

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 protecting
20 caps of chromosomes, are thought to be involved in ageing, cancer risks and to modulate life-history
21 strategies. They shorten with cell division and age in somatic tissues of most species, possibly limiting
22 lifespan. The resource allocation trade-off hypothesis predicts that short telomeres have thus co-evolved
23 with early reproduction, proactive behaviour and reduced lifespan, i.e. a fast Pace-of-Life Syndrome (POLS).
24 Conversely, since short telomeres may also reduce the risks of cancer, the anti-cancer hypothesis advances
25 that they should be associated with slow POLS. Conclusion on which hypothesis best supports the role of

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

36

37 **Introduction**

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

49 One of the current and dominant thinking in life-history is based on the fact that resources are limited, such
50 that allocation trade-offs occur between three key functions: growth, reproduction and self-maintenance
51 (Kirkwood, 2005; Stearns, 1992). These functions are associated with physiological costs, such as oxidative
52 stress and accumulation of oxidative damage, or costs associated with telomere maintenance (Alonso-
53 Alvarez et al., 2004; Metcalfe & Monaghan, 2001; Monaghan, Metcalfe, & Torres, 2009; S. M. Smith, Nager,
54 & Costantini, 2016; von Zglinicki, 2002). These costs limit the ability of an organism to allocate energy to all
55 three functions and potentially cause ageing, driving life-history strategies (Metcalfe & Monaghan, 2003;
56 Monaghan & Haussmann, 2006; Monaghan et al., 2009). In terms of telomeres, the resource allocation

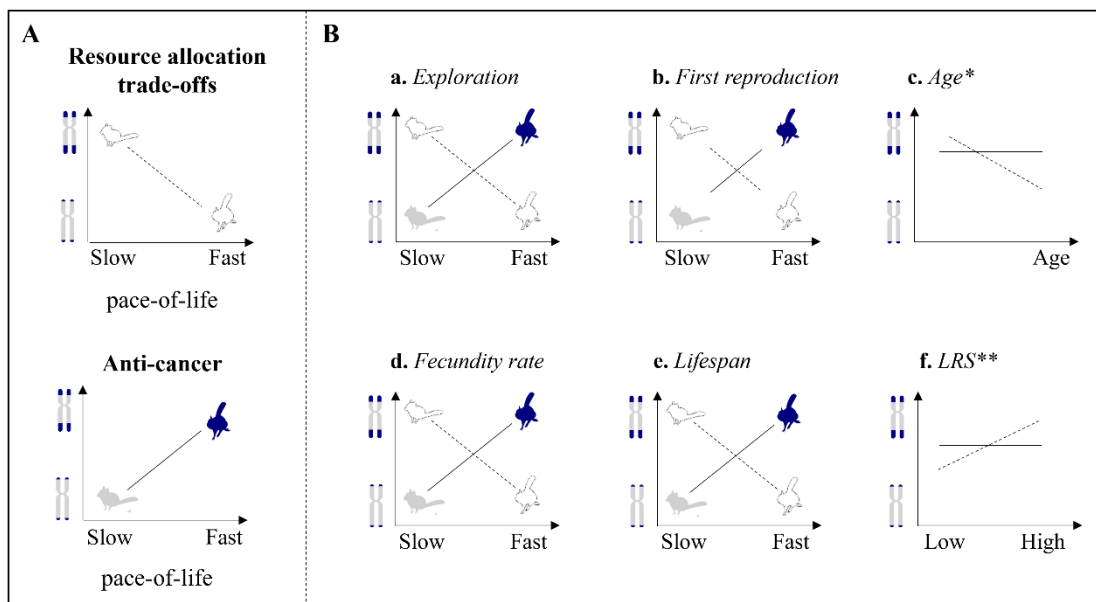
57 trade-offs hypothesis predicts that long telomeric sequences during pre-reproductive life, or at the time of
58 reproduction, are associated with prolonged lifespan (Eastwood et al., 2019; Heidinger et al., 2012). Such
59 long telomeric sequences are also associated to reduced investment into early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

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

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

116 A better understanding of the roles of telomeres in the evolution of ageing can only be achieved through
117 the study of the greatest diversity of taxa. However, studies on reptiles, amphibians and wild, small and
118 short-lived mammals are particularly rare (Pepke & Eisenberg, 2021; Remot et al., 2021; Wilbourn et al.,
119 2018). Capitalizing on a long-term monitoring of a wild population of Eastern chipmunks (*Tamias striatus*),

120 we investigated 1) the role of telomeres as biomarkers of the POLS and 2) whether the link between
 121 telomere length and life history is best supported by the resource allocation trade-offs hypothesis or the
 122 anti-cancer hypothesis in this species. Studies on this population revealed that behavioural traits, such as
 123 exploration profile, are an integral part of the POLS as they correlate with several life-history traits; i.e. fast
 124 explorers display fast life histories, namely an early age at first reproduction and a high fecundity (Montiglio,
 125 Garant, Bergeron, Messier, & Réale, 2014; Montiglio, Garant, Pelletier, & Réale, 2015). We thus tested
 126 whether telomere length at first breeding opportunity was linked to speed of exploration and age at first
 127 breeding (Figure 1B.a & b). We also investigated if telomeres shorten with age, as observed in most
 128 vertebrates studied (Bichet et al., 2020; Monaghan & Hausmann, 2006; Pauliny et al., 2006), or not, as
 129 predicted under the anti-cancer hypothesis(Figure 1B.c). We then assessed the presence of a correlation
 130 between telomere length and two common measures of the pace-of-life: fecundity rate and lifespan
 131 (Figure 1B.d & e). Finally, the “phenotypic quality” hypothesis has received a lot of attention from studies
 132 looking at the role of telomeres as mediators of life-history trade-offs (Eastwood et al., 2019; Giraudeau et
 133 al., 2019; Heidinger et al., 2012), but see (Bergeron, Baeta, Pelletier, Réale, & Garant, 2011). This hypothesis
 134 derivates from the resource allocation trade-offs hypothesis and states that individuals who inherited long
 135 telomeres or benefited from good environmental conditions during key life history stages, such as early life,
 136 may be able to invest more energy in all life-history traits. In addition to predictions made under the life-
 137 history trade-offs hypothesis, the phenotypic quality hypothesis thus posits that a positive relationship
 138 should exist between telomere length in early life and LRS, as individuals with long telomeres are assumed
 139 to successfully cope with the costs of reproduction (Sudyka, 2019). We therefore tested this by looking at
 140 the relationship between telomere length and LRS (Fig. 1B.f).



