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Recent advances in computational phylodynamics

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Time-stamped, trait-annotated phylogenetic trees built from virus genome data are increasingly used for outbreak investigation and monitoring ongoing epidemics. This routinely involves reconstructing the spatial and demographic processes from large data sets to help unveil the patterns and drivers of virus spread. Such phylodynamic inferences can however become quite time-consuming as the dimensions of the data increase, which has led to a myriad of approaches that aim to tackle this complexity. To elucidate the current state of the art in the field of phylodynamics, we discuss recent developments in Bayesian inference and accompanying software, highlight methods for improving computational efficiency and relevant visualisation tools. As an alternative to fully Bayesian approaches, we touch upon conditional software pipelines that compromise between statistical coherence and turn-around-time, and we highlight the available software packages. Finally, we outline future directions that may facilitate the large-scale tracking of epidemics in near real time.

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Introduction

Omics technologies the collective technologies used to explore the roles, relationships and behaviour of large families of cellular molecules such as genes and proteins [1] — have gained a strong foothold in virology and have brought about a considerable intensification of computational research. The wealth of genomic data produced by modern sequencing technologies dramatically increases our capacity to gain insights into the origins, evolution and epidemiology of viruses. Technological innovations have stimulated bioinformatics developments in support of efficiently producing high-quality genomes and deep sequencing data from targeted or metagenomic sequencing experiments, with many tools specifically developed for viral applications. Inference techniques that build on phylogenetic concepts to analyse the resulting genomic data in an evolutionary context have enjoyed a rich and longstanding history of development, and for rapidly evolving pathogens, this has evolved into a specific field called 'phylodynamics'. However, various challenges in analysing viral genomic data have emerged, such as the need to rapidly produce good-quality phylogenetic estimates for large virus data sets and the desire to meld different types of data and models in a phylogenetic framework in order to extract integrated information on epidemiological processes.

Pathogen phylodynamics was originally defined as a framework to study how epidemiological, immunological, and evolutionary processes shape pathogen phylogenies [2,3]. Owing to their rapid rate of evolution, epidemiological and immunological processes can impact viral evolutionary dynamics on short time scales, making their genomes sampled over time a rich resource for outbreak investigation. Developments in the field of pathogen phylodynamics now target various questions regarding the evolution and spread of pathogens. While such questions have been addressed for different viruses with a longstanding circulation history in humans (e.g. HIV [4–6], HCV [7,8] and influenza [9–11]), the ability to rapidly generate genomic data in a 'real-time' fashion now opens up such opportunities for viral outbreaks on smaller time-scales [12••,13]. These scenarios not only require rapid generation, but also rapid analysis, of the genomic data in order to inform outbreak responses in a timely fashion. For particular viruses with a longstanding transmission history in humans, large amounts of sequence data have accumulated in continuously growing databases (e.g. http://www.hiv.lanl.gov/ for HIV and https://www.gisaid.org/ for influenza), of which the analysis requires scaleable phylogenetic methodology.

Whether the interest is to reconstruct evolution on long or short time scales, there is always considerable benefit in calibrating the time scale in calendar time units. Therefore, time-stamped or 'heterochronous' data are the

