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Telomere dynamics in female Columbian ground squirrels: recovery after emergence and loss after reproduction

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Abstract :

Telomeres are specialized non-coding DNA sequences located at the end of chromosomes and that protect genetic information. Telomere loss over lifespan is generally viewed as a phenomenon associated with aging in animals. Recently, telomere elongation after hibernation has been described in several mammals. Whether this pattern is an adaptation to repair DNA damage caused during rewarming from torpor or if it coevolved as a mechanism to promote somatic maintenance in preparation for the upcoming reproductive effort remains unclear. In a longitudinal study measuring telomere length using buccal swabs, we tested if telomere elongation was related to reproductive success in wild adult female Columbian ground squirrels (Urocitellus columbianus) that were monitored from emergence from hibernation to the end of the reproductive season. We found three key results. First, female telomere length increased at the start of the breeding season, both in breeding and non-breeding individuals. Second, post-emergence telomere lengthening was unrelated to female future reproductive output. Third, telomere length decreased in breeding females during lactation, but remained stable in non-breeding females over a similar period. Within breeders, telomeres shortened more in females producing larger and heavier litters. We concluded that telomere lengthening after hibernation did not constrain immediate female reproductive capacities. It was more likely to be part of the body recovery process that takes place after hibernation. Telomere erosion that occurs after birth may constitute a physiological cost of female reproduction.

Keywords : Aging, Telomeres, Cost of reproduction, Hibernation, Telomerase, Stress, Reproduction, Mammals

INTRODUCTION

Telomeres are sequences of non-coding DNA that cap the end of linear chromosomes and protect the integrity of coding DNA where key genetic information resides (Greider, 1991). As cells replicate, telomeres progressively shorten due to the end-replication problem of DNA (viz., the extremities of DNA on the lagging-strand are lost due to a lack of template) (Harley, Futcher, & Greider, 1990; Watson, 1972). Although part of this replication problem is counterbalanced by telomerase, an enzyme that rebuilds otherwise dwindling telomeres (Greider & Blackburn, 1985), critically short telomeres lead to cells leaving the normal replicative cycle through cell apoptosis (Blackburn, 1991). From an organismal perspective, telomere length, and maybe more so the rate of telomere loss, are increasingly considered reliable proxies of individual or species lifespan, both in captive (*e.g.* Heidinger et al., 2012; Whittemore et al., 2019) and free-living vertebrates (reviewed by Wilbourn et al. 2018). Telomeres are associated with individual fitness in numerous species (Bichet et al., 2020; Dupoué et al., 2017; van Lieshout et al., 2019).

Beside the end-replication problem, a growing number of studies have highlighted how telomere length may be an integrative proxy of individual stress, as telomere length integrates the accumulation of stress-related damages with age, and thus reflects individual life experiences (Dupoué et al., 2020; Monaghan & Haussmann, 2006). For instance, environmental harshness (*e.g.* pollution levels, disease prevalence, oxidative stress, environmental and social stressors; reviewed by Chatelain, Drobniak, & Szulkin, 2020) has been shown to negatively affect telomere length in groups as diverse as fish (Molbert et al., 2021), birds (Asghar et al., 2015; Aydinonat et al., 2014; Grunst et al., 2020), and mammals (Kesäniemi et al., 2019), including humans (Blackburn & Epel, 2012). As data grow, so does our understanding of the importance of these highly conserved DNA structures in shaping life-history trade-offs and tactics across species (Dantzer & Fletcher, 2015; Young, 2018).

Telomere characteristics are likely to shed useful mechanistic insights on life histories. This is because telomere loss may reflect the physiological costs that an individual has to pay when limited energy is invested in potentially competing, resource-demanding life-history functions (such as reproduction and self-maintenance). This is based on the hypothesis that telomere maintenance is an energetically costly mechanism (Young, 2018). Whereas we have no direct evidence for this hypothesis so far, indirect evidence has been accumulating from studies focusing on reproductive costs and from hibernating species (*e.g.* Hoelzl et al., 2016; Nowack et al., 2019).

In fact, experimental increases in long-term reproductive effort and actuarial senescence (increased parental mortality rates) have been positively correlated (Boonekamp, Bauch, & Verhulst, 2020), suggesting that reproduction somehow triggers ageing mechanisms in adults (Flatt & Heyland, 2011). Experimentally increasing reproductive effort was found to hasten telomere shortening of breeding birds (Reichert et al., 2014; Sudyka et al., 2014; for contrasting results see Sudyka et al., 2019). While most experimental studies published to date supported the hypothesis of accelerated telomere erosion as a cost of reproduction, negative associations between reproductive traits and telomere dynamics were only found in roughly half of the studies conducted in wild vertebrates (Sudyka et al. 2019). Thus, whether the negative impact of reproduction on telomeres is a general phenomenon across vertebrates remains to be determined (*e.g.* Sudyka et al. 2016; Sudyka et al., 2019), and further evidence is needed, particularly in non-avian species.

Previous studies on hibernating rodents convincingly demonstrate that telomere maintenance is achieved at energetic costs. Hibernation is composed of periods of decreased body temperature (torpor) and rewarming events, and the frequency of these torpor events or rewarming events and associated metabolic activation have been related to telomere dynamics (Turbill et al., 2012; Turbill et al., 2013; Hoelzl et al. 2016). Interestingly, experimental

96 manipulation of food supply or of ambiant temperature during or after hibernation were shown
97 to impact both telomere shortening or elongation, suggesting an energy cost of telomere
98 maintenance (Hoelzl et al. 2016, Nowack et al. 2019). In any case, hibernating species appear
99 to retain the seasonal ability to reconstruct their telomeres after hibernation (*i.e.* at the start of
100 the active season, Hoelzl et al., 2016; Turbill et al., 2012; Turbill et al., 2013). Such a
101 phenomenon may characterize the switch of adult physiology from a non-active hibernating
102 state to an active reproductive state (Hoelzl et al., 2016), and has been suggested as an
103 immediate post-hibernating and pre-reproductive strategy (Nowack et al., 2019), but its actual
104 benefits in terms of fitness remain unclear. Telomere elongation at emergence from hibernation
105 could occur as part of a somatic maintenance process related to hibernation (Turbill, Bieber, &
106 Ruf, 2011), as an anticipatory process in order to buffer the oxidative (telomere-shortening)
107 costs of upcoming reproduction (*i.e*. oxidative shielding hypothesis; Blount et al. 2016, Viblanc
108 et al. 2018), or both. Teasing apart those alternatives requires information from hibernating
109 species in which seasonal telomere dynamics at the individual level are assessed at emergence
110 from hibernation and then longitudinally throughout the breeding season. By comparing
111 breeding and non-breeding individuals sampled at similar time periods, it is possible to test
112 whether telomere length, telomere lengthening or telomere loss predict individual reproductive
113 success, or reflect a cost of reproduction, while controlling for individual differences in age
114 and condition (*i.e.,* inter-individual differences in body mass at the onset of the breeding
115 season).

116 We tested the hypothesis that telomere dynamics (both lengthening and shortening)
117 varied as a response to hibernation and reproduction in free-living female Columbian ground
118 squirrel (*Urocitellus columbianus*). Columbian ground squirrels provide a robust model system
119 for addressing these questions. These montane rodents have an especially short active season
120 (3-4 months) during which females emerge, soon mate, gestate (ca. 24 days), lactate and raise

121 their young (ca. 27 days), molt and fatten before subsequent hibernation (Dobson, Badry, &
122 Geddes, 1992; Dobson & Murie, 1987). The reproductive season starts shortly after emergence
123 from hibernation, and females typically enter oestrus 3-4 days following emergence (Lane et
124 al. 2011). Thus, this is a short and highly active period following a long period of metabolic
125 inactivity. For females, lactation is an energy-demanding period when oxidative metabolism is
126 high (Skibiel, Speakman, & Hood, 2013; Speakman, 2008) and when the oxidative costs of
127 reproduction are expected to be highest. Interestingly, these females seem to have evolved
128 increased antioxidant defenses, which are highest during lactation (Viblanc et al. 2018),
129 allowing them to buffer the oxidative costs of reproduction (*i.e.* the oxidative shielding
130 hypothesis; Blount et al. 2016). This suggest that lactation entails physiological costs, but
131 whether those costs extend to damage to telomeres remains to be tested.
132 We hypothesized that, at the start of the breeding season (from emergence of
133 hibernation to parturition), an increase in telomere length might be expected both with regards
134 to somatic maintenance/reconstruction following hibernation and as part of a mechanistic
135 process that buffers the later oxidative costs of lactation (Blount et al., 2016). On one hand,
136 and after controlling for individual age and condition, a positive association between starting
137 telomere length and reproductive output would suggest that telomere length acts as a constraint
138 on reproduction (*i.e.,* females with low somatic condition would not invest in reproduction).
139 On the other, a positive relationship between reproductive output and telomere loss over
140 lactation would suggest that reproductive effort indeed entails physiological costs (*i.e.,* females
141 with high reproductive outputs exhibit greater reduction in telomere length over the breeding
142 season). To test this, we followed known-aged females at emergence from hibernation and over
143 the course of the breeding season. Since nothing is known so far about how variable telomere
144 length is in this species, or whether telomere length is related to individual age or body
145 condition, we first evaluated the individual repeatability of telomere length over the breeding

