Evolutionary stasis of viruses?

Edward C. Holmes¹ and Sebastián Duchêne²

In their recent Opinion article (Prisoners of war — host adaptation and its constraints on virus evolution. *Nat. Rev. Microbiol.* <https://doi.org/10.1038/s41579-018-0120-2> (2018))¹, Simmonds et al. use studies of hepatitis B virus (HBV)² and B19 parvovirus³ from Bronze Age remains, along with endogenous viruses⁴, to propose that evolutionary rates in viruses decline massively as they adapt to hosts. Although these data have changed our perspective on evolutionary timescales, we disagree that they predict long-term evolutionary stasis.

Simmonds et al. claim that there is “increasing evidence for extreme genetic conservation of viruses over longer periods of evolution”. However, most families and genera of RNA viruses exhibit limited sequence similarity⁵, and as expected with rapid evolution over long time periods, even the amino acid sequences of the most conserved proteins can be difficult to align⁵. We believe that the regression analysis of rates of viral evolution performed by Simmonds et al. is misleading as it combines single host associations with multi-host comparisons. Importantly, the deeper the timescale of analysis the more virus–host associations are compared, such that the lowest rate estimates coincide with the highest frequency of host-jumping, counter to their adaptive model.

Isolates of HBV dating back to the sixteenth century lack temporal structure⁶. Accurately estimating an evolutionary rate therefore requires a longer sampling period⁶, as confirmed by the Bronze Age samples³. Hence, higher rate estimates for HBV are erroneous and likely reflect counts of transient mutations, as expected under time-dependent virus evolution^{7,8}. Although there is temporal structure among recent B19 isolates^{9,10}, this may again reflect time dependence or the impact of a small number of ancient samples in the regression. Simmonds et al. also claim that our earlier paper¹⁰ “predicted a time of origin of current genotype 1 strains to the 1960s or 1970s”. However, no divergence times were presented in this paper, but were previously by Simmonds and colleagues⁹. Moreover, no data are presented for the lowest rate of spumavirus evolution covering ~750 million years. The relatively slow evolution of spumaviruses may reflect low rates of replication¹¹ and the occurrence of nonsynonymous substitutions argues against extreme purifying selection¹¹.

We believe that there is no biological reason why the evolutionary rate in RNA viruses, which encode their own RNA polymerase, should decline to that of hosts that use entirely different replication enzymes. The likely changing nature of the complex environments faced by viruses, combined with the size of sequence space (~4^{10,000} for a typical RNA virus), make evolutionary stasis unrealistic. For example, although influenza viruses have probably been associated with wild bird species for millennia, their evolutionary rates are of the same magnitude as the mammals in which they periodically emerge^{12,13}. Finally, the high mutation rates in RNA viruses ensure that evolutionary stasis would result in massive purifying selection and an enormous mutational load.

Viruses are ancient entities and their remarkable sequence diversity reflects a long evolutionary history characterized by high rates of genetic change. Along with host adaptation, much of the apparent discrepancy between short-term and long-term evolution in viruses may be an illusion caused by the inappropriate use of molecular clock dating without temporal structure, incorrect calibration points, differences in replication rates, site saturation that is even apparent at shallow genetic distances¹⁴ and the inherent time dependence of evolutionary rates⁸.

There is a reply to this letter by Simmonds, P., Aiewsakun, P. & Katourakis, A. *Nat. Rev. Microbiol.* <https://doi.org/10.1038/s41579-019-0169-6> (2019).

Reply to ‘Evolutionary stasis of viruses?’

Peter Simmonds, Pakorn Aiewsakun and Aris Katourakis

Our Opinion article (Prisoners of war — host adaptation and its constraints on virus evolution. *Nat. Rev. Microbiol.* <https://doi.org/10.1038/s41579-018-0120-2> (2018))¹ describes host adaptation and its potential role in shaping long-term evolution of viruses. Holmes and Duchêne’s correspondence (Evolutionary stasis of viruses? *Nat. Rev. Microbiol.* <https://doi.org/10.1038/s41579-019-0168-7> (2019))² closely concurs that viruses are fast-evolving and diverse ancient entities, constrained by host adaptation and that

Edward C. Holmes¹* and Sebastián Duchêne²
¹Marie Bashir Institute for Infectious Diseases and Biosecurity, Charles Perkins Centre, School of Life and Environmental Sciences and Sydney Medical School, The University of Sydney, Sydney, Australia.
²Department of Biochemistry and Molecular Biology, Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Melbourne, Australia.
 *e-mail: edward.holmes@sydney.edu.au
<https://doi.org/10.1038/s41579-019-0168-7>

