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1 **Telomere length positively correlates with pace-of-life in a sex- and cohort-specific**  
2 **way and elongates with age in a wild mammal**

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16 **Keywords**

17 Aging, life-history strategies, evolutionary trade-offs, reproduction, personality, telomere length

18 **Abstract**

19 Understanding ageing and the diversity of life histories is a cornerstone in biology. Telomeres, the  
20 protecting caps of chromosomes, are thought to be involved in ageing, cancer risks and to modulate life-  
21 history strategies. They shorten with cell division and age in somatic tissues of most species, possibly  
22 limiting lifespan. The resource allocation trade-off hypothesis predicts that short telomeres have thus co-  
23 evolved with early reproduction, proactive behaviour and reduced lifespan, i.e. a fast Pace-of-Life  
24 Syndrome (POLS). Conversely, since short telomeres may also reduce the risks of cancer, the anti-cancer

25 hypothesis advances that they should be associated with slow POLS. Conclusion on which hypothesis best  
26 supports the role of telomeres as mediators of life-history strategies is hampered by a lack of study on  
27 wild short-lived vertebrates, apart from birds. Using seven years of data on wild Eastern chipmunks  
28 *Tamias striatus*, we highlighted that telomeres elongate with age and do not limit lifespan in this species.  
29 Furthermore, short telomeres correlated with a slow POLS in a sex-specific way. Females with short  
30 telomeres had a delayed age at first breeding and a lower fecundity rate than females with long  
31 telomeres, whereas those differences were not recorded in males. Our findings support most predictions  
32 adapted from the anti-cancer hypothesis, but none of those made under the resource allocation trade-off  
33 hypothesis. Results are in line with an increasing body of evidence suggesting that other evolutionary  
34 forces than resource allocation trade-offs shape the diversity of telomere length in adult somatic cells and  
35 the relationships between telomere length and life-histories.

36

## 37 **Introduction**

38 Biologists are striving to comprehend the physiological and evolutionary mechanisms that underlie the  
39 wide range of life-history strategies and the diversity of ageing patterns observed across the tree of life  
40 (Jones et al., 2014; Kirkwood, 2005; Ricklefs & Wikelski, 2002). According to the life-history theory,  
41 individuals trade resources devoted towards current reproduction for those devoted towards  
42 maintenance and survival (Clutton-Brock, 1984; Kirkwood, 2005; Stearns, 1992), such that early breeders  
43 may age faster than late ones. Still, some species escape that pattern (Jones et al., 2014). Yet, the  
44 physiological mechanisms underlying ageing remain poorly understood and are still debated (Kirkwood,  
45 2005; Lemaître et al., 2020; Ricklefs & Wikelski, 2002; Sahin & DePinho, 2010). Among diverse interacting  
46 molecular markers, telomeres, the protective caps at the end of chromosomes, are widely considered as  
47 biomarkers of organismal ageing and longevity (Fairlie et al., 2016; Heidinger et al., 2012; Monaghan &  
48 Haussmann, 2006; Sahin & DePinho, 2010).

49 One of the current and dominant thinking in life-history is based on the fact that resources are limited,  
50 such that allocation trade-offs occur between three key functions: growth, reproduction and self-  
51 maintenance (Kirkwood, 2005; Stearns, 1992) . These functions are associated with physiological costs,  
52 such as oxidative stress and accumulation of oxidative damage, or costs associated with telomere  
53 maintenance (Alonso-Alvarez et al., 2004; Metcalfe & Monaghan, 2001; Monaghan, Metcalfe, & Torres,  
54 2009; S. M. Smith, Nager, & Costantini, 2016; von Zglinicki, 2002). These costs limit the ability of an  
55 organism to allocate energy to all three functions and potentially cause ageing, driving life-history

56 strategies (Metcalf & Monaghan, 2003; Monaghan & Haussmann, 2006; Monaghan et al., 2009). In terms  
57 of telomeres, the resource allocation trade-offs hypothesis predicts that long telomeric sequences during  
58 pre-reproductive life, or at the time of reproduction, are associated with prolonged lifespan (Eastwood et  
59 al., 2019; Heidinger et al., 2012). Such long telomeric sequences are also associated to reduced  
60 investment into early reproduction, including reduced first litters and delayed reproduction [(Bauch,  
61 Becker, & Verhulst, 2013; Bauer et al., 2018; Ouyang, Lendvai, Moore, Bonier, & Haussmann, 2016),  
62 reviewed in (Giraudeau, Angelier, & Sepp, 2019; Sudyka, 2019)]; but see (Le Vaillant et al., 2015)] or to a  
63 greater lifetime reproductive success [i.e. LRS (Eastwood et al., 2019; Heidinger, Kucera, Kittilson, &  
64 Westneat, 2021); reviewed in (Sudyka, 2019)]. The Pace-of-Life Syndrome hypothesis (POLS) posits that  
65 physiological and behavioural traits (e.g. exploration profile) have co-evolved with life-history traits by  
66 mediating the trade-offs between current and future reproduction (Montiglio, Dammhahn, Dubuc  
67 Messier, & Réale, 2018; Réale et al., 2010; Ricklefs & Wikelski, 2002). Based on differential resource  
68 allocation trade-offs at the individual level, the POLS hypothesis proposes that telomeres may mediate  
69 individual variation in pace-of-life along a slow-fast continuum (Giraudeau et al., 2019). Within a  
70 population, individuals with long telomeres in their pre-reproductive life are thus expected to display a  
71 slower POLS than individuals with short telomeres (Figure 1, Giraudeau et al., 2019).

72 Positive relationships between telomere length or telomere dynamics and longevity, fecundity rate or LRS  
73 have been confirmed at the intraspecific level (Bichet et al., 2020; Bize, Criscuolo, Metcalfe, Nasir, &  
74 Monaghan, 2009; Eastwood et al., 2019; Heidinger et al., 2012; Monaghan & Haussmann, 2006; Sudyka,  
75 2019). However, when considering inter-specific comparisons, the resource allocation trade-offs  
76 hypothesis fails to explain the relationship between telomere length and lifespan (Haussmann et al., 2003;  
77 Risques & Promislow, 2018). While it would predict that telomere length increases with species size and  
78 longevity, the reverse pattern is observed: large and long-lived species have shorter telomeres than small  
79 and short-lived ones (Risques & Promislow, 2018; Seluanov et al., 2008). In addition, although telomeres  
80 are widely reported to shorten with age, due to cell division or oxidative stress, in birds and humans  
81 (Bichet et al., 2020; Monaghan & Haussmann, 2006; Pauliny, Wagner, Augustin, Szép, & Blomqvist, 2006),  
82 an increasing body of evidence show that this pattern is not universal among vertebrates (Remot et al.,  
83 2021) and even that telomere elongation may occur seasonally or in older individuals in several species  
84 (Criscuolo, Pillay, Zahn, & Schradin, 2020; Hoelzl, Smith, et al., 2016; Panasiak, Dobosz, & Ocalewicz, 2020;  
85 Spurgin et al., 2018). Furthermore, the negative relationship between age and telomere length is absent  
86 in some species (Fairlie et al., 2016; Foley et al., 2018; Hoelzl, Smith, et al., 2016; Monaghan &

87 Haussmann, 2006), questioning the generality of this pattern and on the role of telomeres as biomarkers  
88 of senescence under the resource allocation trade-offs hypothesis.