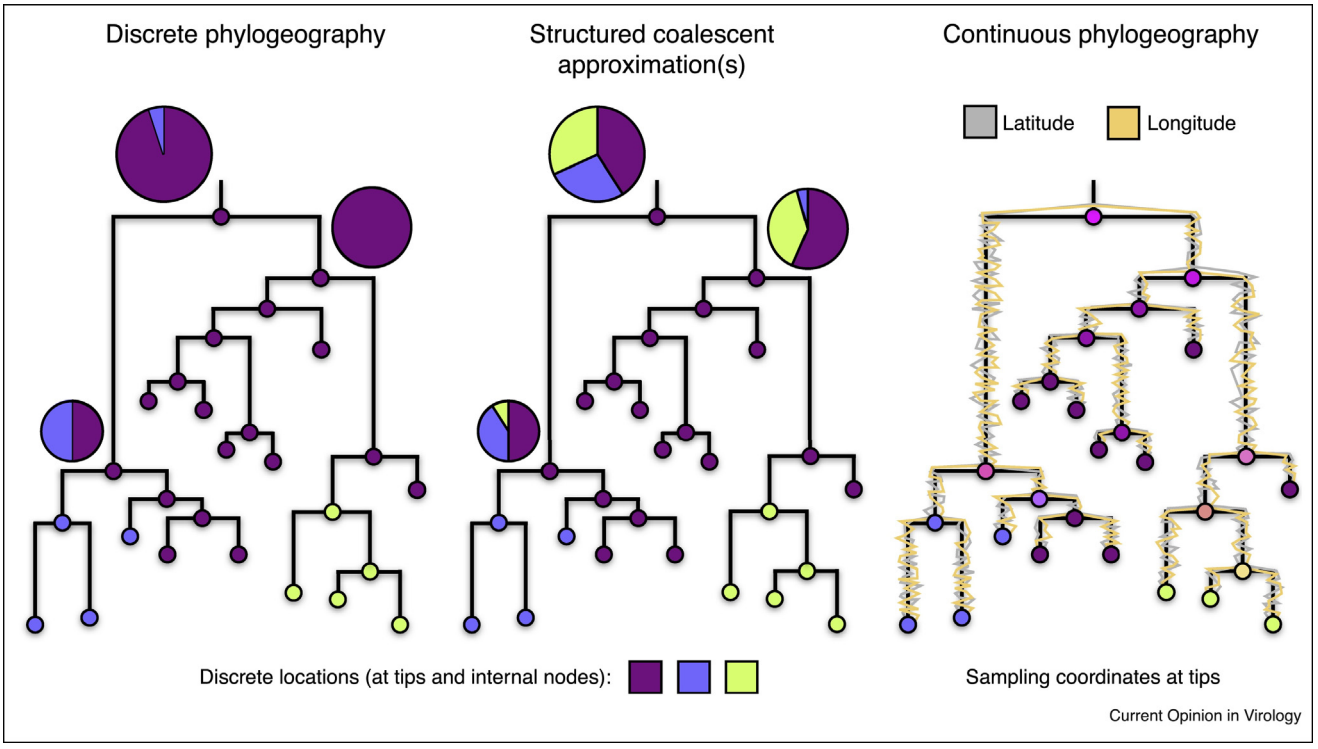
primary ingredients for pathogen phylodynamics because they inform molecular clock models, provide estimates of time-measured trees and date epidemic events [14]. Further, by connecting sampling locations to genetic data, phylodynamic approaches may elucidate the spatial pattern of connectivity underlying viral transmission that may be difficult to assess from surveillance data alone. Phylogeographic models used for this purpose may also find their use in reconstructing the evolution of other viral characteristics, such as host [15] and phenotype [16], and this is rapidly spurring novel developments in Bayesian evolutionary inference methodology. This has resulted for example in the ability to incorporate covariates of different phylodynamic processes, which shifts the focus from reconstructing patterns to testing possible explanatory processes (for an overview, see [17]). These are only few of the novel statistical and computational approaches that have emerged and that allow to extract evolutionary and epidemiological information from pathogen genomes. Here, we review the main inference methods currently in use in the field of pathogen phylodynamics, the software packages in which they are implemented, and tools to visualise the resulting estimates. We first discuss fully Bayesian inference frameworks, which are well known for their statistically coherent handling of sequence and associated trait data, and then consider conditional approaches involving software or pipelines that assume a given topology to obtain divergence time or other phylodynamic estimates.

Bayesian inference of pathogen phylodynamics

Phylogeographic inference

Bayesian phylogeographic inference is a widely used approach to estimate epidemic origins and track geographic spread of pathogens based on discrete or continuous location data [18–20]. The most popular of the discrete phylogeographic approaches (or more generally 'discrete trait analyses'; DTA; Figure 1) consider migration as instantaneous movements (or jumps, [21]) occurring at different frequencies between the set of observed discrete sampling locations. DTA rely on continuous-time Markov chain models, which are also used to model sequence substitution, and can be extended through generalized linear modeling (GLM; [22]) to enable evaluating the relevance and impact of covariates on the dispersal *frequency*. This approach has proven useful in a number of settings, from demonstrating the complex interaction between influenza virus migration and host mobility [22,23] and identifying the drivers of rabies host jumping in American bats [15], to testing the association between virus movements and geographic, administrative

Figure 1



Conceptual representation of the most popular phylogeographic inference approaches. Left: discrete trait analysis for three sampled locations, placing much confidence on particular states on ancestral nodes; middle: structured coalescent approximations showing large uncertainty when reconstructing ancestral nodes; right: continuous phylogeographic inference employs principles from Brownian motion to reconstruct geographic coordinates (that differ from the sampled locations) at ancestral nodes.

and demographic variables during the 2013-2016 West African Ebola virus epidemic [24••].

