Telomere length in relation to colour polymorphism across life stages in the tawny owl

Chiara Morosinotto[1,2], Staffan Bensch[2] and Patrik Karell[1,2]

[1]Bioeconomy Research Team, Novia University of Applied Sciences, Raseborgsvägen 9, FI-10600 Raseborg

[2]Department of Biology, Lund University, Sölvegatan 39 (Ecology Building), SE-223 62 Lund

Corresponding author: Chiara Morosinotto, Bioeconomy Research Team, Novia University of Applied Sciences, Raseborgsvägen 9, FI-10600 Raseborg. E-mail: chiara.morosinotto@novia.fi

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Abstract

Telomere erosion has been proposed to be tightly associated with senescence, environmental stressors and life history trade-offs. How telomere dynamics vary across life stages and especially in relation to (heritable) phenotypic traits is still unclear. The tawny owl (*Strix aluco*) display a highly heritable melanin-based colour polymorphism, a grey and a brown morph, linked to several fitness traits including morph-specific telomere dynamics. As adults, brown tawny owls have shorter relative telomere length (RTL) and exhibit faster telomere shortening rate than grey owls. Here we test if these morph-specific telomere dynamics emerge already during growth, or if they are induced only in adult life through differential physiological costs associated with the life history of the morphs. We analysed RTL from 287 tawny owl offspring and 81 first breeding adults to evaluate at what life stage morph-specific patterns emerge. We found no differences in RTL between the two morphs during the nestling period nor at the first breeding attempt. Sex, brood size or size rank in the nest did not affect offspring RTL. Among first-breeders, females had shorter telomeres than males suggesting a sampling-time dependent difference in reproductive costs between sexes, due to the prominent sex roles in tawny owls in the early nestling period. The probability to return to breed after the first breeding attempt was not affected by RTL, sex or colour morph. The lack of morph-specific difference in RTL among nestlings and first breeders suggests that previously observed morph-specific differences in RTL dynamics in adults emerge at the onset of the breeding career and is likely due to different physiological profiles and life-history strategies adopted by adults. We conclude that different telomere dynamics and senescence patterns among highly heritable phenotypes (colour morphs) are likely to be a result of differential costs of reproduction and self-maintenance.

Keywords:

telomere, colour polymorphism, life-history strategies, melanism, pace-of-life, development

Introduction

Species-specific life history traits in wild vertebrates evolved in response to several ecological factors affecting animal fitness, from survival to reproduction. Telomeres have been suggested to be molecular proxies of the physiology underlying life-history trade-offs and selection processes (Haussmann et al. 2005, Monaghan and Haussmann 2006, Haussmann and Marchetto 2010, Monaghan et al. 2010, Angelier et al. 2018). Telomeres are repetitive non-coding sequences present at the end of the chromosomes of eukaryotes (Monaghan and Haussmann 2006, Monaghan 2014). In wild birds, short telomere length and fast telomere shortening have been associated to lower survival and shorter lifespan (Haussmann et al. 2005, Bize et al. 2009, Salomons et al. 2009, Angelier et al. 2013, Boonekamp et al. 2014, Asghar et al. 2015a, Wilbourn et al. 2018). Telomeres are known to shorten with age and can be susceptible to oxidative stress (Haussmann and Marchetto 2010, Angelier et al. 2018, but see Reichert and Stier 2017) and could be used as biomarker not only of aging but also of adverse conditions during different life stages (reviewed in Angelier et al. 2018, Chatelain et al. 2020). Telomere lengths have thus been suggested to mirror individual quality (reproductive success and survival: e.g. Wilbourn et al. 2018, Angelier et al. 2019) as well as parental quality (via parental care, Viblanc et al. 2020).

In birds, an individual's telomere length and rate of shortening is determined by both intrinsic and extrinsic factors throughout its life. Telomere length is heritable (Asghar et al. 2015b, Reichert et al. 2015) but is also strongly affected by maternal and epigenetic effects, as well as environmental factors perceived during early life (Dugdale et al. 2018, Bauch et al. 2019). During its life the individual will face several life stages, from development and growth, in early life, to breeding and survival in adverse or favourable conditions during adult life, which will have specific physiological costs and may affect

