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Seasonal variation in telomere dynamics in African striped mice

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ABSTRACT

Telomere shortening has been used as an indicator of aging and is believed to accelerate under harsh environmental conditions. This can be attributed to the fact that telomere shortening has often been regarded as non-reversible and negatively impacting fitness. However, studies of laboratory mice indicate that they may be able to repair telomere loss to recover from environmental harshness, as indicated by recent studies in hibernating rodents. We studied seasonal variation in telomere dynamics in African striped mice (*Rhabdomys pumilio*) living in a highly seasonal environment. In our annual species, individuals born in the moist spring (high food availability) need to survive the harsh dry summer (low food availability) to be able to reproduce in the following spring. We studied the effect of the harsh dry vs. the benign moist season on telomere dynamics. We also tested whether telomere length and changes in telomere length were associated with the probability that individuals disappeared from the population during the dry season. Male but not female striped mice showed age-related telomere erosion. Telomeres were longer at the beginning of the dry season compared to the rest of the year. Telomeres increased significantly in length during the moist season. Neither telomere length at the onset of the dry season nor telomere loss over the dry season predicted whether or not individuals disappeared. In conclusion, our data suggest that seasonal attrition and restoring of telomeres also occurs in non-hibernating wild rodents living in hot food restricted environments.

1 INTRODUCTION

2 Telomeres are DNA sequences that cap the end of linear chromosomes and protect genome integrity
3 (Chan and Blackburn 2004). Telomeres erode progressively with cell replication and accumulation of
4 stress-derived damages (Ilmonen et al. 2008; Kotrschal et al. 2007; Olonikov 1973), which is considered
5 as one main indicator of the ageing rate of cells and of organisms. Hence, a straightforward initial
6 telomere-ageing hypothesis was that telomere length is shortened progressively with age, and that
7 faster shortening rate actually reflects senescence, and reduced individual fitness (Dantzer and Fletcher
8 2015; Salomons et al. 2009). Accordingly, both telomere length and telomere shortening have been
9 suggested to predict survival and/or longevity in different taxa (Fairlie et al. 2016; Heidinger et al. 2012;
10 Whittemore et al. 2019).

11
12 A potential causal role of telomeres on life history trajectories was supported by studies showing that
13 telomere length / shortening may reflect life stress effects like the costs of reproduction (Ilmonen et al.
14 2008; Kotrschal et al. 2007), and are related to immune status and response (Criscuolo et al. 2018b).
15 However, contradictory results have been published challenging the idea of continuous telomere
16 shortening with age or its link with higher mortality (Cerchiara et al. 2017; Martin-Ruiz et al. 2005; Seeker
17 et al. 2018). This highlighted a key element for our understanding of telomere role in animal ageing,
18 which is that the telomere – fitness relationships are indeed more complex than initially thought,
19 possibly because telomere dynamics evolved differently under distinct environmental pressures (Tian et
20 al. 2018). Whether telomeres are causal in ageing or rather just a biomarker of somatic maintenance is
21 still an open question (Simons 2015; Young 2018). This seems to be especially true for small mammals in
22 which telomere shortening is often not part of the cell replicative ageing process (Gorbunova et al. 2008;
23 Tian et al. 2018). For example, in laboratory mice, telomeres do not always shorten with cell divisions,
24 (Gomes et al. 2010; Gorbunova et al. 2008). However, other recent studies in laboratory mouse strains

(e.g. C57BL/6 mice housed under *ad libitum* in standard laboratory conditions), showed some shortening in telomere length as mice aged, at rates predictive of individual longevity (Vera et al. 2012). In addition, sex differences exist in telomere length (Barrett and Richardson 2011), resulting in additional complexity related to sex-specific telomere length inheritance or aging. Field studies of telomere dynamics on small mammals to test these contrasting results obtained from controlled laboratory strains are mostly absent.

