On the comparative biology of mammalian telomeres: Telomere length co-evolves with body mass and lifespan

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Summary

Telomeres, the short repetitive DNA sequences that cap the ends of linear chromosomes, shorten during cell division and are implicated in senescence in most species. The enzyme telomerase can rebuild telomeres but is repressed in many mammals that exhibit replicative senescence, presumably as a tumor suppression mechanism. It is therefore important that we have an accurate understanding of the (co-)evolution of telomere biology and life-history traits that has shaped the diversity of senescence patterns across species. Gomes et al. (2011) produced a large data set on telomere length (TL), telomerase activity, body mass and lifespan among 57 mammal species. We re-analyzed their data set using the same phylogenetic multiple regressions presented in the original publication and several sensitivity analyses. We found substantial inconsistencies in our results compared to Gomes et al.'s. Consistent with Gomes et al. we found an inverse association between TL and lifespan. Contrary to the analyses reported in Gomes et al., we found that there was a generally robust inverse association between TL and mass, and only weak non-robust evidence for an association between telomerase activity and

mass. These results suggest that shorter TL may have been selected for in larger and longer-lived species – likely as a mechanism to suppress cancer. However, our results call into question past results suggesting that high telomerase activity has been selected against in larger species and stress the need for careful attention to model construction and sensitivity analyses.

Introduction, Results and Discussion

Telomeres shorten during cell replication and may be further degraded due to oxidative stress caused by acquiring and maintaining body mass (Monaghan & Ozanne, 2018). Telomerase can rebuild telomeres but is repressed in somatic cells of many mammals, presumably as a tumor suppression mechanism (Gomes et al., 2010; Tian et al., 2018). In the absence of telomerase, telomere shortening triggers cell senescence, which can prevent proliferation of potential cancer cells (Campisi, 2001). Gomes et al. (2011), hereafter GEA, measured mean telomere length (TL measured in kb) and telomerase activity (TA measured in cultured fibroblasts relative to an adenocarcinoma cell line) among 57 mammal species. GEA's work created the largest cross-species dataset on telomere biology assembled in a single lab. GEA examined relationships between TL, TA, body mass and maximum lifespan. In re-analyzing their dataset, we found substantially different results from GEA. Here we present these results along with additional sensitivity analyses. We then discuss possible reasons for these discrepancies and the biological implications of our findings.

Presented in Table 1 are the results reported by GEA and our re-analysis – both using two phylogenetic multiple regression models. Following GEA, mass, lifespan, and TL were $\log_{10}$ -transformed. Consistent with GEA, we found that both mass and TL significantly predicted lifespan. However, the remaining results are all substantially discordant. While GEA found no association between TA and lifespan, we observed a significantly positive association. In predicting body mass, GEA found that TA was a significant predictor, but TL was not. We found the opposite: TA did not predict mass, but longer TL predicted decreased body mass.

GEA reported a series of bivariate plots (their Fig. 4) for key variables in their analyses along with p-values. These p-values are identical to those presented in their Fig. 2 (our Table 1), strongly suggesting that these significance values were from phylogenetic multiple regressions, not phylogenetic bivariate regressions. A casual reader is likely to interpret these p-values as bivariate associations. Here we present phylogenetic bivariate regression analyses of these associations to examine the sensitivity of the multiple regression results and more clearly define

these bivariate associations. The bivariate results (Fig. 1) are qualitatively consistent with our multiple regression results, except lifespan did not show any association with TA (Fig. 1d). Based on these bivariate results, a 1% increase in body mass predicts a 0.08% decrease in TL (Fig. 1a), while a 1% increase in lifespan predicts a 0.43% reduction in TL (Fig. 1b).

To establish the robustness of findings we conducted additional sensitivity analyses. There is some ambiguity in how analyses were modeled in GEA. For example, in GEA's Fig. 4, TA was shown on a log-scale while their methods suggested TA was not log-transformed. Log-transformed TA might better reflect aspects of telomerase biology (e.g. if increasing TA has diminishing marginal effects on cancer risk). Since the logarithm is undefined for zero, we used a $\log_{10}(x+1)$ transformation of TA. The results produced are qualitatively consistent with our re-analysis of untransformed TA, except the associations between TA and lifespan is considerably attenuated ($p=0.103$, Table S2) and there is a negative bivariate association between TA and mass ($\beta=-0.1162\pm0.0330$, $p=0.001$). This bivariate association is not robust to controlling for TL (Table S2).