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

156 **Materials and Methods**

157 Model species, study system and site

158 We monitored a population of Eastern chipmunks in a deciduous forest of southern Québec, Canada (Mont-
159 Sutton; 45°05' N, 72°25' W), on three sites from 2013 to 2019 (Sites 1 and 2 of 7.84 ha each, and site 3 of 4
160 ha). Eastern chipmunks are omnivorous, food-hoarding Sciurid rodents that display periodic torpor events
161 in winter (Elliott, 1978; Humphries, Thomas, & Speakman, 2002). Each year, from May to September, we
162 conducted daily trapping from 8:00 a.m. until dusk, using Longworth traps (Bergeron, Réale, Humphries, &
163 Garant, 2011a). Upon capture, chipmunks were marked with ear tags and a Trovan PIT tag and we recorded
164 their body mass, sex, reproductive status and determined their age class (juveniles or adults; see
165 supplementary methods). Individuals weighing > 80 g at first capture in May or displaying reproductive signs
166 over the entire reproductive season were considered as adults. We classified as juvenile individuals
167 weighing less than 70 g at first capture and showing no signs of sexual maturity over the entire season, i.e.
168 absence of darkened scrotum for males and undeveloped mammae for females (Bergeron et al., 2011;
169 Tissier et al., 2020). Chipmunks weighing between 70 g and 80 g at first capture and that could not be
170 classified as juveniles or adults were considered as NA (Bergeron, Réale, et al., 2011a; Tissier et al., 2020).

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

188 Tissue collection and DNA extraction

189 We collected ear tissue punches upon capture per individual during their first two years of life. Since we
190 usually do not collect DNA samples on individuals older than two years, a sample was specifically collected
191 on a subsample of 3–6-year-old individuals (n=12), among which 7 individuals for which we had a measure
192 of rTL at first breeding opportunity, to investigate changes in telomere length with age (see below). Upon
193 collection, ear tissue punches were transferred to 95% ethanol Eppendorf tubes, transferred from the field
194 to laboratory conditions within 2-4 hours where they were stored at +4°C until extraction, which occurred
195 within two months after collection. Upon extraction, DNA samples were stored at -80°C. DNA extraction
196 was carried out using a modified version of a validated salting-out method (Aljanabi & Martinez, 1997; see
197 supplementary methods). Briefly, after allowing ethanol to evaporate from the tissue sample, saline
198 extraction buffer (2 mM ethylenediaminetetraacetic acid, 10 mM Tris-HCl, 0.4 M NaCl), sodium dodecyl
199 sulphate and proteinase K were added to the sample and incubated overnight at 60 °C. The following
200 morning, more proteinase K was added; the mixture was vortexed and incubated for 2 additional hours at
201 60 °C. Once digested, 300 µl of 6 M saline solution was added and the mixture was vortexed and centrifuged
202 for 30 min at 6000 g. We then transferred 600 µl of the aqueous solution into a clean 1.5-ml microtube. The

203 DNA was precipitated by a 1-h incubation at 20 °C with 600 µl isopropanol and pelleted by centrifugation
204 for 20 min at 10000 g. Pellet was rinsed with 200 µl of 70% ethanol, spun again at 10000 g for 10 min, and
205 left to air dry overnight. The next day, the pellet was resuspended using 200 µl of distilled water. After
206 having determined the DNA concentration using gel electrophoresis, samples were diluted to a
207 concentration of 5 ng/µl for polymerase chain reaction (PCR) amplification and frozen at -80°C until being
208 processed for genotyping or telomeres measurements.

209 Genotyping and parental assignment

210 We conducted microsatellite genotyping using 23 loci previously employed in the study system (Bergeron,
211 Réale, Humphries, & Garant, 2011b; Chambers & Garant, 2010; Vandal, Houle, Archambault, Réale, &
212 Garant, 2020). The detailed genotyping protocol, is described in (Chambers & Garant, 2010; Vandal et al.,
213 2020). The parentage assignment protocol is fully described in (Bergeron, Réale, et al., 2011b). Briefly, we
214 captured weaned juveniles that emerged from their known maternal burrow. We used CERVUS (Kalinowski,
215 Taper, & Marshall, 2007) to corroborate the maternal identity and assigned paternity based on
216 microsatellite analyses (Chambers and Garant 2010). When we had no information on the maternal burrow
217 (i.e. when juveniles were captured during systematic trapping on the grid), we used COLONY (Wang &
218 Santure, 2009) to reconstruct family groups (Bergeron et al., 2011b).

