MOLECULAR ECOLOGY

Molecular Ecology (2015) **24**, 6007–6012

NEWS AND VIEWS

COMMENT

Time-dependent estimates of molecular evolutionary rates: evidence and causes

SIMON Y. W. HO,* SEBASTIÁN DUCHÊNE,* MARTYNA MOLAK† and BETH SHAPIRO‡ §

School of Biological Sciences, University of Sydney, Sydney, NSW, Australia; †Museum and Institute of Zoology, Polish Academy of Sciences, Warsaw, Poland; ‡Department of Ecology and Evolutionary Biology, University of California, Santa Cruz, California, USA; §UCSC Genomics Institute, University of California, Santa Cruz, California, USA

We are writing in response to a recent critique by Emerson & Hickerson (2015), who challenge the evidence of a time-dependent bias in molecular rate estimates. This bias takes the form of a negative relationship between inferred evolutionary rates and the ages of the calibrations on which these estimates are based. Here, we present a summary of the evidence obtained from a broad range of taxa that supports a time-dependent bias in rate estimates, with a consideration of the potential causes of these observed trends. We also describe recent progress in improving the reliability of evolutionary rate estimation and respond to the concerns raised by Emerson & Hickerson (2015) about the validity of rates estimated from time-structured sequence data. In doing so, we hope to dispel some misconceptions and to highlight several research directions that will improve our understanding of time-dependent biases in rate estimates.

Keywords: mutation rate, substitution rate, pedigree, ancient DNA, Bayesian phylogenetics, calibration

Received 12 April 2015; revision received 30 June 2015; accepted 17 July 2015

Introduction

Estimating rates of molecular evolution forms an important basis for resolving evolutionary and demographic timescales. However, heterogeneity in substitution rates is an intrinsic feature of molecular evolution. Rate variation across sites and among lineages is well recognized (e.g. Gaut *et al.* 2011), but estimates of rates also appear to scale negatively with the age of the calibration on which they are based (Ho *et al.* 2005, 2011a). The most striking examples of this pattern have been the disparities between mitochondrial estimates of rates of spontaneous mutation and of the rates at which these mutations are fixed as substitutions over time (Ho & Larson 2006). Here, we respond to a recent critique by Emerson & Hickerson (2015), who claim that there is a lack of support for such a time-dependent pattern in rate estimates. Despite making this claim, the authors present a detailed consideration of factors that can cause time-dependent biases in rate estimates. We take this opportunity to summarize the evidence for time-dependent patterns in rate estimates and present an update on recent work that has addressed this issue.

Correspondence: Simon Y. W. Ho, Fax: +61 2 93512175; E-mail: simon.ho@sydney.edu.au

Evidence of a time-dependent bias in rate estimates

Time-dependent biases appear to be a widespread property of molecular rate estimates, with studies finding evidence of such a pattern across a variety of taxonomic groups. The earliest findings were obtained in genetic studies of carnivores (Wayne *et al.* 1991), humans (Howell *et al.* 2003) and birds (García-Moreno 2004). Subsequent large-scale, data-rich analyses of mitochondrial DNA have revealed time-dependent biases in rate estimates from a broad cross section of taxa (Fig. 1), including insects (Papadopoulou *et al.* 2010; Ho & Lo 2013), primates and birds (Ho *et al.* 2005; Subramanian *et al.* 2009), fish (Genner *et al.* 2007; Burridge *et al.* 2008) and amniotes (Molak & Ho 2015).

A number of investigations of mitochondrial genome evolution within species have yielded evidence of time-dependent rate estimates. These include analyses of humans (e.g. Endicott & Ho 2008; Henn *et al.* 2009; Subramanian & Lambert 2011) and Adélie penguins (Subramanian *et al.* 2009). In their argument against the time dependence of molecular rates, Emerson & Hickerson (2015) point out that mean pedigree-based estimates of mitogenomic mutation rates in Adélie penguins are lower than those inferred from ancient DNA, but they did not report the substantial overlap in 95% credibility intervals between these estimates. Emerson & Hickerson (2015) acknowledge, however, that both of these evolutionary rate estimates greatly exceed those inferred from fossil-calibrated analyses of birds.