To explore other possible ways GEA may have analyzed the data, we also modeled telomerase as a binary variable (0=no activity, 1=some activity). These multiple regression results (Table S3) were qualitatively the same as GEA's results (Table 1). It is notable that as TA is first log-transformed (Table S2) and then binary transformed (Table S3), TL becomes less and less predictive of either mass or lifespan. This is likely driven by the fact that as TA is so transformed the phylogenetic correlation between TL and TA increases (Table S4). The bivariate association of mass predicting binary TA can be modelled in a phylogenetic logistic regression, which shows that telomerase repression is significantly associated with higher body mass with an inflection point at 0.8 kg (Figure S1). However, the biological meaning of binary coded TA is unclear. Perhaps having any TA increases the level of cancer risk, but the particular level of TA greater than zero does not change cancer risk – although it is not clear to us why this should be so. In any case, these associations are sensitive to modeling strategies.

Our re-analysis demonstrates that TL has evolved to be shorter with increasing body mass and lifespan, across 57 species of mammals. This supports the hypothesis that short telomeres evolved in large, long-lived mammals to enable replicative senescence as a mechanism to ameliorate cancer risk conferred by a larger number of cells and longer time to accumulate oncogenic mutations (Gorbunova et al., 2014; Risques & Promislow, 2018). In contrast, past studies have shown no significant associations between TL, mass or lifespan of 15 rodent

species (Seluanov et al., 2007) or between TL and lifespan of 19 bird species (Tricola et al., 2018). These differences might be explained by these rodents and birds being relatively small and short-lived compared to many of the species included here. Among 8 species of relatively large mammals and birds (all >0.5 kg), longer early-life TL predicted longer lifespan (the opposite of our findings), when accounting for mass and telomere shortening rate (Pepke & Eisenberg, 2020). Broader taxonomic studies that are attentive to model construction are needed to unravel the generality of these patterns.

Our re-analysis showed that TA had only inconsistent associations with lifespan and body mass. We found some hints that TA was inversely associated with mass when telomerase was either log-transformed or binary transformed. This might suggest that TA variation greater than zero has no meaningful effects on the phenotype under selection with changing body mass. In contrast, Seluanov et al. (2007) found a strong negative correlation between TA and body mass across rodent species. Seluanov et al.'s analysis differed from this study in that they measured TA in spleen, liver, and kidney tissues, while this study depends on telomerase from cultured cells. Additionally, the rodents in Seluanov et al.'s analysis all expressed telomerase and had long telomeres (most species >20 kb), while this analysis includes many species without detectable TA and with shorter TLs. The fact that Seluanov et al.'s analysis showed non-transformed TA had an association with mass among animals with only non-zero TA argues against using TA as a binary variable.

Our re-analyses of the valuable dataset generated by GEA show considerably divergent results from those shown by GEA and that many results are not robust to varying modeling strategies. Unlike GEA, we show that telomere length decreases with increasing body mass. While GEA's results suggest a reduction in telomerase activity with increasing body mass, we only found weak evidence for this. Our results suggest that the co-evolution of telomere biology with lifespan and body mass across species needs to be reconsidered and the sensitivity of results to modeling strategies needs to be more carefully thought through.

Experimental Procedures

Following Gomes et al. (2011) we used the best species-level mammal supertree from Bininda-Emonds et al. (2007) pruned to match the dataset. We used the phylogenetic generalized least squares method (Grafen, 1989) implemented in the R package 'caper' (Orme et al., 2018) to perform the phylogenetic regressions. Pagel's λ was estimated with default bounds (0-1) using maximum likelihood. Analyses were performed in R (R Core Team, 2020).

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Conflict of Interest: None.

Author Contributions: M.L.P. analyzed the data and both authors wrote the paper.

Data Availability Statement: Species trait data from Gomes et al. (2011) is included in Table S5 the Supporting information. The phylogenetic data from Bininda-Emonds et al. (2007) is readily available within the R package ‘PhyloOrchard’ (O’Meara et al., 2012).

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183 **Tables and figures**

184 **Table 1:** Phylogenetic multiple regressions with results from Gomes et al. (2011) compared to
185 our re-analysis.¹

	Gomes et al.	Our re-analysis
Response: log ₁₀ (mass)	(p-value)	β (p-value)
telomerase activity	(0.0082)**	-0.0014 (0.89)
log ₁₀ (telomere length)	(0.71)	-1.8435 (0.007)**
Response: log ₁₀ (lifespan)		
telomerase activity	(0.34)	0.0039 (0.029)*
log ₁₀ (telomere length)	(0.0032)**	-0.4118 (0.002)**
log ₁₀ (mass)	(<0.0001)***	0.1401 (<0.001)***
* p < 0.05, ** p < 0.01, *** p < 0.001		

186 ¹Gomes et al. do not explicitly describe their model parameters, but these are our best guess at
187 a reconstruction. More complete results from our re-analyses are given in Table S1 in the
188 Supporting information.

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192 **Figure 1:** Associations between telomere length, maximum lifespan and body mass (all log₁₀-
193 transformed) and telomerase activity (untransformed). Bivariate phylogenetic regressions of
194 these associations are shown. Scatter plots do not depict the phylogenetic controls.