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To the Editors of *PLOS Computational Biology*

We would like to submit our article entitled 'Assessing the effect of model specification and prior sensitivity on Bayesian tests of temporal signal' for consideration as a *Research Article* to *PLOS Computational Biology*.

Phylogenetic analyses of rapidly evolving microbes and those involving ancient DNA often use molecular sequence sampling times for calibrating the molecular clock and estimating evolutionary rates and time scales. The key requirement for this approach is that the period of time over which the molecular samples were collected captures a sufficient amount of evolutionary change to inform the resulting estimates.

BETS (Bayesian evaluation of temporal signal) is one of the most popular approaches to determine whether a data set has temporal signal. Its premise is to compare the statistical fit of two models, one with and one without sampling times. In our study we investigate the sensitivity of BETS to the choice of prior distribution, with the following key results:

- Using empirical data sets from two bacteria (*Vibrio cholerae*, *Treponema pallidium*), and one virus (*Powassan virus*) we demonstrate that BETS can be highly sensitive to the choice of prior distribution. This situation sometimes also impacts the Bayesian selection of the molecular clock model.

- BETS has an high false positive rate under some prior distributions that are commonly used in empirical studies.

- False positives result in a dramatic overestimation of the evolutionary time scale and a corresponding underestimation of substitution rates.

- Choosing a reasonable prior distribution can eliminate classification errors in BETS.

- We demonstrate that conducting prior predictive simulations and prior sensitivity analyses is essential for choosing appropriate priors in empirical studies.

We believe that our study will become a key reference for improving our understanding of the long-term evolution of microbes, particularly when ancient DNA data are used, and that it will be highly relevant to readers of *PLOS Computational Biology*.

Yours sincerely,

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