89 An alternative hypothesis has recently emerged, seeking to explain why large and long-lived species have  
90 shorter telomeres than small and short-lived ones: the anti-cancer hypothesis (Pepke & Eisenberg, 2021;  
91 Seluanov et al., 2008; Tian et al., 2018). This evolutionary hypothesis advances that long lifespans have co-  
92 evolved with short telomeres (Risques & Promislow, 2018; Seluanov et al., 2008; Tian et al., 2018) because  
93 of a trade-off between preventing senescence and avoiding cancer (Lemaître et al., 2020; Pepke &  
94 Eisenberg, 2021; Risques & Promislow, 2018). Long-lived species do not invest in costly mechanisms to  
95 prevent the shortening of telomeres as a mechanism to limit cancer, but pay a senescence cost (Tian et  
96 al., 2018). In contrast, because they have long telomeres, short-lived species have a low probability to live  
97 old due to higher risks of developing cancer, and thus follow a “live fast and die young strategy”. Short-  
98 lived species have thus evolved different types of telomere maintenance compared to large species and  
99 do not show replicative senescence as a mechanism to protect from cancer (Lemaître et al., 2020; Risques  
100 & Promislow, 2018; Seluanov et al., 2007; Seluanov et al., 2008). Hence, they can overcome this  
101 senescence-cancer trade-off and have longer telomeres than long-lived species (Risques & Promislow,  
102 2018; Seluanov et al., 2008; Tian et al., 2018). So far, this anti-cancer hypothesis has only been applied to  
103 species comparisons and mostly focuses on mammals (Pepke & Eisenberg, 2021; Risques & Promislow,  
104 2018). It has not been used to explain variation of telomere dynamics at the inter-individual level. To do  
105 so, the patterns observed must be cleaned from any age effect, since even species that maintain an active  
106 telomerase in the adult soma, display telomere shortening with age (François Criscuolo et al., 2018). Still,  
107 selective forces and evolutionary mechanisms leading to the negative link between telomere length and  
108 lifespan at the inter-species level, should find their origin at the within-species level, and may vary  
109 between large and small-bodied species (Tian et al., 2018). In addition, intra-individual differences in  
110 telomere mechanisms should be detectable already early in life (i.e. as they would be associated with  
111 short or long-lived individual trajectories) (Noguera, Metcalfe, Reichert, & Monaghan, 2016). Under high  
112 cancer-risks, when applied to the within-species level, the anti-cancer hypothesis predicts opposite  
113 relationships between telomere length and POL than the resource allocation trade-offs hypothesis, with  
114 long telomeres being associated with a fast pace-of-life (Figure 1). The resource allocation trade-offs and  
115 the anti-cancer hypotheses are not mutually exclusive but could translate into different, and opposite,  
116 selective pressures on telomere length (Gomes et al., 2011; Pepke & Eisenberg, 2021; Risques &  
117 Promislow, 2018; Young, 2018).

118 A better understanding of the roles of telomeres in the evolution of ageing can only be achieved through

119 the study of the greatest diversity of taxa. However, studies on reptiles, amphibians and wild, small and

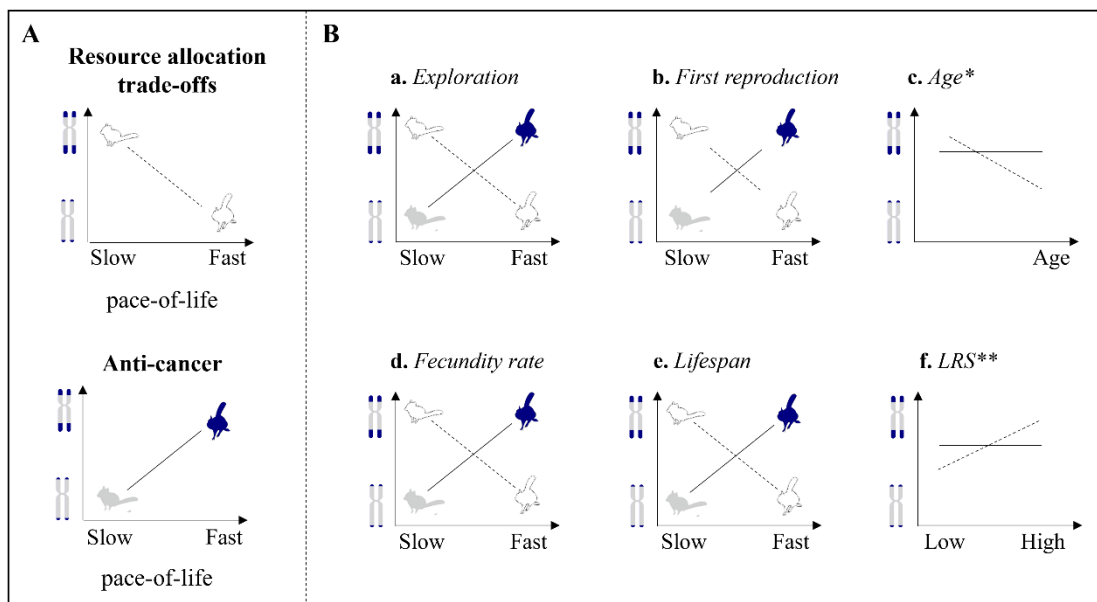
120 short-lived mammals are particularly rare (Pepke & Eisenberg, 2021; Remot et al., 2021; Wilbourn et al.,



121 2018). Capitalizing on a long-term monitoring of a wild population of Eastern chipmunks (*Tamias striatus*),

122 we investigated 1) the role of telomeres as biomarkers of the POLS and 2) whether the link between

123 telomere length and life history is best supported by the resource allocation trade-offs hypothesis or the  
 124 anti-cancer hypothesis in this species. Studies on this population revealed that behavioural traits, such as  
 125 exploration profile, are an integral part of the POLS as they correlate with several life-history traits; i.e. fast  
 126 explorers display fast life histories, namely an early age at first reproduction and a high fecundity  
 127 (Montiglio, Garant, Bergeron, Messier, & Réale, 2014; Montiglio, Garant, Pelletier, & Réale, 2015). We  
 128 thus tested whether telomere length at first breeding opportunity was linked to speed of exploration and  
 129 age at first breeding (Figure 1B.a & b). We also investigated if telomeres shorten with age, as observed in  
 130 most vertebrates studied (Bichet et al., 2020; Monaghan & Haussmann, 2006; Pauliny et al., 2006), or not,  
 131 as predicted under the anti-cancer hypothesis(Figure 1B.c). We then assessed the presence of a  
 132 correlation between telomere length and two common measures of the pace-of-life: fecundity rate and  
 133 lifespan (Figure 1B.d & e). Finally, the “phenotypic quality” hypothesis has received a lot of attention from  
 134 studies looking at the role of telomeres as mediators of life-history trade-offs (Eastwood et al., 2019;  
 135 Giraudeau et al., 2019; Heidinger et al., 2012), but see (Bergeron, Baeta, Pelletier, Réale, & Garant, 2011).  
 136 This hypothesis derivates from the resource allocation trade-offs hypothesis and states that individuals  
 137 who inherited long telomeres or benefited from good environmental conditions during key life history  
 138 stages, such as early life, may be able to invest more energy in all life-history traits. In addition to  
 139 predictions made under the life-history trade-offs hypothesis, the phenotypic quality hypothesis thus  
 140 posits that a positive relationship should exist between telomere length in early life and LRS, as individuals  
 141 with long telomeres are assumed to successfully cope with the costs of reproduction (Sudyka, 2019). We  
 142 therefore tested this by looking at the relationship between telomere length and LRS (Fig. 1B.f).



143 Figure 1. Expected relationships between telomere length (TL) and life histories under the resource  
144 allocation trade-offs and anti-cancer hypotheses (A) and detailed predictions on the expected relationships  
145 between pre-reproductive TL, life-history and behavioural traits (B). We define pre-reproductive telomere  
146 length here as telomere length before reproduction (early life TL measured in juveniles) or at first  
147 reproduction (TL at first breeding opportunity). Traits characterizing the pace-of-life (a, b, d, e) and the  
148 expected relationship between TL and age (c) or TL and LRS (f) are shown. Empty white symbols and  
149 dashed lines are attributed to the resource allocation trade-off hypothesis, filled coloured symbols and  
150 solid lines to the anti-cancer hypothesis (slow individuals are in grey and fast individuals are in blue, for  
151 references on other figures). The shape of the chipmunk symbols represents the speed of the pace-of-life  
152 (slow VS fast). \* According to the anti-cancer hypothesis, no significant erosion of telomeres would be  
153 expected with age in small-bodied species under high cancer risk, considering that small-bodied species  
154 have evolved different telomere maintenance and anti-cancer mechanisms than large species (Tian et al.,  
155 2018). \*\* Within the framework of the resource allocations trade-offs hypothesis, the phenotypic quality  
156 hypothesis posits that individuals with long telomeres cope well with the costs of reproduction and display  
157 a greater LRS. Under these hypotheses, LRS will depend on the outcome between fecundity rate and  
158 lifespan.