Even in other taxa than rodents, some studies found that telomeres may either be stable (cows: (Seeker et al. 2018)) or even increase over time (great tits: (Atema et al. 2018)). In other mammals, such as wild Soay sheep, telomere lengthening over an individual's lifetime was described (Fairlie et al. 2016) (for a bird example, see also (Spurgin et al. 2018)). Remarkably, a recent study on hibernating rodents have highlighted compensatory telomere lengthening when environmental conditions are becoming favorable after winter (Hoelzl et al. 2016a). Three explanations have been proposed for an increase of telomere length over time. Although (1) measurement errors or statistical adjustment in telomere length assessment need to be checked (Nettle and Bateson 2017; Simons et al. 2014; Verhulst et al. 2013), biological explanations have to be considered (Bateson and Nettle 2016), such as (2) variation in cell population on which telomere lengths are measured, or (3) in activated telomere maintenance processes (e.g. telomerase activity or alternative lengthening pathway) leading to actual elongation of telomere ends (Blackburn 1991; Cesare and Reddel 2010; Criscuolo et al. 2018a), a process which may be energetically costly (Hoelzl et al. 2016a; Nowack et al. 2019). If telomere size elongation in juvenile or adult somatic cells is more common than initially believed (Fairlie et al. 2016; Haussmann et al. 2004; Olsson et al. 2018), this would have important consequences for our understanding of how telomeres interact with fitness-related traits. For instance, it may highlight why long telomeres correlate better with individual fitness than short telomeres (Bauch et al. 2014). Importantly, we should test whether there is further evidence for an increase in telomere length in individuals living under different harsh environmental conditions than studied so far and whether this is related to fitness.

In wild-derived house mice strains (*Mus musculus*) kept under laboratory conditions, individuals showed accelerated telomere loss with immune, reproductive or social stress but non-significant relationships with mortality (Ilmonen et al. 2008; Kotrschal et al. 2007). Thus, one important emerging question is whether the idea that the shortening of telomeres under environmentally harsh conditions is long-lasting (De Punder et al. 2018; Reichert et al. 2014), or whether shortened telomeres can remain stable in size or even increase when environmental conditions become favorable. Whether telomere lengthening is a selected maintenance process improving individual fitness *via* increased lifespan, for example in hibernating mammals (Hoelzl et al. 2016b; Turbill et al. 2012), remains an unanswered question. To our knowledge, only four published studies described seasonal variation in telomere length (one on small wild mammals) (Fairlie et al. 2016; Hoelzl et al. 2016a; Quirici et al. 2016b; Stier et al. 2014). Two studies approached this question indirectly through cross-sectional sampling, assessing differences in telomere length between individuals experiencing different environments during growth or reproduction (Quirici et al. 2016a; Stier et al. 2014). Studies in Soay sheep and edible dormice provided some evidence that changes in telomere length are more related to seasonal variation in environmental conditions (like high population densities and feeding conditions before and/or during the winter) than to age (Fairlie et al. 2016; Hoelzl et al. 2016a).

To better understand how telomere dynamics are affected by environmental harshness, longitudinal studies are needed, where individuals are sampled repeatedly under different environmental conditions. Here, we investigated telomere dynamics in African striped mice (*Rhabdomys pumilio*). Striped mice inhabit a highly seasonal environment with high food abundance in the moist spring being followed by the food restricted harsh dry season in summer. We sampled individuals repeatedly, at the onset of the dry season (January), after they experienced 3 months of harshness in the middle of the dry season (March), 7 months after they experienced harshness at the end of the onset of the moist season (July), and at the end of the favourable moist season with high food abundance

73 (October). We predicted telomere shortening to be faster during the harsh food-restricted dry season
74 than during the favourable moist season, and that telomere length at the onset of the dry season is
75 related to disappearance risk of individuals, which we used as proxy for dry season survival and thus
76 fitness.