1. Simmonds, P., Aiewsakun, P. & Katourakis, A. Prisoners of war — host adaptation and its constraints on virus evolution. *Nat. Rev. Microbiol.* <https://doi.org/10.1038/s41579-018-0120-2> (2018).
2. Muhlemann, B. et al. Ancient hepatitis B viruses from the bronze age to the medieval period. *Nature* **557**, 418–423 (2018).
3. Muhlemann, B. et al. Ancient human parvovirus B19 in Eurasia reveals its long-term association with humans. *Proc. Natl Acad. Sci. USA* **115**, 7557–7562 (2018).
4. Katourakis, A. & Gifford, R. J. Endogenous viral elements in animal genomes. *PLOS Genet.* **6**, e1001191 (2010).
5. Shi, M. et al. Redefining the invertebrate virosphere. *Nature* **540**, 539–543 (2016).
6. Patterson Ross, Z. et al. The paradox of HBV evolution as revealed from a 16th century mummy. *PLOS Pathog.* **14**, e1006750 (2018).
7. Duchêne, S. et al. Analyses of evolutionary dynamics in viruses are hindered by a time-dependent bias in rate estimates. *Proc. R. Soc. B* **281**, 20140732 (2014).
8. Ho, S. Y. et al. Time-dependent rates of molecular evolution. *Mol. Ecol.* **20**, 3087–3101 (2011).
9. Norja, P. et al. Rapid sequence change and geographical spread of human parvovirus B19: comparison of B19 virus evolution in acute and persistent infections. *J. Virol.* **82**, 6427–6433 (2008).
10. Shackleton, L. A. & Holmes, E. C. Phylogenetic evidence for the rapid evolution of human B19 erythrovirus. *J. Virol.* **80**, 3666–3669 (2006).
11. Switzer, W. M. et al. Ancient co-speciation of simian foamy viruses and primates. *Nature* **434**, 376–380 (2005).
12. Fourment, M. & Holmes, E. C. Avian influenza virus exhibits distinct evolutionary dynamics in poultry and wild birds. *BMC Evol. Biol.* **15**, 120 (2015).
13. Worobey, M. et al. A synchronized global sweep of the internal genes of modern avian influenza virus. *Nature* **508**, 254–257 (2014).
14. Duchêne, S. et al. Declining transition/transversion ratios through time reveal limitations to the accuracy of nucleotide substitution models. *BMC Evol. Biol.* **15**, 36 (2015).

Competing interests

The authors declare no competing interests.

longest timescales, rates approach those of their hosts as viral adaptation tracks their evolution.

Holmes and Duchêne note that many RNA viruses exhibit limited sequence similarity. It is, however, remarkable how conserved viral genomes can be given the ancient integration times of many endogenous viruses. Their sequences should be completely unrecognizable if their longer-term evolutionary rates were similar to their short-term rates¹.

Holmes and Duchêne critique the regression analysis in our paper. We did not perform a regression, but simply annotated a previously published analysis⁵ by superimposing rates derived from ancient DNA (aDNA) sequences. We find it quite extraordinary how well these match relationships observed from other data and provide a new window into intermediate time periods (1–10 Kya) where other methods are uninformative.

Holmes and Duchêne correctly propose that over longer timescales, frequent host jumps would prevent a reduction in evolutionary rates. This is, however, encapsulated within our model and indeed represents an empirically testable prediction from it. However, the deeper time points in the time-dependent rate phenomenon (TDRP) regressions arise from observations where codivergence is known to have occurred and there have been long periods of virus–host adaptation.

We agree with Holmes and Duchêne that isolates of HBV dating back to the sixteenth century lack temporal structure. That does not prevent us from calculating a maximum substitution rate from evolutionary distances between aDNA sequences and the closest contemporary HBV variant. Nevertheless, as Holmes and Duchêne indicate, temporal structure is recoverable when more divergent Bronze Age aDNA sequences are included⁶ and the rate calculated fits remarkably closely to the TDRP regression for retrotranscribing viruses.

We cited Shackelton and Holmes' seminal paper on B19V⁷ as one of the first demonstrations of high rates of evolution in a small DNA virus and a clear association between root distance and time over their sampling period. Confirming this study,

we recovered a similar temporal signal for exogenous B19V variants, with both regression and BEAST estimating origin times of genotype 1 to the late 1950s⁸. Neither analysis^{7,8} provides any reason to doubt high short-term substitution rates of B19V or, as we proposed then, recent dates for the origin of B19V genotypes. This is why the separate identity of genotype 1 nearly 7,000 years ago⁹ is so remarkable.