Despite their popularity, DTA make a number of restrictive assumptions that can be inappropriate when applied to the migration of lineages between geographic locations. DTA potentially under-represent ancestral trait uncertainty and are known to be sensitive to biased sampling of subpopulations [25••] (Figure 1). An alternative approach to discrete phylogeography involves the structured coalescent, an extension of Kingman's coalescent [26] that builds on the standard Wright-Fisher model by specifying a number of discrete subpopulations, allowing location to explicitly affect the coalescent rate. While computational inference under such models remained restricted to only a few populations for a long time, various developments paved the way towards structured coalescent inference with improved statistical properties [27,28]. Recently, De Maio *et al.* [25••] have made inference under the structured coalescent far more efficient when confronted with larger numbers of subpopulations and migration events, although performing inference — including ancestral reconstruction — for high state spaces remains challenging. Their Bayesian structured coalescent approximation (BASTA; available in BEAST 2; [29]) employs a new model-based approach that achieves a close approximation to the structured coalescent, while still integrating over all possible migration histories in a computationally efficient manner. In a study of the validity and robustness of approximations to the structured coalescent, Müller *et al.* [30] present an exact numerical solution to the structured coalescent that — while currently computationally intractable for more than a few lineages and states — led to the development of an improved structured coalescent approximation and an accompanying implementation in BEAST 2 [29]. Müller *et al.* [30] show that, in cases of biased sampling, their method allows for unbiased inferences of migration rates as opposed to previous approximations.

Different from DTA and structured coalescent approaches, continuous diffusion models allow ancestral reconstruction of the spatial history that is not limited to the observed sampling locations (Figure 1). Extensions of the standard Brownian diffusion process that model branch-specific variation in dispersal rates [19] can provide a more realistic description of the spatial diffusion process, which contributed to the popularity of continuous diffusion models (e.g. [31,32]). While the use of such relaxed random walk (RRW) diffusion processes is also very useful for modeling other continuous trait data, they may not appropriately describe the evolutionary process in certain situations. For example, the evolution of influenza antigenic change towards different optima through time or the population evolutionary process towards increased resistance of HIV-1 against neutralization may invalidate the assumption of a zero-mean displacement of Brownian diffusion and its RRW extension. To address this by incorporating directional trends, Gill *et al.* [33] provide a continuous diffusion model that permits permits multiple directional trends on a phylogenetic tree and allows to infer the number of directional trends supported by the data as well as the locations of trend changes.

Complementary to discrete phylogeographic testing, modeling migration as a continuous process allows to test for an impact of external features on the virus dispersal *velocity*. This has recently been achieved by comparing the time elapsed over the branches of the phylogeny with the environmental distances computed between the corresponding edge nodes in a *post hoc* fashion [34••]. The environmental distances are derived from maps that reflect the spatial heterogeneity of the tested feature, and the procedure is repeated over a set of plausible trees to accommodate phylogenetic uncertainty. Similarly, Jacquot *et al.* [35] show how to accommodate variables that lack a spatial component. These approaches have for instance been used to investigate the environmental drivers of rabies virus (RABV) spread in the North-East American raccoon population (Figure 2) [36] and elsewhere [37] and bluetongue virus spread in Europe [35].