77

MATERIAL AND METHODS

Study species

The striped mouse is an omnivorous annual species: individuals are born in the moist spring (August to December) with peak food availability, then have to survive the following hot summer dry season (January to May) with very low food availability, before reproducing in the following spring; less than 1% of striped mice survive to reproduce in a second spring (Schradin et al. 2012). Depending on the harshness of the dry season, 1% to 50% of the striped mice survive the dry season to reproduce, such that dry season survival is a key determinant of individual fitness. In the breeding season, striped mice can live solitarily but more often live in communally breeding groups with 2-4 breeding females and 1 breeding male, and adult offspring remain philopatric, such that all striped mice live in social groups during the dry season (Schradin et al. 2010; Schradin et al. 2019).

Data collection

The study was conducted in Goegap Nature Reserve in South Africa (S 29 41.56, E 18 1.60). Goegap lies within the semi-desert Succulent Karoo biome, which is characterized by cold and moist winters followed by spring with high food abundance and hot dry summers with low food abundance. During the current study, food availability measured as the number of food plant species per 4 m² monitoring plot changed from 2.8 in January to 2.0 in March, 6.4 in August (after the July sampling), and 3.6 in October. Our field site was 7 ha and contained 12 social groups. Because the population was monitored throughout the year, we knew all individuals present on the field site, their life history, and their group affiliation (Schradin et al. 2019). Trapping continued even after our study such that we knew which individuals disappeared during the study and did not re-appear afterwards. During our normal monitoring, every group was trapped for 3 days 2 to 4 times every month (i.e. 6 to 12 days per month).

Tail tissue can be used to evaluate somatic telomere dynamics and are representative of telomere dynamics of overall body soma (Daniali et al. 2013). Collecting tail tissue has the advantage of being non-lethal and allowing repeated sampling, which is essential for longitudinal studies. We repeatedly collected tissue samples (1-2mm tail snips) from all individuals present on the field site (n=80, age 11 – 737 days) during extensive trapping sessions (always for 7 days) in 2018 at: (i) the beginning of January (onset dry season); (ii) March (middle dry season); (iii) End of July, beginning of August (onset of the moist and breeding season); and (iv) October (end of the moist / breeding season). Individuals were born in the moist season (August and December) in 2016 (n=9) or 2017 (n=48), with 14 individuals being born between January and July 2018. Season of birth was unknown for 9 immigrants born in 2016 (n=2), 2017 (n=6) and undefined (n=1). Tail clips were stored in 95% ethanol and transported to Strasbourg, France, for analysis.

For the onset of the dry season (January), we had samples from 54 individuals (29 females, 25 males, age 15-501 days). For the onset of the moist season (end of July), we had samples from 26 individuals (16 females, 10 males, age 9-652 days). In addition, intra-individual changes in telomere length was evaluated through repeated tissue sampling of the same individual in (i) January and March for 36 individuals (21 females, 15 males, individual variation in telomere dynamics over the dry season), (ii) July and October for 26 individuals (16 females, 10 males, individual variation in telomere dynamics over the moist season), and (iii) 6 individuals sampled monthly during all four months (Figure 1).

Fieldwork and tissue sampling were carried out under the necessary licenses. All data were collected following protocols approved by the Animal Ethics Screening Committee of the University of the Witwatersrand (AESC 2012/37/2A, AESC 2014/40/B).

Telomere analysis

Striped mice DNA was extracted from tail tissue using Nucleospin Tissue kit (Macherey-Nagel, Düren, Germany). DNA quality and concentration were assessed with a Nano-Drop spectrophotometer (NanoDrop Technologies, Thermo Fisher Scientific) and diluted to the concentration of 2.5 ng/mL. Relative telomere length was measured in striped mice on extracted DNA from tail tissue, using the methodology of real-time qPCR that originated from the work of Cawthon and collaborators on human samples (Cawthon 2002). For our study, the qPCR amplification protocol has been adapted as follows:

(i) qPCR gives us as a final results a ratio (T/S) of amplification cycles (Cq) of the telomere sequence (T) and of a genomic gene sequence (S) that must be specific or preferably a non-variable copy number (non-VCN) gene (Smith et al. 2011). Final T/S values were calculated by taking into account each plate efficiencies, following (Pfaffl 2001). For the present study, we designed amplification primers for a region using aligned sequences of PGK1 genes from other closely related organisms extracted from the NCBI data base (Huchon et al. 2017). We used a 150 bp portion of the phosphoglycerate gene (PGK1), which was found non-variable in copy number within our population of striped mice (Cq values ranged between 24-25.6 for 90% of the samples). Primer sequences for the non-VCN gene were: *Forward primer*: 5'-CGGAGACACCGCCACTTG-3' and *Reverse primer*: 5'-AAGGCAGGAAAATACTAAACAT-3'. The forward and reverse primers for the telomere sequence were: 5'-CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT-3' and 5'-GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT-3', respectively.

(ii) Amplifications of T and S were done on distinct 96-wells plates due to differences in amplification temperatures using an automated thermocycler (Master-cycler Realplex; Eppendorf, Hamburg, Germany). The reactions conditions were set as follows: for the telomere amplification, 95°C for 2 min – followed by 30 cycles of 95°C for 15 sec, 56°C for 15 sec, 72°C for 30 sec; for the PGK1 amplification, 95°C for 2 min – followed by 30 cycles of 95°C for 15 sec, 60°C for 15 sec, 72°C for 30 sec. All amplification runs were ended by a melting curve program (steady increase in amplification temperature from 56°C (T)

or 60°C (S)) to verify the absence of non-specific amplification events (*i.e.* lack of specificity of the primers or primer-dimer artefacts, see below). A negative control (without DNA) was also present on each plate to check for potential contamination.

(iii) Amplifications were performed with 5 ng of DNA (2 uL) per well, in a final volume of 10 uL containing 5 uL of GoTaq qPCR mix (Promega, Madison, WI, USA), 0.5 uL of each primers at an initial concentration of 5 nM and 2 uL of ultra-pure water.

(iv) Samples were amplified in duplicates, on 4 different runs (each comprising 1 telomere plate and 1 PGK1 plate). Each 96-wells plate included a dilution curve of the same randomly-chosen reference sample, serially diluted from 10 to 0.625 ng/mL of DNA, to check for consistency in amplification efficiency among plates (r^2 of the dilution curves were comprised between 0.931 – 0.998 (telomere) and 0.995 – 0.998 (PGK1)). In addition, 5 samples were repeated over all plates. Mean amplification efficiencies were of 96.0 ± 0.44 % for PGK1 and 97.8 ± 0.97 % for telomere. We did not include any plate effect in our models since there was no indications for an inter-plate variation in the T/S ratio values (Linear model, estimates -0.002 ± 0.017 , $t = -0.13$, $P = 0.896$).

qPCR repeatabilities and effect of storage time

As suggested by (Nettle et al. 2019), we evaluated the repeatability of our measures using intra-class correlation coefficients (ICC, *R* package ICC, (Wolak et al. 2012)) both for intra-plate (duplicates) and inter-plates variability in T/S ratio (see also (Eisenberg 2016)). Intra-plate ICC was of 0.864 and inter-plate ICC was of 0.836 (Cicchetti 1994). We also checked whether ICC varied with time of storage, *i.e.* time of sampling (from January to October): all samples were analysed together in May 2018, such that samples from January had the longest storage time and those samples from October had shortest storage time. Measurement repeatability (intra-individual) did not change with time from January (ICC = 0.851), March (0.835), July (0.769) and October 2018 (0.901). In addition, we found that T/S ratios were correlated

within individuals measured both in January and March ($n=35$, $t_{1,34} = 2.413$, $P = 0.021$), and both in July and October ($n=30$, $t_{1,29} = 2.198$, $P = 0.036$; see Figure1 in ESM 1). Additional qPCR methodological details are presented in ESM1.

