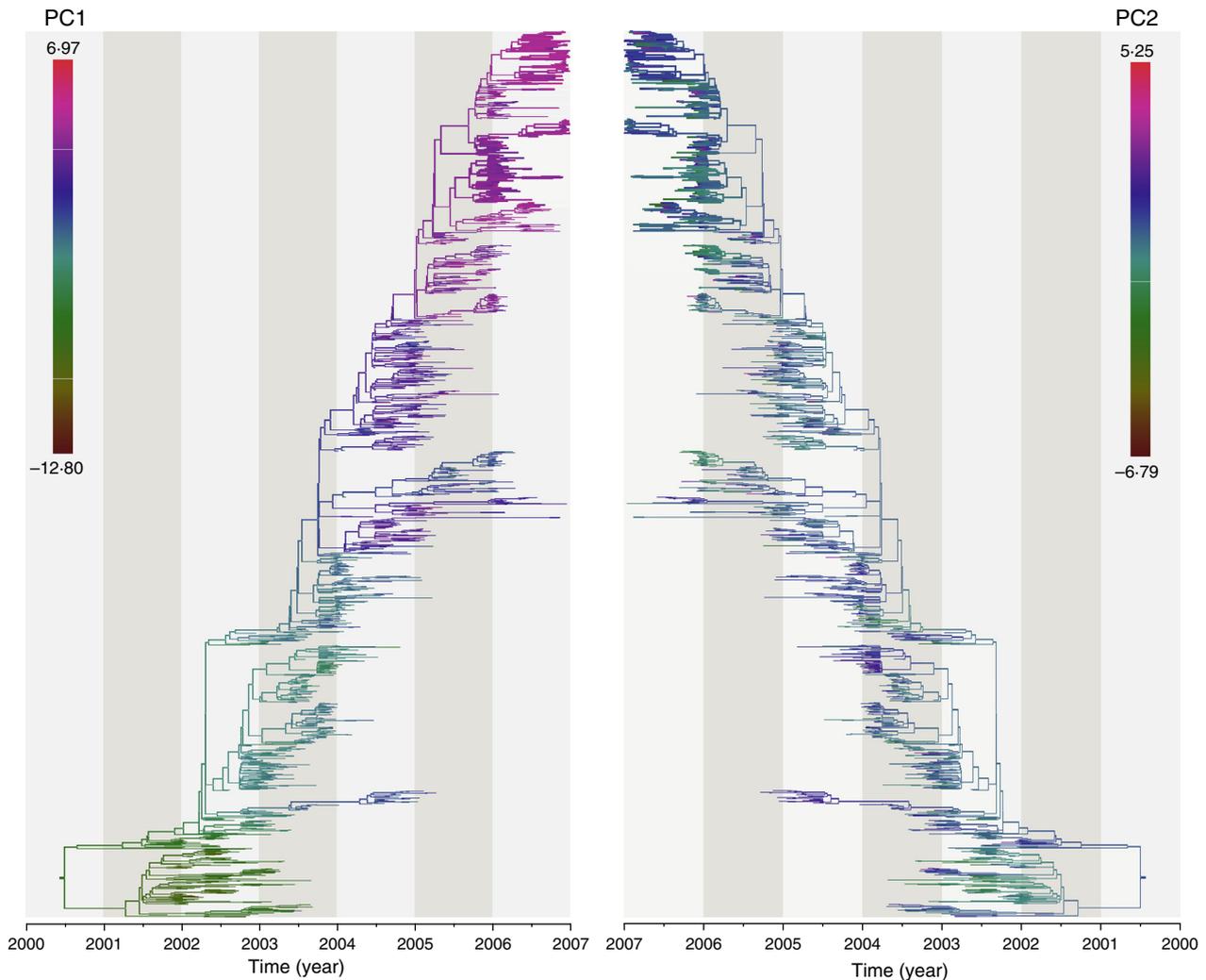


Fig. 5. Influenza H3N2 antigenic evolution based on two MDS coordinates derived from HI assay data. Ancestral reconstructions for two MDS coordinates (PC1 and PC2) are represented by a colour gradient on the same maximum clade credibility (MCC) tree summary.