146 season, and the potential effect of female age and condition (mass) on telomere length. Then,
147 we evaluated: (i) if female telomere lengthening occurred at the start of the breeding season,
148 from emergence of hibernation to parturition; (ii) if telomere length at emergence from
149 hibernation (and potential subsequent reconstruction from emergence to parturition) predicted
150 female reproductive output (assessed by litter size and mass measured at weaning); and (iii) if
151 female reproductive output was associated with increased telomere loss during lactation.
152
153 **METHODS**
154 **Study site and population monitoring**
155 Female Columbian ground squirrels were monitored in a 2.6 ha subalpine meadow from 1992
156 to 2019 in the Sheep River Provincial Park, Alberta, Canada (50 38' 10.73" N; 114° 39'
157 56.52"W; 1520 m), as part of a long-term study on their ecology, behavior, and physiology
158 (Dobson et al. 2020). Reproduction takes place over the short summer season (3-4 months),
159 during which sexually mature females (2-14 years old) produce a single litter (2-7 pups) over
160 51 days (24 days of gestation and *ca* 27 days of lactation and post-weaning parental care, Murie
161 and Harris 1982). In each year, the entire population was trapped at emergence from
162 hibernation, using 13 x 13 x 40 cm traps (Tomahawk Live Traps; Hazelhurst, WI, USA) baited
163 with a small amount of peanut butter. Upon trapping, each ground squirrel was weighed to the
164 nearest 5 g (Pesola spring-slide scale; Pesola® Ag; Baar; Switzerland), tagged if unmarked
165 (#1-Monel metal tag; National Band and Tag Company, Newport; KY) and painted with a
166 unique dorsal mark for visual identification with black hair dye (Clairol, Stamford, CT).
167 Consequently, all females in the population were of known age. Buccal tissue for telomere
168 length (see below) was collected during the 2019 active period.
169 We observed animals daily (from ~ 7:00 am to 2:00 pm), starting at emergence from
170 hibernation and continuing throughout the reproductive season. We determined mating dates

for each female from male-female interactions, the occurrence of below-ground consortships or above-ground mating events (Raveh et al., 2010, 2011). All females were surveyed daily until we were certain that they had (or had not) reproduced, as confirmed by mating behavior. When in doubt, we inspected female genitalia for the presence of copulatory plug material and dried sperm as indicators of successful mating events (Murie & Harris, 1982). We flagged the location of female nest-burrows (single-entrance burrows where females raise and lactate to feed their young), identified by daily observations of female's morning emergences from these burrows and observations of females stocking them with loads of dry grass. Mothers and their entire litters were trapped at about the time of weaning, when offspring first emerged above ground, approximately 51 days after mating and 27 days after parturition. All offspring were weighed to the nearest 1 g and given unique ear tags and dye markings.

Tissue collection and telomere length measurement

Tissue collection

Cell tissue was collected from the buccal mucosa of each ground squirrel by gently twirling a Gynobrush® brush (Heinz Herenz Medizinalbedarf, Hamburg) on the inside of each cheek. This technique is particularly adapted for repeated non-invasive sampling (Hoelzl et al. 2016), and buccal cell DNA to reflect individual health status (e.g. in humans, Thomas et al. 2008) The collected tissue was immediately transferred to 96% ethanol Eppendorf tubes and kept at 4°C until DNA extraction. For breeding females (N = 19; age = 4.4 ± 1.9 years old, range = 2 – 8 years old), tissue was collected at four points calculated for each individual: emergence from hibernation (or the day after), and then 26, 38 and 54 days later (around the times of parturition, mid-lactation, and offspring weaning). Tissue was collected from non-breeding females (non-breeding females: N = 24; age = 2.6 ± 2.0 years old, range = 1 – 7 years old) following a similar schedule. Overall, we were able to acquire tissue for 43 females including

19 breeding and 24 non-breeding individuals all sampled four times (i.e. 172 samples). However, differences in sample sizes between our different analyses are due to varying success at acquiring samples in the field or telomere amplification in the laboratory, or missing data in other parameters on some occasions (*e.g.,* female mass).

DNA extraction

DNA extraction was carried out using the Nucleospin Tissue kit, Macherey-Nagel, Düren, Germany, and checked for quality using gel-migration (DNA integrity) and a NanoDrop 1000 (ThermoFisher Scientific, Waltham, MA, USA) spectrophotometer (absorbance ratio A260/280; A260/230, DNA quality). Briefly, after allowing ethanol to evaporate from the tissue sample, lysis was achieved by incubation of the sample material in a proteinase K / SDS solution. Appropriate conditions for DNA binding to the silica membrane in the NucleoSpin® Tissue Columns were achieved by adding chaotropic salts and ethanol to the lysate. The binding process was reversible and specific to nucleic acids. Contaminants were removed by subsequent washing with two different buffers as indicated by the kit's protocol. Pure genomic DNA was finally eluted under low ionic strength conditions in a slightly alkaline elution buffer.

Telomere qPCR amplification

Extracted DNA was used to amplify both the telomere sequence and a control gene by quantitative real-time amplification (qPCR) based on Cawthon's (2002) original publication. As a reference gene that was invariant in copy number (non-VCN, Smith et al. 2011), we used a 54 bp portion of the c-myc proto-oncogene, which was tested for non-variability in copy number in our population using amplicon gel migration (see Online Supplementary Materials). Forward and reverse telomeric primers were 5'- CGG TTT GTT TGG GTT TGG GTT TGG GTT TGG GTT TGG GTT - 3' and 5'- GGC TTG CCT TAC CCT TAC CCT TAC CCT TAC

CCT TAC CCT - 3'. Primer sequences for the non-VCN gene were 5'- GAG GGC CAA GTT GGA CAG TG - 3', and 5' - TTG CGG TTG TTG CTG ATC TG -3'. A master mix was prepared for each primer set containing 5 µL GoTaq® qPCR Master Mix (Promega, Madison, WI, USA).

We used telomere primers at a concentration of 200 nM and non-VCN primers at 400 nM in a 10 µL reaction. Each sample of DNA was diluted to 2.5 ng/µL with double-distilled H_2O just prior to running the reactions, and 2 µL of this sample is used in each 10 µL reaction. qPCR conditions for telomeres were 2 min at 95°C followed by 30 cycles of 15 s at 95°C, 30 s at 56°C and 30 s at 72°C. qPCR conditions for the non-VCN gene were 2 min at 95°C followed by 40 cycles of 15 s at 95°C, 30 s at 59°C and 1 min at 72°C. Amplifications were done on a 384-well thermocycler (CFX-384 Touch Real-Time PCR Detection System, Biorad, USA). Duplicates of each sample's telomere and non-VCN qPCR amplifications were performed on separate plates (*i.e.* forming a qPCR run), the amplification conditions being different between telomere and non-VCN sequences (see above). In total, all samples of our experiment were measured over 2 runs.

In addition to our ground squirrel samples, all plates included a no template control (water) and a 'calibrator' sample in duplicate. The calibrator was DNA extracted from a single individual (golden sample randomly chosen among those for which a large quantity of DNA was available), diluted to the same concentration as other ground squirrel samples (2.5 ng/µL). Both a negative control (water) and melting curves were run for each plate to control for the absence of (i) non-specific amplification and of (ii) primer-dimer artefact. On each plate, we also included a calibrator sample dilution curve (from 10 ng/µL to 0.3125 ng/µL) to evaluate plate amplification efficiencies of the telomere sequence and the non-VCN gene, and to check that the Cq values produced declined in a log-linear fashion ($r^2 > 0.98$) before proceeding to statistical analysis.