Reconstructing viral population dynamics

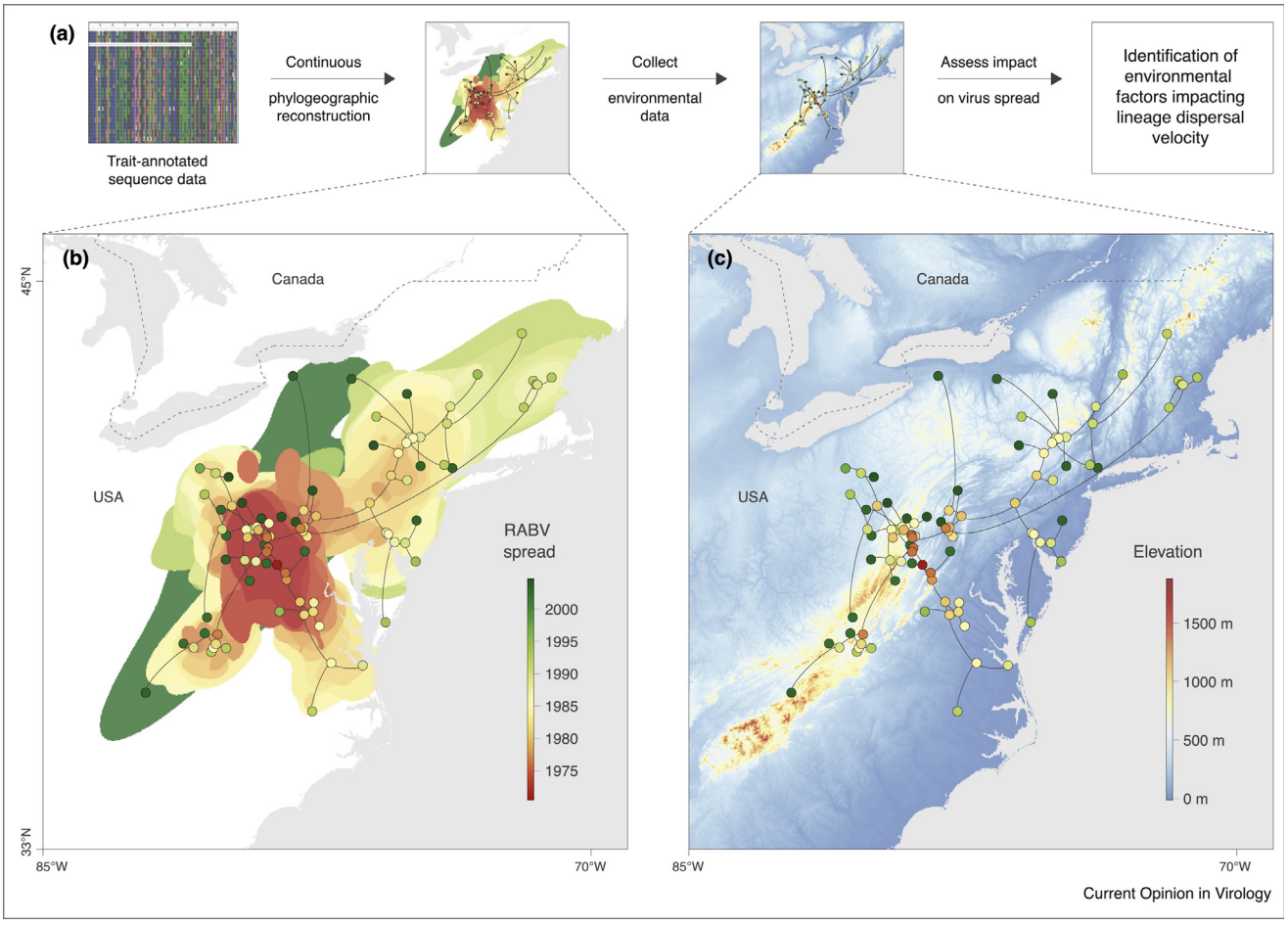
The shape of time-measured phylogenies reflects the dynamic process of pathogen transmission through time. This is the premise of coalescent or birth-death models that are widely used to extract phylodynamic information from viral genetic data in a quantitative manner. A rich history of coalescent development has culminated in flexible models that can accommodate complex dynamics (i.e. the 'sky' models, [39–41]). In several studies, these approaches have allowed interpreting viral spread in a historical setting, pointing at factors that could have contributed to transmission [42,43]. Recent developments seek to more formally examine explanatory variables of viral population dynamics, for example by incorporating covariates into the inference procedure [44,45].

Another promising line of research involves coupling coalescent or birth-death models to complex models of infectious disease dynamics. This builds on earlier work that connects tree generative models to compartmental models (i.e. SI, SIS or SIR models) in order to estimate fundamental quantities like the basic reproductive number (R_0, [46–50]). By adopting novel statistical techniques such as particle filtering (or Sequential Monte Carlo, SMC), prevalence and incidence trajectories [51] or parameters of tailored transmission processes can now be estimated from genetic data [52••].