Statistical analysis

Data were analysed using R version 3.5.1 (R Foundation for statistical Computing Platform, 2018), using the packages *lme4* (function *lmer*) (Bates et al. 2014) and *Factominer* (Lê et al. 2008). We tested for normality with both a Shapiro-Wilk's test and QQ plots distribution (*fitdistrplus* package, (Delignette-Muller and Dutang 2015)). We used a Linear Mixed Model (LMM) to analyse the relationship between telomere length ($n=154$ measures) and the following explanatory variables: *Age*, *sex*, and *season* and all interactions. Season was coded in 4 categories : beginning of the dry season (January), middle of the dry season (March), beginning of the moist season (July) and middle of the moist season (October). So, our statistical analysis considers the 4 sampling points and tested for differences among 4 season categories. Posthoc multiple comparisons among the 4 season categories were conducted using the function *glht* from the *multcomp* package (Hothorn et al. 2008). Individual ID was included as random factor (to control for repeated sampling). Because age, body mass and body size are interdependent, we also used a principal component analysis (PCA) to merge those variables into one PC axis (ESM2). PCA1 turned out to be non-significant in all models, so we kept only *Age* as a fixed factor in the final analysis. The date of birth was initially used as a fixed factor when testing variance in individual relative telomere length, but it was not significant (LMM, estimates 0.002 ± 0.001 , $t = 1.556$, $P = 0.124$) and not considered in our final models. Individual changes in telomere length over the dry and moist seasons were tested using paired *t*-tests. To test the predictive value of relative telomere length on disappearance risk of individual striped mice from the population (Vuarin et al. 2019) , we used a linear mixed model with binomial distribution of the survival variable (0 and 1, the latter indicating the presence of the individual). Individuals were

regarded as not having disappeared when they were still trapped in July or afterwards (monthly trapping of groups continued for 1.5 years). Of the 54 individuals sampled in January, 19 survived until July and 35 disappeared. July was chosen as the censoring date, since this is when breeding dispersal peaks, such that most cases of disappearance that occur before this time can be assumed to be due to mortality (Vuarin et al. 2019). Explanatory variables included in the models were telomere lengths measured in January, March and July (n=52 measures, with Individual ID as random factor). In addition, telomere length rate of change (*i.e.* the within individual differences in telomere lengths measured within the dry season or TROC, n=92 measures) was tested separately. Using the correction for the regression to the mean (RtM) effect ((Verhulst et al. 2013), ESM4) or using, as we present in the main text, uncorrected TROC with the initial telomere length as a covariate (Bateson et al. 2019; Hoelzl et al. 2016b) did not change the output of the models . We choose to present models obtained after model selection using the Akaike Information Criterion corrected for small sample sizes (*Mumin* R package, (Barton 2020), ESM3).

RESULTS

There was an overall significant variation in relative telomere length over the seasons (Table 1, Figure 2). Posthoc Tukey contrasts indicated that, overall, relative telomere lengths were longest at the start of the dry season in January and shortest in March and July. Relative telomere lengths were significantly longer in October than in July ($P = 0.035$; while there was no significant difference between March and October, $P = 0.994$). Individual telomere dynamics (Figure 3) demonstrated that, during the dry season, telomere length of most individuals declined (paired t -test, $t_{35} = 3.486$, $P = 0.001$, mean difference in relative telomere length = -0.238 T/S ratio) whereas during the moist season, telomere lengths of most individuals increased (paired t -test, $t_{31} = 4.373$, $P < 0.001$, mean difference in relative telomere length = 0.170 T/S ratio).

The difference in relative telomere length between sexes depended on age (interaction term Age x Sex: $P = 0.031$, Table 1). Telomeres of males shortened significantly during ageing while female telomeres did not (Figure 4, linear regression, males: $df = 62$, $t = -2.91$, $P = 0.005$; females: $df = 88$, $t = -0.09$, $P = 0.930$).

Individual disappearance risk during the dry season (*i.e.* from January to July) was not significantly predicted by the relative telomere length measured at the beginning of the dry season ($n = 52$, Table 2A) nor by TROC within the dry season (January to March, Table 2B). TROC corrected for the regression to the mean stayed non-significant for predicting disappearance risk (0.956 ± 0.866 , $z = 0.110$, $P = 0.912$). None of the other explanatory variables (sex, age, and interaction terms, all $P > 0.308$) predicted individual disappearance.