telomere dynamics (Chatelain et al. 2020). During early life, most wild vertebrates undergo quick telomere shortening (Angelier et al. 2018) when the metabolic modification and oxidative stress associated with growth take place (Haussmann and Marchetto 2010, Geiger et al. 2012, Smith et al. 2016), and which can be accentuated for example by sibling competition (Boonekamp et al. 2014, Nettle et al. 2015). During adult life, intraspecific difference in telomere length may result from the effect of several stressors (Chatelain et al. 2020): from differential reproductive investment (Bauch et al. 2013, Sudyka et al. 2014, Bauch et al. 2016) and diseases (Asghar et al. 2015a, Karell et al. 2017) to environmental conditions (Angelier et al. 2013, Angelier et al. 2018). For example, parents of experimentally enlarged broods had faster telomere shortening than parents with unmanipulated or reduced broods (Sudyka et al. 2014, but see Sudyka et al. 2016) and these costs of reproduction could persist up to a year after the breeding event (Reichert et al. 2014).

Telomere dynamics may also vary between individuals due to variation in melanin-based colouration (Costanzo et al. 2017, Karell et al. 2017, Parolini et al. 2017). Such associations between melanism and physiology has been proposed to result from pleiotropic effects of the genes involved in the expression of colour that give rise to different physiological profiles (Ducrest et al. 2008). The link between melanogenesis and telomeres is still unclear, but could be an indirect consequence of pleiotropic effects within the melanocortin systems (Ducrest et al. 2008), affecting for example stress response and thus indirectly telomeres. Alternatively, melanin coloration and telomere dynamics could also be linked via the interactions between the enzyme telomerase and those involved in the melanin biosynthesis (Bagheri et al. 2006, Costanzo et al. 2017), or via an overproduction of reactive oxygen species (ROS) during melanin biosynthesis (Galvan and Solano 2015, Galvan and Solano 2016). An increase in ROS production

and oxidative stress could ultimately lead to telomere shortening (Geiger et al. 2012, Stier et al. 2015, review in Angelier et al. 2018) although a direct link between stress and telomere erosion is still not clear *in vivo* (Boonekamp et al. 2017, Reichert and Stier 2017).

In the tawny owl (*Strix aluco*) plumage colouration varies depending on the level of pheomelanin in the feathers. Colour spectrum follow a bimodal distribution, meaning that individuals can be categorised as grey or reddish-brown (hereafter "brown morph"; Brommer et al. 2005). Colour morphs are genetically determined and highly heritable (Gasparini et al. 2009, Karell et al. 2011a, Morosinotto et al. 2020). Brown tawny owls have overall shorter relative telomere length and faster telomere shortening across breeding events than individuals of the grey morph (Karell et al. 2017). Interesting, such morph-specific telomere dynamics is in line with the morph-specific lifespan observed in this species. Indeed, at northern latitudes, adults of the grey morph have a longer breeding lifespan resulting in a higher lifetime offspring production (Brommer et al. 2005). Grey individuals also survive better to harsh, cold and snow-rich winters, whereas in mild winters the morphs survive equally well (Karell et al. 2011a).

It is however unclear whether the morph-specific telomere dynamics observed in tawny owls (Karell et al. 2017) are due to intrinsic physiological differences between morphs, or whether it is mediated through differential costs of reproduction and self-maintenance due to morph-specific life-history strategies (Emaresi et al. 2014). Comparing morph-specific telomere length and dynamics at different life stages appears a relevant way to gain insight on these mechanisms. If the telomere dynamics observed are due to either direct melanin production or morph-specific differences in metabolism and growth, we expect that brown nestlings will exhibit consistently shorter telomeres than grey individuals. Morph-specific growth patterns (Piault et al. 2009, Morosinotto et

al. 2020) would support this hypothesis. On the other hand, if the shorter telomeres observed in brown adults are a direct consequence of morph-specific differential reproductive effort and / or somatic maintenance in harsh winter conditions (as suggested in Karell et al. 2017 and Grunst et al. 2019), we would expect to find no morph-specific differences in telomere length of offspring (at any age).

