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Comment

Are rates of molecular evolution in mammals substantially accelerated in warmer environments?

Gillman *et al.* [1] test the notion that rates of molecular evolution are accelerated in small-bodied mammal species inhabiting geographical areas with warmer ambient temperatures. Their methodology involved estimation of many phylogenetic trees each consisting of a mammal sister species pair and two closely related outgroup species. One member of each sister species pair occurred at lower latitudes or altitudes (the 'warmer' species) than the other (the 'cooler' species). If rates of molecular evolution are accelerated in the warmer species, then the branch length leading to it on the phylogeny should usually be longer than that leading to the cooler species. Remarkably, Gillman *et al.* [1] report that the branch lengths of the warmer species are on average 1.47 times longer than that of the cooler species. While we don't disagree with the statistical significance of their finding, our reanalysis of their data suggests that the magnitude of the effect of latitude is much weaker than reported by Gillman *et al.* and would have minimal impact on molecular clock dating across latitudinal gradients.

Our first concern is that the model of molecular evolution fitted by Gillman *et al.* [1] was over-parameterized. To calculate the branch lengths, they fitted the general time reversible model of sequence evolution with correction for invariant sites and among-site rate variation (GTR-I-Γ) to cytochrome b sequences. They fit all 10 model parameters separately to each pair of sister species (separate-parameter model, hereafter), even though each pair provided only four DNA sequences (the sister species and two outgroup species). However, it is unlikely that stable parameter estimates can be obtained using only the four DNA sequences included in each sister species set (e.g. [2,3]).

We used the methods reported by Gillman *et al.* to calculate parameter estimates and the branch lengths under their separate-parameter model and compared these to estimates under an alternative model in which parameters were estimated from the entire dataset of 516 sequences (joint-parameter model, hereafter; see electronic supplementary material, appendix). Resulting estimates of the branch lengths and distances separating members of each sister species pair differed greatly between these two approaches, with 19 pairs exhibiting genetic distances more than 150 per cent greater under the separate-parameter model than under the joint-parameter model (figure 1*a*). Separate-parameter model distances between the sister species were as large as 144 per cent sequence divergence, a value far higher than is typical for sister species of mammals, which in our earlier analysis never exceeded 34 per cent sequence divergence [4]. Under the joint-parameter model, distances between the sister species were all less than 46 per cent sequence divergence (30% after excluding the *Perognathus fasciatus* and *P. flavescens* pair, which may not be each other's closest relatives [5]). Compared with published studies of small mammals, the separate-parameter model estimated excessively low (i.e. correction for among-site rate variation) or high (i.e. substitution rates) parameter values for many sister species while parameter values for the joint-parameter model were consistent with published estimates (see electronic supplementary material, appendix). Determination of variable and invariable sites in DNA sequences, levels of among-site rate variation and other model parameters requires many sequences [6]. Low parameter values for the correction for among-site rate variation and high values for substitution rates, as obtained in the separate-parameter model for a number of sister pairs, are likely to over-stretch long branches (i.e. overcorrect for saturation of substitutions; figure 1*a*). Shorter branches should have less saturation and as a result would be stretched to a lesser degree by unstable parameter estimates, resulting in an overestimate of the difference in rate of molecular evolution between warmer and cooler species in affected pairs.

We re-estimated the magnitude of effect of temperature using the slope of the major axis regression of warmer on cooler branch lengths fitted through the origin (see electronic supplementary material, appendix; unlike least-squares regression, major axis regression yields the same relationship between the variables regardless of which is designated the x variable and which the y variable). This approach is preferable to estimating the mean of ratios, as done by Gillman *et al.* [1], because ratios may be undefined and are often excessively large if the denominator branch length is estimated with error and its realized value is zero or close to zero.