Among the amplification values of the control gene, we had 23 samples (13.4% of 172 samples,) which were delayed of 1 Cq compared to the mean Cq value. Running the analyses with or without these samples yielded similar results, and thus we chose to keep them in the present analysis. Efficiencies for the two reactions were 0.997 (range 0.996 – 0.999) for non-VCN genes and 0.996 (range 0.991- 1.001) for telomeres. We calculated relative telomere length (RTL) following Pfaffl (2001), as:

$$RTL = \frac{E_{TEL}^{\,Cq_{TEL[calibrator]} - Cq_{TEL[sample]}}}{E_{non-VCN}^{\,Cq_{non-VCN[calibrator]} - Cq_{non-VCN[sample]}}}$$

where E_{TEL} and $E_{non\text{-}VCN}$ are the mean plate efficiencies for each sequences, and $Cq_{TEL[calibrator]}$ and $Cq_{TEL[sample]}$ are the mean Cq for telomere calibrator and sample, respectively. Similarly, $Cq_{non\text{-}VCN[calibrator]}$ and $Cq_{non\text{-}VCN[sample]}$ are the mean Cq for non-VCN calibrator and sample, respectively. The intraclass correlation coefficient (ICC) was calculated for intra-run and inter-run variation of T/S ratio following (Cicchetti 1994), and was recorded at 0.783 and 0.805, respectively.

Data analyses

Telomere length repeatability and relationship with female age and body mass

First, we used a linear mixed model (LMM) approach to estimate individual repeatability in telomere length over the course of a breeding season as

$$R = \frac{V_G}{V_P} = \frac{V_G}{V_G + V_R} \;;$$

V_G is the among-individual variance in telomere length, V_P the total phenotypic variance in telomere length, and V_R the within-individual (or residual) variance in telomere length (Nakagawa and Schielzeth 2010, Stoffel et al. 2017). We accounted for potential error measurement associated with qPCR assays by including assay-related terms (plate, row within plate) as random factors in the model. Confidence intervals around repeatability estimates were

calculated by parametric bootstrapping (10,000 iterations) using the 'rptR' R cran package (Stoffel et al., 2017). The statistical significance of repeatability estimates was calculated using likelihood ratio tests, comparing the model fit to models where group-level variance was constrained to zero.

Second, we ran a LMM ('lme4' package in R) to test whether female telomere length was related to female age or condition (estimated as mass) at the time of measurement. For this, female telomere length was specified as the dependent variable, and female age and mass as independent variables. We included female ID as well as assay-related terms (plate, row within plate) as random factors in the model to account for repeated measures on individual females over the breeding season, and potential measurement error associated with qPCR assays. Telomere length and independent variables were systematically standardized (z-scores) prior to analyses to facilitate future comparisons between q-PCR-based telomere studies (Verhulst, 2020), and so that estimates of models for independent variables could be directly compared as effect sizes.

Seasonal changes in telomere length

Seasonal changes in telomere length were investigated separately in females using a LMM, with telomere length specified as the dependent variable, and seasonal timing (emergence *vs.* birth *vs.* lactation *vs.* weaning), breeding status (breeder *vs.* non-breeder), and their interaction as independent variables. Breeding females were those observed lactating and raising a litter. We specifically tested for the interaction between breeding status and seasonal timing, to test whether seasonal dynamics in telomere length were different between breeding and non-breeding females. Because our previous analyses showed significant influences of female age and mass on telomere length, we controlled for those variables, as well as for female ID (repeated measures over the season) and assay-related terms (plate, row within plate) as random

295 factors in the model. As above, all variables were standardized. As a measure of effect size, we
296 calculated percent changes in telomere length between seasonal time periods from marginal
297 means obtained from a similar LMM but where telomere length was not standardized (so that
298 actual percent differences between time periods could be calculated). Significant differences
299 between time periods were determined by Tukey Honest Significant Difference post-hoc tests
300 (HSD), which control for multiple testing, using the '*emmeans*' package. Marginal model
301 means are presented along with their 95% confidence intervals in the figures and significant
302 differences along with their 95% confidence intervals are presented in the tables.
303
304 *Telomere length in relation to subsequent reproduction*
305 For those females that bred, we tested whether telomere length at the start of the breeding
306 season predicted female reproductive output. Litter size (N = 18, litter size (± SD) = 2.5 ± 1.0,
307 range 1 – 5) or total litter mass (N = 18, total litter mass = 247.2 ± 89.6 g, range 58 – 410 g)
308 were regressed on telomere length at emergence. We further accounted for female mass at the
309 start of the breeding season as a covariate in the model for known influences of female starting
310 capital on reproduction (Broussard, Dobson, & Murie, 2005; Dobson, Risch, & Murie, 1999;
311 Rubach et al., 2016; Skibiel, Dobson, & Murie, 2009), as well as for assay-related terms (plate,
312 row within plate) as random variables. We initially controlled for female age as well, but
313 removed it from the final model since it explained virtually no variation and prevented models
314 from properly converging. In addition, we tested whether the increase in telomere length at the
315 start of the season (from emergence to birth) predicted litter size and mass at weaning (good
316 proxies of lactational investment; Skibiel, Dobson, & Murie, 2009). For this, we specified total
317 litter mass or total litter size at weaning as the dependent variables in separate models, and the
318 change in telomere length from emergence to parturition (hereafter birth) ($TL_{birth} - TL_{emergence}$)

as the independent variable of interest. Here also, we controlled for the influence of female mass at the start of the breeding season by entering it as a covariate in the model.

Telomere loss following reproduction

We tested whether reproductive output was reflected in telomere loss. For this analysis, we focused on female telomere dynamics during lactation, *i.e.* the period of highest energy commitment to reproduction in mammals (Speakman, 2008), and regressed telomere change from mid lactation to weaning ($TL_{weaning} - TL_{lactation}$, unscaled values) on litter mass and litter size at weaning. Here also, we initially included assay-related terms (plate, row within plate) and female age as random factors in the models. However, those variables explained virtually no variation in telomere change, and we removed them from the final model as they prevented models from properly converging. Then, we tested the correlation between female telomere change from emergence to parturition ($TL_{birth} - TL_{emergence}$) and female telomere change from mid lactation to weaning ($TL_{weaning} - TL_{lactation}$). This allowed us to determine if the magnitude of maternal telomere change over lactation was related to the magnitude of maternal telomere change at the start of the season, *i.e.* if females showing the greatest telomere loss during lactation were also those that showed the highest amount of telomere reconstruction prior to parturition.

Statistical analyses

All analyses were done in R v 4.0.2 (R Core Team 2021). We inspected model residuals for normality using density distribution, Q-Q plots, cumulative distribution functions and P-P plots obtained with the "fitdistrplus" package in R (Delignette-Muller & Dutang, 2015). Sample sizes are indicated as the number of observations (n) and number of individuals (N).

RESULTS

Telomere length repeatability and relationship with female age and body mass

Controlling for assay-related terms (plate and row within plate), the repeatability of female relative telomere length over the season was low, but significant (r = 0.11; CI_{95} = [0.00, 0.26]; P = 0.044; Fig 1). Relative telomere length was significantly and negatively associated with female age (LMM; z-age estimate = -0.28, CI_{95} = [-0.51; -0.06], t = -2.51, P = 0.013), and positively associated with female mass (z-mass estimate = 0.27, CI_{95} = [0.08; 0.46], t = 2.79, P = 0.006).

Seasonal changes in telomere length

Controlling for female ID, age, mass and assay-related terms in our model, female relative telomere length varied in a quadratic fashion with advancing breeding season, and differed between females that did or did not raise a litter (LMM; period x status interaction; $F_{3,107.6}$ = 3.16, p = 0.028, n = 154 observations, N = 43 females; Fig 1, Table 1). For females that raised a litter, relative telomere length increased by 23%, though not quite significantly (Tukey HSD; t = -2.34, p = 0.096, Table 2), between emergence from hibernation to around the time of birth (i.e., during gestation). Relative telomere length then decreased significantly, by 28% between birth and weaning (Fig 1, Table 2). In females that did not raise a litter, relative telomere length also increased by 17% from emergence to what would correspond to around the time of birth for breeding females, and by another 10% to around the time of mid-lactation for breeding females (the increase from emergence to mid-lactation being significant; Fig 1, Table 2). In contrast to breeders, these females did not show a significant decrease in relative telomere length from the time of mid-lactation to the time of weaning of the offspring (Fig 1, Table 2). Interestingly, non-breeding females had significantly longer telomeres than breeding females