Table 6. Phylogenetic signal estimates for HIV evolution towards resistance to antibodies

Trait	λ_B	KL	BF_{f_0}	BF_{f_1}
$IC50_{PG9}$	0.468 (0.079,0.841)	0.005	-0.459	5.207
$IC50_{PG16}$	0.570 (0.175,0.958)	0.044	2.359	0.243
$IC50_{VRC01}$	0.558 (0.184,0.870)	0.048	2.232	9.241
$IC50_{PG9,PG16,VRC01}$	0.364 (0.075,0.677)	0.230	-5.052	15.743

Discussion

We present a Bayesian implementation of Pagel's λ (λ_B) to quantify phylogenetic signal of multivariate traits. The estimator accommodates the different sources of uncertainty associated with both the sequence and (repeated) trait evolutionary processes and outperforms other estimators, including its maximum likelihood equivalent λ_{ML} , in terms of accuracy and precision on simulation data under different degrees of heritability. Further exploration of prior specification on λ_B suggests that a bell-shaped prior [$\beta(2,2)$], preferring intermediate phylogenetic signal *a priori*, further improves

estimator performance. This prior may help to linearize the sigmoidal relationship between λ and the known heritability spectrum as well as reduce the generally large variance of λ estimates for intermediate heritability values. These characteristics are not specific to the relationship between simulated heritability and phylogenetic signal as they have been observed in other simulation studies as well (Münkemüller *et al.* 2012). Instead of prior specification, it may therefore be useful to examine transformations like to logit function for this type of phylogenetic signal estimators. We did not perform an exhaustive evaluation of different phylogenetic simulators, but more comprehensive simulation studies have shown that λ_{ML} and Abouheif's C_{mean} fulfilled most of the criteria for a good performance (Münkemüller *et al.* 2012). This, and the fact that λ_{ML} was also the most robust and sensitive among different methods to estimate spVL heritability (Shirreff *et al.* 2013), reassures that the λ_B estimator will generally perform well.

We here focus on different traits related to viral virulence, infectivity and phenotypic evolution. Only recently, phylogenetic signal estimators have been proposed to examine the heritability of viral traits throughout transmission history