## 159 **Materials and Methods**

### 160 Model species, study system and site

161 We monitored a population of Eastern chipmunks in a deciduous forest of southern Québec, Canada  
162 (Mont-Sutton; 45°05' N, 72°25' W), on three sites from 2013 to 2019 (Sites 1 and 2 of 7.84 ha each, and  
163 site 3 of 4 ha). Eastern chipmunks are omnivorous, food-hoarding Sciurid rodents that display periodic  
164 torpor events in winter (Elliott, 1978; Humphries, Thomas, & Speakman, 2002). Each year, from May to  
165 September, we conducted daily trapping from 8:00 a.m. until dusk, using Longworth traps (Bergeron,  
166 Réale, Humphries, & Garant, 2011a). Upon capture, chipmunks were marked with ear tags and a Trovan  
167 PIT tag and we recorded their body mass, sex, reproductive status and determined their age class  
168 (juveniles or adults; see supplementary methods). Individuals weighing > 80 g at first capture in May or  
169 displaying reproductive signs over the entire reproductive season were considered as adults. We classified  
170 as juvenile individuals weighing less than 70 g at first capture and showing no signs of sexual maturity over  
171 the entire season, i.e. absence of darkened scrotum for males and undeveloped mammae for females  
172 (Bergeron et al., 2011; Tissier et al., 2020). Chipmunks weighing between 70 g and 80 g at first capture

173 and that could not be classified as juveniles or adults were considered as NA (Bergeron, Réale, et al.,  
174 2011a; Tissier et al., 2020).

175 From 2013 to 2019, we captured a total of 819 chipmunks, including 209 adults at their first breeding  
176 opportunity (Table S1). Ear tissue punches were collected on each individual for genotyping. We  
177 measured relative telomere length (rTL) on all the individuals for which untouched tissue or DNA samples  
178 were remaining after genotyping, resulting in 70 individuals with a rTL measurement at first breeding  
179 opportunity (27 females and 43 males; see Table S1). Capitalizing on a long-term monitoring of the  
180 population, we also had data on behavioural and life-history traits for a portion of these individuals,  
181 namely on their exploration profile (n=48), fecundity rate (n=37), LRS (n=35) and minimum lifespan  
182 (n=51). Chipmunks live on average 30 months (Bergeron, Réale, et al., 2011a). The maximum recorded  
183 lifespan is 7 years in this population (*unpublished results*). Previous studies showed that chipmunks  
184 exclusively reproduce in the summer before and in the spring after a beech mast in autumn in this system  
185 (Bergeron, Réale, et al., 2011a; Tissier et al., 2020). Individuals cannot reproduce before their first winter.  
186 Therefore, first breeding opportunity either occurs at 7 or 15 months-old for the summer and spring  
187 cohorts, respectively (Montiglio, Garant, Bergeron, Dubuc Messier, & Réale, 2014). First breeding  
188 opportunity corresponds to the first time for which we can have a pre-reproductive measure on most  
189 residents, considering the low recapture rate of juveniles. Note that we make the distinction between first  
190 breeding opportunity, which occurs at either 7 or 15 months old, and age at first effective reproduction,  
191 which may occur at 22 months old, for individuals that failed to reproduce at their first opportunity  
192 (Montiglio et al., 2014).

### 193 Tissue collection and DNA extraction

194 We collected ear tissue punches upon capture per individual during their first two years of life. Since we  
195 usually do not collect DNA samples on individuals older than two years, a sample was specifically collected  
196 on a subsample of 3–6-year-old individuals (n=12), among which 7 individuals for which we had a measure  
197 of rTL at first breeding opportunity, to investigate changes in telomere length with age (see below). Upon  
198 collection, ear tissue punches were transferred to 95% ethanol Eppendorf tubes, transferred from the  
199 field to laboratory conditions within 2-4 hours where they were stored at +4°C until extraction, which  
200 occurred within two months after collection. Upon extraction, DNA samples were stored at -80°C. DNA  
201 extraction was carried out using a modified version of a validated salting-out method (Aljanabi &  
202 Martinez, 1997; see supplementary methods). Briefly, after allowing ethanol to evaporate from the tissue  
203 sample, saline extraction buffer (2 mM ethylenediaminetetraacetic acid, 10 mM Tris-HCl, 0.4 M NaCl),

204 sodium dodecyl sulphate and proteinase K were added to the sample and incubated overnight at 60 °C.  
205 The following morning, more proteinase K was added; the mixture was vortexed and incubated for 2  
206 additional hours at 60 °C. Once digested, 300 µl of 6 M saline solution was added and the mixture was  
207 vortexed and centrifuged for 30 min at 6000 g. We then transferred 600 µl of the aqueous solution into a  
208 clean 1.5-ml microtube. The DNA was precipitated by a 1-h incubation at 20 °C with 600 µl isopropanol  
209 and pelleted by centrifugation for 20 min at 10000 g. Pellet was rinsed with 200 µl of 70% ethanol, spun  
210 again at 10000 g for 10 min, and left to air dry overnight. The next day, the pellet was resuspended using  
211 200 µl of distilled water. After having determined the DNA concentration using gel electrophoresis,  
212 samples were diluted to a concentration of 5 ng/µl for polymerase chain reaction (PCR) amplification and  
213 frozen at -80°C until being processed for genotyping or telomeres measurements.

#### 214 Genotyping and parental assignment

215 We conducted microsatellite genotyping using 23 loci previously employed in the study system (Bergeron,  
216 Réale, Humphries, & Garant, 2011b; Chambers & Garant, 2010; Vandal, Houle, Archambault, Réale, &  
217 Garant, 2020). The detailed genotyping protocol, is described in (Chambers & Garant, 2010; Vandal et al.,  
218 2020). The parentage assignment protocol is fully described in (Bergeron, Réale, et al., 2011b). Briefly, we  
219 captured weaned juveniles that emerged from their known maternal burrow. We used CERVUS  
220 (Kalinowski, Taper, & Marshall, 2007) to corroborate the maternal identity and assigned paternity based  
221 on microsatellite analyses (Chambers and Garant 2010). When we had no information on the maternal  
222 burrow (i.e. when juveniles were captured during systematic trapping on the grid), we used COLONY  
223 (Wang & Santure, 2009) to reconstruct family groups (Bergeron et al., 2011b).

224 Assessment of relative telomere length (rTL)We used DNA extracted from ear tissue punches collected in  
225 spring to measure relative telomere length (rTL, expressed as T/S ratio). Telomeres length is commonly  
226 measured on ear tissues in rodents (Hehar & Mychasiuk, 2016; Turbill et al., 2013), and strongly correlates  
227 with telomere length in other tissues, including brain tissues such as hippocampus and prefrontal cortex  
228 (Hehar & Mychasiuk, 2016) or buccal mucosa (Turbill et al., 2013). In total, we obtained rTL on 204  
229 chipmunks of known age, 74 at their first breeding opportunity (among which 70 for which we had life-  
230 history data as detailed above), 106 at emergence from the maternal burrow, 12 individuals aged 18-24  
231 months, and 12 individuals aged 3-6 years. There were 20 individuals for which we had two rTL  
232 measurements in spring. At their first breeding opportunity, individuals were either 7 months or 15  
233 months of age (Montiglio et al. 2014). In both cases, individuals were captured in spring after their first  
234 winter. Individuals aged 7 months were about to initiate reproduction at that time whereas individuals

235 aged 15 months already initiated it. To investigate change in telomere length with age, we randomly  
236 selected 20 out of 30 individuals for which we had sufficient remaining DNA to measure rTL at two ages in  
237 spring, ensuring to have a representative sampling of our population (samples from different years, sexes,  
238 and ages; see Table S1). We also measured rTL on juveniles sampled upon emergence from the maternal  
239 burrow (i.e. weaning, ~30-45 days of age). The average recapture rate of juveniles after their first winter is  
240 14.5% in our population (9% for males and 20% for females; *unpublished data*, see also (Loew, 1999)  
241 reporting similar proportions in other chipmunk populations). Combined with the exclusion of 3  
242 individuals that were aged of 1 year-old at the time of the study, this reduced our initial sample of 106 to  
243 a subset of 12 juveniles for which we had data on both telomere length and lifespan.