368 from around the time of mid-lactation (+20%; Tukey HSD; t = -2.1, p = 0.04) to the time when
369 breeding females weaned their offspring (+38%; Tukey HSD; t = -3.3, p = 0.001).
370
371 **Telomere length in relation to subsequent reproduction**
372 Controlling for maternal mass at emergence and assay-related terms, female telomere length at
373 emergence from hibernation neither significantly affected the size (LMM; z-telomere length =
374 0.08; CI_{95} = [-0.45; 0.60], t = 0.32, P = 0.753, N = 17 females) or mass (z-telomere length =
375 0.20; CI_{95} = [-36.73; 31.13], t = 0.01, P = 0.991, N=17 females) of the litter at weaning.
376 Similarly, controlling for maternal mass, telomere change at the start of the active season (from
377 emergence to birth; see Fig 1) did not significantly affect either litter size (z-telomere change
378 = 0.11; CI_{95} = [-0.65; 0.87], t = 0.32, P = 0.758, N = 16 females) or litter mass at weaning (z-
379 telomere change = 30.28; CI_{95} = [-17.47; 78.02], t = 1.41, P = 0.188, N = 16)
380
381 **Telomere loss following reproduction**
382 For females that raised a litter, telomere loss over lactation was significantly and negatively
383 related to both litter size (LM; z-litter size = -0.28; CI_{95} = [-0.53; -0.03], t = -2.38, P = 0.031;
384 R^2 = 0.23; N = 17; Fig 2A) and litter mass at weaning (z-litter mass = -0.32; CI_{95} = [-0.57; -
385 0.08], t = -2.83, P = 0.013; R^2 = 0.31; N = 17; Fig 2B). Female telomere changes at the start
386 and end of the breeding season were positively, but not significantly, correlated (Pearson's r =
387 0.39; CI_{95} = [-0.15; 0.75], t = 1.53, P = 0.149).
388
389 **DISCUSSION**
390 In Columbian ground squirrels, we found that telomere lengthening occurred between
391 emergence of hibernation through to about the time of births, some 26 days later, both for
392 breeding and non-breeding females (see Fig 1A). Afterwards, only breeding females

393 experienced significant shortening of their telomeres during the reproductive season, the
394 amplitude of telomere erosion being negatively related to litter size and mass (Fig. 2).
395 It has been suggested that telomere lengthening at emergence from hibernation might
396 occur to counteract the deleterious effects of hibernation (Hoelzl et al., 2016; Ruf & Bieber,
397 2020). The physiological costs of hibernation extend from impaired immune function (Cooper
398 et al., 1992) to enhanced oxidative stress, the latter being particularly problematic during
399 euthermic arousals associated with metabolic boosts and high production of reactive oxygen
400 species (ROS) (Orr et al., 2009). Telomeres may be a target of ROS (von Zglinicki 2002,
401 Reichert and Stier 2017; but see Boonekamp et al. 2017) and particularly vulnerable to
402 rewarming processes during euthermic arousals, due to sudden increases in oxidative
403 respiration (Murín et al., 2001). For instance, telomere shortening rates in hibernating edible
404 or garden dormice were positively related to the time spent euthermic during the inactive period
405 (Giroud et al., 2014), and to the frequency of arousals (Hoelzl et al., 2016). Reconstructing
406 chromosome ends at the start of the active period would be of particular importance if long
407 telomeres (or reduced annual shortening) are a seasonal prerequisite for individual survival
408 (Bize et al., 2009; Wood & Young, 2019). Though this must still be determined in Columbian
409 ground squirrels, associations between telomere length and survival have been documented
410 across vertebrates (Haussmann et al., 2003; Tricola et al., 2018; Vera et al., 2012; Whittemore
411 et al., 2019). Telomere maintenance (and rebuilding) may have coevolved with the relatively
412 slow pace of life that is characteristic of some hibernating rodents (*e.g.*, reduced mortality due
413 to winter inactivity; Turbill et al. 2011, Constant et al. 2020). In our study, although most
414 squirrels were captured on the day of emergence from hibernation (as known from the daily
415 survey of the study site and by the condition of the animal upon capture), we cannot exclude
416 the possibility that a few animals were caught slightly after the day of emergence. Thus, if
417 anything, telomere reconstruction following hibernation may have been slightly

418 underestimated in the present study, and may be even more pronounced than our current data
419 suggest.

420 In addition to counteracting the potentially deleterious effects of hibernation, findings
421 of telomere lengthening at the end of hibernation and onset of the active season (Hoelzl et al.,
422 2016; Ruf & Bieber, 2020; Turbill et al., 2013) question the importance of this process in
423 determining reproductive success and individual fitness in the wild. In this regards, telomere
424 elongation might be critical in at least two aspects: (*i*) by favoring reproduction, since greater
425 telomere length (or reduced telomere erosion) has been associated with greater reproductive
426 success (Angelier et al., 2019); or (ii) by serving as a pre-emptive change that mitigates the
427 physiological costs of reproduction (*i.e.*, shortened telomeres or higher telomere loss due to
428 reproduction; Bauch et al. 2012, Sudyka et al. 2014, Bichet et al. 2020, but see Sudyka 2019).

429 In our study, we found no clear relationship between telomere lengthening at the start
430 of the reproductive season (between emergence and birth) and female reproductive effort
431 measured as the litter size or mass produced at weaning. These results suggest that telomere
432 length at emergence of hibernation does not constitute a physiological constraint on
433 reproduction for females in this species. This is perhaps not surprising, given that female
434 Columbian ground squirrels are primarily income breeders: current reproductive effort depends
435 more strongly on current environmental conditions (Dobson & Oli, 2001), than on carried over
436 reserves and somatic condition from the previous year (Broussard et al., 2005; Rubach et al.,
437 2016). In contrast, female telomere length of somatic cells decreased from lactation to weaning
438 in breeding – but not in non-breeding (same relative chronological dates, see Methods) –
439 individuals. In addition, in breeding females, telomere length decreased in relation to
440 reproductive output: females producing larger and heavier litters experienced a higher telomere
441 loss (see Figs 1 and 2).

Telomere loss may be the ultimate cellular consequence of increased oxidative metabolism and its inevitable production of reactive oxygen species ROS due to reproduction (Kirkwood and Kowald 2012; but see Speakman et al. 2015). Several studies have found faster telomere deterioration in breeding adults (Kotrschal et al. 2007, Reichert et al. 2014, Sudyka et al. 2014, 2019, Bichet et al. 2020), even if the link between oxidative stress and telomere loss remains unclear (Boonekamp et al., 2017). The present results seem to support the hypothesis that reproduction entails physiological costs such as increased telomere loss (but see Sudyka et al. 2019 for counter-examples). This is evidenced both by the difference between breeding and non-breeding females, and by the positive association between telomere loss and the increasing reproductive output by breeding females (*i.e.* litter size or mass).

Although ROS production during reproduction may contribute to shorter telomeres, other mechanisms, such as different changes in hormonal levels in breeding and non-breeding females across the reproductive cycle, might explain differential changes in telomere length. For instance, elevated testosterone has been found to increase telomere loss (Heidinger et al. 2021), whereas increased progesterone has been found to increase telomerase activation (Kyo et al., 1999). Both testosterone and oestrogen are likely to be higher in breeding females which are territorial during lactation (Murie and Harris 1988), and their effects on telomere dynamics (together with those of other hormones such as progesterone or prolactin) remain to be investigated.

Of interest is the result that telomere elongation prior to reproduction may perhaps play a role in mitigating the physiological costs of reproduction by pre-emptively reconstructing telomeres before the costly period of lactation, as we previously documented with regards to oxidative stress in this species (increased oxidative defences and reduced oxidative damage during lactation; Viblanc et al. 2018). We indeed found a positive correlation ($r = 0.39$), between pre-reproductive telomere elongation and post-reproductive telomere loss. However,

it important to note that the correlation was not significant, and additional data are therefore needed to conclude if the reproductive physiology of females is geared towards mitigating all of the physiological costs of lactation. One interesting question is how efficient this system actually is: previous studies have failed to evidence long-term fitness costs to reproduction in this species (Murie and Dobson 1987, Neuhaus 2000, Rubach et al. 2016; but see Neuhaus and Pelletier 2001). Variability in individual ability to compensate for somatic and telomere damage accumulated over hibernation also suggests that females of better individual quality may better buffer the negative effects of high reproductive investments (Angelier et al., 2019; Bauch et al., 2012; Sudyka, 2019). In line with this suggestion, we found a positive relationship between female emergence mass and telomere length. Such an observation is consistent with the idea that individual telomere length over the active period in female ground squirrels is partly explained by differences in individual condition or quality (Sudyka, 2019). More work is needed to clearly establish how far the consequences of reproduction on telomere length result from a trade-off between actual costs on one hand (telomere attrition due to reproduction) and variability in individual capacity to buffer these costs on the other.