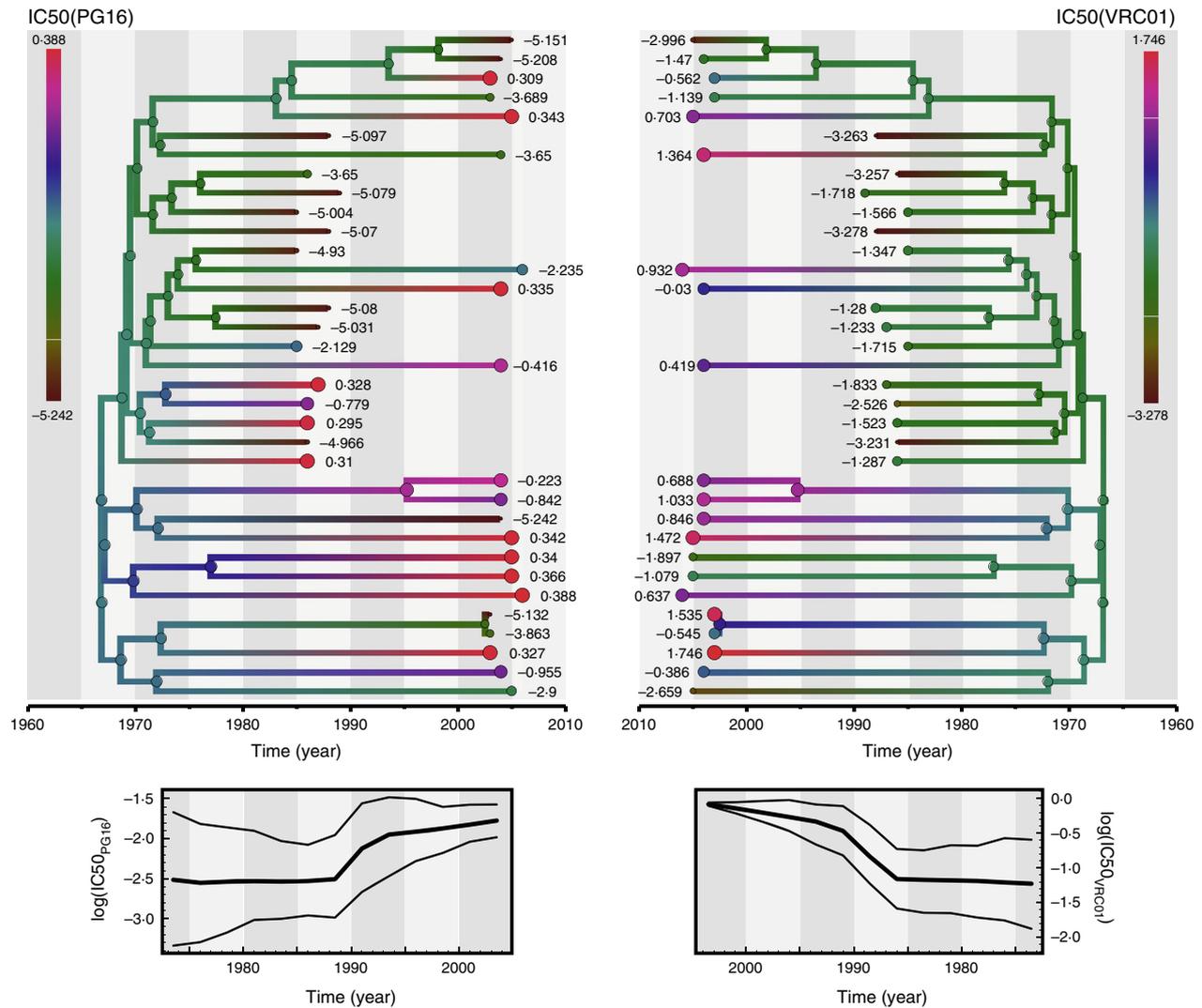


Fig. 6. HIV-1 sensitivity to the broadly neutralizing monoclonal antibodies. Log IC₅₀ measurements for two MABs, PG16 and VRC01, are shown at the tips of the same tree (left and right for PG16 and VRC01, respectively), with tip circles areas proportional to these values. Higher log IC₅₀ values represent lower sensitivity of the virus to neutralization. The ancestral reconstruction of the sensitivity to MAB neutralization is depicted using a colour gradient along the branches. Below the trees, we plot the average evolution in log IC₅₀ with credible intervals (95% highest posterior density intervals) by slicing the tree branches at particular time points and imputing the trait values at that time point [cf. Bielejec *et al.* (2011)].

Table 7. Sigma virus infectivity in *Drosophila*

Trait	λ_B	KL	BF_{j0}	BF_{j1}
Wing size	0.790 (0.588,0.938)	1.307	34.951	24.264
DAfSV log ₂ (VL)	0.770 (0.529,0.969)	0.971	37.811	45.629
DMelSV log ₂ (VL)	0.786 (0.567,0.971)	1.152	24.523	7.654
DObsSV log ₂ (VL)	0.711 (0.494,0.896)	0.842	29.608	21.715
DAfSV/DMelSV/ DObsSV log ₂ (VL)	0.781 (0.616,0.921)	1.313	32.813	38.680

[e.g. Alizon *et al.* (2010)]. Although we can reproduce a heritability of about 50% for HIV-1 spVL, a predictor of disease progression, in the MSM strict data set from Alizon *et al.* (2010), we acknowledge that this estimate is not strongly informed by the data. As for the example of HIV-1 resistance to antibody neutralization, the fact that HIV trees represent exponentially growing populations resulting in starlike tree topologies

(Lemey, Rambaut & Pybus 2006), may offer little opportunity to quantify phylogenetic trait association with great precision. However, when focusing on the subset of taxa in clusters with relatively recent common ancestors, we retrieve similar, uncertain estimates for spVL phylogenetic signal in the MSM strict data set, but still lower signal in the other data sets. While in general, phylogenetic signal estimates are likely to benefit from higher phylogenetic accuracy, the potentially higher phylogenetic accuracy gained by focusing on transmission clusters in the SHCS does not appear to explain the higher spVL phylogenetic signal for the MSM strict data set. Other data set-specific characteristics may therefore be more important. Adhering to a more strict definition for spVL may reduce measurement error, and focusing on the MSM risk group may remove the effect of patient sex and to a large extent also age as confounding factors (Alizon *et al.* 2010). It is for example well established that spVL is affected by patient sex, with males tend-