There is also compelling evidence of time dependence in rate estimates from the genomes of viruses (Li *et al.* 2007; Gibbs *et al.* 2010; Duchêne *et al.* 2014; Aiewsakun & Katzourakis 2015a,b) and bacteria (Comas *et al.* 2013; Biek *et al.* 2015) (Fig. 1), and it seems clear that accounting for these disparities in rates can reconcile some of the conflicting estimates of the evolutionary timescales of pathogens (Aiewsakun & Katzourakis 2015a). In viruses, a pattern of time-dependent molecular rates appears to hold across taxonomic groups and within lineages (Duchêne *et al.*

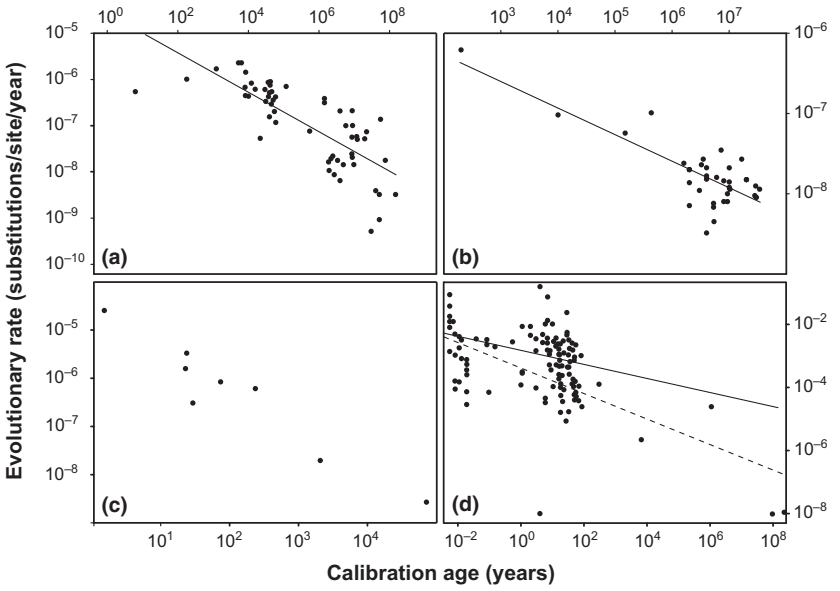

Fig. 1 Time-dependent patterns in rate estimates have been observed in a variety of taxonomic groups, including: (a) non-coding mitochondrial DNA from amniotes (data from Fig. 1b in Molak & Ho 2015), (b) mitochondrial DNA from insects (data from Fig. 2 in Ho & Lo 2013), (c) genomic DNA from bacteria (data from Fig. 4 in Comas *et al.* 2013) and (d) genomic RNA and DNA from viruses (data from Fig. 1a in Duchêne *et al.* 2014). Trend lines are based on those estimated in the original analyses of these data sets. In panel d, separate trend lines are given for RNA viruses (solid) and DNA viruses (dashed).

2014). For example, Duchêne *et al.* (2015b) found a time-dependent pattern of rate estimates within ten different lineages of RNA and DNA viruses, with similar trends observed in recent studies of Ebola virus (Park *et al.* 2015) and foamy viruses (Aiewsakun & Katzourakis 2015b). This might explain why rate estimates based on tip calibrations support evolutionary timescales that are much shorter than those suggested by other lines of evidence, such as codivergence between viruses and their hosts (e.g. Gifford 2012; Wertheim *et al.* 2013) and phylogeny-based comparison with ancient endogenous viral elements (Gifford *et al.* 2008).

Meta-analyses of evolutionary rate estimates have collectively revealed not only the taxonomic breadth of time-dependent rates, but also the temporal extent across which this bias is evident. Specifically, a relationship between rate estimates and calibration times has been found to span many orders of magnitude of time depth. This relationship is best described by a power law (Duchêne *et al.* 2014; Aiewsakun & Katzourakis 2015b; Molak & Ho 2015), rather than by a translated exponential function as previously believed (Ho *et al.* 2005; Ho & Larson 2006). Although quantifying the time-dependent biases in rate estimates has not directly enabled resolution of its specific causes, the temporal span of the trend suggests that there are likely to be multiple drivers (Molak & Ho 2015).