DISCUSSION

Here we showed that (i) the degree of telomere length degradation differed between the harsh dry and the favorable moist season in African striped mice, and (ii) that this effect appeared independent of an age effect which was only evident in males. In fact, on average, telomere length even increased in individuals from July to October (more so in females), demonstrating that the telomere loss during the preceding dry season cannot be simply explained by age (Figure 3). This indicates that environmental harshness influences telomere dynamics: telomeres degrade under harsh conditions but can increase afterwards, at least in females. Thus, our study indicates that a small mammal living under harsh conditions characterized by heat and low food abundance shows similar telomere dynamics as small hibernating mammals after winter but can be subsequently restored, at least partially, during periods of higher food availability (Hoelzl et al. 2016a).

Telomere shortening might indicate energetic costs (Criscuolo et al. 2018b; Ilmonen et al. 2008; Nettle et al. 2015; Reichert et al. 2014). In our study, food was two times more abundant during the peak of the moist season than during the dry season, such that the dry season was much harsher than the moist season. Accordingly, telomeres shortened significantly during the dry season. Physiological factors that are suspected to negatively affect telomeres in other species include high metabolic rate via increased oxidative stress ((Reichert and Stier 2017) but see (Boonekamp *et al.* 2017) and (Turbill et al. 2012)) and corticosterone levels ((Hausmann et al. 2012; Tissier et al. 2014) but see (Gil *et al.* 2019)). Also, in striped mice, the food restricted dry season triggers several physiological mechanisms (Rimbach et al. 2016; Schradin 2008a; Schradin 2008b; Schradin et al. 2014), enabling them to reduce both resting metabolic rate and daily energy expenditure (Rimbach et al. 2018a). Metabolic rate and corticosterone levels in striped mice are increased during the moist season (Rimbach et al. 2018a; Rimbach et al. 2018b; Schradin 2008c) which would predict a decrease in telomere length, while in opposite we observed an increase. Instead, the low energy availability in the dry season might lead to a decreased investment in

cell maintenance and telomere degradation. In the harsh summer, striped mice might rather invest in mechanisms that improve immediate dry season survival while increased food abundance in the subsequent spring favours energy investment into telomere maintenance.

We found age-related telomere erosion in males but not in females. Males also tended to have slightly longer telomeres than females (Table 1, $P = 0.076$). If males start their yearly cycle with longer telomeres (Figure 3), this may give a mechanistic explanation for faster shortening thereafter, since long telomeres are expected to be more susceptible to damage and less to maintenance than short telomeres (Bauch et al. 2014). A trade-off based effect due to sex-specific investment in reproduction vs. telomere maintenance should be explored in the future, based, for instance, on an earlier reproductive investment in males during the dry season (Schradin and Pillay 2005). Further, females may better protect their telomeres or invest in soma maintenance in order to transfer longer telomeres to the next generation, as suggested for another ageing-related mechanism, oxidative stress, in two distinct mammal species (*Mungos mungo* and *Urocitellus columbianus*, (Viblanco et al. 2018; Vitikainen et al. 2016). Future studies should investigate sex specific telomere dynamics depending on sex specific investment into reproduction and whether the shield hypothesis protecting offspring from maternal oxidative stress (Blount et al. 2016) extends to telomere inheritance.

Only 37% of the individuals sampled at the onset of the dry season were still present on the field site after the dry season, the rest having disappeared. In our current study, individuals with shorter telomeres were not more likely to disappear during the food restricted dry season. This leads to the hypothesis that telomere length in striped mice is uncoupled from short-term survival because of high extrinsic mortality risks. An alternative may be that, like other small rodents (Seluanov et al. 2007), striped mice maintain a high telomerase activity throughout their lives and consequently telomere length is not a good cell ageing marker. However, in wild mice strains (*Mus musculus* (Criscuolo et al. 2018b; Kotrschal et al. 2007)) and in a laboratory study conducted on Sudanian grass rat (*Arvicanthis*

ansorgei, (Grosbellet et al. 2015)), the rate of telomere shortening increased with stress caused by circadian desynchronization. Telomere shortening in mice should then be more closely related to survival (Ilmonen et al. 2008), since a faster telomere loss may reflect a concomitant deterioration of the organism's functioning (*i.e.* as an intrinsic ageing process, (Harris et al. 2016; Whittemore et al. 2019)). We did not find such a relationship since individuals with increased telomere shortening during the dry season were not more likely to disappear. In future, we need more data to test whether telomere length is correlated with reproductive success in striped mice.