The regression slope of warmer on cooler branch lengths should be greater than 1.0 if rates of molecular evolution are accelerated at low latitude and elevation. The calculated slope was only 1.17 (95% CI: 1.07 to 1.26) or 1.18 (95% CI: 1.07 to 1.31) after standardizing each measurement by the square root of the average of the two branch lengths of the sister species, to accommodate an expected increase in the variance of residuals with time. The calculated slope under the separate-parameter model was 1.27 (95% CI: 1.13 to 1.43; after standardizing: slope = 1.27, 95% CI = 1.14 to 1.42), considerably higher than under the joint-parameter model. These values—whether obtained from the joint- or separate-parameter model—are considerably less than the mean ratio of 1.47 reported by Gillman *et al.*

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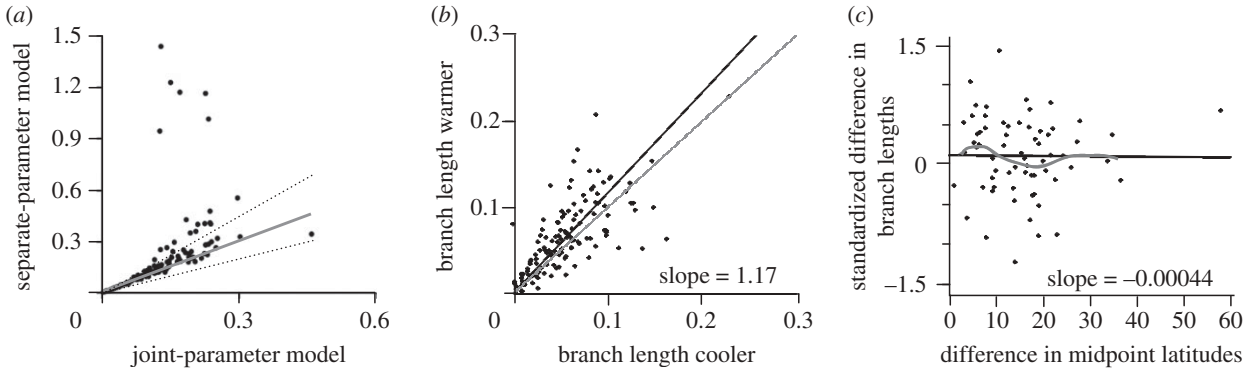

Figure 1. Branch lengths of the sister species. (*a*) Comparison of genetic distances between the sister species (sum of branch lengths separating the pair) under the GTR-I-Γ model estimated under the separate-parameters model and the joint-parameters model. (*b*) Branch lengths under the GTR-Γ-I model of sequence evolution with parameters estimated under the joint-parameter model. (*c*) Standardized differences in branch length compared with distances between midpoint latitudes of sister species excluding those with overlap in their latitudinal ranges greater than 25%. Slope in (*b*) was estimated with major axis regression fit through the origin. Slope in (*c*) was estimated with least-squares regression and with a Loess smoothed curve (grey line). Grey lines in (*a*,*b*) indicated 1 : 1 relationship. Points between the dashed lines in (*a*) indicate sister species with genetic distances estimated under the joint-parameter model to be less than 1.5 and greater than 0.66 times the value of the distances under the separate-parameter model.

The magnitude of the effect, specifically of latitude on rates of molecular evolution, is crucial to estimating latitudinal gradients in the ages of species. For example, we previously estimated genetic distances between the mammalian sister species in the New World to be three times greater on average near the equator than at 50° latitude [4]. Gillman *et al.* [1] suggested that faster rates of molecular evolution at lower latitudes could drive this pattern, rather than slower rates of speciation and extinction in the tropics as we suggested. To investigate the effect of latitude on rates of molecular evolution we focused on the Gillman *et al.*'s [1] 101 comparisons involving latitude (leaving out the altitudinal comparisons). Gillman *et al.* [1] stated that they excluded any species pair for which the latitudinal range of the larger-ranged species overlapped that of the smaller-ranged species by more than 25 per cent to insure that species being compared are sufficiently different in latitude for temperature differences to occur. However, we found that 45 of the 101 pairs included in their analysis, almost half of the data, did not meet their criterion, having excessive overlap in absolute latitudinal ranges (13 pairs overlapped by more than 60% and three had complete overlap). The inclusion of high-overlap sister pairs might be considered conservative in the tests of Gillman *et al.* However, the resulting mix of data is not a complete sample of species pairs: almost all available sister pairs whose ranges overlap in latitude by less than 25 per cent were included in the Gillman *et al.* dataset, whereas only some sister pairs that overlap in latitude by more than 25 per cent were included. In any case, we analysed the data both with and without the high-overlap pairs. Dropping the 45 high-overlap pairs reduced the slope of the major axis regression slightly to 1.10 (95% CI: 0.98 to 1.25) from 1.14 (95% CI: 1.04 to 1.25) for the joint-parameter model, or to 1.13 (95% CI = 0.99 to 1.28) from 1.15 (95% CI = 1.02 to 1.30) after standardizing. These results indicate that the effect of latitude on rates of molecular evolution is much smaller than reported by Gillman *et al.*