Our findings add to the growing evidence that telomere length does not always reflect unidirectional cellular ageing processes in the organism, including in adult animals. We found that telomere dynamics in breeding females followed a seasonal pattern of reconstruction – and shortening as a cost of reproduction. Our results suggest (*i*) that telomere reconstruction occurs following hibernation in female Columbian ground squirrels, as in other hibernating mammals (*ii*) that telomere loss over during the breeding season at least partly reflects a physiological cost to reproduction, but (*iii*) we did not detect any effect of post-hibernating telomere elongation on future reproductive success. Disentangling the importance of the (likely co-occurring) processes of reproductive costs and variation in individual quality in buffering these

491 costs will require longitudinal surveys of life-long individual telomere dynamics and
492 reproductive success in animals, indicating the necessity of long-term studies in the wild.
493
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FIGURE CAPTIONS

Fig 1. Relative telomere length dynamics in female Columbian ground squirrels over the course of the breeding season. Results are marginal means ± 95% confidence intervals. Differences between means were tested for relevant contrasts using Tukey HSD. Longitudinal differences are indicated Table 2. Cross-sectional differences between groups at specific time points are indicated by asterisks (**P < 0.01). Note that time intervals among sampling events are not identical (see Methods). Sample sizes are given below the means for breeding (black) and non-breeding (grey) categories. Relative telomere length was standardized (see Methods).

Fig 2. Relationship between relative telomere change over lactation and reproductive output in female Columbian ground squirrels, measured as (a) litter size and (b) total litter mass (g) at weaning (both variables standardized). The predictions and 95% confidence interval are given. Relative telomere length was standardized (see Methods).

TABLES

Table 1. Mixed model estimates for the effects of breeding status (breeding [B] vs. non-breeding [NB]), period in the breeding season (emergence from hibernation, shortly before births, at mid-lactation and at weaning – see Methods) and their interaction on female relative telomere length in female Columbian ground squirrels. Individual ID, age, mass, and assay-related terms (plate, row within plate) were included as random factors in the models. Relative telomere length and independent variables are standardized (z-scores). N refers to individual and n to TL sample size (repeated measurements on individuals).

Predictors	*Estimates*	*CI*	*t*	*p*	*n(N)*	R^2_{mar}/R^2_{cond}
(Intercept)	-0.50	-1.12 – 0.11	-1.62	0.108		
$Period_{[Birth]}$	0.67	0.12 – 1.22	2.39	0.018*		
$Period_{[Lactation]}$	0.33	-0.24 – 0.91	1.14	0.257		
$Period_{[Weaning]}$	-0.36	-0.91 – 0.19	-1.28	0.201		
$Status_{[NB]}$	0.11	-0.48 – 0.69	0.36	0.721		
$Period_{[Birth]}$ x $Status_{[NB]}$	-0.15	-0.90 – 0.60	-0.40	0.693	154(43)	0.15 / 0.49
$Period_{[Lactation]}$ x $Status_{[NB]}$	0.52	-0.25 – 1.30	1.34	0.183		
$Period_{[Weaning]}$ x $Status_{[NB]}$	0.87	0.11 – 1.63	2.27	0.025*		

Table 2. Tukey Honest Significant Differences for longitudinal mean comparisons in relative telomere length (standardized, z-scores) for female Columbian ground squirrels at different time points in the breeding season. Significant differences for $P < 0.05$ are indicated by asterisks.

Status	*Difference*	*Estimate ± SE*	*t ratio*	*P*
Breeders	Emergence - Birth	-0.64 ± 0.27	-2.34	0.096
	Emergence - Lactation	-0.31 ± 0.28	-1.10	0.689
	Emergence - Weaning	0.34 ± 0.27	1.25	0.596
	Birth - Lactation	0.32 ± 0.27	1.20	0.629
	Birth - Weaning	0.98 ± 0.26	3.80	0.002*
	Lactation - Weaning	0.65 ± 0.27	2.46	0.073
Non-breeders	Emergence - Birth	-0.49 ± 0.25	-1.97	0.205
	Emergence - Lactation	-0.81 ± 0.25	-3.17	0.011*
	Emergence - Weaning	-0.48 ± 0.26	-1.87	0.246
	Birth - Lactation	-0.32 ± 0.25	-1.27	0.585
	Birth - Weaning	0.01 ± 0.25	0.05	1.000
	Lactation - Weaning	0.33 ± 0.26	1.29	0.571

FIGURES

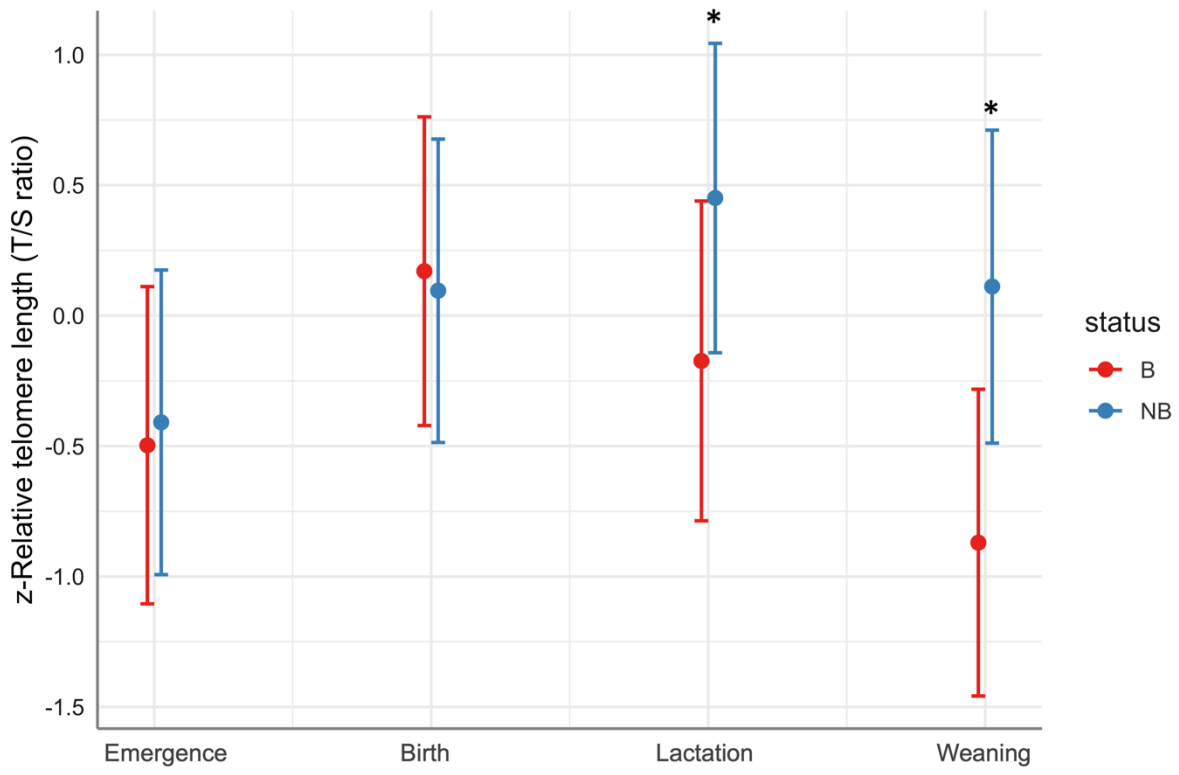

Fig 1.