Software packages

Many phylodynamic inference approaches have been implemented in a Bayesian inference framework,

Figure 2



Workflow to assess the impact of environmental factors on lineage dispersal velocity, with an example of rabies spread in North-East American raccoons. **(a)** Software pipeline to determine environmental impact on lineage dispersal velocity, based on continuous diffusion modeling (either through joint inference or conditional on an existing tree). **(b)** Reconstruction of spatio-temporal diffusion of rabies virus spread in North-East American raccoons, based on a data set of 47 viral sequences [38]: mapped maximum clade credibility (MCC) tree and 95% highest posterior density (HPD) regions obtained from continuous phylogeographic inference; tree nodes and corresponding HPD regions are coloured according to time. **(c)** The MCC tree mapped over environmental data (in this case an elevation map).

allowing to produce estimates with adequately quantified uncertainty for full probabilistic models, which can integrate timed evolutionary histories of sequence evolution under different molecular clock models with trait evolutionary and demographic processes. As indicated earlier, these processes can also be extended to take into account covariates for efficient hypothesis testing. The Bayesian Evolutionary Analysis by Sampling Trees (BEAST) 1 (recently released version 1.10; [53•]) and 2 (currently at version 2.5.0; [29]) software packages have become widely adapted for performing Bayesian phylogenetic and phylodynamic inference from genetic sequence data. Both versions are now developed largely independently, with somewhat different interests such as the modularity and extensibility of the code base through a plug-in package system in BEAST 2 and a focus on data integration in BEAST 1 (we refer to [53•] for more details on the relation of BEAST 1 to BEAST 2).

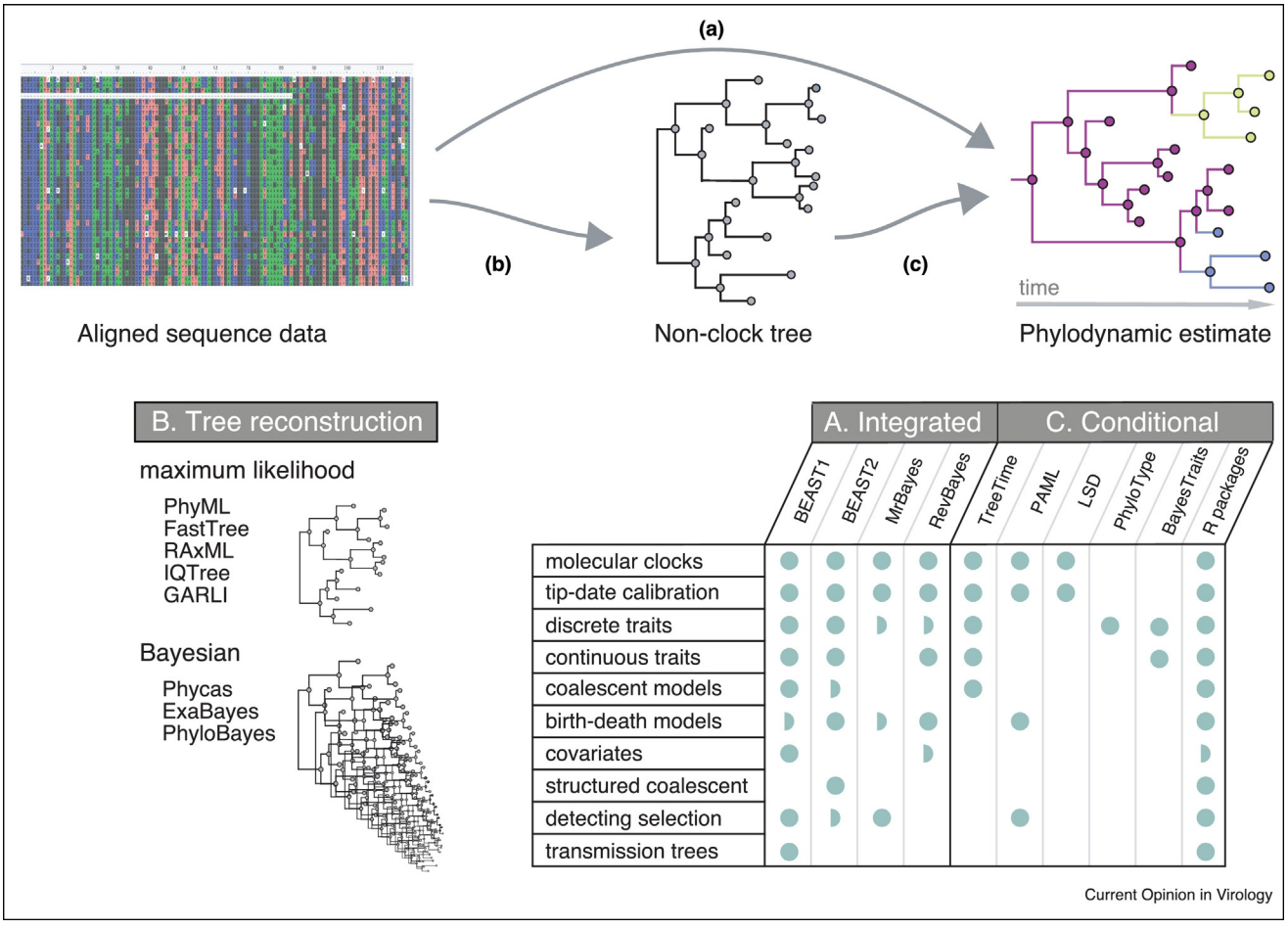
MrBayes [54]) and RevBayes [55] also offer strict and relaxed clock models to estimate divergence times from serially sampled sequences, but present fewer options for reconstructing trait evolutionary processes and coalescent and birth-death models for phylodynamic inference (Figure 3).