To fill in this gap, we made a cross-sectional comparison of relative telomere length (hereafter RTL) between tawny owls colour morphs in Finland at three life stages: young nestlings, fledglings and first-breeders (i.e. young adults that have overcome the juvenile stage and have survived one, or few, winter but had never bred before). Comparing the rate of telomere shortening between grey and brown nestlings allow us to also distinguish between patterns present at birth with those potentially caused by the rapid skeletal and physiological development throughout nestling period, since in this species offspring grow from a few grams at hatching to almost half a kilo before fledging. On the other hand, investigating telomere length in first breeders allows us to separate between the impact of morph-specific life-history strategies during reproduction, i.e. that would then accumulate and be evident with increasing number of breeding events, from post-fledging maintenance costs. Indeed, if telomere dynamics are induced mostly by the conditions perceived during the early post-fledging phase (for example due to morph-specific costs in foraging or somatic maintenance during winter), then we would expect a morph-specific difference at first-breeder stage but not among nestlings. Finally, to test whether telomere length among adults of the species is indicative of condition, we tested if first breeders with shorter relative telomere length would have lower probability to return to breed the following season than individuals with longer telomeres.

Materials and methods

Tawny owl blood samples were collected between 2009 and 2019 from a well-established population with approximately 200 nest-boxes spread in a *ca.* 500 km^2 in western Uusimaa, Southern Finland (60˚15' N, 24˚ 15' E). In early April, all the nest-boxes were checked to detect breeding attempts and collect information on clutch size, brood size and hatching date. Both parents were trapped at the nest few days after hatching; they were aged, measured and ringed to allow individual identification (Karell et al. 2009) and a blood sample was collected from the brachial vein. Plumage colour was scored in facial disc, breast, back and as overall appearance, resulting in a total score between 4 to 14 points. This continuous colour score displays a bimodal curve distribution and individuals can thus be categorised to either grey or brown morph (see detailed methods in Brommer et al. 2005). Adults that were unringed when first trapped, or that had been ringed as nestling but had not bred before in the study area, were considered as "first-breeders". Depending on the prevailing food conditions tawny owls can start their breeding career as one-, two- or over three-year-olds (Karell et al. 2009), which are also the age classes that can be identified based on their partial moult (Karell et al. 2013). All unringed birds were considered as immigrants to the population because an extensive search for natural nests was consistently performed in the study area (see methods in Karell et al. 2009). All offspring were ringed when they were approximately 25 days old (hereafter "fledglings", n=287; mean ± stdev 25.74 ± 3.27 days, min-max: 14-32 days due to substantial hatching asynchrony within broods); age was estimated through a growth curve calculated from wing length. At ringing, all fledglings were weighed and a blood sample was collected from the brachial vein using heparinised capillary tubes. At this age colour was also scored as either grey or brown morph based on characteristics in the plumage: brown fledglings have clear

development of reddish colour in facial disc, tertiaries and an overall reddish-brown appearance (Morosinotto et al. 2020). In 2019, nestlings were measured (wing and mass) also at *ca*. 12 days of age (n=57; mean ± stdev 11.75 ± 3.14 days, min-max: 5-17 days; age estimated based on their wing length, hereafter "young nestlings"), and a blood sample was collected. All offspring in 2019 were marked with a non-toxic marker for individual recognition to allow to measure growth and telomere shortening between the two blood samplings (mean ± stdev 13.00 ± 1.85 days, min-max: 10-16 days between measurements, see below).

Laboratory analyses

Blood samples were preserved in ethanol (2009, 2011, 2014, 2016-2018) or SET buffer (2019) and then kept at -20°C, or as red blood cells directly in -80°C (2013, 2015). There were no samples collected for this analysis in 2010 and 2012, whereas in 2015 only samples from first breeders were collected. During spring 2019, DNA was extracted from all samples using ammonium acetate (NH_4Ac), following a modification of the protocol by (Nicholls et al. 2000). From samples kept in ethanol, or directly frozen, a small piece of dried blood, or few µl of blood, were collected and placed in a tube with 125 µl of SET buffer. For samples already in SET buffer, 125 µl were selected. To all the samples, 3.5 µl of 20% SDS and 2.5 µl of 20mg/ml Proteinase K were added and then digested overnight at 56°C. 125 µl of NH_4Ac were then added and incubated for 60 minutes at room temperature. The samples were centrifuged for 15 minutes at 13000 rpm, the supernatant was collected and 500 µl of ice cold 95% ethanol was added to it. The samples were centrifuged again for 15 minutes at the 13000 rpm and 250 µl of ice cold 70% ethanol was added to the pellet and quickly removed. The samples were left overnight to air dry, 50 µl of TE buffer was then added and the

samples were left for a few days at 4°C to dissolve the pellet until DNA concentration and purity was measured with Nanodrop (see Table 1 in Appendix).

