

# Phylotocol: Promoting transparency and overcoming bias in phylogenetics

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The following report is based on our experience using phylotocol over the past 12 months. We are actively soliciting opinions on phylotocol and will not submit this manuscript to a journal before April 1, 2018 to give readers time to weigh in on the idea.

# Abstract

Research products that lack transparency and are influenced by confirmation bias lead to barriers that, when left unchecked, propagate throughout the scientific record and lead to wasted research effort. Phylogenetics is particularly vulnerable given its ever-evolving methodology and wide choice of options for conducting analyses. Great strides in transparency have been achieved in clinical research by the implementation of *a priori* protocols. Here we propose a similar approach—phylotocol—a straightforward, protocol-driven strategy tailored to the needs of phylogenetic studies. We provide a simple template and offer a flexible range of implementation frameworks, including preregistration options. Besides increasing transparency and accountability, phylotocol has the added benefits of improving study design and reproducibility, enhancing collaboration and education, and increasing the likelihood of project completion. The increased transparency afforded by wide adoption of an *a priori* system like phylotocol would have extensive benefits to science.

# Introduction

The importance of reproducibility in science has been written about extensively over the past decade (Ioannidis et al., 2014; Markowetz, 2015; Nosek et al., 2015; Ihle et al., 2017), but its counterpart, transparency, has received considerably less attention. While reproducibility is important to ensure that a study's methodology is sound, transparency is critical to avoid confirmation bias. Reproducibility without transparency is not sufficient to guarantee reliable scientific output (Parker et al., 2016). For example, if confirmation bias leads to the reporting of only a subset of results along with the methods required to generate those results (reporting bias), the study is technically reproducible, but lacks transparency. This is a problem across scientific disciplines, and is particularly important in phylogenetics.

Inferring relationships between genes, genomes, and species is essential for a fundamental understanding of biology. In the nearly 70 years since Hennig formalized phylogenetics (Hennig, 1950; Hennig, 1965), the field has matured immensely through the continuous development and improvement of algorithms, models, and data manipulation strategies (Whelan et al., 2001). This continuous development has led to many advances in phylogenetic methodology. However, the lack of methodological standardization has left open the door to selective reporting driven by confirmation bias.

For most phylogenetic analyses it is possible to justify a multitude of approaches from a seemingly infinite combination of algorithms, models, and data manipulation techniques. Tree reconstruction involves optimization algorithms that can employ a number of different criteria to measure the fit between tree and data including distance, parsimony, maximum likelihood, and Bayesian inference (Felsenstein, 2004). Similarly, model choice is far from standardized. There are a large number of single-matrix models (e.g. JTT) and several criteria to determine the most appropriate (e.g. AIC and BIC) (Page & Holmes, 2009). Likewise, partitioning and mixture models, which are increasingly favored over single-matrix approaches,

involve a plethora of decisions for which many seemingly sound arguments can be invoked (Blair & Murphy, 2010). Data manipulation, in particular, is far from standardized; common practices include: removing unstable and quickly evolving taxa or genes, using only characters that recover 'known' clades, including only slowly evolving characters, and using conserved amino acid substitutions or indels (Salichos and Rokas, 2013). Finally, this issue is not exclusive to tree reconstruction; other phylogenetic applications (e.g., molecular clock analyses, ancestral state reconstruction, hypothesis testing, and detection of selection) each involve several competing approaches (Baum & Smith, 2012). These myriad choices in the field exacerbate susceptibility to confirmation bias.

Decisions during the course of a phylogenetic study are often made immediately prior to a particular analysis rather than at the conception of the project. Even more problematic is the temptation to adjust methods immediately *after* an analysis in reaction to an unexpected result. A common assumption is that unanticipated phylogenetic outcomes are the result of an error in some aspect of the analysis. This can lead to reactionary adjustments in algorithm, model choice, or data manipulation. These types of decisions are problematic because they are greatly influenced by investigators' biases.