In their critique, Emerson & Hickerson (2015) do not draw a distinction between rate estimates derived from mitochondrial and nuclear genomes. We feel that this significantly biases the interpretation of their analysis because, compared with the abundant evidence of time-dependent rate estimates from mitochondrial DNA, results from metazoan nuclear genomes have been less clear. Many of the estimates of short-term nuclear mutation rates from pedigrees, parent–offspring trios and mutation–accumulation lines have been based on small numbers of mutations (Kondrashov & Kondrashov 2010) and are therefore associated with considerable error. As pointed out by Emerson & Hickerson (2015), estimates of spontaneous mutation rates in human nuclear genomes appear to be similar to, or lower than, those of phylogenetic rates inferred using a human–chimpanzee calibration (Scally & Durbin 2012; Subramanian & Lambert 2012). However, the estimates of spontaneous mutation rates might represent minima because of biases in high-throughput sequencing and filtering to reduce the frequency of false-positive calls (Campbell & Eichler 2013), whereas several factors might be causing the phylogenetic rate to be overestimated (Keightley 2012). Although there remains considerable uncertainty about nuclear mutation rates in humans (Gibb & Hills 2013; Ségurel *et al.* 2014; Thomas & Hahn 2014; Callaway 2015; Harris 2015), it does appear that rates are time-dependent in regions of the genome that are under purifying selection (Subramanian & Lambert 2012).

There have been few short-term rate estimates from the nuclear genomes of nonhuman animals. Estimates of spontaneous mutation rates in the invertebrates *Caenorhabditis elegans* (Denver *et al.* 2004), *Drosophila melanogaster* (Keightley *et al.* 2014) and *Heliconius melpomene* (Keightley *et al.* 2015) are all higher than corresponding phylogenetic estimates, but by less than an order of magnitude in each case. In addition, an analysis of ancient nuclear genomes from woolly mammoths yielded a rate estimate higher than that based on a much older fossil calibration (Palkopoulou *et al.* 2015).

These independent lines of evidence provide overwhelming support for a time-dependent pattern in rate estimates, at least for viruses, bacteria and mitochondrial DNA in animals. Therefore, we do not believe that Emerson & Hickerson (2015) have in any way demonstrated a lack of support for time-dependent rate estimates.

Causes of time dependence

A variety of biological and statistical factors can lead to time-dependent biases in rate estimates. These factors, which include purifying selection, ancestral polymorphism, calibration errors, sequence errors and misspecification of demographic models, were discussed at length in our most comprehensive review of the topic (Ho *et al.* 2011a). In their critique, Emerson & Hickerson (2015) claim that the time-dependent pattern in rate estimates can be entirely explained as an artefact of data analysis, focusing on the confounding effects of factors such as ancestral polymorphism and 'nonbiological phenomena'. However, they imply that because some of these causes have the *potential* to explain time-dependent patterns in rates, then they are *sufficient*. This is misleading because most of the proposed causes are unlikely to apply across a broad range of timescales and taxa or to affect all of the different methods that have been used to estimate rates. In any case, most of the potential causes of time-dependent rates cannot readily be designated as being exclusively biological, statistical or artefactual in nature. For example, purifying selection is a biological process, but a failure to model it accurately could be deemed a statistical shortcoming.

Time-dependent rate estimates are probably the result of multiple factors (Ho *et al.* 2011a). Ho *et al.* (2005) originally ascribed a prominent role to purifying selection in driving a time-dependent pattern in rate estimates. Purifying selection removes deleterious mutations over time, such that younger branches in a gene tree tend to carry an excess of mutations (Williamson & Orive 2002). This inevitably leads to time-dependent rates (Soares *et al.* 2009). However, some studies have shown that this effect is insufficient to explain the magnitude of the decline in rate estimates with calibration age (Woodhams 2006; Duchêne *et al.* 2014; Molak & Ho 2015). As stated previously (e.g. Ho *et al.* 2007b, 2011a; Soubrier *et al.* 2012), we agree with the suggestion by Emerson & Hickerson (2015) that purifying selection alone is unlikely to provide a full explanation of time-dependent biases in rate estimates. Some of the potential causes can be disentangled using carefully designed analyses. For example, separate analyses of sites that are subject to differing selective constraints have provided useful insights into the impacts of purifying selection on rates estimated on different timescales (Endicott & Ho 2008; Subramanian *et al.* 2009; Subramanian & Lambert 2011, 2012).