Faster methods for large-scale or near real-time phylodynamics

Methodology

Alternatives to integrated Bayesian approaches consist of software pipelines that reduce computation time by combining non-clocklike (fast) maximum-likelihood (ML) or

Figure 3



Schematic overview of phylodynamic estimation procedures and popular software implementations. Integrated approaches **(a)** combine a wide range of models to produce time-stamped phylogenetic trees along with other estimates, such as ancestral trait reconstruction. Conditional approaches first estimate a phylogeny **(b)** that serves as input for software packages that require a fixed phylogenetic tree for phylodynamic inference **(c)**. Maximum likelihood methods produce a point estimate of the topology whereas Bayesian methods estimate a set of evolutionary histories that are all plausible explanations of the sequence relationships. We list different modeling and analysis options for integrated and conditional approaches; half circles indicate limited capacity for the corresponding feature relative to other software packages.

Bayesian tree estimation with divergence time estimation using molecular clocks, ancestral trait reconstruction and estimation of effective population size over time, to name a few (see Figure 3). This entails the initial estimation of one or more unrooted non-clocklike phylogenetic trees from solely the sequence data, which are then treated as known without error in further downstream analyses. By combining bootstrap resampling [56] with ML methods or by taking a Bayesian approach to the tree estimation it is possible to incorporate phylogenetic variability in the *post hoc* analyses. Although quantifying uncertainty is desirable in many hypothesis testing settings as it avoids the information loss and bias associated with the use of a single tree estimate, procedures that produce point estimates may be the only practical way to analyse very large data sets.

Software packages

Popular maximum likelihood and Bayesian software packages geared towards tree inference are listed in Figure 3. A number of these exploit high-performance computational libraries to more efficiently use the available computer hardware (see Box 1). Different conditional approaches take a tree as input to produce a similar estimate, but they may employ different models or estimation procedures. For instance, TreeTime [57], PAML [58] and LSD [59••] can use time-stamped sequences to rescale branch lengths from substitutions per site into calendar time units. Likewise, both PhyloType [60] and BayesTraits [61] may prove useful for reconstructing for example the spatial spread of an epidemic. While these software tools focus on a selected subset of inference options, the modular setup of the *R* software has

Box 1 High-performance computational libraries

The past decades have seen the rise of multi-core processors, both in the CPU and GPU processor markets, enabling massively parallel computations that can be exploited by phylogenetic and phylodynamic software packages. Applications such as IQ-TREE [65] and RaxML/ExaML [66,67] are well known for their use of such architectures to boost performance using a parallel MPI (Message Passing Interface) system. While each of these applications contain their own application-specific software routines to exploit multi-core hardware systems, high-performance computational libraries remove the need for such application-specific code to parallelise computations across multiple processor cores and graphics processing units (GPUs). The BEAGLE (Broad-platform Evolutionary Analysis General Likelihood Evaluator) library is aimed at performing phylogenetic calculations for existing software packages to make more effective use of available multi-core hardware, including GPUs [68]. Since its release, BEAGLE has been adopted by BEAST 1 [53•], BEAST 2 [29], MrBayes [54], PhyML [69] and GARLI [70].