If this effect on rate stems from latitude, then we would expect that the difference in rate between the sister species would be larger, the greater is their difference in latitude. The slope of the relationship between rate difference and latitude difference would also yield the necessary correction to any analysis attempting to estimate diversification rates along the latitudinal gradient (e.g. [4]). We compared the rate difference, calculated as the difference in branch length between the warmer and cooler species divided by the average of the branch lengths for both species, with the difference in the midpoints of the latitudinal ranges of the sister species (figure 1*c*). The slope was estimated as −0.00045 units per degree latitude (95% CI: −0.0136 to 0.0127; *y*-intercept = 0.116), when the 45 high-overlap sister-species pairs are excluded (slope = 0.0028, 95% CI: −0.0096 to 0.0152; *y*-intercept = 0.079, for all 101 pairs). Such a flat relationship (also suggested by a smoothed Loess curve; figure 1*c*) suggests that latitudinal distance separating species has almost no effect on rates of molecular evolution. For example, the regression line suggests rates of molecular evolution are on average 1.11 times faster in the warmer species when midpoint latitudes are separated by just 10° and 1.10 times faster when separated by 50°. While, we are uncertain what is driving this pattern, what is clear is that the threefold latitudinal difference in age of mammal species across the latitudinal gradient [4] would not be greatly affected by these results.

Our re-analysis of the Gillman *et al.* [1] data indicate that they overestimated the effect of latitude and elevation on rates of molecular evolution as a result of reporting mean ratios, fitting an over-parameterized model, and by errors in the dataset. While a statistically detectable increase in rates of molecular evolution at warmer latitudes (or altitudes) may remain, the effect is much smaller than suggested by the mean ratio of branch lengths of warmer to cooler species reported by Gillman *et al.* Importantly, we found a flat relationship between the difference in molecular rate and the difference in latitude separating species. While the notion that accelerated molecular rates towards the equator may drive the build

up of high species diversity in the tropics seems appealing, a strong connection between rates of molecular evolution and speciation rate has not been established [7].

Jason T. Weir[1,2,*] and Dolph Schluter[3]
[1]*Department of Ecology and Evolution, University of Chicago, Chicago 60637, USA*
[2]*Department of Biological Sciences, University of Toronto Scarborough, Toronto, Canada MIC1A4*
[3]*Department of Zoology and Center for Biodiversity Research, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z4*
[*]Author for correspondence (jtweir@uchicago.edu).

REFERENCES

1 Gillman, L. N., Keeling, D. J. & Ross, H. A. 2009 Latitude, elevation and the tempo of molecular evolution in mammals. *Proc. R. Soc. B.* **276**, 3353–3359. (doi:10.1098/rspb.2009.0674)
2 Sullivan, J., Swofford, D. L. & Naylor, G. J. P. 1999 The effect of taxon sampling on estimating rate heterogeneity parameters of maximum-likelihood models. *Mol. Biol. Evol.* **16**, 1347–1356.
3 Blouin, C., Butt, D. & Roger, A. J. 2005 The impact of taxon sampling on the estimation of rate of evolution at sites. *Mol. Biol. Evol.* **22**, 784–791. (doi:10.1093/molbev/msi065)
4 Weir, J. T. & Schluter, D. 2007 The latitudinal gradient in recent speciation and extinction rates in birds and mammals. *Science* **315**, 1574–1576. (doi:10.1126/science.1135590)
5 Alexander, L. F. & Riddle, B. R. 2005 Phylogenetics of the New World rodent family Heteromyidae. *J. Mammal.* **86**, 366–379. (doi:10.1644/BER-120.1)
6 Yang, Z. 1996 Among-site rate variation and its impact on phylogenetic analysis. *Trends Ecol. Evol.* **11**, 367–373. (doi:10.1016/0169-5347(96)10041-0)
7 Mittelbach, G. G. *et al.* 2007 Evolution and the latitudinal diversity gradient: speciation, extinction and biogeography. *Ecol. Lett.* **10**, 315–331. (doi:10.1111/j.1461-0248.2007.01020.x)