219 Assessment of relative telomere length (rTL) We used DNA extracted from ear tissue punches collected in
220 spring to measure relative telomere length (rTL, expressed as T/S ratio). Telomeres length is commonly
221 measured on ear tissues in rodents (Hehar & Mychasiuk, 2016; Turbill et al., 2013), and strongly correlates
222 with telomere length in other tissues, including brain tissues such as hippocampus and prefrontal cortex
223 (Hehar & Mychasiuk, 2016) or buccal mucosa (Turbill et al., 2013). In total, we obtained rTL on 204
224 chipmunks of known age, 74 at their first breeding opportunity (among which 70 for which we had life-
225 history data as detailed above), 106 at emergence from the maternal burrow, 12 individuals aged 18-24
226 months, and 12 individuals aged 3-6 years. There were 20 individuals for which we had two rTL
227 measurements in spring. At their first breeding opportunity, individuals were either 7 months or 15 months
228 of age (Montiglio et al. 2014). In both cases, individuals were captured in spring after their first winter.
229 Individuals aged 7 months were about to initiate reproduction at that time whereas individuals aged 15
230 months already initiated it. To investigate change in telomere length with age, we randomly selected 20 out
231 of 30 individuals for which we had sufficient remaining DNA to measure rTL at two ages in spring, ensuring
232 to have a representative sampling of our population (samples from different years, sexes, and ages; see
233 Table S1). We also measured rTL on juveniles sampled upon emergence from the maternal burrow (i.e.

234 weaning, ~30-45 days of age). The average recapture rate of juveniles after their first winter is 14.5% in our
235 population (9% for males and 20% for females; *unpublished data*, see also (Loew, 1999) reporting similar
236 proportions in other chipmunk populations). Combined with the exclusion of 3 individuals that were aged
237 of 1 year-old at the time of the study, this reduced our initial sample of 106 to a subset of 12 juveniles for
238 which we had data on both telomere length and lifespan.

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

258 Measurements were done on two 384-well plates. We added 10 ng (2 μl) of DNA in each well, together with
259 8 μl of GoTaq QPCR Mix (Promega, Madison, WI, USA); with an extra 100 μM final concentration of dNTPs;
260 and forward and reverse primers at 5 μM. Cycling conditions included: 1) an initial hold at 95 °C for 2 min;
261 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-VCN) of
262 95 °C for 15 sec, 60 °C for 30 sec and 72°C for 1 min, with fluorescence readings recorded at this step; and
263 3) a final melt step was set at 60 °C to 95 °C in 0.5 °C increments (see Figure S1). Cycling was performed on
264 an Automated Thermal Cycler, Q real-time PCR instrument (CFX-384, Biorad, Hercules, CA, USA). Each plate

265 contained samples ran in duplicates, a no-template control (sterile water used for dilution of samples),
266 duplicates of 4 individuals and duplicates of a control well of 10 ng of 100 pooled individuals' DNA sample
267 (i.e. repeated on each qPCR and used as the "golden sample", the reference sample with a value of T/S = 1,
268 used to calculate the values of the other points). Calculation of rTL (T/S ratio) was carried-out using the
269 software LinRegPCR (V.12.5) as described in (Pfaffl, 2001).. Efficiencies of qPCR reactions were of 99.9% for
270 the control and 99.8% for telomeres in plate 1 and of 100% for the control and 99.9% for telomeres in Plate
271 2. Intraclass correlation coefficient for intra- and inter-runs (ICC; based on 12 samples repeated over the
272 two 385-well plates; see (Eisenberg, Nettle, & Verhulst, 2020; Verhulst et al., 2016)) were of 0.976 and
273 0.992, respectively. Regarding measurements on change in rTL with age, samples were spread over the two
274 plates as follows: we measured the two rTL of a same individual on two different plates for 9 individuals
275 (three on plate 1 and six on plate 2) and on the same plate for 11 individuals (three on plate 1, eight on
276 plate 2), thus preventing that plate effect would influence the results.

277 Life-history and exploration variables

278 We considered two measures of fecundity: the average number of offspring per reproductive event as a
279 measure of fecundity rate, and the lifetime number of assigned offspring at weaning as a measure of the
280 lifetime reproductive success (LRS). We calculated the minimum lifespan in months, as the difference
281 between the date of last capture and either the date of first capture (i.e. emergence from the maternal
282 burrow) or the date of latest possible birth for the cohort (i.e. for individuals missed at emergence from the
283 burrow and only captured after their first winter). For instance, given the recorded patterns of reproduction
284 on our study sites since 2012 (Tissier et al., 2020), an unmarked adult first caught in spring 2013 would be
285 assigned to an emergence date in June 2012 (spring cohort). An individual first captured in June 2016 would
286 be assigned to an emergence date in September 2015 (summer cohort). We considered only residents for
287 this analysis and thus excluded 7 individuals based on this criterion, considering that we had data on
288 telomere length at first breeding opportunity and exploration profile for those individuals but no
289 information about their life-history. We also excluded 12 individuals that were still alive and aged one year-
290 old for the same reasons. We considered as "residents" individuals that were present on the sites at their
291 first reproductive opportunity (dispersion rarely occur afterwards; Elliott 1978) and that were caught more
292 than 5 times during their life/over several seasons (to exclude potential dispersers or individuals at the
293 periphery of the sites, for which we would not have accurate life-history data).

294 We quantified chipmunk behavioural response to a novel environment, as a measure of their exploratory
295 behaviour and their activity, by conducting open-field tests (Gharnit, Bergeron, Garant, & Réale, 2020;

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

306 Data Analyses

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

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

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

321 We investigated the links between rTL at first breeding opportunity and fecundity (response variable,
322 expressed as the total number of offspring weaned or sired /number of reproductive event) using a LM
323 (model 2), assuming the normality of the residuals, which was confirmed by a Kolmogorov-Smirnov test.
324 We investigated the relationship between rTL and LRS using a Generalized Linear Model (GLM; model 3)
325 with a Poisson distribution, after verifying the absence of overdispersion. Finally, we used a LM to
326 investigate the relationship between rTL at first breeding opportunity and minimum lifespan. We assessed

327 the normality of the residuals with a Kolmogorov-Smirnov test (LM, model 4). As normality was not met,
328 lifespan was expressed in $\text{Log}_{10}(\text{months}+1)$ (Eastwood et al., 2019). We included age at first breeding
329 opportunity (7 or 15 months), average adult body mass (linear term in models 2 and 3 and both the linear
330 and quadratic terms in model 4 to account for the quadratic relationship between body mass and lifespan
331 in our data; *unpublished results*), sex, site, rTL and the double-way interactions (rTL*sex, rTL*age at first
332 breeding opportunity and sex*age at first breeding opportunity) as fixed effects in the three models. We
333 faced issues of model convergence when including the year of birth as a random effect in two of the three
334 models. We thus included year as a fixed continuous effect in all full models. We also included the minimum
335 lifespan as fixed effect in models 2 to account for the duration of breeding opportunities. Body mass, year,
336 and exploration score (mean individual exploration BLUP) were standardized (Z-transformed) before
337 inclusion in the models.