244 We first assessed the quality of each DNA sample by gel electrophoresis (DNA integrity: samples were  
245 conserved only in absence of smears). Using a Nanodrop ND1000 (ThermoFisher Scientific, Waltham, MA,  
246 USA) we then measured DNA concentration and purity of each sample looking at the ratios of absorbance  
247 at 260nm (ratio 260/280) and at 230nm (ratio 230/260). We fixed exclusion criteria at a minimum  
248 concentration of 10ng/μL, and ratios at 1.7-2.0 (260/280) and >1.5 (230/260). Regarding the qPCR  
249 reaction, the rTL (T/S ratio) measurement is based on the determination of a number of amplification  
250 cycles necessary to detect a lower threshold of fluorescent signal. Cycle number is proportional to the  
251 sample telomere length (T), or to the number of copies of a non-variable copy number gene (or control  
252 gene S used as a reference). As a reference non-variable copy number (non-VCN) gene, we used a 54 bp  
253 portion of the c-myc proto-oncogene, which was tested for non-variability in copy number (i.e. we  
254 checked in the genome that there were not 2 copies of the gene in chipmunks), as described for other  
255 species (Nussey et al., 2014; Smith, Turbill, & Penn, 2011). We first select 2 (2-5) non-VCN genes candidate  
256 sequences. Then we conducted a pilot study comprising a subset of 10 (10-20) samples (on a range of  
257 samples representing both sexes and all our populations used in the final sample set) to allow pairwise  
258 correlation of rTLs estimated from the different non-VCN gene. We then conducted rTL measurements by  
259 quantitative real-time amplification (Cawthon, 2002) adapted for ear tissues and for Eastern chipmunks.  
260 Forward and reverse telomeric primers were 5'- CGG TTT GTT TGG GTT TGG GTT TGG GTT TGG -  
261 3' and 5'- GGC TTG CCT TAC CCT TAC CCT TAC CCT TAC CCT - 3', respectively. Primer sequences  
262 for the non-VCN gene were 5'- GAG GGC CAA GTT GGA CAG TG - 3', and 5' - TTG CGG TTG TTG CTG ATC  
263 TG -3', respectively.

264 Measurements were done on two 384-well plates. We added 10 ng (2 μl) of DNA in each well, together  
265 with 8 μl of GoTaq QPCR Mix (Promega, Madison, WI, USA); with an extra 100 μM final concentration of

266 dNTPs; and forward and reverse primers at 5  $\mu$ M. Cycling conditions included: 1) an initial hold at 95 °C for  
267 2 min; 2) 30 cycles (telomere) of 95 °C for 15 sec, 56 °C for 30 sec, and 72 °C for 30 sec or 40 cycles (non-  
268 VCN) of 95 °C for 15 sec, 60 °C for 30 sec and 72°C for 1 min, with fluorescence readings recorded at this  
269 step; and 3) a final melt step was set at 60 °C to 95 °C in 0.5 °C increments (see Figure S1). Cycling was  
270 performed on an Automated Thermal Cycler, Q real-time PCR instrument (CFX-384, Biorad, Hercules, CA,  
271 USA). Each plate contained samples ran in duplicates, a no-template control (sterile water used for  
272 dilution of samples), duplicates of 4 individuals and duplicates of a control well of 10 ng of 100 pooled  
273 individuals' DNA sample (i.e. repeated on each qPCR and used as the "golden sample", the reference  
274 sample with a value of T/S = 1, used to calculate the values of the other points). Calculation of rTL (T/S  
275 ratio) was carried-out using the software LinRegPCR (V.12.5) as described in (Pfaffl, 2001).. Efficiencies of  
276 qPCR reactions were of 99.9% for the control and 99.8% for telomeres in plate 1 and of 100% for the  
277 control and 99.9% for telomeres in Plate 2. Intraclass correlation coefficient for intra- and inter-runs (ICC;  
278 based on 12 samples repeated over the two 385-well plates; see (Eisenberg, Nettle, & Verhulst, 2020;  
279 Verhulst et al., 2016)) were of 0.976 and 0.992, respectively. Regarding measurements on change in rTL  
280 with age, samples were spread over the two plates as follows: we measured the two rTL of a same  
281 individual on two different plates for 9 individuals (three on plate 1 and six on plate 2) and on the same  
282 plate for 11 individuals (three on plate 1, eight on plate 2), thus preventing that plate effect would  
283 influence the results.

#### 284 Life-history and exploration variables

285 We considered two measures of fecundity: the average number of offspring per reproductive event as a  
286 measure of fecundity rate, and the lifetime number of assigned offspring at weaning as a measure of the  
287 lifetime reproductive success (LRS). We calculated the minimum lifespan in months, as the difference  
288 between the date of last capture and either the date of first capture (i.e. emergence from the maternal  
289 burrow) or the date of latest possible birth for the cohort (i.e. for individuals missed at emergence from  
290 the burrow and only captured after their first winter). For instance, given the recorded patterns of  
291 reproduction on our study sites since 2012 (Tissier et al., 2020), an unmarked adult first caught in spring  
292 2013 would be assigned to an emergence date in June 2012 (spring cohort). An individual first captured in  
293 June 2016 would be assigned to an emergence date in September 2015 (summer cohort). We considered  
294 only residents for this analysis and thus excluded 7 individuals based on this criterion, considering that we  
295 had data on telomere length at first breeding opportunity and exploration profile for those individuals but  
296 no information about their life-history. We also excluded 12 individuals that were still alive and aged one  
297 year-old for the same reasons. We considered as "residents" individuals that were present on the sites at



298 their first reproductive opportunity (dispersion rarely occur afterwards; Elliott 1978) and that were caught  
299 more than 5 times during their life/over several seasons (to exclude potential dispersers or individuals at  
300 the periphery of the sites, for which we would not have accurate life-history data).

301 We quantified chipmunk behavioural response to a novel environment, as a measure of their exploratory  
302 behaviour and their activity, by conducting open-field tests (Gharnit, Bergeron, Garant, & Réale, 2020;  
303 Montiglio, Garant, Thomas, & Réale, 2010). All the open-field tests were conducted on site. Upon capture,  
304 individuals were placed in an opaque tubular entrance for one minute for acclimation before the test.  
305 Then, we gently pushed them inside the open-field arena (80 x 80 x 40 cm, without any refuge and  
306 gridded at the base) and closed the entrance with a trapping grid (Montiglio et al., 2014, 2010). Chipmunk  
307 behaviour was videotaped for 90 s and we hereafter counted the number of lines crossed during this  
308 period (using The Observer XT11 software; Nodus). Individuals were tested twice, allowing us to calculate  
309 individual's best linear unbiased predictor (BLUPs) for exploration (Gharnit et al., 2020). We subsequently  
310 simulated 1000 series of BLUPs and used the standardized mean individual exploration BLUPs of the 1000  
311 estimates to characterize the exploration profile (from slow/thorough to fast/superficial) of each  
312 individual in our analyses (Gharnit et al., 2020).

### 313 Data Analyses

#### 314 *Model 1 - Determinants of the rTL at first breeding opportunity*

315 We investigated which variables influenced rTL at first breeding opportunity using a Linear Model (LM).  
316 The rTL was log-transformed and Z-transformed before being included in the models (Heidinger et al.,  
317 2012; Verhulst, 2020). Considering the reported effects of age and sex, alone or in interaction, on TL  
318 (Barrett & Richardson, 2011; Bauch, Gatt, Granadeiro, Verhulst, & Catry, 2020; Heidinger et al., 2021), we  
319 included age at first breeding opportunity (categorical variable), sex and their interaction as fixed effects  
320 in our model. Note that age at first breeding opportunity is a life-history stage, which occurs either at 7 or  
321 15 months old, depending on whether individuals were born in the previous spring or summer (Bergeron,  
322 Réale, et al., 2011a; Montiglio et al., 2014). Site, exploration score and the age\*exploration and  
323 sex\*exploration interactions were also included as fixed effects. These two-way interactions were  
324 reported to affect other life-history traits in this species (Bergeron, Réale, et al., 2011a; Montiglio et al.,  
325 2014). Finally, individual body mass was included as a covariate, as a proxy of growth and because  
326 telomere length covaries with body mass (Pepke & Eisenberg, 2021).

#### 327 *Models 2, 3 and 4 - Relationship between rTL and life-history traits*

328 We investigated the links between rTL at first breeding opportunity and fecundity (response variable,  
329 expressed as the total number of offspring weaned or sired /number of reproductive event) using a LM  
330 (model 2), assuming the normality of the residuals, which was confirmed by a Kolmogorov-Smirnov test.  
331 We investigated the relationship between rTL and LRS using a Generalized Linear Model (GLM; model 3)  
332 with a Poisson distribution, after verifying the absence of overdispersion. Finally, we used a LM to  
333 investigate the relationship between rTL at first breeding opportunity and minimum lifespan. We assessed  
334 the normality of the residuals with a Kolmogorov-Smirnov test (LM, model 4). As normality was not met,  
335 lifespan was expressed in  $\text{Log}_{10}(\text{months}+1)$  (Eastwood et al., 2019). We included age at first breeding  
336 opportunity (7 or 15 months), average adult body mass (linear term in models 2 and 3 and both the linear  
337 and quadratic terms in model 4 to account for the quadratic relationship between body mass and lifespan  
338 in our data; *unpublished results*), sex, site, rTL and the double-way interactions (rTL\*sex, rTL\*age at first  
339 breeding opportunity and sex\*age at first breeding opportunity) as fixed effects in the three models. We  
340 faced issues of model convergence when including the year of birth as a random effect in two of the three  
341 models. We thus included year as a fixed continuous effect in all full models. We also included the  
342 minimum lifespan as fixed effect in models 2 to account for the duration of breeding opportunities. Body  
343 mass, year, and exploration score (mean individual exploration BLUP) were standardized (Z-transformed)  
344 before inclusion in the models.