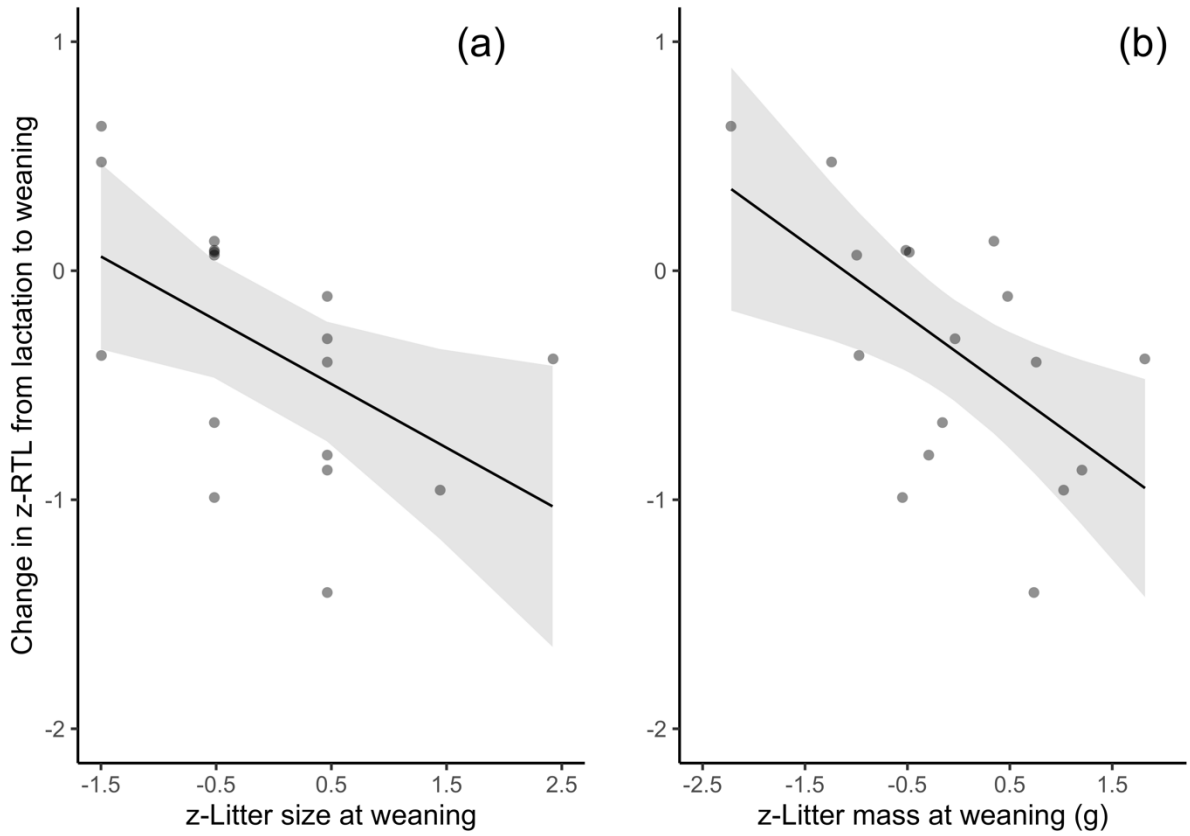
(a)
(b)
Change in z-RTL from lactation to weaning
z-Litter size at weaning
z-Litter mass at weaning (g)
1
0
-1
-2
-1.5
-0.5
0.5
1.5
2.5
-2.5

Fig 2.

REFERENCES

Angelier, F., Weimerskirch, H., Barbraud, C., & Chastel, O. (2019). Is telomere length a molecular marker of individual quality? Insights from a long-lived bird. *Functional Ecology*, *33*(6), 1076–1087.

Asghar, M., Hasselquist, D., Hansson, B., Zehtindjiev, P., Westerdahl, H., & Bensch, S. (2015). Hidden costs of infection: Chronic malaria accelerates telomere degradation and senescence in wild birds. *Science*, *347*(6220), 436–438.

Aydinonat, D., Penn, D. J., Smith, S., Moodley, Y., Hoelzl, F., Knauer, F., & Schwarzenberger, F. (2014). Social Isolation Shortens Telomeres in African Grey Parrots (*Psittacus erithacus erithacus*). *PLoS ONE*, *9*(4), e93839.

Badás, E. P., Martínez, J., Rivero de Aguilar Cachafeiro, J., Miranda, F., Figuerola, J., & Merino, S. (2015). Ageing and reproduction: Antioxidant supplementation alleviates telomere loss in wild birds. *Journal of Evolutionary Biology*, *28*(4), 896–905.

Bauch, C., Becker, P. H., & Verhulst, S. (2012). Telomere length reflects phenotypic quality and costs of reproduction in a long-lived seabird. *Proceedings of the Royal Society B: Biological Sciences*, *280*(1752), 20122540.

Bichet, C., Bouwhuis, S., Bauch, C., Verhulst, S., Becker, P. H., & Vedder, O. (2020). Telomere length is repeatable, shortens with age and reproductive success, and predicts remaining lifespan in a long-lived seabird. *Molecular Ecology*. *29*(2), 429–441.

Bize, P., Criscuolo, F., Metcalfe, N. B., Nasir, L., & Monaghan, P. (2009). Telomere dynamics rather than age predict life expectancy in the wild. *Proceedings of the Royal Society B: Biological Sciences*, *276*(1662), 1679–1683.

Blackburn, E. H. (1991). Structure and function of telomeres. *Nature*, *350*(6319), 569–573.

Blackburn, E. H., & Epel, E. S. (2012). Telomeres and adversity: Too toxic to ignore. *Nature*, *490*(7419), 169–171.

Blount, J. D., Vitikainen, E. I. K., Stott, I., & Cant, M. A. (2016). Oxidative shielding and the cost of reproduction. *Biological Reviews*, *91*(2), 483–497.

Boonekamp, J. J., Bauch, C., Mulder, E., & Verhulst, S. (2017). Does oxidative stress shorten telomeres? *Biology Letters*, *13*(5), 20170164.

Boonekamp, J. J., Bauch, C., & Verhulst, S. (2020). Experimentally increased brood size accelerates actuarial senescence and increases subsequent reproductive effort in a wild bird population. *Journal of Animal Ecology*, *89*(6), 1395–1407.

Broussard, D. R., Dobson, F. S., & Murie, J. O. (2005). The effects of capital on an income

583 breeder: evidence from female Columbian ground squirrels. *Canadian Journal of Zoology*,
584 *83*(4), 546–552.
585 Cawthon, R. M. (2002). Telomere measurement by quantitative PCR. *Nucleic Acids*
586 *Research*, *30*(10), 47e – 47.
587 Chatelain, M., Drobniak, S. M., & Szulkin, M. (2020). The association between stressors
588 and telomeres in non-human vertebrates: a meta-analysis. *Ecology Letters*, 23(2)381-398.
589 Constant, T., Giroud, S., Viblanc, V. A., Tissier, M. L., Bergeron, P., Dobson, F. S., &
590 Habold, C. (2020). Integrating Mortality Risk and the Adaptiveness of Hibernation.
591 *Frontiers in Physiology*, *11*, 706.
592 Cooper, E. L., Wright, R. K., Klempau, A. E., & Smith, C. T. (1992). Hibernation alters
593 the frog's immune system. *Cryobiology*, *29*(5), 616–631.
594 Cram, D. L., Monaghan, P., Gillespie, R., & Clutton-Brock, T. (2017). Effects of early-life
595 competition and maternal nutrition on telomere lengths in wild meerkats. *Proceedings of*
596 *the Royal Society B: Biological Sciences*, *284*(1861), 20171383.
597 Criscuolo, F., Sorci, G., Behaim-Delarbre, M., Zahn, S., Faivre, B., & Bertile, F. (2018).
598 Age-related response to an acute innate immune challenge in mice: proteomics reveals a
599 telomere maintenance-related cost. *Proceedings of the Royal Society B: Biological*
600 *Sciences*, *285*(1892), 20181877.
601 Dantzer, B., & Fletcher, Q. E. (2015). Telomeres shorten more slowly in slow-aging wild
602 animals than in fast-aging ones. *Experimental Gerontology*, *71*, 38–47.
603 Delehanty, B., & Boonstra, R. (2011). Coping with Intense Reproductive Aggression in
604 Male Arctic Ground Squirrels: The Stress Axis and Its Signature Tell Divergent Stories.
605 *Physiological and Biochemical Zoology*, *84*(4), 417–428.
606 Delignette-Muller, M. L., & Dutang, C. (2015). fitdistrplus: An R package for fitting
607 distributions. *Journal of Statistical Software*, *64*(4), 1–34.
608 Dobson, F. S., Badry, M. J., & Geddes, C. (1992). Seasonal activity and body mass of
609 Columbian ground squirrels. *Canadian Journal of Zoology*, *70*(7), 1364–1368.
610 Dobson, F. S., & Murie, J. O. (1987). Interpretation of Intraspecific Life History Patterns:
611 Evidence from Columbian Ground Squirrels. *The American Naturalist*, *129*(3), 382–397.
612 Dobson, F .S., Murie, J. O. &Viblanc V. A.. 2020. Fitness estimation for ecological studies:
613 an evaluation in Columbian ground squirrels. *Frontiers in Ecology and Evolution* 8: 216.
614 Dobson, F. S., & Oli, M. K. (2001). The Demographic Basis of Population Regulation in
615 Columbian Ground Squirrels. *The American Naturalist*, *158*(3), 236–247.
616 Dobson, F. S., Risch, T. S., & Murie, J. O. (1999). Increasing returns in the life history of