338 *Models 5, 6 and 7: relationships between age, rTL and telomere dynamic and between rTL at weaning and* 339 *lifespan*

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

354 *Model selection and parsimony*

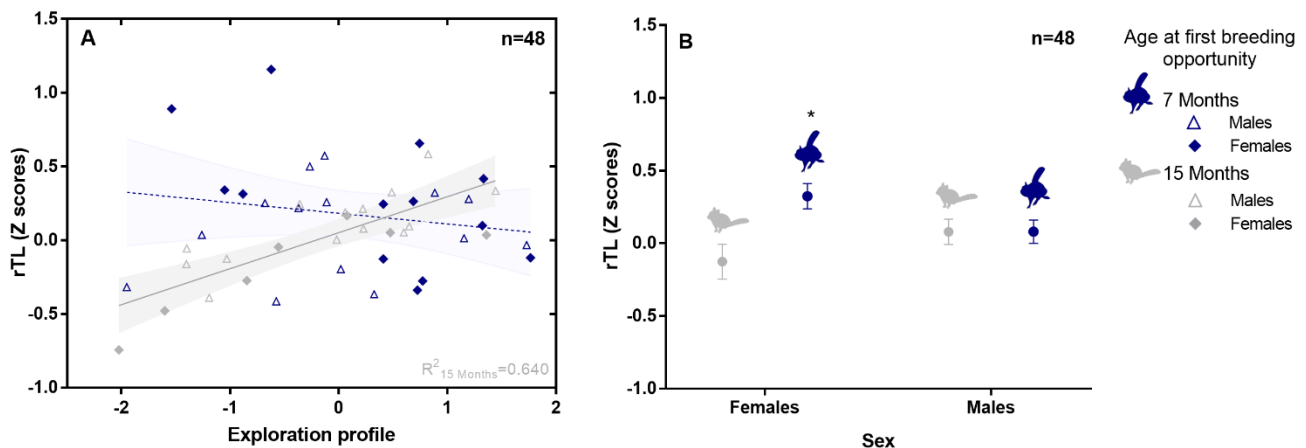
355 Starting from the full models, we removed one by one the least significant variables, only if their removal
356 reduced the AICc. Both full and reduced models are presented in the online supplementary material (Tables
357 S2-S6). We implemented false discovery rates to correct for multiple comparisons (Pike, 2011) in models 2,

358 3 and 4, using the SDM project spreadsheet available at:
359 <https://www.sdmproject.com/utilities/?show=FDR>. Analyses were conducted using SPSS software (IBM
360 SPSS Statistics for WINDOWS v. 24.0. Armonk, NY: IBM Corp.).

361 Results

362 *Correlates of telomere length at first breeding opportunity*

363 We found no effect of age on rTL at first breeding opportunity ($t=0.34$, $p=0.74$) but found a significant
364 exploration*age interaction (LM, $t=3.42$, $p=0.001$; model 1, Table S2B): rTL increased with speed of
365 exploration, but only for individuals experiencing a first breeding opportunity at 15 months of age ($R^2 = 0.64$,
366 Figure 2A, Table S2B). We also found an effect of the sex*age interaction, with females that had a first
367 breeding opportunity at 7 months of age showing longer telomeres than females having a first breeding
368 opportunity at 15 months of age (LM, $t=2.62$, $p=0.01$), whereas we found no differences in males (Figure
369 2B, Table S2B).



370 **Figure 2.** Determinants of relative Telomere Length (rTL) at first breeding opportunity. In (A) interaction
371 between exploration and age at first breeding opportunity on rTL (Table S2, n=48, sample sizes by age at
372 first breeding, 7 months: n=14 males and 13 females; 15 months: n=14 males and 7 females; sex symbols
373 are solely represented on the figures for informative purposes; no interaction with sex was found). The
374 dashed line represents a non-significant relationship in individuals aged 7 months. In (B) the interaction
375 between sex and age at first breeding opportunity on rTL is shown (means \pm SE; Table S2). Females whose
376 first breeding opportunity occurred at 7 months old had significantly longer telomeres than females with a
377 first breeding opportunity at 15 months old, whereas no significant difference was observed in males. The