345 *Models 5, 6 and 7: relationships between age, rTL and telomere dynamic and between rTL at weaning and*  
346 *lifespan*

347 Using Linear Mixed Models (LMMs, model 5), we investigated the effect of age on telomere length. We  
348 first tested the relationship between age and rTL in spring at the population level (n=204), with age, sex,  
349 site, cohort of birth and sex\*cohort of birth as fixed effects, and the year of birth as a random effect to  
350 account for measurements on individuals born within the same years. Then, we looked at the relationship  
351 between age and rTL at the individual level, using the 20 individuals for which we had repeated spring  
352 measurements of telomere length (model 6). We included sex and age as fixed effects and the ID  
353 (intercept) and age (slope) as random effects in this model to separate within- from between-subject  
354 effects (van de Pol & Wright, 2009). We tested for within-individual repeatability in rTL by estimating the  
355 marginal  $R^2$  using a LMM as detailed in (Spurgin et al., 2018). A recent meta-analysis highlighted that  
356 within individual repeatability in telomere length depends on the duration of the study (time between the  
357 two measures of telomere length in a same individual; see (Kärkkäinen, Briga, Laaksonen, & Stier, 2021).  
358 Considering that it varied from 7 to 428 months in our study, we also conducted the same analysis on  
359 individuals for which there was <12 months between the two measures of telomere length (n=11). We

360 finally assessed the relationship between early life rTL and lifespan on the subset of 12 juveniles, using a  
361 LM (model 7).

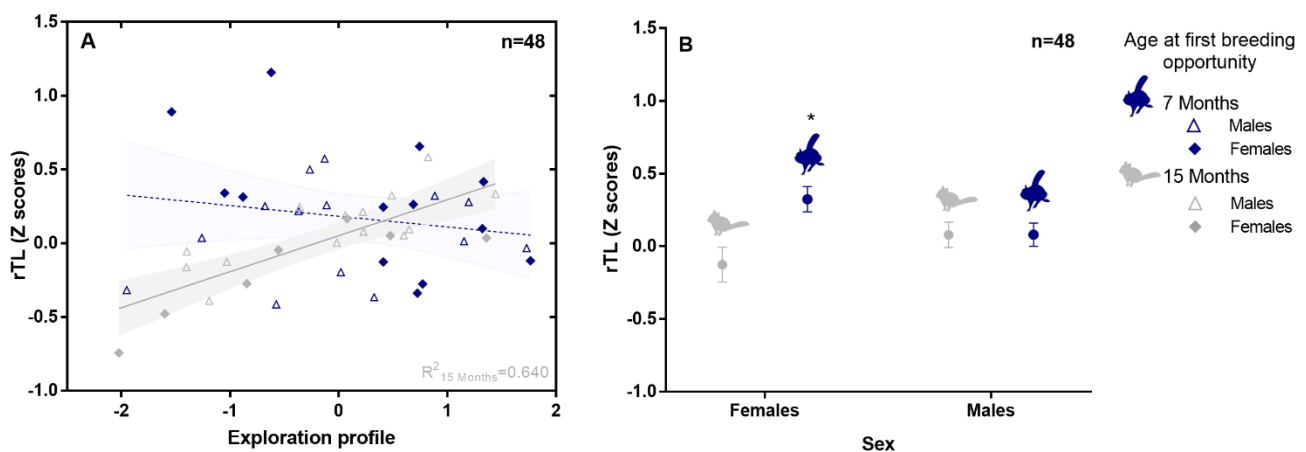
### 362 *Model selection and parsimony*

363 Starting from the full models, we removed one by one the least significant variables, only if their removal  
364 reduced the AICc . Both full and reduced models are presented in the online supplementary material  
365 (Tables S2-S6). We implemented false discovery rates to correct for multiple comparisons (Pike, 2011) in  
366 models 2, 3 and 4, using the SDM project spreadsheet available at:  
367 <https://www.sdmproject.com/utilities/?show=FDR>. Analyses were conducted using SPSS software (IBM  
368 SPSS Statistics for WINDOWS v. 24.0. Armonk, NY: IBM Corp.).

## 369 Results

### 370 *Correlates of telomere length at first breeding opportunity*

371 We found no effect of age on rTL at first breeding opportunity ( $t=0.34$ ,  $p=0.74$ ) but found a significant  
372 exploration\*age interaction (LM,  $t=3.42$ ,  $p=0.001$ ; model 1, Table S2B): rTL increased with speed of  
373 exploration, but only for individuals experiencing a first breeding opportunity at 15 months of age ( $R^2 =$   
374  $0.64$ , Figure 2A, Table S2B). We also found an effect of the sex\*age interaction, with females that had a  
375 first breeding opportunity at 7 months of age showing longer telomeres than females having a first  
376 breeding opportunity at 15 months of age (LM,  $t=2.62$ ,  $p=0.01$ ), whereas we found no differences in males  
377 (Figure 2B, Table S2B).



378 **Figure 2.** Determinants of relative Telomere Length (rTL) at first breeding opportunity. In (A) interaction  
379 between exploration and age at first breeding opportunity on rTL (Table S2, n=48, sample sizes by age at

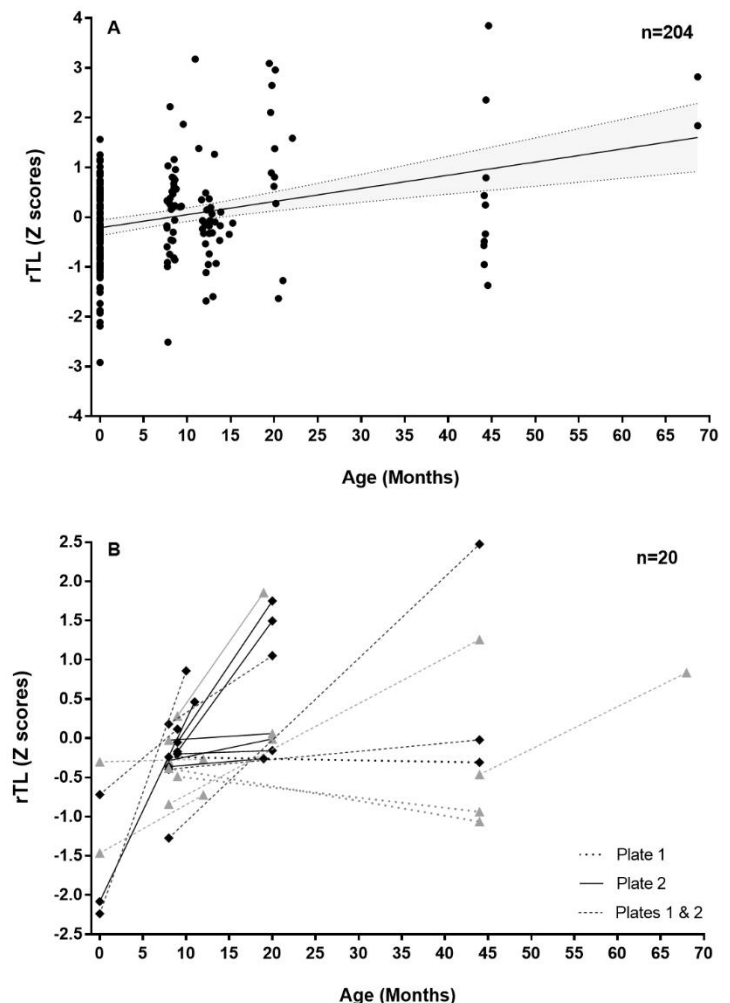
380 first breeding, 7 months: n=14 males and 13 females; 15 months: n=14 males and 7 females; sex symbols  
 381 are solely represented on the figures for informative purposes; no interaction with sex was found). The  
 382 dashed line represents a non-significant relationship in individuals aged 7 months. In (B) the interaction  
 383 between sex and age at first breeding opportunity on rTL is shown (means  $\pm$  SE; Table S2). Females whose  
 384 first breeding opportunity occurred at 7 months old had significantly longer telomeres than females with a  
 385 first breeding opportunity at 15 months old, whereas no significant difference was observed in males. The  
 386 rTL, expressed as T/S ratio, has been LN transformed before conducting the analyses. Z-scores are  
 387 presented for exploration and rTL.