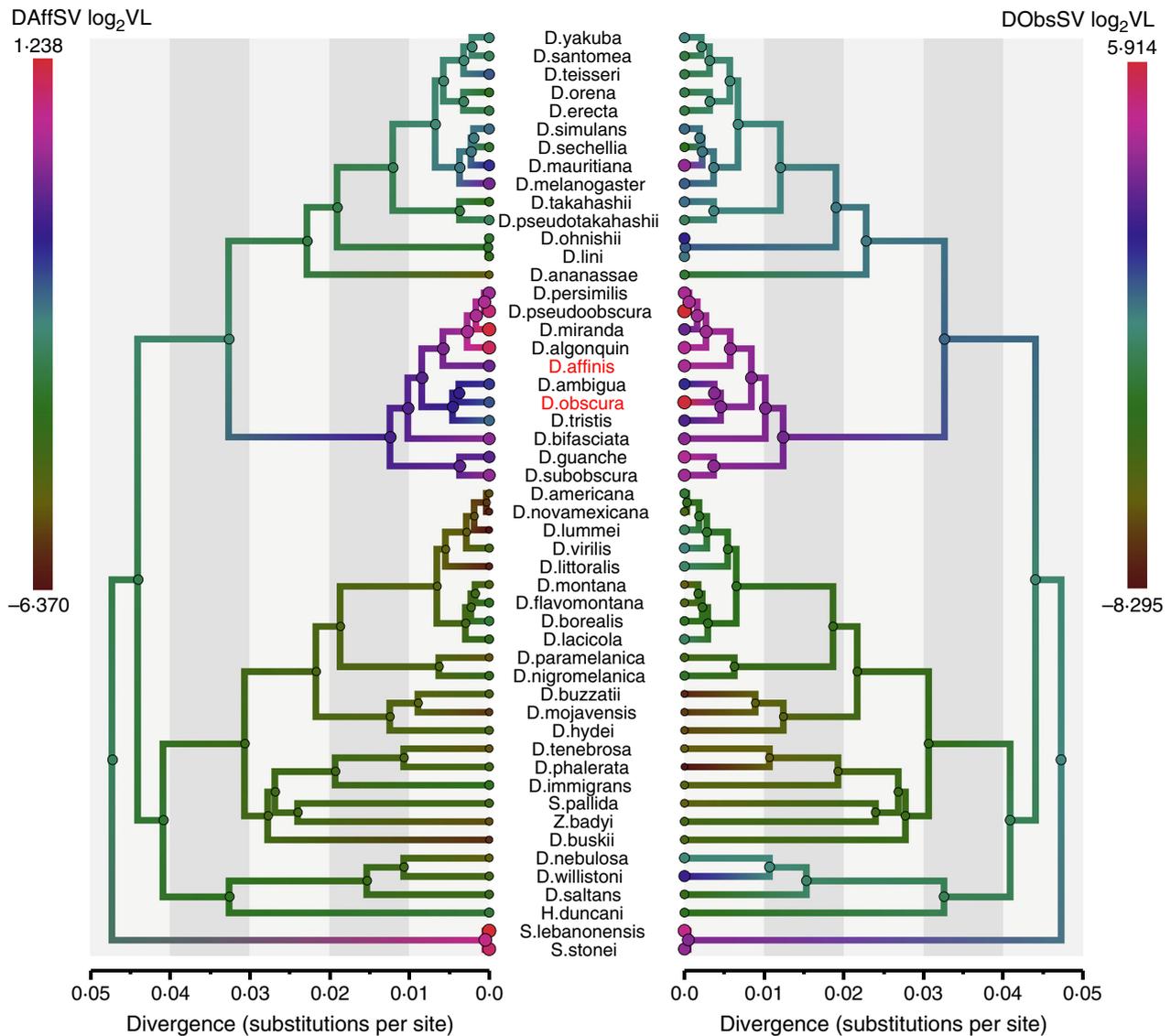


Fig. 7. *Drosophila* evolutionary history with reconstructed susceptibility to infection with different host-specific sigma viruses. The ancestral reconstruction of $\log_2(\text{VL})$ measurements for two host-specific viruses (DAffSV in *D. affinis*, left; DObsSV in *D. obscura*, right) is shown using a colour gradient along the branches of the same MCC tree. Higher $\log_2(\text{VL})$ values reflect higher levels of viral replication; circles are proportional to these values.