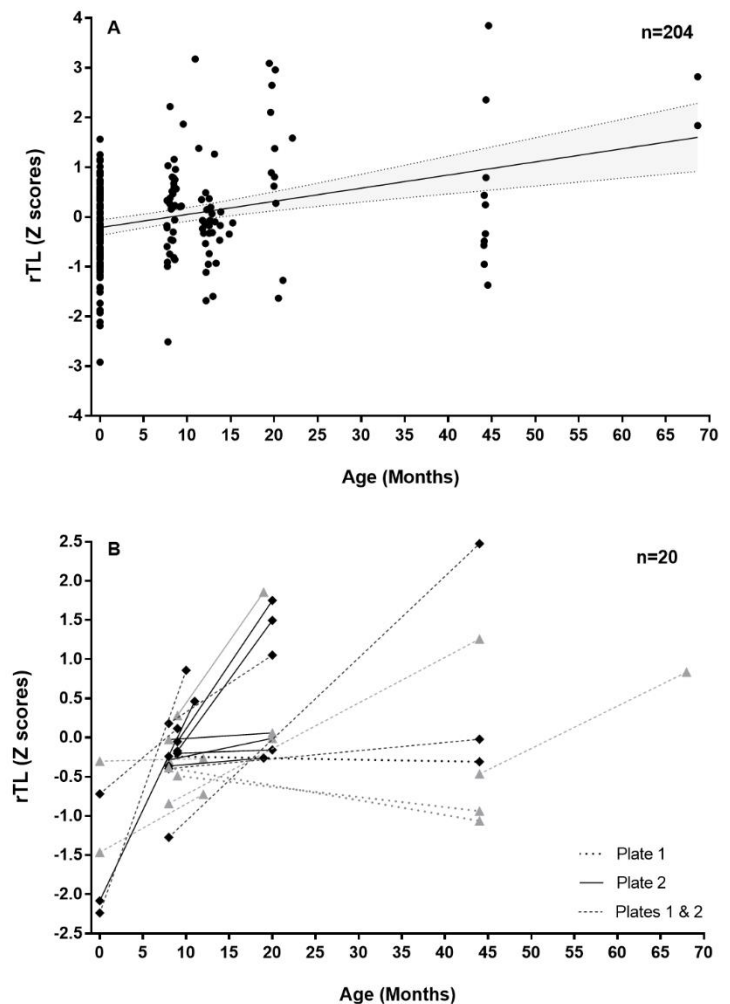
378 rTL, expressed as T/S ratio, has been LN transformed before conducting the analyses. Z-scores are presented
379 for exploration and rTL.

380 *Change in telomere length with age*

381 We found a within-individual repeatability in rTL of $R = 0.126$ for all 20 chipmunks for which we had to
382 measures of rTL. Repeatability for the subset of individuals sampled at <12 months was $R = 0.378$. We found
383 a positive relationship between age and rTL at the population level (model 5, LMM, $n=204$, $F_{1,190}=37.57$, $t=6.13$,
384 $p<0.001$, Figure 3A), but no effect of the sex ($F_{2,169.6}=1.26$, $p=0.71$), cohort of birth ($F_{2,28.5}=0.56$, $p=0.85$) or their
385 two-way interaction ($F_{3,169.7}=1.00$, $p=0.32$; Table S3A).

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

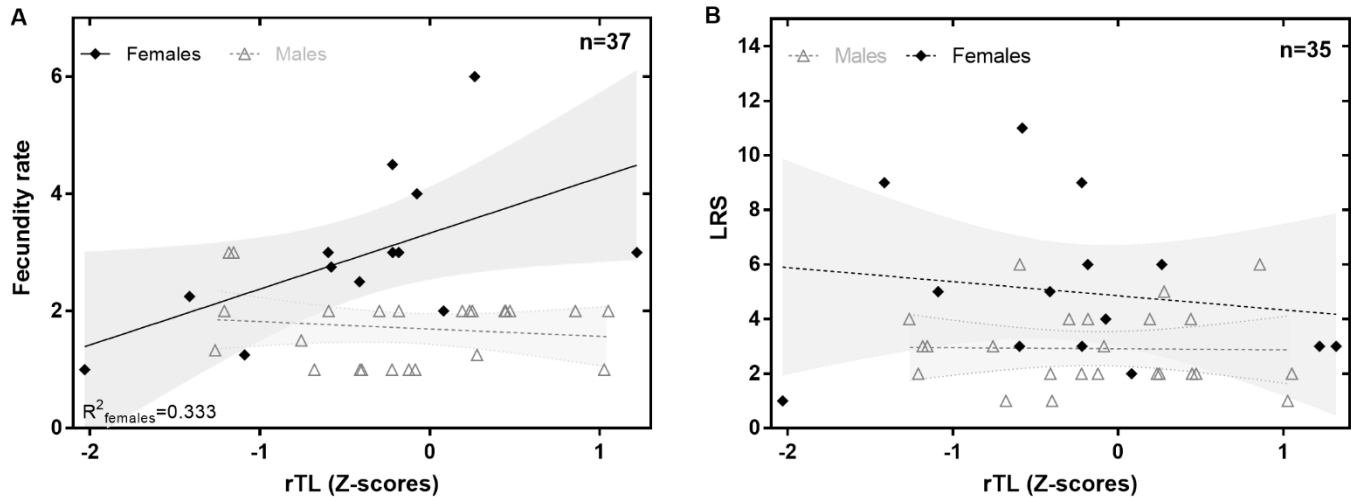
390 Figure 3. Relationships between relative telomere
 391 length (Z scores of LN(T/S ratio)) and age (months since
 392 the emergence from the maternal burrow) in Eastern
 393 chipmunks. (A) Relative telomere length in spring
 394 according to age at the population level (n=204). A
 395 positive relationship between age and rTL was
 396 recorded ($R^2=0.12$, $t=6.18$, $p<0.001$, Table S3A), with an
 397 appearance of long telomeres (i.e. with a T/S ratio >
 398 2.70) in individuals of >7 months of age (these long
 399 telomeres were not recorded in juveniles sampled at
 400 emergence from the maternal burrow, 0 months,
 401 n=106). (B) Repeated measurements of relative
 402 telomere length in individual chipmunks sampled twice
 403 in their life in spring. Telomeres increased significantly
 404 with age ($t=3.98$, $p<0.001$, Table S3B). Data are
 405 displayed for females (diamonds and black lines) and
 406 males (triangles and grey lines) as informative purposes
 407 (we found no significant difference between the sexes,
 408 Table S3B); plates are also represented by dotted,
 409 dashed and solid lines to highlight that elongation was
 410 not caused by methodological artefacts.