Citing the work of Peterson & Masel (2009), Emerson & Hickerson (2015) propose that ancestral polymorphism can also result in time-dependent biases in rate estimates. This effect is essentially a type of calibration error, because it arises from the temporal discrepancy between population divergence and the corresponding divergence of two alleles in the ancestral population. The relationship between these can be difficult to resolve, because it depends on the size and structure of the ancestral population. It is possible to reduce the impact of ancestral polymorphism in analyses of molecular data, for example by correlating the timing of geological and climatic events with relevant demographic events rather than with specific nodes in the gene tree (Crandall *et al.* 2012). Alternatively, rates can be estimated from time-structured sequence data, a process that is based on calibrations at the tips of the tree and avoids the estimation error caused by ancestral polymorphism.

Rate estimates from time-structured data

Improving the reliability of rate estimates is an important step towards understanding the causes of time-dependent rates. There has been considerable progress in rate-estimation methods over the past decade (Ho & Duchêne 2014). Time-structured data sets, in which the sequences have different ages, have an important role in elucidating the patterns and causes of time-dependent rate estimates. In particular, ancient DNA sequences have the potential to provide clock calibrations for time depths that are intermediate between those of pedigree studies and fossil-calibrated analyses (Ho *et al.* 2011a). However, as pointed out by Emerson & Hickerson (2015) and others (Debruyne & Poinar 2009; Navascués & Emerson 2009; Ho *et al.* 2011b), rate estimates from time-structured data are subject to various potential sources of error. There are several methods of addressing these potential problems.

Most importantly, the reliability of estimates from time-structured data depends on the information content in the sequences and their sampling times. Tip calibrations should only be used when the population is *measurably evolving*: when there is an appreciable amount of evolutionary change during the sampling window (Drummond *et al.* 2003). Even a small number of ancient sequences can be sufficient for calibration, provided that they are old enough to produce a wide sampling window (Molak *et al.* 2013).

Analyses of time-structured mitochondrial data have often yielded elevated estimates of substitution rates relative to phylogenetic estimates (Ho *et al.* 2007a, 2011b). However, Emerson & Hickerson (2015), referring to ancient DNA (aDNA), claim that 'endorsement of a given rate estimate from aDNA often seems to be that other rate estimates from aDNA are similarly high' (p. 705). This is demonstrably incorrect, because there has been growing use of methods to confirm the validity of rate estimates made using Bayesian phylogenetic methods. For example, a regression of the root-to-tip distance (in mutations per site) against sampling time (Fitch *et al.* 1991) can be used to evaluate the appropriateness of a particular time-structured data set for informing an evolutionary rate estimate. A strong relationship between these two quantities reflects clocklike evolution and pronounced temporal structure in the sampling times, and the slope of the regression provides an estimate of the rate (Korber *et al.* 2000). However, this method incorrectly treats the individual root-to-tip distances as mutually independent and is unable to provide clear insight into the degree of temporal structure when there is rate variation among branches.

A second approach to determine whether a time-structured data set has adequate temporal structure to inform a rate estimate is the date-randomization test (Ramsden *et al.* 2009). In a Bayesian phylogenetic context, the tip dates are

randomized a number of times and rate estimates are obtained from the date-randomized replicates. If the rate estimate from the original data set is excluded from the 95% credibility intervals of the rate estimates from at least 95% of the date-randomized replicates, the data set is deemed to contain adequate temporal structure to allow a meaningful estimate of the rate. Many, but not all, published ancient DNA and virus data sets satisfy this condition (Firth *et al.* 2010; Ho *et al.* 2011b; Duchêne *et al.* 2014). Modifications of the test, including the use of a more conservative criterion, were proposed recently (Duchêne *et al.* 2015a). Data sets that fail the date-randomization test tend to yield overestimates of evolutionary rates, but careful application of the test can act as a filter to identify data sets with poor temporal structure.