In clinical trials, where the outcomes of a study can lead to decisions that put human lives at risk, biases have been explicitly controlled for and transparency and reproducibility ensured through the requirement of *a priori* protocols that outline objective(s), design, methodology, statistical considerations, and study organization (Laine et al., 2007; Zarin and Tse, 2013; Zarin et al., 2017). Protocols must be registered to a governmental regulatory agency, funding agency and/or an institutional review board prior to the start of a study. Any changes to a protocol require explicit justification and an updated version of the protocol. Many journals require protocols to be published with clinical trial publications, providing further motivation for their implementation. Protocols greatly reduce, if not eliminate, the potential for researcher bias and in the process ensure the safety of subjects and the integrity of the trial.

Recently, preregistration of research designs has been put forth as a framework for promoting transparency in the fields of Behavioral Ecology (Ihle et al. 2017), Ecology and Evolution (Parker et al 2016), and Psychology (Hartgerink and Wicherts 2016). The proposed measures are largely comparable to protocol registration in clinical trials and provide effective means to promote transparency in each particular field. Responses to these efforts have been positive (e.g. Blumstein, 2017; Parker and Nakawaga, 2017; Forstmeier, 2017), negative (Koenig, 2017), and mixed (Cockburn, 2017; Hatchwell, 2017). The biggest barriers to widespread adoption to preregistration is the administrative effort associated with its implementation, perceived restrictions on scientific creativity and exploratory analyses, and the potential for project ideas to be scooped. A major challenge for preregistration and similar approaches moving forward is balancing the increase in transparency with the ease of implementation and flexibility.

Here we argue that the field of phylogenetics would benefit tremendously from increased transparency and propose an *a priori* protocol-driven approach—phylotocol—that can be easily incorporated into phylogenetic studies. Below we

describe a phylotocol template in detail, propose a set of guidelines for its use, and discuss how it can reduce bias and improve transparency and reproducibility in phylogenetics with minimal burdens on researchers' time.

The primary objectives of phylotocol is to add transparency to phylogenetic studies by front-loading decisions and foster accountability by requiring that changes made during the course of a study are documented and justified. There are also several ancillary benefits associated with phylotocol that we describe below. The goal of this manuscript is to initiate a dialogue about transparency in phylogenetics and present a framework that may help the community to implement research transparency. To this end, we solicit feedback on our proposed phylotocol and encourage suggestions on how to formalize an unobtrusive, yet effective, system for improving transparency, accountability, and reproducibility in our work.

The figure displays a two-page Microsoft Word template for a phylotocol. The left page includes a header for 'Protocol Title' and 'Version v.<v> <DD Month YYYY>'. It features a 'LIST OF ABBREVIATIONS' table with columns for '<abbreviation>' and '<full word or phrase>'. Below this is section '1 INTRODUCTION: BACKGROUND & SCIENTIFIC RATIONALE', which contains sub-sections '1.1 BACKGROUND INFORMATION' (with a placeholder '<insert background>'), '1.2 RATIONALE' (with a placeholder '<insert why the study is needed>'), and '1.3 OBJECTIVES' (with a placeholder '<insert goals to acheive by the end of the study>'). The right page continues with section '2 STUDY DESIGN & ENDPOINTS' (with placeholders for '<insert all proposed analyses>', '<bulleted list of command lines>', and '<include custom scripts in the repo>'), section '3 WORK COMPLETED SO FAR WITH DATES' (with a placeholder '<insert prelim analyses performed as part of this study>'), section '4 LITERATURE REFERENCES' (with a placeholder '<insert references>'), and section '5 PHYLOTOCOL AMMENDMENT HISTORY' (with a table for tracking changes, including columns for '<version>', '<date>', and '<significant revisions>').

**Figure 1: Phylotocol template.** Based on the NIH clinical trial protocol, phylotocol layout has been tailored to match the needs of phylogenetic research. The format and the amount of information required are flexible. The figure displays the Microsoft Word version of the template, but there is also a markdown version. A phylotocol can be uploaded to any online repository or used as the basis for preregistration (see Implementation section below).