388 *Change in telomere length with age*

389 We found a within-individual repeatability in rTL of  $R = 0.126$  for all 20 chipmunks for which we had to  
 390 measures of rTL. Repeatability for the subset of individuals sampled at <12 months was  $R = 0.378$ . We found  
 391 a positive relationship between age and rTL at the population level (model 5, LMM, n=204,  $F_{1;190}=37.57$ ,  
 392  $t=6.13$ ,  $p<0.001$ , Figure 3A), but no effect of the sex ( $F_{2;169.6}=1.26$ ,  $p=0.71$ ), cohort of birth ( $F_{2;28.5}=0.56$ ,  
 393  $p=0.85$ ) or their two-way interaction ( $F_{3;169.7}=1.00$ ,  $p=0.32$ ; Table S3A).

394 We also found that telomere length of both male and  
 395 female chipmunks increased with age at the within  
 396 individual level (model 6, LMM, n=20,  $t=3.981$ ,  
 397  $p<0.001$ , Table S3B), although two individuals  
 398 displayed a slight reduction in rTL with age and one  
 399 female showed no elongation or shortening (Figure  
 400 3B). Our LMM revealed no sex effect on change in  
 401 telomere length (n= 9 males and 11 females ,  $t=0.42$ ,  
 402  $p=0.67$ , Table S3B).

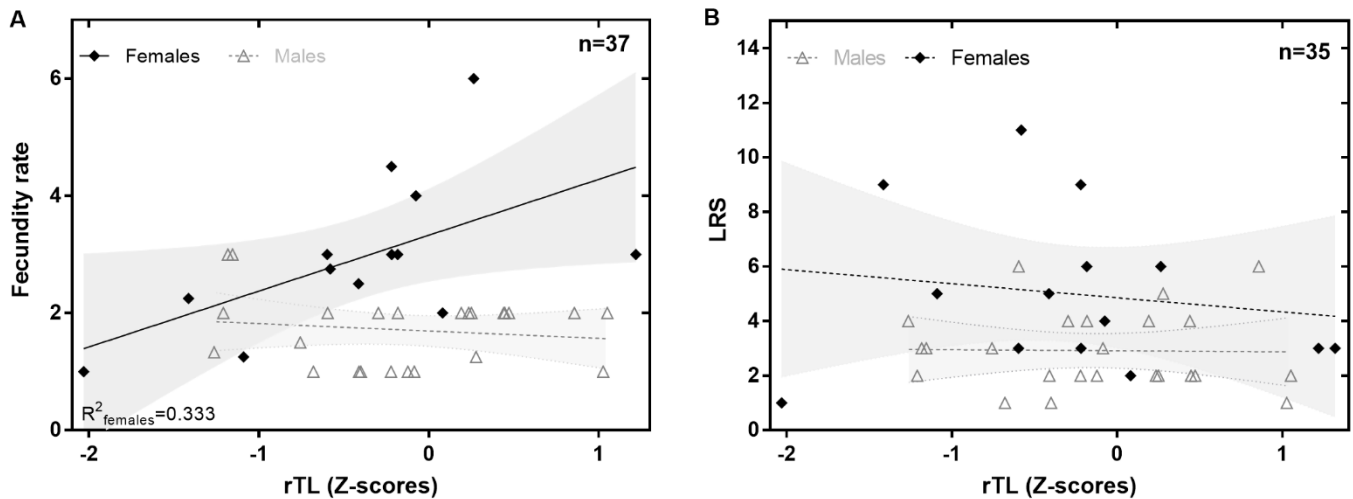
403 **Figure 3. Relationships between relative telomere**  
 404 **length (Z scores of LN(T/S ratio)) and age (months since**  
 405 **the emergence from the maternal burrow) in Eastern**  
 406 **chipmunks. (A) Relative telomere length in spring**  
 407 **according to age at the population level (n=204). A**  
 408 **positive relationship between age and rTL was**  
 409 **recorded ( $R^2=0.12$ ,  $t=6.18$ ,  $p<0.001$ , Table S3A), with**



410 an appearance of long telomeres (i.e. with a T/S ratio > 2.70) in individuals of >7 months of age (these long  
 411 telomeres were not recorded in juveniles sampled at emergence from the maternal burrow, 0 months,  
 412 n=106). (B) Repeated measurements of relative telomere length in individual chipmunks sampled twice in  
 413 their life in spring. Telomeres increased significantly with age ( $t=3.98$ ,  $p<0.001$ , Table S3B). Data are  
 414 displayed for females (diamonds and black lines) and males (triangles and grey lines) as informative purposes  
 415 (we found no significant difference between the sexes, Table S3B); plates are also represented by dotted,  
 416 dashed and solid lines to highlight that elongation was not caused by methodological artefacts.

417 *Telomere length at first breeding opportunity and life-history traits*

418 We found no effect of age or body mass on fecundity rate, expressed as the average number of offspring per  
 419 reproductive event (model 2,  $p>0.05$ ; Table S4). However, we found that the sex\*rTL interaction had a



420 significant effect on this trait (model 2,  $t=2.75$ , FDR-adjusted  $p$ -value = 0.02; Table S4): fecundity rate  
 421 positively covaried with rTL at first breeding opportunity in females, but not in males (Figure 4A,  
 422  $R^2_{females}=0.33$ ,  $p=0.001$ ). Regarding LRS, however, we found no significant link between rTL at first breeding  
 423 opportunity and LRS, nor of the sex\*rTL interaction, that were both excluded from the final selected model  
 424 (model 3, Figure 4B & Table S5). Our model, however, revealed a positive and significant correlation  
 425 between lifespan and LRS ( $\chi^2=4.50$ ,  $p=0.03$ ; Table S5). No effects of the cohort of birth (spring or summer)  
 426 were found on either the fecundity rate or the LRS ( $p>0.05$ , Table S4 & Table S5).

427 **Figure 4. Relationship between relative Telomere length (rTL) at first breeding opportunity and (A) fecundity rate**  
 428 **rate (average number of offspring per reproductive event) or (B) LRS, in males (triangles) and in females**

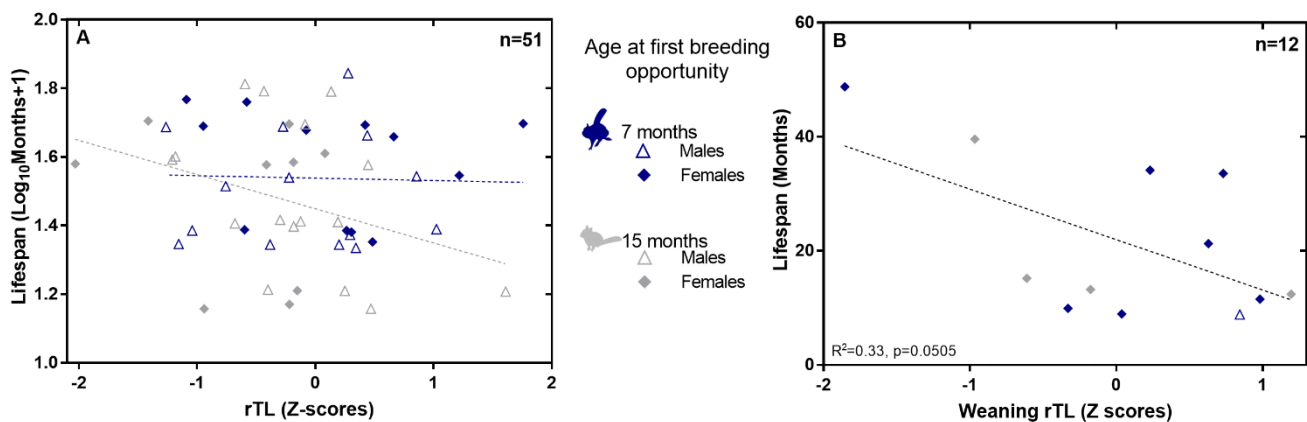
429 (diamonds). We found a sex\*rTL interaction effect on fecundity rate (Table S4) but no effect of rTL or the  
430 interaction on LRS (Table S5). The full line represents a significant relationship between rTL and fecundity  
431 rate in females (A; Table S4), whereas the dashed lines represent no significant relationships.