The lowest rate of spumavirus evolution (3.6×10^{-9} substitutions per site per year (SSY)) calculated at 465.5 Myr arose from dividing the inferred number of substitutions from the prototype foamy virus down to the common ancestor of all foamy viruses¹⁰. Importantly, slow long-term rates of foamy virus evolution are not the result of low rates of replication — spumaviruses establish persistent infections with active virus excretion in oral mucosae¹¹. They furthermore possess an error-prone reverse transcriptase¹² and show a short-term substitution rate of $\sim 3 \times 10^{-4}$ SSY¹³. Furthermore, we have verified the time dependence in substitution rates of spumaviruses over the time spans we are considering¹⁴.

RNA viruses possess their own RNA polymerases for replication and, as Holmes and Duchêne state, they are therefore not subject to the error rates of their hosts. However, mutation and substitution rates are distinct. Over short timescales, high mutation rates facilitate crucial evolutionary events such as immune, antiviral or vaccine escape. Over longer timescales, mutation rates are not the limiting factor that drives virus sequence change. Similarity in TDRP regression curves between viruses in different Baltimore groups⁵ demonstrates that differences in replication mechanisms and error rates become increasingly irrelevant over long evolutionary periods.

We fully acknowledge and have reviewed the methodological issues that influence rate measurements alluded to by Holmes and Duchêne. However, these appear inadequate to account for the more than 1 million-fold difference in rates over different evolutionary timescales. We agree that viral evolution does not come to a standstill — viruses have likely

been churning away in their sequence spaces from the beginning. However, the process of fitness optimization to a niche imposes constraints that drastically influence the extent to which they can change over longer periods. This is the essence of the niche-filling perspective that we advance in our Opinion article.

Peter Simmonds^{1*}, Pakorn Aiewsakun² and Aris Katzourakis^{3*}

¹Nuffield Department of Medicine, University of Oxford, Oxford, UK.

²Department of Microbiology, Faculty of Science, Mahidol University, Bangkok, Thailand.

³Department of Zoology, University of Oxford, Oxford, UK.

*e-mail: peter.simmonds@ndm.ox.ac.uk;

aris.katzourakis@zoo.ox.ac.uk

<https://doi.org/10.1038/s41579-019-0169-6>

1. Simmonds, P., Aiewsakun, P. & Katzourakis, A. Prisoners of war — host adaptation and its constraints on virus evolution. *Nat. Rev. Microbiol.* <https://doi.org/10.1038/s41579-018-0120-2> (2018).
2. Holmes, E. C. & Duchêne, S. Evolutionary stasis of viruses? *Nat. Rev. Microbiol.* <https://doi.org/10.1038/s41579-019-0168-7> (2019).
3. Ho, S. Y. et al. Time-dependent rates of molecular evolution. *Mol. Ecol.* **20**, 3087–3101 (2011).
4. Aiewsakun, P. & Katzourakis, A. Endogenous viruses: connecting recent and ancient viral evolution. *Virology* **479–480**, 26–37 (2015).
5. Aiewsakun, P. & Katzourakis, A. Time-dependent rate phenomenon in viruses. *J. Virol.* **90**, 7184–7195 (2016).
6. Muhlemann, B. et al. Ancient hepatitis B viruses from the bronze age to the medieval period. *Nature* **557**, 418–423 (2018).
7. Shackelton, L. A. & Holmes, E. C. Phylogenetic evidence for the rapid evolution of human B19 erythrovirus. *J. Virol.* **80**, 3666–3669 (2006).
8. Norja, P. et al. Rapid sequence change and geographical spread of human parvovirus B19; comparison of B19 evolution in acute and persistent infections. *J. Virol.* **82**, 6427–6433 (2008).
9. Muhlemann, B. et al. Ancient human parvovirus B19 in Eurasia reveals its long-term association with humans. *Proc. Natl Acad. Sci. USA* **115**, 7557–7562 (2018).
10. Aiewsakun, P. & Katzourakis, A. Marine origin of retroviruses in the early palaeozoic era. *Nat. Commun.* **8**, 13954 (2017).
11. Murray, S. M. et al. Expanded tissue targets for foamy virus replication with simian immunodeficiency virus-induced immunosuppression. *J. Virol.* **80**, 663–670 (2006).
12. Boyer, P. L. et al. In vitro fidelity of the prototype primate foamy virus (PFV) RT compared to HIV-1 RT. *Virology* **367**, 253–264 (2007).
13. Schweizer, M. et al. Genetic stability of foamy viruses: long-term study in an African green monkey population. *J. Virol.* **73**, 9256–9265 (1999).
14. Aiewsakun, P. & Katzourakis, A. Time dependency of foamy virus evolutionary rate estimates. *BMC Evol. Biol.* **15**, 119 (2015).

Competing interests

The authors declare no competing interests.