## Anatomy of phylotocol

The template phylotocol is based off the clinical trial protocol established by the National Institutes of Health (Hudson et al., 2016; National Institutes of Health, 2017a; National Institutes of Health, 2017b) and has seven major sections: 1) Title, 2) Abbreviations, 3) Introduction, 4) Study design, 5) Steps completed, 6)

References, and 7) Appendix with version history (Figure 1). While there are certainly other sections that could be added, the minimalist approach of phylotocol reduces unnecessary burdens and is therefore an important motivator for potential users. The format is flexible and can be customized to the requirements, preference, and computational expertise of a particular user. We have created versions of the template in both Microsoft Word and markdown formats.

Phylotocol is not a duplication or replacement for a methods section. Instead, it outlines all decisions that could affect the final outcome of a set of studies. Some common decisions include: (1) central hypotheses, (2) which taxa will be included, (3) which methods will be applied, and (4) which models will be implemented. In addition, many parameter settings (e.g. number of starting trees, seeds used for programs with random processes, minimum occupancy of phylogenomic matrices) can influence the outcome of a study, and therefore should be considered for inclusion in a phylotocol. It is also important to anticipate difficult decisions; for example, when applying different algorithms, models, etc. to the same data matrix, it is imperative to provide explicit criteria for how to evaluate conflicting results.

## Primary objectives of phylotocol

### *Transparency*

By specifying the objectives and outlining the full methodology before any analyses are started, the phylotocol promotes transparency and reduces biases on the part of researchers. Once the project is underway, any changes to the analyses are documented in the phylotocol. This ensures that all steps in the analysis pipeline are made available, even those that failed, were replaced by other methods, or that motivated a downstream analysis but were not themselves included in the final manuscript. When decision-making processes are transparent, readers, reviewers, and editors are better able to contextualize, interpret, and evaluate the merits of a study.

### *Accountability*

Transparency generates accountability (Mellor et al., 2018). In phylogenetics, as in other fields, it can be tempting to discount results that conflict with prior assumptions and then perform additional analyses until an expected result is realized. The implementation of phylotocol helps alleviate this temptation by holding researchers that make these types of decisions publically accountable.

## Auxiliary benefits of phylotocol

Although the primary goals of phylotocol are transparency and accountability, the process offers a number of additional benefits.

### *Designing a better study*

Outlining each step of a study in a phylotocol *a priori* can bring about a more robust plan. The process of explicitly transcribing procedures and guidelines for the interpretation of results can identify important steps that may have previously been

overlooked. In addition, logical flaws in experimental design can be recognized. Catching these obstacles early in the process can lead to huge savings in time and/or money.

### *Reproducibility*

Although a phylotocol need not contain all the details necessary to carry out a study, it can be helpful in producing a highly reproducible set of methods. Unlike in wet-lab based experimental biology, keeping a detailed notebook is less commonplace in phylogenetics. When a phylotocol is implemented, all steps in the analyses are documented, and therefore reported more accurately. Furthermore, at the conclusion of a study, a phylotocol serves as a key reference document for constructing the methods section of a manuscript.

### *Collaboration*

Creating and following a phylotocol can facilitate seamless collaborations among research groups. When designing a project, early drafts of a phylotocol can help with the planning phases. Getting input early from collaborators can strengthen a study while also ensuring that effort between collaborators does not overlap. Listing all steps also allows computational, personnel, budgetary, and/or other resource needs to be assessed. Using phylotocol leads to efficient planning and distribution of effort (e.g. computation allocation) across collaborators. Finalizing the steps for analyses before a project is initiated ensures that all members of a team are in agreement and on task, potentially avoiding misunderstandings and/or conflicts down the line.

### *Education*

Phylotocol provides an excellent framework from which to train early career scientists. By making all decisions at the start of a project, it becomes natural to either collectively draft a phylotocol or for students to prepare a first draft and discuss all decisions before the work is initiated. During this process, students gain a deeper understanding of the components of the study and have a roadmap from which to work throughout the project. Previous phylotocols are also useful references for new lab members who want to quickly get up to speed on how the lab performs particular analyses. These previous phylotocols can act as a template from which to start new analyses, particularly when they include command lines for commonly used programs. Phylotocols can easily be incorporated into undergraduate and graduate courses as a tool to teach methodology, the importance of robust experimental design, and to reinforce the concepts of transparency and reproducibility in science.