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

412 We found no effect of age or body mass on fecundity rate, expressed as the average number of offspring per
 413 reproductive event (model 2, $p>0.05$; Table S4). However, we found that the sex*rTL interaction had a
 414 significant effect on this trait (model 2, $t=2.75$, FDR-adjusted p-value = 0.02; Table S4): fecundity rate positively
 415 covaried with rTL at first breeding opportunity in females, but not in males (Figure 4A, $R^2_{\text{females}}=0.33$, $p=0.001$).
 416 Regarding LRS, however, we found no significant link between rTL at first breeding opportunity and LRS, nor
 417 of the sex*rTL interaction, that were both excluded from the final selected model (model 3, Figure 4B &
 418 Table S5). Our model, however, revealed a positive and significant correlation between lifespan and LRS

419 ($\chi^2=4.50$, $p=0.03$; Table S5). No effects of the cohort of birth (spring or summer) were found on either the
 420 fecundity rate or the LRS ($p>0.05$, Table S4 & Table S5).



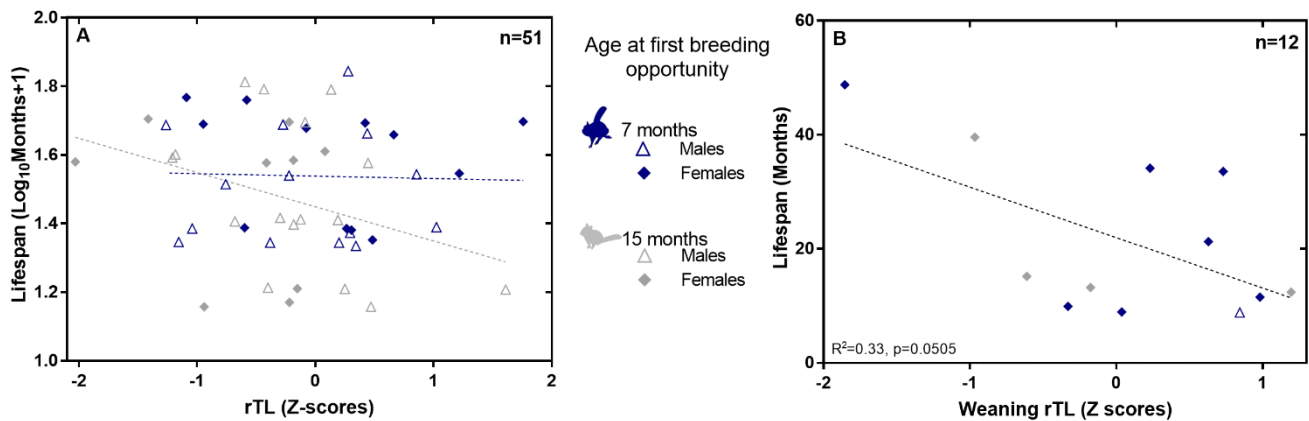
421 **Figure 4. Relationship between relative Telomere length (rTL) at first breeding opportunity and (A) fecundity rate**
 422 **(average number of offspring per reproductive event) or (B) LRS, in males (triangles) and in females (diamonds).**
 423 We found a sex*rTL interaction effect on fecundity rate (Table S4) but no effect of rTL or the interaction on
 424 LRS (Table S5). The full line represents a significant relationship between rTL and fecundity rate in females (A;
 425 Table S4), whereas the dashed lines represent no significant relationships.

426

427 Regarding lifespan, we found no significant effect of rTL nor of the age at first breeding opportunity*rTL
 428 interaction (model 4, $t=0.63$ & $t=1.43$, FDR-adjusted p -values = 0.60 and $p= 0.24$, respectively; Table S6). We
 429 only observed a significant effect of the year of birth on lifespan ($t=3.07$, FDR-adjusted $p = 0.02$; Table S6). A
 430 supplementary analysis on a subset of 12 juveniles revealed that telomere length at the emergence from the
 431 maternal burrow (i.e. weaning) also tended to be negatively associated with longevity (model 7, $R^2=0.33$,
 432 $p=0.05$; Figure 5B).

433

434



435 **Figure 5. Relationship between relative telomere length (rTL) and lifespan.** In (A) rTL of adults according to their
 436 age at their first breeding opportunity is represented with lifespan expressed in $\text{Log}_{10}(\text{Months}+1)$. Individuals
 437 with a first breeding opportunity at 7 months old (fast) are shown in dark blue, whereas individuals first mating
 438 at 15 months old (slow) are shown in light grey; both males (triangles) and females (diamonds) are represented
 439 for informative purposes. We found no effects of rTL on lifespan and no effects of sex or age at first breeding
 440 opportunity on the relationship between rTL and lifespan (Table S6). In (B) early life rTL, measured at
 441 emergence from the maternal burrow, is shown with lifespan expressed in months. The dashed line represents
 442 a non-significant linear regression. The rTL, expressed as the T/S ratio, is presented as Z scores of LN
 443 transformed data.

