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To the Editors of *Molecular Biology and Evolution*,

We would like to submit our article entitled 'The phylodynamic threshold of measurably evolving populations' for consideration as a *Methods* article.

The molecular clock is an essential tool for understanding the pace and time scale over which organisms evolve. All molecular clock methods require calibration information to disentangle genetic distance and time. Many bacteria and viruses accrue mutations so rapidly that their sampling times can be used for such molecular clock calibration. That is, there is a quantifiable amount of molecular evolutionary change over the course of the sampling period, typically months or years.

Two concepts have been widely adopted to refer to organisms for which sampling times can be used for molecular clock calibration: those of measurably evolving populations and the phylodynamic threshold. Both have become widely popular in the research community. The original study using the term 'phylodynamic threshold' has over 440 citations since 2020, whereas that introducing the concept of a 'measurably evolving population' has 447 citations since 2003.

Importantly, measurably evolving populations and the phylodynamic threshold are intertwined concepts whose practical consequences are poorly understood. In our study, we address this problem with the following key outcomes:

- We demonstrate that temporal signal—whether the population is measurably evolving or has attained its phylodynamic threshold—determines the information content of the data, not necessarily the reliability of the inferences.
- Contrary to the dogma in the field, some forms of sampling bias increase uncertainty but do not necessarily result in systematically biased estimates.
- In empirical data of Hepatitis B virus, we find that increasing the number of ancient samples can improve precision and accuracy, even when the data are subsampled.

Overall, our study provides important guidelines for improving the practice of molecular clock analyses of rapidly evolving organisms. We believe that this work will become a key reference in molecular evolution and that it will be highly relevant to readers of *Molecular Biology and Evolution*.

Yours sincerely,

Ariane Weber, Julia Kende, Sanni Översti, and Sebastian Duchene