### *Project completion*

The inherent open-endedness of scientific endeavors can often be intimidating and create a barrier to project completion. Implementing a phylotocol can remove this barrier by providing explicit starting and stopping points for a project and the motivation to complete the study as planned. The phylotocol quantifies the number of objectives a project requires and helps researchers prioritize each step. Beginning and completing a manuscript for the project will also be less daunting because the



background information, study justification, methods, and references will already be compiled in the phylotocol. Starting new projects hinders the ability to complete existing projects; a phylotocol serves as a gentle impediment to spontaneously starting tangential projects and therefore increases productivity.

## Implementation

Scientists can use several strategies to implement phylotocol. The simplest is to create a document on one's computer at the beginning of a study and use it as a private guide for the study. This can be incredibly powerful, but does not maximize transparency since others have to trust that the analyses were planned *a priori*. Another strategy is to post a phylotocol to a public data repository (e.g. FigShare, Zenodo) or a software repository (e.g. GitHub) before beginning a study. An advantage of online data repositories is that a digital object identifier (DOI) can be issued for a phylotocol (versioning is also available for phylotocol updates). These platforms also provide free private space that can later be made public with a timestamp. A development platform like GitHub includes timestamps and makes updates to a phylotocol a more natural process (especially with the markdown version of phylotocol), but GitHub currently charges for private space and does not include the built-in ability to generate a DOI (although this can be achieved in conjunction with Zenodo or FigShare).

Another option is to post a phylotocol within the Open Science Framework (OSF) (Nosek et al. 2015). This platform has a specific interface for preregistration; we recommend choosing the "Open-Ended Registration" option and pasting a text version of phylotocol into the box. Preregistration includes advantages in addition to those provided in other repositories. The OSF registry has a built-in embargo system allowing a registered project to remain private for up to four years. One particular advantage of the OSF is the ability to connect to other workflow management tools (e.g. Dataverse, Dropbox, figshare, Github, and others, see: <http://help.osf.io/m/addons>), so that work from disparate members of a research team can be connected, persistently stored, and cited in one location. When it is ready to disseminate early findings, any file on the OSF can be shared as a preprint prior to formal publication in a journal (<https://osf.io/preprints>).

## Discussion

### *Motivations for Individual Researchers*

The ultimate goal of researchers is to make discoveries and formulate theories that stand up to rigorous testing, and eventually become widely accepted as truth. The suspicion of bias, especially for controversial topics where two or more research groups report conflicting results, impedes this goal. By adopting transparent practices, researchers reduce the perception of manipulation and help cultivate confidence in the reliability and robustness of their science. In this way, transparent research practices like phylotocol help maximize research impact.

## 276 *Implications for Science*

277 Two main trajectories in science are building upon prior knowledge and  
 278 overturning existing perspectives. When inaccuracies in the scientific record are due  
 279 to confirmation bias, the progress of science is impeded. First, research built upon  
 280 published inaccuracies is typically doomed from the outset. Likewise, overturning  
 281 false conclusions requires considerable work, reducing time and funds that can be  
 282 applied to forward-looking efforts. It is difficult to estimate how much of the  
 283 scientific record is inaccurate due to confirmation bias in phylogenetics, but in the  
 284 absence of transparency, the potential for bias is enormous, as is the potential for  
 285 falsities and wasted research effort. As such, efforts like phylotocol to decrease bias  
 286 in phylogenetics could potentially lead to greater productivity in our field by  
 287 reducing superfluous research.

## 289 *Conclusion*

290 Phylotocol is a powerful tool to increase transparency and accountability in  
 291 phylogenetics. It has great potential to improve how phylogenetic research is  
 292 conducted, interpreted, communicated, and perceived. The implementation that we  
 293 outline is straightforward and offers a range of auxiliary benefits, including making  
 294 contributions to study design, reproducibility, collaboration, and education.  
 295 Phylotocol can bolster scientific productivity both at the level of the individual  
 296 researcher as well as in the broader context of the scientific record. While  
 297 phylotocol is a simple idea, its repercussions could be far reaching if widely  
 298 implemented.

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