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Abstract

Our understanding of the evolution of many microbes has been revolutionised by the molecular clock, a statistical tool to infer evolutionary rates and timescales. The fundamental assumption of molecular clock models is that the rate at which substitutions accumulate can be described by a statistical process. In all molecular clock models evolutionary rates and times are unidentifiable, and therefore 'calibration' information is essential to obtain estimates in calendar time.

For many microbes, the sequence sampling times themselves can be often used for calibration. Phylogenetic tests of temporal signal are often used to decide whether such calibrations are reliable. Critically, in addition to the calibration information, the full Bayesian phylogenetic model also includes the molecular clock model and a branching process (tree prior). As a result, there are multiple sources of information that are difficult to untangle.

We assessed temporal signal in three microbial data sets of human and animal diseases with a range of evolutionary characteristics and with ancient DNA sequences; *Powassan virus* (POWV), the cholera bacetrium (*Vibrio cholerae*), and the syphilis bacterium (*Treponema palladium*). We found that the tree prior can have a substantial impact on whether temporal signal is detected. To investigate this problem we conducted extensive simulations and calculated the sensitivity and specificity of these tests under several tree priors.

We find that highly informative sequence data sets are generally robust to the tree prior. In contrast, in data sets with low information content, choosing a prior that is highly informative and inconsistent with the data can result in the false rejection of temporal signal.

We propose prior sensitivity analyses and prior predictive simulations to determine whether the prior is reasonable and to improve the detection of temporal signal and maximise the information that can be drawn from molecular sequence data sets.

Author summary

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Introduction

Molecular sequence data have been essential to unravel the evolutionary history of many organisms. The molecular clock is a statistical tool that posits that molecular evolution,

in the form of substitutions, follows a statistical process. For example, under the simplest molecular clock model, known as the strict clock, substitutions accumulate linearly over time along a lineage, such that the evolutionary rate is constant over time [1]. At the other end of the spectrum, relaxed molecular clocks allow every lineage in a phylogenetic tree to display a different evolutionary rate ([2] and reviewed in [3]).

All molecular clock models have a fundamental limitation, where evolutionary rates and times are unidentifiable. That is, there exist an infinite number of combinations of evolutionary rates and times that are compatible with an amount of evolutionary divergence [4,5]. For this reason, external information, known as a molecular clock calibration is necessary to produce estimates in calendar units. As a case in point, consider two sequences whose genetic divergence from their most recent common ancestor is 10 subs/site. In the absence of calibrating information it is impossible to know *how rapidly* they evolve and *when* they diverged. The calibration can be a known evolutionary rate, such as 1 subs/site/year, or a divergence date, such as 1 year before present. The genetic distance can be divided by the evolutionary rate to infer the divergence time to infer a time to the most recent common ancestor of 10 years, or the genetic distance can be divided by the divergence date to infer the evolutionary rate, 10/subs/site/year in this case.

The finding that some organisms accumulate substitutions in a measurable timescale prompted the use of sequence sampling times for calibration [6]. The motivation behind this practice is that sequence data collected at different points in time would have accumulated a corresponding number of substitutions. With the example above, a sequence collected six months of the common ancestor would have accumulated 5 subs/site (10 subs/site/year \times 0.5 years = 5 subs/site), whereas one collected after 1 year would have accrued 10 subs/site (10 subs/site/year \times 1 year = 10 subs/site). As a result, sequence sampling times act as a time-calibration that is intuitively informative about the evolutionary rate.

A fundamental question to warrant the use of sampling times for molecular calibration is the extent to which a data set behaves as a measurably evolving population.

Introduction up to here. We need more information on measurably evolving populations, tests of temporal signal (including regression), and their relevance in light of genome data

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Discussion 66

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Materials and methods 73

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