444 Discussion

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

454 *Correlates of telomere length at first breeding opportunity*

455 We found that fast and superficial explorers, reported to have a higher fecundity rate than slow and thorough
456 explorers in this population (Montiglio et al., 2014), also had longer telomeres, but solely in late breeders (i.e.
457 individuals whose first breeding opportunity occurred at 15 months old). This result confirms predictions
458 derived from the anti-cancer hypothesis. It echoes findings from a previous study on wild chipmunks,
459 highlighting that fast explorers showed lower daily energy expenditure levels than did slow explorers, contrary
460 to predictions made under the resource-allocation trade-offs hypothesis (Careau et al., 2015). The absence of
461 relationship between telomere length and exploration profile in early breeders in our study could be explained
462 by a greater variation in their exploration profile or telomere length compared to late breeders, as already
463 reported for exploration in this species (Montiglio et al., 2014). This greater variation and the absence of a
464 significant relationship with telomere length could represent different behavioural/life-history strategies that
465 could be genetically fixed in late breeders compared to early ones. Alternatively, it could reflect a greater
466 plasticity of early breeders compared to late ones. Early-breeding female chipmunks (7 months of age) were
467 shown to be faster explorers than late-breeding ones in the studied population (Montiglio et al., 2014). Here,
468 we show that these early-breeding females have longer telomeres than late-breeding females, as predicted
469 by the anti-cancer hypothesis. Because telomeres elongated with age in this population, this result cannot be
470 explained by a simple age difference between females of the two groups (i.e. with older females having shorter
471 telomeres because of a greater erosion with age). This result suggests that, for the same age, females with a
472 first breeding opportunity before 15 months of age had longer telomeres than females with a first breeding
473 opportunity at 15 months of age. However, given the intrinsic link between cohort of birth and age at first
474 breeding opportunity in this system, early breeding and late-breeding females would never have the same age
475 at the same period of their life cycle (Bergeron et al. 2011, Montiglio et al. 2014, Tissier et al. 2020). Comparing
476 their telomere length at the same age would thus be biased by life-cycle stage and season, and therefore by
477 varying intrinsic and environmental factors that could have an influence on their telomeres. Together, our
478 findings support the idea that rTL early in life positively covaries, in a cohort- and sex-specific manner, with
479 speed of exploration and age at first breeding opportunity, two metrics commonly used to map individuals
480 along the slow-fast continuum of the POLS (Araya-Ajoy et al., 2018; Réale et al., 2010). These results also
481 suggest that resource allocation trade-offs alone are unlikely to drive the evolution of life-histories in this
482 system and that other forces, such as cancer risks, are at play (see (Cohen, Coste, Li, Bourg, & Pavard, 2020;
483 Kokko & Hochberg, 2015; Lemaître et al., 2020; Pepke & Eisenberg, 2021)).

484 *Telomere elongation with age*

485 Following the anti-cancer hypothesis, telomeres were not predicted to shorten with age, but were not
486 expected to significantly elongate at the within individual and population levels (Figure 3). Most studies
487 conducted so far found that telomeres do shorten with age in humans, captive-bred animals and wild, long-
488 lived vertebrates (Bichet et al., 2019; Bize et al., 2009; Eastwood et al., 2019; Heidinger et al., 2012; Monaghan
489 & Hausmann, 2006; Sudyka, 2019). This usually translates into shorter telomeres being measured in older
490 than in younger individuals at the population level (Hausmann et al., 2003; Monaghan & Hausmann, 2006;
491 Pauliny et al., 2006). Here, we found the reversed patterns, with a positive relationship between age and rTL
492 (Figure 3A) and a within individual elongation of telomeres with age (Figure 3B). This positive relationship
493 cannot solely be explained by a selective disappearance of individuals with short telomeres (Dantzer &
494 Fletcher, 2015), as an appearance of long telomeres (with a T/S ratio >2.70, which is above the maximum
495 recorded in the 106 juveniles sampled) seem to appear with age in this population (Figure 3A). Within
496 individual repeatability in telomere length was $R=0.128$ (whole sample) and $R=0.378$ (subset of individuals
497 sampled at <12 months apart), which is equivalent or above repeatability recorded in other mammal studies
498 using qPCR essays (Fairlie et al., 2016; van Lieshout et al., 2019). However, repeatability within individuals is
499 based on several hypotheses: 1) telomere length on year n is predictive of what will happen in year $n+1$; 2)
500 telomere length shorten with age and 3) the rate of shortening is similar between individuals of a same cohort.
501 In our case, however, these hypotheses are not met, especially considering that we did not record a no
502 reduction in telomere length with age but a lengthening.

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

515 small wild mammals because it requires high concentrations of DNA difficult to obtain with least-invasive
516 approaches (Dantzer et al., 2020; Nussey et al., 2014; Smith et al., 2011).

517 Few other studies also found a positive relationship between age and telomere length (Hausmann et al.,
518 2003; Hoelzl, Smith, et al., 2016). In edible dormice (*Glis glis*), young individuals significantly shorten their
519 telomeres with age while old individuals (>6 years old) slightly elongate their telomeres when approaching the
520 end of their life (Hoelzl, Smith, et al., 2016). This pattern has been attributed to the peculiar life-history strategy
521 of this species, that may skip reproduction and hibernate for up to almost a year (Hoelzl, Cornils, Smith,
522 Moodley, & Ruf, 2016; Hoelzl, Smith, et al., 2016). Here, we found that telomere elongation occurred in
523 chipmunks of all ages. A within-individual seasonal elongation of telomeres has also been recorded in few
524 rodent species (Criscuolo et al., 2020; Hoelzl, Cornils, et al., 2016). In our study, telomere length was always
525 measured in spring. We can, thus, exclude a potential seasonal effect on the elongation pattern observed,
526 which was recorded both in young and in old individuals. However, we cannot exclude that an environmental
527 effect – e.g. pulsed availability of food resources that have a strong impact on behaviour, reproduction and
528 life-history strategies in this system (Bergeron, Réale, Humphries, & Garant, 2011a; Montiglio et al., 2014;
529 Tissier, Réale, Garant, & Bergeron, 2020) – may partially explain this elongation (Hoelzl, Cornils, et al., 2016).
530 Nonetheless, the observation of telomere elongation with age under controlled conditions with unlimited food
531 resources and using the Q-FISH procedure (see Panasiak et al., 2020), highlights that food alone unlikely
532 explains these patterns of telomere elongation.