432

433 Regarding lifespan, we found no significant effect of rTL nor of the age at first breeding opportunity\*rTL  
434 interaction (model 4,  $t=0.63$  &  $t=1.43$ , FDR-adjusted  $p$ -values = 0.60 and  $p=0.24$ , respectively; Table S6). We  
435 only observed a significant effect of the year of birth on lifespan ( $t=3.07$ , FDR-adjusted  $p=0.02$ ; Table S6). A  
436 supplementary analysis on a subset of 12 juveniles revealed that telomere length at the emergence from the  
437 maternal burrow (i.e. weaning) also tended to be negatively associated with longevity (model 7,  $R^2=0.33$ ,  
438  $p=0.05$ ; Figure 5B).

439

440



441 **Figure 5. Relationship between relative telomere length (rTL) and lifespan.** In (A) rTL of adults according to  
442 their age at their first breeding opportunity is represented with lifespan expressed in  $\text{Log}_{10}(\text{Months}+1)$ .  
443 Individuals with a first breeding opportunity at 7 months old (fast) are shown in dark blue, whereas  
444 individuals first mating at 15 months old (slow) are shown in light grey; both males (triangles) and females  
445 (diamonds) are represented for informative purposes. We found no effects of rTL on lifespan and no effects  
446 of sex or age at first breeding opportunity on the relationship between rTL and lifespan (Table S6). In (B)  
447 early life rTL, measured at emergence from the maternal burrow, is shown with lifespan expressed in

448 months. The dashed line represents a non-significant linear regression. The rTL, expressed as the T/S ratio, is  
449 presented as Z scores of LN transformed data.

## 450 Discussion

451 We found that telomeres elongated with age both at the population level and within individuals, although  
452 we initially predicted that telomeres length would either shortened with age or would be maintained , under  
453 the resource allocation trade-offs and the anti-cancer hypotheses, respectively. Telomere length at first  
454 breeding opportunity positively correlated with speed of exploration, age and subsequent fecundity rate  
455 (thus with a fast pace-of-life), but did so in a sex-specific or cohort-specific manner. However, we found no  
456 correlation between telomere length and LRS or lifespan. Our results, thus, supported three of the five  
457 predictions from the anti-cancer hypothesis, but none of the predictions from the resource allocations trade-  
458 offs or the phenotypic quality hypotheses (Figure 1). Our findings suggest that resource allocation trade-offs  
459 alone are unlikely to drive ageing and the evolution of life-histories in this species.

### 460 *Correlates of telomere length at first breeding opportunity*

461 We found that fast and superficial explorers, reported to have a higher fecundity rate than slow and  
462 thorough explorers in this population (Montiglio et al., 2014), also had longer telomeres, but solely in late  
463 breeders (i.e. individuals whose first breeding opportunity occurred at 15 months old). This result confirms  
464 predictions derived from the anti-cancer hypothesis. It echoes findings from a previous study on wild  
465 chipmunks, highlighting that fast explorers showed lower daily energy expenditure levels than did slow  
466 explorers, contrary to predictions made under the resource-allocation trade-offs hypothesis (Careau et al.,  
467 2015). The absence of relationship between telomere length and exploration profile in early breeders in our  
468 study could be explained by a greater variation in their exploration profile or telomere length compared to  
469 late breeders, as already reported for exploration in this species (Montiglio et al., 2014). This greater  
470 variation and the absence of a significant relationship with telomere length could represent different  
471 behavioural/life-history strategies that could be genetically fixed in late breeders compared to early ones.  
472 Alternatively, it could reflect a greater plasticity of early breeders compared to late ones. Early-breeding  
473 female chipmunks (7 months of age) were shown to be faster explorers than late-breeding ones in the  
474 studied population (Montiglio et al., 2014). Here, we show that these early-breeding females have longer  
475 telomeres than late-breeding females, as predicted by the anti-cancer hypothesis. Because telomeres  
476 elongated with age in this population, this result cannot be explained by a simple age difference between

477 females of the two groups (i.e. with older females having shorter telomeres because of a greater erosion  
478 with age). This result suggests that, for the same age, females with a first breeding opportunity before 15  
479 months of age had longer telomeres than females with a first breeding opportunity at 15 months of age.  
480 However, given the intrinsic link between cohort of birth and age at first breeding opportunity in this system,  
481 early breeding and late-breeding females would never have the same age at the same period of their life  
482 cycle (Bergeron et al. 2011, Montiglio et al. 2014, Tissier et al. 2020). Comparing their telomere length at the  
483 same age would thus be biased by life-cycle stage and season, and therefore by varying intrinsic and  
484 environmental factors that could have an influence on their telomeres. Together, our findings support the  
485 idea that rTL early in life positively covaries, in a cohort- and sex-specific manner, with speed of exploration  
486 and age at first breeding opportunity, two metrics commonly used to map individuals along the slow-fast  
487 continuum of the POLS (Araya-Ajoy et al., 2018; Réale et al., 2010). These results also suggest that resource  
488 allocation trade-offs alone are unlikely to drive the evolution of life-histories in this system and that other  
489 forces, such as cancer risks, are at play (see (Cohen, Coste, Li, Bourg, & Pavard, 2020; Kokko & Hochberg,  
490 2015; Lemaître et al., 2020; Pepke & Eisenberg, 2021)).

#### 491 *Telomere elongation with age*

492 Following the anti-cancer hypothesis, telomeres were not predicted to shorten with age, but were not  
493 expected to significantly elongate at the within individual and population levels (Figure 3). Most studies  
494 conducted so far found that telomeres do shorten with age in humans, captive-bred animals and wild, long-  
495 lived vertebrates (Bichet et al., 2019; Bize et al., 2009; Eastwood et al., 2019; Heidinger et al., 2012;  
496 Monaghan & Hausmann, 2006; Sudyka, 2019). This usually translates into shorter telomeres being  
497 measured in older than in younger individuals at the population level (Hausmann et al., 2003; Monaghan &  
498 Hausmann, 2006; Pauliny et al., 2006). Here, we found the reversed patterns, with a positive relationship  
499 between age and rTL (Figure 3A) and a within individual elongation of telomeres with age (Figure 3B). This  
500 positive relationship cannot solely be explained by a selective disappearance of individuals with short  
501 telomeres (Dantzer & Fletcher, 2015), as an appearance of long telomeres (with a T/S ratio >2.70, which is  
502 above the maximum recorded in the 106 juveniles sampled) seem to appear with age in this population  
503 (Figure 3A). Within individual repeatability in telomere length was  $R=0.128$  (whole sample) and  $R=0.378$   
504 (subset of individuals sampled at <12 months apart), which is equivalent or above repeatability recorded in  
505 other mammal studies using qPCR essays (Fairlie et al., 2016; van Lieshout et al., 2019). However,  
506 repeatability within individuals is based on several hypotheses: 1) telomere length on year  $n$  is predictive of



507 what will happen in year  $n+1$ ; 2) telomere length shorten with age and 3) the rate of shortening is similar  
508 between individuals of a same cohort. In our case, however, these hypotheses are not met, especially  
509 considering that we did not record a no reduction in telomere length with age but a lengthening.

510 Considering the significant elongation of telomeres recorded at the within individual level combined with the  
511 significant increase observed at the population level ( $n=204$ ), also reported in other species (Hoelzl, Smith, et  
512 al., 2016; Spurgin et al., 2018), telomere elongation with age is unlikely to be due to a methodological  
513 artefact of the qPCR method. It is also unlikely to be caused by a simple increase in interstitial telomeric  
514 sequences as suggested in previous work (Aksenova & Mirkin, 2019). One FISH-labelling study conducted in a  
515 squirrel species indeed found no specific staining of telomere probes at the interstitial location and  
516 highlighted that the occurrence of multiple interstitial nucleolar organizer regions is rare in rodents  
517 (Fagundes, Christoff, Amaro-Ghilard, Scheibler, & Yonenaga-Yassuda, 2003). However, using the native  
518 telomeric restriction fragment assay (TRF) to measure the length of terminal telomeres would allow to  
519 confirm these statements. The native TRF assay allows to obtain an absolute telomere length measurement  
520 (without interstitial telomeric sequences), unlike the qPCR assay, which only provides an estimated relative  
521 telomere length compared to a control gene (S. Smith, Turbill, & Penn, 2011). However, the TRF assay is  
522 difficult to implement on longitudinal studies of small wild mammals because it requires high concentrations  
523 of DNA difficult to obtain with least-invasive approaches (Dantzer et al., 2020; Nussey et al., 2014; Smith et  
524 al., 2011).