ing to have a higher spVL (Alizon *et al.* 2010) [reviewed in Langford, Ananworanich & Cooper (2007)]. Ignoring this variability as well as spVL measurement error may of course result in lower estimates of phylogenetic signal. In agreement with the low phylogenetic signal for the larger data sets, Hodcroft *et al.* (2014) recently found small but significant spVL heritability in a large UK data set by making use of a phylogenetic mixed modelling, which can also be extended to accommodate intraspecific variation (Lynch 1991; Housworth, Martins & Lynch 2004). The heritability of spVL therefore requires further investigation, in particular because it has important implications for understanding HIV dynamics (Hool, Leventhal & Bonhoeffer 2013).

Despite the different assays involved, it is clear that the antigenic evolutionary patterns are associated with different phylogenetic signals in human influenza and HIV-1. Although also

governed by a limited number of genetic changes (Koel *et al.* 2013), escaping the antibody response has a strong effect on the influenza population dynamics as reflected by the ladder-like trees for sequences sampled throughout different epidemic seasons. This pattern reflects a continual turnover and a relatively low standing genetic variation at any point in time [akin to the within-host HIV-1 phylodynamics, Lemey, Rambaut & Pybus (2006)](Grenfell *et al.* 2004). It is therefore not surprising that this tree structure translates into a strong phylogenetic signal and that major MDS coordinate shows a clear drift pattern across the tree (Fig. 5). On the other hand, the phylogenetic structure of HIV at the population level, with multiple cocirculating lineages within a particle subtype, does not reflect the action of (humoral) immune selection (Grenfell *et al.* 2004). It is therefore remarkable to find population evolution towards increased resistance (Bunnik *et al.* 2010; Euler *et al.* 2011;

Bouvin-Pley *et al.* 2013). Despite the uncertain phylogenetic signal estimates, the apparently non-random clustering of early and late time point viruses in the example we examined hints at the importance of using a phylogenetic approach to address such questions and advocates for caution against potential sampling artefacts.

According to the support against $\lambda_B = 1$ (Table 5), the influenza antigenic evolutionary patterns do not adhere to a perfect Brownian motion process. On the one hand, measurement error may be partly responsible for this because the MDS coordinates are based on sparse HI tables with interval and truncated measurements susceptible to experimental noise. On the other hand, a Brownian trait evolutionary model is unlikely to be appropriate for antigenic drift processes because it assumes zero-mean displacement. Therefore, it would be useful to relax this assumption and allow for an unknown estimable drift vector for the mean displacement in the multivariate diffusion model. Such a model extension may also prove useful for the example of HIV-1 resistance against neutralization if there would indeed be a need to model a population evolution process towards increased resistance.

To quantify phylogenetic signal in influenza antigenic evolution, we here focus on the MDS coordinates that were readily available for the data set under investigation (Russell *et al.* 2008). We note however that the process of mapping antigenic phenotypes, referred to as 'antigenic cartography' (Smith *et al.* 2004), can be integrated with the genetic information by modelling the diffusion of antigenic phenotype over a shared virus phylogeny using the diffusion framework we also adopt here (Bedford *et al.* 2014). It would therefore be straightforward to use the tree transformation approach, resulting in the phylogenetic signal estimates, in this integrated genetic-antigenic framework. Perhaps the phylogenetic signal estimators may assist in selecting the number of dimensions in the Bayesian MDS approach. We have recently shown that a 2D model yielded optimal predictive power for a different H3N2 data set (Bedford *et al.* 2014), which seems to be in agreement with the phylogenetic signal estimates we obtain here. The second dimension still results in reasonable phylogenetic signal, but with drastically reduced support compared to the first dimension. It is therefore questionable that a third dimension would still be characterized by tree-based evolutionary patterns.