It is currently widely debated whether reported increases in telomere length represents measurement errors or are of biological relevance (Bateson and Nettle 2016; Steenstrup et al. 2013). To date, negative and positive variation in telomere length over life have been found in free-living mammals and birds sampled longitudinally (Fairlie et al. 2016; Spurgin et al. 2018), illustrating the potential biological relevance of telomere lengthening. In striped mice, telomeres even increased in many individuals during the moist season (Figure 3). Although a long-term study with several dry and moist seasons differing in harshness is needed to definitively show an effect of harshness on telomere dynamics, the observed lengthening of telomeres raises questions about both its ultimate function (*i.e.* link with reproductive success) and proximate mechanism (*i.e.* seasonal changes in telomerase activity or changes in the rate of cell renewal). It is important to note that even though we were not able to statistically test for true lengthening (which needs at least 3 repeated samples, (Simons et al. 2014), because we used repeated tail sampling for telomere measurements, attributing telomere lengthening to a change in cell population is less likely than in studies using repeated blood sampling. One ultimate hypothesis may be related to breeding. Since the moist season is also the breeding season for striped mice, the lengthening of somatic telomeres may improve reproductive success either because it is associated with enhanced adult physiological processes or because it ensures better offspring quality. Therefore, elongating telomeres in breeding adults may be of key importance for fitness. Such an

increase in telomere length was previously found in the edible dormouse (*Glis glis*), also during the active breeding season (Turbill *et al.* 2013). Our study sheds lights on the fact that telomere elongation is not specific to hibernating rodents. It suggests that telomere dynamics change seasonally in a sex specific way in free-living striped mice, and that, as previously found in hibernating small mammals (Hoelzl *et al.* 2016a; Nowack *et al.* 2019), telomeres can even lengthen after a harsh season when environmental conditions become favourable. This indicates that telomere dynamics are plastic and future studies need to focus on the ultimate function and proximate mechanisms of increasing telomere length in striped mice specifically and free-living animals generally.

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317 **FIGURES legends**

318 **Figure1**

319 Schematic representation of the repeated sampling design of the study. In total, we collected 154
320 samples from 80 individuals (starting with 54 mice in January and adding 26 mice in July), but not all
321 individuals were sampled repeatedly. 36 individuals were sampled both at the start (January) and the
322 middle (March) of the dry season. 32 individuals were sampled both at the start (July) and the middle
323 (October) of the moist season. Altogether 6 individuals were sampled during all four months.

324 **Figure 2**

325 Year-round variation in relative telomere length in adult striped mice (156 samples from 80 individuals).
326 Relative telomere length was repeatedly measured in 36 individuals over part of the dry season (two
327 sampling sessions in January and March) and in 32 individuals over the moist season (July and October).
328 * indicates significant levels of posthoc Tukey contrasts: *** < 0.001, ** < 0.01, * < 0.05. Boxplots
329 indicate minimum and maximum, median (black line), first and third quartile and mean (diamonds)
330 values. See text for details.

331

332 **Figure 3**

333 Individual variation in relative telomere length in male (left panel) and female (right panel) adult striped
334 mice over the beginning and middle of the dry season (January and February, black circles, 36
335 individuals) and beginning and middle of the moist season (July and October, grey circles, 26
336 individuals). Increases in telomere length were found in 24 of 32 individuals in the moist season.
337 Numbers for the dry season was 11 of 36 individuals. Dashed lines indicate the 6 individuals that were
338 sampled 4 times over the year.