525 Few other studies also found a positive relationship between age and telomere length (Hausmann et al.,  
526 2003; Hoelzl, Smith, et al., 2016). In edible dormice (*Glis glis*), young individuals significantly shorten their  
527 telomeres with age while old individuals (>6 years old) slightly elongate their telomeres when approaching  
528 the end of their life (Hoelzl, Smith, et al., 2016). This pattern has been attributed to the peculiar life-history  
529 strategy of this species, that may skip reproduction and hibernate for up to almost a year (Hoelzl, Cornils,  
530 Smith, Moodley, & Ruf, 2016; Hoelzl, Smith, et al., 2016). Here, we found that telomere elongation occurred  
531 in chipmunks of all ages. A within-individual seasonal elongation of telomeres has also been recorded in few  
532 rodent species (Criscuolo et al., 2020; Hoelzl, Cornils, et al., 2016). In our study, telomere length was always  
533 measured in spring. We can, thus, exclude a potential seasonal effect on the elongation pattern observed,  
534 which was recorded both in young and in old individuals. However, we cannot exclude that an  
535 environmental effect – e.g. pulsed availability of food resources that have a strong impact on behaviour,  
536 reproduction and life-history strategies in this system (Bergeron, Réale, Humphries, & Garant, 2011a;

537 Montiglio et al., 2014; Tissier, Réale, Garant, & Bergeron, 2020) – may partially explain this elongation  
538 (Hoelzl, Cornils, et al., 2016). Nonetheless, the observation of telomere elongation with age under controlled  
539 conditions with unlimited food resources and using the Q-FISH procedure (see Panasiak et al., 2020),  
540 highlights that food alone unlikely explains these patterns of telomere elongation.

541 Our results, combined with recent literature on telomere dynamics in small (Criscuolo et al., 2020; Foley et  
542 al., 2018; Hoelzl, Smith, et al., 2016) and large mammals (Kirby, Johnson, Alldredge, & Pauli, 2019; Lemaître  
543 et al., 2021), as well as in fishes (Panasiak et al., 2020) and birds (Spurgin et al., 2018), pinpoint that a much  
544 greater variety of telomere dynamics patterns may exist than previously reported, including within-individual  
545 telomere elongation, and that these patterns likely correlate with pace of life. Proximal mechanisms  
546 explaining telomere elongation in chipmunks will have to be investigated in the future, but we can  
547 hypothesize that telomerase activity is involved (S. Smith, Hoelzl, Zahn, & Criscuolo, 2021). Telomerase is the  
548 enzyme that repairs telomeres (Chan & Blackburn, 2004). It is repressed after growth in the somatic tissues  
549 of large and long-lived vertebrates, but not in the somatic tissues of small and short-lived ones (Chan &  
550 Blackburn, 2004; Tian et al., 2018). Among 18 rodent species, Eastern chipmunks show the highest body-  
551 mass specific telomerase activity in their somatic tissues (Seluanov et al., 2007; Tian et al., 2018). We initially  
552 expected that this high activity of telomerase would prevent telomere erosion with age. However, it could in  
553 fact be involved in the significant and consistent telomere elongation that we recorded at the within  
554 individual and population levels, as observed, for instance, in sperm of large mammals, including humans,  
555 where telomerase is maintained active (Kimura et al., 2008; Kozik, Bradbury, & Zalensky, 1998; Ozturk, 2015;  
556 Tian et al., 2018). However, the qPCR assay did not allow us to assess the strength of this elongation, nor to  
557 compare the absolute length of telomeres recorded in this population with other studies in rodents, which  
558 could be done using the TRF or Q-FISH approaches (Lai, Wright, & Shay, 2018; Nussey et al., 2014). Future  
559 studies could tackle those issues by optimizing DNA extraction from buccal swabs, which offers a promising  
560 avenue to obtain sufficient DNA in small mammals with a non-invasive technique (Hoelzl, Cornils, Smith,  
561 Moodley, & Ruf, 2016; Lai et al., 2018), while assessing whether telomerase, beyond its impact on telomere  
562 length or dynamics, acts as a mediator of life-history strategies (Criscuolo, Smith, Zahn, Heidinger, &  
563 Haussmann, 2018; S. Smith et al., 2021).

564 *Early-life telomere length and life-history traits*

565 Fecundity rates positively co-varied with telomere length, as predicted by the anti-cancer hypothesis, but did  
566 do in a sex-specific manner. This absence of relationship between rTL and fecundity rate in males is not  
567 surprising, considering the differences of parental investment between sexes. In chipmunks, males engage in  
568 scrambling competitions for mates but do not provide parental care (Bergeron, Réale, Humphries, & Garant,  
569 2011b), whilst females bear the costs of gestation and lactation, which are high in small mammals  
570 (Speakman, 2008). Previous studies revealed sex-differences in stress reactivity and behaviour, likely  
571 associated to differential investment into reproduction (Montiglio et al., 2014; Montiglio et al., 2015). The  
572 sex-specific relationship observed could reflect the sexually discordant selection on telomeres reported in  
573 other vertebrate species, through sex-specific telomere dynamics or sex-specific relationships between  
574 telomere length and life-history traits (Barrett & Richardson, 2011; Bauch et al., 2020; Brown et al., 2021;  
575 Heidinger et al., 2021), or a well-reported sex-specific differences in POLS (Tarka, Guenther, Niemelä,  
576 Nakagawa, & Noble, 2018). Since fecundity rate is considered as one of the best metrics of individual pace-  
577 of-life (Araya-Ajoy et al., 2018), this positive relationship between rTL and fecundity rate in females,  
578 independently of their cohort of birth, provides another support for the anti-cancer hypothesis. Finally, we  
579 found no link between early life telomere length and LRS, neither in males nor in females, as expected under  
580 the “phenotypic quality” hypothesis (Sudyka, 2019).

581 We failed to find any significant relationship between lifespan and rTL in Eastern chipmunks. An absence of  
582 correlation between telomere length and lifespan has previously been reported at the between-species level  
583 in rodents (Seluanov et al., 2007). Nonetheless, the supplementary analysis on a subset of 12 juveniles shows  
584 a trend for a negative correlation between telomere length and lifespan . It also highlights that the two  
585 juveniles with the shortest telomeres at weaning displayed an above-average lifespan of more than 40  
586 months, i.e. the average lifespan is of ~30 months in this population (Bergeron, Réale, et al., 2011a).  
587 Although anecdotal, this observation goes against the idea of a selective disappearance of individuals with  
588 short telomeres, supported by our results on telomere elongation with age. Lifespan, commonly linked to  
589 telomere length in birds and humans (Bichet et al., 2019; Eastwood et al., 2019; Heidinger et al., 2012; Sahin  
590 & Depinho, 2010), is commonly used as a metric of the POLS (Giraudeau et al., 2019; Ricklefs & Wikelski,  
591 2002). However, it could be a less valuable metric than fecundity rate to assess the pace of life of species  
592 with low survival probabilities such as chipmunks, as it displays less inter-individual variation compared to  
593 fecundity rate and generation time (Araya-Ajoy et al., 2018). This could potentially explain why we found no  
594 significant relationship in adults and solely a trend in juveniles, together with a lack of statistical power.

595 Consequently, we found no support for a positive relationship between rTL and lifespan or LRS, as expected  
596 under the phenotypic quality hypothesis. We argue that the concept of phenotypic “quality” should be used  
597 and interpreted with caution, especially when fitness is used to both define and assess quality (Bergeron,  
598 Baeta, Pelletier, Réale, & Garant, 2011).

## 599 **Conclusion**

600 To our knowledge, this study is the first to test the anti-cancer hypothesis at the within-species level, within  
601 the framework of the life-history theory. Our results, based on a long-term monitoring of a wild rodent,  
602 support several predictions following the anti-cancer hypothesis, but not the resource-allocation trade-offs  
603 hypothesis. We also show within-individual elongation of telomeres with age, in individuals from all age  
604 classes. Previous studies revealed that Eastern chipmunks show the highest body-mass specific telomerase  
605 activity in their somatic tissues. The elongation of telomeres recorded with age in chipmunks could thus be  
606 compared to closely related species. Recent literature suggests that patterns of telomere elongation exist in  
607 other vertebrate species, especially in non-model and non-avian species. Further investigations are thus  
608 needed to disentangle to which extent life histories and telomere dynamics of species are more likely to be  
609 driven by resource allocation trade-offs alone or by higher order constraints that depend on species size and  
610 taxa.

611

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634  
635 **Supplementary material**

636 Tables S1 to S6

637 Figures S1 and S2

638 Captions for external databases S1-S3

639 External databases S1-S3

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