Emerson & Hickerson (2015) present two hypothetical examples of time-structured data sets that would yield misleading estimates of rates when analysed using a Bayesian phylogenetic approach (panels c and d of their Fig. 2). However, these two data sets clearly do not represent measurably evolving populations. Data sets that have exhibited no genetic change during the sampling window would fail the date-randomization test, indicating that any rate estimates obtained from these data sets would be unreliable. As previously suggested by Emerson and colleagues (Navascués *et al.* 2010), the first step in an analysis of time-structured data should be to 'test if the data show measurable evolution' (p. 762).

Comment on the case study of bison

Emerson & Hickerson (2015) revisit the bison mitochondrial data set that we analysed previously (Ho *et al.* 2007b), which comprises a combination of modern and ancient D-loop sequences. In our original analysis, we found that mean rate estimates from this data set scaled negatively with increasing calibration age, although all of the rate estimates had wide 95% credibility intervals. In their reanalysis, Emerson & Hickerson (2015) estimate the substitution rate while fixing the age of the root, the effective population size, or both. They find that the mean rate estimate increased when older sequences were removed from the data set, contrary to the time-dependent patterns seen in our original analysis. However, fixing the age of the root means that the width of the calibration window is fixed. Under these circumstances, any changes in the rate estimate are primarily driven by changes in the sequence data rather than in the calibrations. The removal of sequences from the data set, including those representing ancient tips, would lead to the removal of terminal branches. In the presence of incomplete purifying selection, these branches carry a disproportionately large number of mutations (Williamson & Orive 2002). On the other hand, removing ancient tips would also tend to cause the removal of other deep branches in the tree, which would be expected to carry fewer mutations. Therefore, removing ancient sequences while fixing the age of the root has unpredictable effects on the resulting rate estimate.

The impacts of fixing the effective population size for different temporal subsamples of the data are less clear. First, Emerson & Hickerson (2015) only fix the population size at time zero (present day), but the cataclysmic demographic model includes two other parameters that allow considerable variation in past population sizes. Second, removal of a biased subsample from the data set can induce changes in the apparent effective population size. Specifically, purifying selection can lead to an apparent decline in effective population size towards the tips of the genealogy (Nicolaisen & Desai 2012).

Therefore, we believe that the results from the bison case study are far less clear than previously suggested, either by ourselves (Ho *et al.* 2007b) or by Emerson & Hickerson (2015). The data set is small by current measures and future work will yield more decisive results by examining patterns of rate variation in larger time-structured data sets, including those from whole mitochondrial genomes, plastid genomes and nuclear loci.

Conclusions

The evidence for a negative relationship between evolutionary rate estimates and calibration times is compelling and comes from a variety of taxonomic groups and using a wide range of estimation methods. This strongly contradicts the claims made by Emerson & Hickerson (2015) that there is a lack of support for time-dependent biases in rate estimates. Nevertheless, further understanding of time-dependent biases in rate estimates will depend on the continued development of calibration methods, including identification of reliable biogeographic calibrations. Refinements of rate-estimation methods, including more biologically realistic models of rate variation, will improve the quantification of rates among lineages. In combination with further research on factors affecting the accuracy of rate estimates, this will lead to a more reliable characterization of molecular evolutionary rates across different timescales.

Further work is needed to improve our understanding of rates in nuclear genomes and of the causes of these patterns across taxa and data sets. With the rapid growth of data from nuclear genomes, a clearer quantification of nuclear evolutionary rates is on the horizon. This will not only enable resolution of some of the anomalous rate estimates from human genomes, but will also provide valuable insights into evolutionary dynamics across broad temporal scales.