Columbian ground squirrels. *Journal of Animal Ecology*, *68*(1), 73–86.
Dupoué, A., Angelier, F., Ribout, C., Meylan, S., Rozen-Rechels, D., Decencière, B., Agostini, S., & Le Galliard, J.-F. (2020). Chronic water restriction triggers sex-specific oxidative stress and telomere shortening in lizards. *Biology Letters*, *16*(2), 20190889.
Dupoué, A., Rutschmann, A., Le Galliard, J. F., Clobert, J., Angelier, F., Marciau, C., Ruault, S., Miles, D. & Meylan, S. (2017). Shorter telomeres precede population extinction in wild lizards. *Scientific Reports*, *7*(1), 16976.
Flatt, T., & Heyland, A. (2011). *Mechanisms of Life History Evolution: The Genetics and Physiology of Life History Traits and Trade-Offs*. Oxford University Press.
Giroud, S., Zahn, S., Criscuolo, F., Chery, I., Blanc, S., Turbill, C., & Ruf, T. (2014). Late-born intermittently fasted juvenile garden dormice use torpor to grow and fatten prior to hibernation: consequences for ageing processes. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1797), 1131.
Greider, C. W. (1991). Telomeres. *Current Opinion in Cell Biology*, *3*(3), 444–451.
Greider, C. W., & Blackburn, E. H. (1985). Identification of a specific telomere terminal transferase activity in tetrahymena extracts. *Cell*, *43*(2), 405–413.
Grunst, M. L., Grunst, A. S., Pinxten, R., & Eens, M. (2020). Anthropogenic noise is associated with telomere length and carotenoid-based coloration in free-living nestling songbirds. *Environmental Pollution*, *260,* 114032.
Harley, C. B., Futcher, A. B., & Greider, C. W. (1990). Telomeres shorten during ageing of human fibroblasts. *Nature*, *345*(6274), 458–460.
Haussmann, M. F., Winkler, D. W., O'Reilly, K. M., Huntington, C. E., Nisbet, I. C. T., & Vleck, C. M. (2003). Telomeres shorten more slowly in long-lived birds and mammals than in short–lived ones. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *270*(1522), 1387–1392.
Heidinger, B. J., Blount, J. D., Boner, W., Griffiths, K., Metcalfe, N. B., & Monaghan, P. (2012). Telomere length in early life predicts lifespan. *Proceedings of the National Academy of Sciences*, *109*(5), 1743–1748.
Hoelzl, F., Cornils, J. S., Smith, S., Moodley, Y., & Ruf, T. (2016). Telomere dynamics in free-living edible dormice (Glis glis): the impact of hibernation and food supply. *The Journal of Experimental Biology*, *219*(16), 2469–2474.
Hoelzl, F., Smith, S., Cornils, J. S., Aydinonat, D., Bieber, C., & Ruf, T. (2016). Telomeres are elongated in older individuals in a hibernating rodent, the edible dormouse (Glis glis). *Scientific Reports*, *6*(1), 36856.

651 Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous inference in general parametric
652 models. *Biometrical Journal*, *50*(3), 346-363.
653 Kesäniemi, J., Lavrinienko, A., Tukalenko, E., Boratyński, Z., Kivisaari, K., Mappes, T.,
654 Milinevsky, G., Møller, A. P., Mousseau, T. A., Watts, P. C. (2019). Exposure to
655 environmental radionuclides associates with tissue-specific impacts on telomerase
656 expression and telomere length. *Scientific Reports*, *9*, 850.
657 Kirkwood, T. B. L., & Kowald, A. (2012). The free-radical theory of ageing - older, wiser
658 and still alive. *BioEssays*, *34*(8), 692–700.
659 Kotrschal, A., Ilmonen, P., & Penn, D. J. (2007). Stress impacts telomere dynamics.
660 *Biology Letters*, *3*(2), 128–130.
661 Kuznetsova, A., Brockhoff, P.B., & Christensen, R.H.B. (2017). "lmerTest" package: tests
662 in linear mixed effects models. *Journal of Statistical Softwar*e, 82(13), 1-26.
663 Lane, J. E., Kruuk, L. E. B., Charmantier, A., Murie, J. O., Coltman, D. W., Buoro, M.,
664 Raveh, S., & Dobson, F. S. (2011). A quantitative genetic analyses of hibernation
665 emergence date in a wild population of Columbian ground squirrels. *Journal of*
666 *Evolutionary Biology*, *24*, 1949-1959.
667 Manno, T. G., & Dobson, F. S. (2008). Why are Male Columbian Ground Squirrels
668 Territorial? *Ethology*, *114*(11), 1049–1060.
669 Manno, T. G., Nesterova, A. P., DeBarbieri, L. M., Kennedy, S. E., Wright, K. S., &
670 Dobson, F. S. (2007). Why do male Columbian ground squirrels give a mating call? *Animal*
671 *Behaviour*, *74*(5), 1319–1327.
672 Molbert, N., Angelier, F., Alliot, F., Ribout, C., & Goutte, A. (2021). Fish from urban rivers
673 and with high pollutant levels have shorter telomeres. *Biology Letters*, *17*(1), 20200819.
674 Monaghan, P., & Haussmann, M. F. (2006). Do telomere dynamics link lifestyle and
675 lifespan? *Trends in Ecology & Evolution*, *21*(1), 47–53.
676 Murie, J. O., & Dobson, F. S. (1987). The costs of reproduction in female Columbian
677 ground squirrels. *Oecologia*, *73*(1), 1–6.
678 Murie, J. O., & Harris, M. A. (1978). Territoriality and dominance in male Columbian
679 ground squirrels (*Spermophilus columbianus*). *Canadian Journal of Zoology*, *56*(11),
680 2402–2412.
681 Murie, J. O., & Harris, M. A. (1982). Annual Variation of Spring Emergence and Breeding
682 in Columbian Ground Squirrels (*Spermophilus columbianus*). *Journal of Mammalogy*,
683 *63*(3), 431–439.
684 Murie, J. O., & Harris, M. A. (1988). Social interactions and dominance relationships

685 between female and male Columbian ground squirrels. *Canadian Journal of Zoology*,
686 *66*(6), 1414–1420.
687 Murín, R., Drgová, A., Kaplán, P., Dobrota, D., & Lehotský, J. (2001).
688 Ischemia/Reperfusion-induced oxidative stress causes structural changes of brain
689 membrane proteins and lipids. *General Physiology and Biophysics*, *20*(4), 431–438.
690 Nakagawa, S., & Schielzeth, H. (2010). Repeatability for Gaussian and non-Gaussian data:
691 a practical guide for biologists. *Biological Reviews*, *85*(4), 935–956.
692 Neuhaus, P. (2000). Weight comparisons and litter size manipulation in Columbian ground
693 squirrels (*Spermophilus columbianus*) show evidence of costs of reproduction. *Behavioral*
694 *Ecology and Sociobiology*, *48*(1), 75–83.
695 Neuhaus, P., & Pelletier, N. (2001). Mortality in relation to season, age, sex, and
696 reproduction in Columbian ground squirrels (*Spermophilus columbianus*) . *Canadian*
697 *Journal of Zoology*, *79*(3), 465–470.
698 Nowack, J., Tarmann, I., Hoelzl, F., Smith, S., Giroud, S., & Ruf, T. (2019). Always a price
699 to pay: hibernation at low temperatures comes with a trade-off between energy savings and
700 telomere damage. *Biology Letters*, *15*(10), 20190466.
701 Orr, A. L., Lohse, L. A., Drew, K. L., & Hermes-Lima, M. (2009). Physiological oxidative
702 stress after arousal from hibernation in Arctic ground squirrel. *Comparative Biochemistry*
703 *and Physiology Part A: Molecular & Integrative Physiology*, *153*(2), 213–221.
704 Raveh, S., Heg, D., Dobson, F. S., Coltman, D. W., Gorrell, J. C., Balmer, A., & Neuhaus,
705 P. (2010). Mating order and reproductive success in male Columbian ground squirrels
706 (*Urocitellus columbianus*). *Behavioral Ecology*, *21*(3), 537–547.
707 Raveh, S., Heg, D., Viblanc, V. A., Coltman, D. W., Gorrell, J. C., Dobson, F. S., Balmer,
708 A. & Neuhaus, P. (2011). Male reproductive tactics to increase paternity in the
709 polygynandrous Columbian ground squirrel (*Urocitellus columbianus*). *Behavioral*
710 *Ecology and Sociobiology*, *65*(4), 695–706.
711 R Core Team (2021). R: A language and environment for statistical computing. R
712 Foundation for Statistical Computing, Vienna, Austria. URL : https://www.R-project.org/.
713 Reichert, S., & Stier, A. (2017). Does oxidative stress shorten telomeres in vivo ? A review.
714 *Biology Letters*, *13*(12), 20170463.
715 Reichert, S., Stier, A., Zahn, S., Arrivé, M., Bize, P., Massemin, S., & Criscuolo, F. (2014).
716 Increased brood size leads to persistent eroded telomeres. *Frontiers in Ecology and*
717 *Evolution*, *2*, 1–11.
718 Remot, F., Ronget, V., Froy, H., Rey, B., Gaillard, J.-M., Nussey, D. H., & Lemaître, J.-F.