533 Our results, combined with recent literature on telomere dynamics in small (Criscuolo et al., 2020; Foley et al.,
534 2018; Hoelzl, Smith, et al., 2016) and large mammals (Kirby, Johnson, Alldredge, & Pauli, 2019; Lemaître et al.,
535 2021), as well as in fishes (Panasiak et al., 2020) and birds (Spurgin et al., 2018), pinpoint that a much greater
536 variety of telomere dynamics patterns may exist than previously reported, including within-individual telomere
537 elongation, and that these patterns likely correlate with pace of life. Proximal mechanisms explaining telomere
538 elongation in chipmunks will have to be investigated in the future, but we can hypothesize that telomerase
539 activity is involved (S. Smith, Hoelzl, Zahn, & Criscuolo, 2021). Telomerase is the enzyme that repairs telomeres
540 (Chan & Blackburn, 2004). It is repressed after growth in the somatic tissues of large and long-lived
541 vertebrates, but not in the somatic tissues of small and short-lived ones (Chan & Blackburn, 2004; Tian et al.,
542 2018). Among 18 rodent species, Eastern chipmunks show the highest body-mass specific telomerase activity
543 in their somatic tissues (Seluanov et al., 2007; Tian et al., 2018). We initially expected that this high activity of
544 telomerase would prevent telomere erosion with age. However, it could in fact be involved in the significant

545 and consistent telomere elongation that we recorded at the within individual and population levels, as
546 observed, for instance, in sperm of large mammals, including humans, where telomerase is maintained active
547 (Kimura et al., 2008; Kozik, Bradbury, & Zalensky, 1998; Ozturk, 2015; Tian et al., 2018). However, the qPCR
548 assay did not allow us to assess the strength of this elongation, nor to compare the absolute length of
549 telomeres recorded in this population with other studies in rodents, which could be done using the TRF or Q-
550 FISH approaches (Lai, Wright, & Shay, 2018; Nussey et al., 2014). Future studies could tackle those issues by
551 optimizing DNA extraction from buccal swabs, which offers a promising avenue to obtain sufficient DNA in
552 small mammals with a non-invasive technique (Hoelzl, Cornils, Smith, Moodley, & Ruf, 2016; Lai et al., 2018),
553 while assessing whether telomerase, beyond its impact on telomere length or dynamics, acts as a mediator of
554 life-history strategies (Crisuolo, Smith, Zahn, Heidinger, & Hausmann, 2018; S. Smith et al., 2021).

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

556 Fecundity rates positively co-varied with telomere length, as predicted by the anti-cancer hypothesis, but did
557 so in a sex-specific manner. This absence of relationship between rTL and fecundity rate in males is not
558 surprising, considering the differences of parental investment between sexes. In chipmunks, males engage in
559 scrambling competitions for mates but do not provide parental care (Bergeron, Réale, Humphries, & Garant,
560 2011b), whilst females bear the costs of gestation and lactation, which are high in small mammals (Speakman,
561 2008). Previous studies revealed sex-differences in stress reactivity and behaviour, likely associated to
562 differential investment into reproduction (Montiglio et al., 2014; Montiglio et al., 2015). The sex-specific
563 relationship observed could reflect the sexually discordant selection on telomeres reported in other vertebrate
564 species, through sex-specific telomere dynamics or sex-specific relationships between telomere length and
565 life-history traits (Barrett & Richardson, 2011; Bauch et al., 2020; Brown et al., 2021; Heidinger et al., 2021),
566 or a well-reported sex-specific differences in POLS (Tarka, Guenther, Niemelä, Nakagawa, & Noble, 2018).
567 Since fecundity rate is considered as one of the best metrics of individual pace-of-life (Araya-Ajoy et al., 2018),
568 this positive relationship between rTL and fecundity rate in females, independently of their cohort of birth,
569 provides another support for the anti-cancer hypothesis. Finally, we found no link between early life telomere
570 length and LRS, neither in males nor in females, as expected under the “phenotypic quality” hypothesis
571 (Sudyka, 2019).

572 We failed to find any significant relationship between lifespan and rTL in Eastern chipmunks. An absence of
573 correlation between telomere length and lifespan has previously been reported at the between-species level

574 in rodents (Seluanov et al., 2007). Nonetheless, the supplementary analysis on a subset of 12 juveniles shows
575 a trend for a negative correlation between telomere length and lifespan . It also highlights that the two
576 juveniles with the shortest telomeres at weaning displayed an above-average lifespan of more than 40 months,
577 i.e. the average lifespan is of ~30 months in this population (Bergeron, Réale, et al., 2011a). Although
578 anecdotal, this observation goes against the idea of a selective disappearance of individuals with short
579 telomeres, supported by our results on telomere elongation with age. Lifespan, commonly linked to telomere
580 length in birds and humans (Bichet et al., 2019; Eastwood et al., 2019; Heidinger et al., 2012; Sahin & Depinho,
581 2010), is commonly used as a metric of the POLS (Giraudeau et al., 2019; Ricklefs & Wikelski, 2002). However,
582 it could be a less valuable metric than fecundity rate to assess the pace of life of species with low survival
583 probabilities such as chipmunks, as it displays less inter-individual variation compared to fecundity rate and
584 generation time (Araya-Ajoy et al., 2018). This could potentially explain why we found no significant
585 relationship in adults and solely a trend in juveniles, together with a lack of statistical power. Consequently,
586 we found no support for a positive relationship between rTL and lifespan or LRS, as expected under the
587 phenotypic quality hypothesis. We argue that the concept of phenotypic “quality” should be used and
588 interpreted with caution, especially when fitness is used to both define and assess quality (Bergeron, Baeta,
589 Pelletier, Réale, & Garant, 2011).

590 **Conclusion**

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

602

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612 (protocol number: DG2015–01-Université de Sherbrooke), Bishop’s University (protocole number: PB2015-02
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616 conceived the theoretical framework of this study. S.Z. and F.C. designed the qPCR measurements, which were
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624

625 **Supplementary material**

626 Tables S1 to S6

627 Figures S1 and S2

628 Captions for external databases S1-S3

629 External databases S1-S3

630 **References**

631

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