Acknowledgements

We thank Greger Larson and Sergios-Orestis Kolokotronis for useful discussions. This work was supported by the Australian Research Council (grant number DP110100383 to S.Y.W.H.) and the Gordon and Betty Moore Foundation (B.S.).

References

Aiewsakun P, Katzourakis A (2015a) Endogenous viruses: connecting recent and ancient viral evolution. *Virology*, **479–480**, 26–37.

Aiewsakun P, Katzourakis A (2015b) Time dependency of foamy virus evolutionary rate estimates. *BMC Evolutionary Biology*, **15**, 119.

Biek R, Pybus OG, Lloyd-Smith JO, Didelot X (2015) Measurably evolving pathogens in the genomic era. *Trends in Ecology and Evolution*, **30**, 306–313.

Burridge CP, Craw D, Fletcher D, Waters JM (2008) Geological dates and molecular rates: fish DNA sheds light on time dependency. *Molecular Biology and Evolution*, **25**, 624–633.

Callaway E (2015) DNA clock proves tough to set. *Nature*, **519**, 139–140.

Campbell CD, Eichler EE (2013) Properties and rates of germline mutations in humans. *Trends in Genetics*, **29**, 575–584.

Comas I, Coscolla M, Luo T *et al.* (2013) Out-of-Africa migration and Neolithic coexpansion of *Mycobacterium tuberculosis* with modern humans. *Nature Genetics*, **45**, 1176–1182.

Crandall ED, Sbrocco EJ, DeBoer TS, Barber PH, Carpenter KE (2012) Expansion dating: calibrating molecular clocks in marine species from expansions onto the Sunda Shelf Following the Last Glacial Maximum. *Molecular Biology and Evolution*, **29**, 707–719.

Debruyne R, Poinar HN (2009) Time dependency of molecular rates in ancient DNA data sets, a sampling artifact? *Systematic Biology*, **58**, 348–359.

Denver DR, Morris K, Lynch M, Thomas WK (2004) High mutation rate and predominance of insertions in the *Caenorhabditis elegans* nuclear genome. *Nature*, **430**, 679–682.

Drummond AJ, Pybus OG, Rambaut A, Forsberg R, Rodrigo AG (2003) Measurably evolving populations. *Trends in Ecology and Evolution*, **18**, 481–488.

Duchêne S, Holmes EC, Ho SYW (2014) Analyses of evolutionary dynamics in viruses are hindered by a time-dependent bias in rate estimates. *Proceedings of the Royal Society London Series B: Biological Sciences*, **281**, 20140732.

Duchêne S, Duchêne D, Holmes EC, Ho SYW (2015a) The performance of the date-randomisation test in phylogenetic analyses of time-structured virus data. *Molecular Biology and Evolution*, **32**, 1895–1906.

Duchêne S, Ho SYW, Holmes EC (2015b) Declining transition/transversion ratios through time reveal limitations to the accuracy of nucleotide substitution models. *BMC Evolutionary Biology*, **15**, 312.

Emerson BC, Hickerson MJ (2015) Lack of support for the time-dependent molecular evolution hypothesis. *Molecular Ecology*, **24**, 702–709.

Endicott P, Ho SYW (2008) A Bayesian evaluation of human mitochondrial substitution rates. *American Journal of Human Genetics*, **82**, 895–902.

Firth C, Kitchen A, Shapiro B *et al.* (2010) Using time-structured data to estimate evolutionary rates of double-stranded DNA viruses. *Molecular Biology and Evolution*, **27**, 2038–2051.

Fitch WM, Leiter JME, Li XQ, Palese P (1991) Positive Darwinian evolution in human influenza A viruses. *Proceedings of the National Academy of Sciences of the United States of America*, **88**, 4270–4274.

García-Moreno J (2004) Is there a universal mtDNA clock for birds? *Journal of Avian Biology*, **35**, 465–468.

Gaut B, Yang L, Takuno S, Eguiarte LE (2011) The patterns and causes of variation in plant nucleotide substitution rates. *Annual Review of Ecology, Evolution, and Systematics*, **42**, 245–266.

Genner MJ, Seehausen O, Lunt DH *et al.* (2007) Age of cichlids: new dates for ancient lake fish radiations. *Molecular Biology and Evolution*, **24**, 1269–1282.