(2020). No sex differences in adult telomere length across vertebrates: a meta-analysis. *Royal Society Open Science*, *7*(11), 200548.

Rollings, N., Uhrig, E. J., Krohmer, R. W., Waye, H. L., Mason, R. T., Olsson, M., Whittington, C. M., & Friesen, C. R. (2017). Age-related sex differences in body condition and telomere dynamics of red-sided garter snakes. *Proceedings of the Royal Society B: Biological Sciences*, *284*(1852), 20162146.

Rubach, K., Wu, M., Abebe, A., Dobson, F. S., Murie, J. O., & Viblanc, V. A. (2016). Testing the reproductive and somatic trade-off in female Columbian ground squirrels. *Ecology and Evolution*, *6*(21), 7586–7595.

Ruf, T., & Bieber, C. (2020). Physiological, Behavioral, and Life-History Adaptations to Environmental Fluctuations in the Edible Dormouse. *Frontiers in Physiology*, *11*, 423.

Skibiel, A. L., Dobson, F. S., & Murie, J. O. (2009). Maternal influences on reproduction in two populations of Columbian ground squirrels. *Ecological Monographs*, *79*(2), 325–341.

Skibiel, A. L., Speakman, J. R., & Hood, W. R. (2013). Testing the predictions of energy allocation decisions in the evolution of life-history trade-offs. *Functional Ecology*, *27*(6), 1382–1391.

Smith, S., Turbill, C., & Penn, D. J. (2011). Chasing telomeres, not red herrings, in evolutionary ecology. *Heredity*, *107*(4), 372–373.

Speakman, J R. (2008). The physiological costs of reproduction in small mammals. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *363*(1490), 375–398.

Speakman, John R, Blount, J. D., Bronikowski, A. M., Buffenstein, R., Isaksson, C., Kirkwood, T. B. L., Monaghan, P., Ozanne, S. E., Beaulieu, M., Briga, M., Carr, S. K., Christensen, L. L., Cochemé, H. L., Cram, D. L., Dantzer, B., Harper, J. M., Jurk, D., King, A., Noguera, J. C., Salin, K., Sild, E., Simons, M. J. P., Smith, S., Stier, A., Tobler, M., Vitikainen, E., Peaker, M., & Selman, C. (2015). Oxidative stress and life histories: unresolved issues and current needs. *Ecology and Evolution*, *5*(24), 5745–5757.

Stoffel, M. A., Nakagawa, S., & Schielzeth, H. (2017). rptR: repeatability estimation and variance decomposition by generalized linear mixed-effects models. *Methods in Ecology and Evolution*, *8*(11), 1639–1644.

Sudyka, J., Arct, A., Drobniak, S., Dubiec, A., Gustafsson, L., & Cichoń, M. (2014a). Experimentally increased reproductive effort alters telomere length in the blue tit (*Cyanistes caeruleus*). *Journal of Evolutionary Biology*, *27*(10), 2258–2264.

753 Sudyka, J. (2019). Does Reproduction Shorten Telomeres? Towards Integrating Individual
754 Quality with Life-History Strategies in Telomere Biology. *BioEssays*, *41*(11), 1900095.
755 Sudyka, J., Arct, A., Drobniak, S. M., Gustafsson, L., & Cichon, M. (2019). Birds with
756 high lifetime reproductive success experience increased telomere loss. *Biology Letters*,
757 *15(1*), 20180637.
758 Sudyka, J., Casasole, G., Rutkowska, J., & Cichoń, M. (2016). Elevated reproduction does
759 not affect telomere dynamics and oxidative stress. *Behavioral Ecology and Sociobiology*,
760 *70(12)*, 2223-2233.
761 Thomas, P., O' Callaghan, N. J., & Fenech, M. (2008). Telomere length in white blood
762 cells, buccal cells and brain tissue and its variation with ageing and Alzheimer's disease.
763 *Mechanisms of Ageing and Development, 129*(4), 183-190.
764 Tricola, G. M., Simons, M. J. P., Atema, E., Boughton, R. K., Brown, J. L., Dearborn, D.
765 C., Divoky, G., Eimes, J. A., Huntington, C. E., Kitaysky, A. S., Juola, F. A., Lank, D. B.,
766 Litwa, H. P., Mulder, E. G. A., Nisbet, I. C. T., Okanoya, K., Safran, R. J., Schoech, S.,
767 Schreiber, E. A., Thompson, P. M., Verhulst, S., Wheelwright, N. T., Winkler, D. W.,
768 Young, R., Vleck, C. M., & Haussmann, M. F. (2018). The rate of telomere loss is related
769 to maximum lifespan in birds. *Philosophical Transactions of the Royal Society B:*
770 *Biological Sciences*, *373(1741)*, 20160445.
771 Turbill, C, Smith, S., Deimel, C., & Ruf, T. (2012). Daily torpor is associated with telomere
772 length change over winter in Djungarian hamsters. *Biology Letters*, *8*(2), 304–307.
773 Turbill, C., Bieber, C., & Ruf, T. (2011). Hibernation is associated with increased survival
774 and the evolution of slow life histories among mammals. *Proceedings of the Royal Society*
775 *B: Biological Sciences*, *278*(1723), 3355–3363.
776 Turbill, C., Ruf, T., Smith, S., & Bieber, C. (2013). Seasonal variation in telomere length
777 of a hibernating rodent. *Biology Letters*, *9*(2), 20121095.
778 van Lieshout, S. H. J., Bretman, A., Newman, C., Buesching, C. D., Macdonald, D. W., &
779 Dugdale, H. L. (2019). Individual variation in early-life telomere length and survival in a
780 wild mammal. *Molecular Ecology*, *28(18)*, 4152–4165.
781 Vera, E., Bernardes de Jesus, B., Foronda, M., Flores, J. M., & Blasco, M. A. (2012). The
782 Rate of Increase of Short Telomeres Predicts Longevity in Mammals. *Cell Reports*, *2*(4),
783 732–737.
784 Verhulst, S. (2020). Improving comparability between qPCR-based telomere studies.
785 *Molecular Ecology Resources*, *20*(1), 11–13.
786 Viblanc, V. A., Schull, Q., Roth, J. D., Rabdeau, J., Saraux, C., Uhlrich, P., Criscuolo, F.,

& Dobson, F. S. (2018). Maternal oxidative stress and reproduction: Testing the constraint, cost and shielding hypotheses in a wild mammal. *Functional Ecology*, *32*(3), 722–735.

von Zglinicki, T. (2002). Oxidative stress shortens telomeres. *Trends in Biochemical Sciences*, *27*(7), 339–344.

Waterman, J. M. (1998). Mating tactics of male Cape ground squirrels, Xerus inauris: consequences of year-round breeding. *Animal Behaviour*, *56*, 459–466.

Watson, J. D. (1972). Origin of Concatemeric T7DNA. *Nature New Biology*, *239*(94), 197–201.

Watson, R. L., Bird, E. J., Underwood, S., Wilbourn, R. V., Fairlie, J., Watt, K., Salvo-Chirnside, E., Pilkington, J. G., Pemberton, J. M., McNeilly, T. N., Froy, H., & Nussey, D. H. (2017). Sex differences in leucocyte telomere length in a free-living mammal. *Molecular Ecology*, *26*(12), 3230 – 3240.

Whittemore, K., Vera, E., Martínez-Nevado, E., Sanpera, C., & Blasco, M. A. (2019). Telomere shortening rate predicts species life span. *Proceedings of the National Academy of Sciences*, *116*(30), 15122–15127.

Wilbourn, R. V., Moatt, J. P., Froy, H., Walling, C. A., Nussey, D. H., & Boonekamp, J. J. (2018). The relationship between telomere length and mortality risk in non-model vertebrate systems: a meta-analysis. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *373*(1741), 20160447.

Wood, E. M., & Young, A. J. (2019). Telomere attrition predicts reduced survival in a wild social bird, but short telomeres do not. *Molecular Ecology*, *28*(16), 3669–3680.

Young, A. J. (2018). The role of telomeres in the mechanisms and evolution of life-history trade-offs and ageing. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *373*(1741), 20160452.

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