Our approach is not restricted to viral traits as shown by the sigma virus virulence study, where we in fact measure phylogenetic signal for a host trait (host susceptibility to viral infection). The phylogenetic signal estimates confirm that host phylogeny explains most of the variation in sigma virus replication and persistence in different *Drosophila* species, in a quantitatively similar way as the host phylogeny controls for the variation in wing sizes. Both measurement error and limitations to the model may again be responsible for deviations from a pure Brownian process, although we attempted to take into account the former by integrating out a mean tip trait value based on the repeated measurements. Longdon *et al.* (2011) showed that in addition to a 'phylogenetic effect', which explains similar levels of susceptibility for related species, there is also a 'distance effect' ensuring that viral titre is higher in species that are more

closely related to the natural host. This systematic change in viral titre as a function of the distance from the natural host implies that drift, perhaps due to viral adaptation to its natural host, can also play a role in this trait evolutionary history. The correlation among the virulence patterns in the host phylogeny for the three different sigma viruses suggests that, despite a relatively high divergence, they may share similar modes of infection and replication in the same host tissues. As a consequence, genetic changes in different host lineages that impact cellular or immune components involved in replication and persistence may impact susceptibility to the three different viruses.

The Bayesian phylogenetic signal estimator has a number of advantages over other approaches. Whereas many implementations of alternative estimators are restricted to single traits, our extension of general-purpose phylogenetic diffusion models can be used to determine the phylogenetic association of multivariate traits of any dimension. As noted by Freckleton, Harvey & Pagel (2002), simultaneously estimating the precision matrix and λ allows quantifying the correlation between pairs of traits that is optimal under a common random effects Brownian process. In addition, our framework is also equipped with ancestral trait reconstruction, which is also naturally achieved under the appropriate degree for statistical dependence. The phylogenetic signal estimator can also be connected to model extensions that relax the Brownian motion assumptions. We have previously presented a relaxed random-walk model that accommodates diffusion rate heterogeneity by rescaling the precision matrix in a branch-specific manner (Lemey *et al.* 2010). Future studies will therefore be able to examine how phylogenetic signal estimates are affected by violations of the constant variance assumption in Brownian processes. Most of the examples we study also demonstrate that there is a need to relax the zero-mean displacement assumption and incorporate some degree of drift. The Ornstein-Uhlenbeck (OU) process has been proposed as a 'mean reverting' extension of Brownian motion (Hansen 1997; Blomberg, Garland & Ives 2003; Butler & King 2004), but perhaps more natural generalizations may be developed through stochastic modelling in our Bayesian framework. While it is important to model trait evolutionary processes more realistically, it may also be useful to accommodate heterogeneity in phylogenetic signal throughout evolutionary history because different lineages in a phylogeny may exhibit different degrees of phylogenetic signal (Münkemüller *et al.* 2012).

Bayesian inference is a natural framework to accommodate different sources uncertainty. In addition to phylogenetic error and uncertainty in the sequence and trait evolutionary process, we also take into account measurement error for our phylogenetic signal estimates when multiple measurements are available for the tip traits. One drawback of adequately accommodating uncertainty through simultaneous estimation is the computation time that may need to be invested, in particular when attempting to average over all plausible evolutionary histories. While we show here that this is still feasible for data sets including more than 600 taxa, we abandoned random trees when comparing model fit using marginal likelihood estimation. We use a stepping stone sampling approach for marginal likelihood estimation, which has proved to provide a relatively

accurate measure of model fit, but at the price of considerable computational burden (Baele *et al.* 2012). Therefore, future studies may need to pursue more efficient testing procedures, for example by allowing λ to shrink to zero with some prior probability in our inference and estimating Bayes factors through comparison of prior and posterior odds [see, e.g., Suchard, Weiss & Sinsheimer (2005)].

In summary, many questions in evolutionary biology need to be addressed using a comparative phylogenetic approach. Although we have focused on phylogenetic signal in this study, we hope to have demonstrated that Bayesian phylogenetic diffusion models offer a flexible framework for evolutionary hypothesis testing. We also hope that future advances will open up more opportunities for unravelling trait evolutionary processes and their underlying genetic determinants, for viral pathogens as well as other organisms in general.

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Data accessibility

Data used in this paper were taken from original publications, made available from the authors upon request. Section 2.4 'Viral Examples' describes in detail which data have been used for which part of the research.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Fig. S1. Marginal prior and posterior densities of λ_B for the MSM strict data set.

Table S1. Comparison of bias, mean squared error (MSE) and coverage for different phylogenetic signal estimators across a range of heritability values.

Fig. S2. Estimator performance of Blombergs K, Pagels λ_{ML} and λ_B on simulated data.

Fig. S3. Performance of the Bayesian phylogenetic signal estimation under various priors on λ_B .