Gibb GC, Hills SFK (2013) Intergenerational mutation rate does not equal long-term evolutionary substitution rate. *Proceedings of the National Academy of Sciences of the United States of America*, **110**, E611.

Gibbs AJ, Fargette D, García-Arenal F, Gibbs MJ (2010) Time – the emerging dimension of plant virus studies. *Journal of General Virology*, **91**, 13–22.

Gifford RJ (2012) Viral evolution in deep time: lentiviruses and mammals. *Trends in Genetics*, **28**, 89–100.

Gifford RJ, Katzourakis A, Tristem M *et al.* (2008) A transitional endogenous lentivirus from the genome of a basal primate and implications for lentivirus evolution. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 20362–20367.

Harris K (2015) Evidence for recent, population-specific evolution of the human mutation rate. *Proceedings of the National Academy of Sciences of the United States of America*, **112**, 3439–3444.

Henn BM, Gignoux CR, Feldman MW, Mountain JL (2009) Characterizing the time dependency of human mitochondrial DNA mutation rate estimates. *Molecular Biology and Evolution*, **26**, 217–230.

Ho SYW, Duchêne S (2014) Molecular-clock methods for estimating evolutionary rates and timescales. *Molecular Ecology*, **23**, 5947–5965.

Ho SYW, Larson G (2006) Molecular clocks: when times are a-changin'. *Trends in Genetics*, **22**, 79–83.

Ho SYW, Lo N (2013) The insect molecular clock. *Australian Journal of Entomology*, **52**, 101–105.

Ho SYW, Phillips MJ, Cooper A, Drummond AJ (2005) Time dependency of molecular rate estimates and systematic overestimation of recent divergence times. *Molecular Biology and Evolution*, **22**, 1561–1568.

Ho SYW, Kolokotronis SO, Allaby RG (2007a) Elevated substitution rates estimated from ancient DNA sequences. *Biology Letters*, **3**, 702–705.

Ho SYW, Shapiro B, Phillips MJ, Cooper A, Drummond AJ (2007b) Evidence for time dependency of molecular rate estimates. *Systematic Biology*, **56**, 515–522.

Ho SYW, Lanfear R, Bromham L *et al.* (2011a) Time-dependent rates of molecular evolution. *Molecular Ecology*, **20**, 3087–3101.

Ho SYW, Lanfear R, Phillips MJ *et al.* (2011b) Bayesian estimation of substitution rates from ancient DNA sequences with low information content. *Systematic Biology*, **60**, 366–375.

Howell N, Smejkal CB, Mackey DA *et al.* (2003) The pedigree rate of sequence divergence in the human mitochondrial genome: there is a difference between phylogenetic and pedigree rates. *American Journal of Human Genetics*, **72**, 659–670.

Keightley PD (2012) Rates and fitness consequences of new mutations in humans. *Genetics*, **190**, 295–304.

Keightley PD, Ness RW, Halligan DL, Haddrill PR (2014) Estimation of the spontaneous mutation rate per nucleotide site in a *Drosophila melanogaster* full-sib family. *Genetics*, **196**, 313–320.

Keightley PD, Pinharanda A, Ness RW *et al.* (2015) Estimation of the spontaneous mutation rate in *Heliconius melpomene*. *Molecular Biology and Evolution*, **32**, 239–243.

Kondrashov FA, Kondrashov AS (2010) Measurements of spontaneous rates of mutations in the recent past and the near future. *Philosophical Transactions of the Royal Society Biological Sciences*, **365**, 1169–1176.

Korber B, Muldoon M, Theiler J *et al.* (2000) Timing the ancestor of the HIV-1 pandemic strains. *Science*, **288**, 1789–1796.

Li Y, Carroll DS, Gardner SN *et al.* (2007) On the origin of smallpox: correlating variola phylogenics with historical smallpox records. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 15787–15792.

Molak M, Ho SYW (2015) Prolonged decay of molecular rate estimates for metazoan mitochondrial DNA. *PeerJ*, **3**, e821.