Alternatively, the Phylogenetic Likelihood Library (PLL; [71]) is a highly optimized application programming interface for performing likelihood-based phylogenetic inference, targeting multi-core processors and host processors such as the Intel Xeon Phi using MPI. The PLL has so far been integrated with two phylogenetic software packages: DPPDiv [72] — a Bayesian tool for estimating divergence times on a fixed tree topology — and IQ-TREE [65]. A recent extension of PLL is currently known as LibPLL, a lower-level redesign of PLL that can make use of advanced instruction set extensions to the x86 architecture to improve computational performance [73].

facilitated the development of several dedicated packages capable of (some of) the various post-processing steps indicated in Figure 3. This includes, but is not limited to, *ape* [61], *phytools* [63], *treedater* [64], *rcolgem* [46], *skygrowth* [45] and *phyland* (Frost *et al.*, unpublished). Other useful packages for phylogenetic and phylodynamic inference can be found at https://cran.r-project.org/web/views/Phylogenetics.html and http://www.repidemicsconsortium.org/.

Visualisation tools

Many software tools have been developed to visualise phylogenetic trees, but discussing these is beyond the scope of this review. Instead, we focus on specific applications within the phylodynamics community. Following the initial development of models that accommodate spatial diffusion in discrete and continuous space (e.g. [18,19]), SPREAD (Spatial Phylogenetic Reconstruction of EvolutionAry Dynamics) was developed as a cross-platform application to summarize and visualize Bayesian phylogeographic reconstructions [74] by producing input for Google Earth. Novel web technologies, such as JavaScript and Data-Driven Documents (D3), have enabled the development of browser-based visualization packages. Follow-up work on SPREAD resulted in SpreaD3, which enables modern and interactive web-based visualization of phylogeographic estimates as well as any phylogenetic trait history of interest [75••].

The popularity of browser-based tools is also illustrated by Microreact [76] and Nextstrain [77••]. The former is a web application that allows visualizing data sets consisting of any combination of trees, geographical, temporal and associated (meta)data [76]. Nextstrain aims to visualise epidemics as they unfold in as close to real time as possible by regularly polling public databases for the latest sequences and using fast maximum-likelihood reconstructions [78,66,57]. The framework consists of a database of viral genomes, a bioinformatics pipeline for phylodynamics analysis, and an interactive visualisation platform based on Python and JavaScript [77••]. Using this pipeline, Nextstrain's accompanying website http://www.nextstrain.org/ keeps track of the current state of emerging infectious diseases such as those caused by Zika and dengue viruses.

The computational and methodological advances that made phylodynamic inference possible for increasingly large data sets have also created a need to visualize large phylogenetic trees as well as any annotated information (e.g. virus and patient data). iTol and PhyloGeoTool are particular examples that address this need by supporting interactive navigation of large phylogenies and exploration associated clinical and epidemiological data [79,80].

Future directions

Future phylodynamic model developments will likely see an increasing emphasis on their scalability, as reflected by our discussion of conditional methods and high-performance computational libraries. For computationally demanding approaches, a promising avenue of research involves the development of techniques for updating existing phylogenies/analyses as new data are added. Various software packages allow to perform a likelihood-based insertion of new data on a branch of an unrooted fixed phylogenetic tree, be it sequences (pplacer; [81]) or short reads (EPA, which is integrated into RaxML; [82]), either by resorting to maximum-likelihood or Bayesian estimates or by performing faster approximate methods.

For Bayesian approaches, sequential Monte Carlo (SMC) may provide more formal approach to accommodate novel data into existing analyses in a flexible way [83]. The basic idea is that new sequences are attached to the growing phylogenetic tree following a proposal that relies on genetic similarity. Recently, Dinh *et al.* [84••] published theoretical results on the consistency and stability of such SMC methods for online Bayesian phylogenetic inference, offering important insights for future development. Fourment *et al.* [85] show that poor placement of new sequences within the existing phylogeny can result in poor results, and guided insertion strategies are needed to tackle this problem. Further research into this direction is needed to establish a flexible and easy-to-use online

Bayesian phylodynamic framework that offers clear performance benefits over current inference approaches.

Conflict of interest statement

Nothing declared.

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