339

340 **Figure 4**

341 Relative telomere length of striped mice in relation to sex over the entire study period. The Sex x Age
342 interaction was significant ($P = 0.031$, Table 1), indicating that males underwent an age-related telomere
343 erosion (linear regression, $P = 0.005$) while females did not (linear regression, $P = 0.930$). See text for
344 details. Confidence intervals 95% are indicated in grey.
345

Table 1: Linear mixed model explaining the effects of season (coded using the 4 monthly sampling points, January / March for the dry season and July / October for the moist season), age, and sex on individual relative telomere length in wild striped mice. The model encompasses repeated telomere data from individuals sequentially sampled within seasons (individual ID as random factor) but includes all individual measurements (in total 154 telomere measurements among 80 individuals). Seasons are defined as dry (January and March) and moist (July and October, see figure 1). Significant fixed effects (in bold) are considered at $P < 0.05$ and tendencies (in italics) are considered at $P < 0.1$, P values being obtained using the *anova* R function. The model presented was selected based on AICc values. Posthoc comparisons were done using the *multcomp* R package.

Response variable: Relative telomere length		Estimates \pm SE	df	Z value	P
<i>Random factors</i>	Individual ID	0.019 \pm 0.014			
	Residual variance	0.063 \pm 0.251			
<i>Fixed factors</i>					
	Intercept	0.935 \pm 0.057	128.2	16.275	< 0.001
	Sex (M)	0.150 \pm 0.084	100.6	1.794	<i>0.076</i>
	Age	0.001 \pm 0.001	114.4	0.436	0.664
	Sex x Age	-0.001 \pm 0.001	100.8	-2.192	0.031
	Season	-0.199 \pm 0.062	104.9	10.723	< 0.001
<i>Tukey Contrast posthoc</i>				Z value	P
	January - March	0.181 \pm 0.056		3.263	0.006
	January - July	0.334 \pm 0.063		5.280	< 0.001
	January - October	0.164 \pm 0.063		2.593	0.047
	March - July	0.152 \pm 0.068		2.234	0.113
	March - October	-0.018 \pm 0.068		-0.259	0.994
	July - October	-0.170 \pm 0.063		-2.692	0.035

Table 2: Predictors of survival of striped mice until the beginning of the 2018 breeding season (*i.e.* July). A generalized linear model was used, following a binary distribution of individual survival (0 = disappearance) using (A) the individual telomere lengths measured at the start of the dry season (*i.e.* January) and (B) the rate of change in telomere length (TROC) over the dry season (*i.e.* January – March). Results were also not significant when using TROC corrected for the regression to the mean effect (see Results). Estimates \pm SE are given for males vs. females. Significant fixed effects (in bold) are considered for $P < 0.05$. The models presented here were selected from AICc values.

A. Response variable: Survival	Estimates \pm SE	Z value	P
<i>Random factors</i> Individual ID	5276 \pm 72.63		
<i>Fixed factors</i>			
Intercept	-18.899 \pm 6.633	-2.849	0.004
Relative telomere length (T/S ratio)	0.664 \pm 6.218	0.107	0.915
Sex (M)	-9.389 \pm 23.168	-0.405	0.685
Age	-0.063 \pm 2.902	-0.180	0.798
Sex x Age	0.039 \pm 0.052	0.749	0.454
Relative telomere length x Sex	6.063 \pm 14.284	0.424	0.671
B. Response variable: Survival	Estimates \pm SE	Z value	P
<i>Random factors</i> Individual ID	NA		
<i>Fixed factors</i>			
Intercept	-1.070 \pm 1.703	-0.629	0.530
Telomere rate of change (T/S ratio)	0.390 \pm 1.926	0.203	0.839
Age	0.002 \pm 0.003	0.781	0.435
Sex (M vs. F)	-2.791 \pm 1.395	-2.001	0.045
Initial relative telomere length	1.144 \pm 1.900	0.602	0.547
Age x Sex	0.017 \pm 0.012	1.363	0.173

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