Molak M, Lorenzen ED, Shapiro B, Ho SYW (2013) Phylogenetic estimation of timescales using ancient DNA: the effects of temporal sampling scheme and uncertainty in sample ages. *Molecular Biology and Evolution*, **30**, 253–262.

Navascués M, Emerson BC (2009) Elevated substitution rate estimates from ancient DNA: model violation and bias of Bayesian methods. *Molecular Ecology*, **18**, 4390–4397.

Navascués M, Depaulis F, Emerson BC (2010) Combining contemporary and ancient DNA in population genetic and phylogeographical studies. *Molecular Ecology Resources*, **10**, 760–772.

Nicolaisen LE, Desai MM (2012) Distortions in genealogies due to purifying selection. *Molecular Biology and Evolution*, **29**, 3589–3600.

Palkopoulou E, Mallick S, Skoglund P *et al.* (2015) Complete genomes reveal signatures of demographic and genetic declines in the woolly mammoth. *Current Biology*, **25**, 1395–1400.

Papadopoulou A, Anastasiou I, Vogler AP (2010) Revisiting the insect mitochondrial molecular clock: the mid-Aegean trench calibration. *Molecular Biology and Evolution*, **27**, 1659–1672.

Park DJ, Dudas G, Wohl S *et al.* (2015) Ebola virus epidemiology, transmission, and evolution during seven months in Sierra Leone. *Cell*, **161**, 1516–1526.

Peterson GI, Masel J (2009) Quantitative prediction of molecular clock and ka/ks at short timescales. *Molecular Biology and Evolution*, **26**, 2595–2603.

Ramsden C, Holmes EC, Charleston MA (2009) Hantavirus evolution in relation to its rodent and insectivore hosts: no evidence for codivergence. *Molecular Biology and Evolution*, **26**, 143–153.

Scally A, Durbin R (2012) Revising the human mutation rate: implications for understanding human evolution. *Nature Reviews Genetics*, **13**, 745–753.

Ségurel L, Wyman MJ, Przeworski M (2014) Determinants of mutation rate variation in the human germline. *Annual Review of Genomics and Human Genetics*, **15**, 47–70.

Soares P, Ermini L, Thomson N *et al.* (2009) Correcting for purifying selection: an improved human mitochondrial molecular clock. *American Journal of Human Genetics*, **84**, 740–759.

Soubrier J, Steel M, Lee MSY *et al.* (2012) The influence of rate heterogeneity among sites on the time dependence of molecular rates. *Molecular Biology and Evolution*, **29**, 3345–3358.

Subramanian S, Lambert DM (2011) Time dependency of molecular evolutionary rates? Yes and no. *Genome Biology and Evolution*, **3**, 1324–1328.

Subramanian S, Lambert DM (2012) Selective constraints determine the time dependency of molecular rates for human nuclear genomes. *Genome Biology and Evolution*, **4**, 1127–1132.

Subramanian S, Denver DR, Millar CD *et al.* (2009) High mitogenomic evolutionary rates and time dependency. *Trends in Genetics*, **25**, 482–486.

Thomas GWC, Hahn MW (2014) The human mutation rate is increasing, even as it slows. *Molecular Biology and Evolution*, **31**, 253–257.

Wayne RK, Vanvalkenburgh B, Obrien SJ (1991) Molecular distance and divergence time in carnivores and primates. *Molecular Biology and Evolution*, **8**, 297–319.

Wertheim JO, Chu DKW, Peiris JSM, Pond SLK, Poon LLM (2013) A case for the ancient origin of coronaviruses. *Journal of Virology*, **87**, 7039–7045.

Williamson S, Orive ME (2002) The genealogy of a sequence subject to purifying selection at multiple sites. *Molecular Biology and Evolution*, **19**, 1376–1384.

Woodhams M (2006) Can deleterious mutations explain the time dependency of molecular rate estimates? *Molecular Biology and Evolution*, **23**, 2271–2273.

All authors contributed to the ideas presented in this article. S.Y.W.H. wrote the article, with input from S.D., M.M. and B.S.

---

doi: 10.1111/mec.13450