Molecular sexing

All the offspring samples were sexed using PCR-based methods (using a modified protocol from Kekkonen et al. 2008) using two primers (2550F 5'-GTTACTGATTCGTCTACGAGA-3' and 2718R 5'-ATTGAAATGATCCAGTGCTTG-3') specific to the CHD gene (Griffiths et al. 1998). Briefly, the PCR mix included 1 µl of DNA at a concentration of ~25 ng/µl, 0.1 µl AmpliTaq ® DNA Polymerase (5U/µl), 2.5 µl PCR Buffer, 1.5 µl 25 mM $MgCl_2$ (all: Applied Biosystems, Foster City, CA), 2.5 µl 1 mM dNTPs (Thermo Fisher Scientific), 1 µl of each primer (10 uM), 3 µl Bovine Serum Albumin (1.00 mg/ml; Invitrogen, Carlsbad, USA) and then ddH_2O to reach a total volume of 25 µl. The analysis was performed in a GeneAMP® PCR System 9700 thermal cycler (Applied Biosystems) using a modified protocol from Fridolfsson and Ellegren (1999): 94°C for 2 min, 10 cycles at 94°C for 30 s, 30 s at 60°C lowered one-half degree per cycle, and 1 min at 72°C, followed by 30 more cycles with a constant annealing temperature of 50°C, and a final 10 min extension at 72°C. The PCR products were then separated by gel electrophoresis on 2% agarose gels.

Relative telomere length

The qPCR protocol for telomere length estimation was modified from Karell et al. 2017. For each sample, we ran two different real-time PCR plates due to the different annealing temperature of primers: one with primers for an ultra-conserved single-copy nuclear non-coding sequence (*sfsr*/3Fb 5'-ACTAGCCCTTTCAGCGTCATGT-3' and *sfsr*/3Rb 5'-CATGCTCGGGAACCAAAGG-3'; hereafter "control gene"; Asghar et al. 2011, Karell et al. 2017) and one with primers for telomeres (*Tel*1b 50 -

CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT-3' and *Tel*2b 5'-GGCTTGCCTTACCCTT ACCCTTACCCTTACCCTTACCCT-3'; Criscuolo et al. 2009, Karell et al. 2017). Samples were incubated at 50°C for 2 minutes and at 95°C for 10 minutes in all the plates. Followed by 40 thermal cycles for *sfsr* primer (95°C for 15s, 58°C for 45s and at 72°C for 45s) and 30 cycles for *Tel* primers (95°C for 15s, 56°C for 30s and 72°C for 30s). Both thermal protocols ended with a melt curve from 95°C reducing of 0.5°C per min to exclude the presence of primer dimers. The qPCR used was C1000 Touch Termal Cycler with CFX96 Real Time system (BioRad). Each well of the qPCR plate contained 25 µl composed of 5 µl of DNA (2ng μl^{-1}), 12.5 µl of Platinum SYBR-green qPCR Supermix-UDG (Invitrogen), 0.1 µl of ROX, the primers (0.3 µl at 10 µM for each *Tel* primer and 1 µl at 10 µM for each *sfsr* primer respectively) and ddH_2O. Each 96-wells plates included samples, two negative controls, serially diluted standards (i.e. a randomly chosen tawny owl sample diluted 2x with ddH_2O from 8 to 0.25 ng μl^{-1}) and an "interplate control", i.e. a reference sample (2ng μl^{-1}) included in all the plates to control for interplate variability; all were loaded in duplicates. All the samples were loaded randomly in the plates, whereas the standards were loaded in the same well positions. The repeatability of Ct values of intraplate sample duplicates was high for both primers (Ct for *sfsr*: R± SE 0.993 ± 0.001, confidence intervals 0.992- 0.993; P=<.001; Ct for *Tel*: R± SE 0.986 ± 0.001; confidence intervals 0.983- 0.988; P=<.001) and was measured using "rptr" R package (Stoffel et al., 2017). qPCR plates were discarded and re-run if the standard curves were outside the 100 ± 15% qPCR efficiency range (mean efficiency ± st dev: SFSR 95.2 ± 4.8, TEL 93.6 ± 3.6). The efficiency was calculated on the regression based on all data points (whole curve fit) rather than on a single threshold, allowing the efficiency

calculation to be only in points with good fluorescence reading (Zhao and Fernald 2005).

We calculated a relative telomere length (T/S ratio; hereafter RTL) following the method described by Criscuolo et al. (2009) and adjusted for intra and interplate assay variability using the interplate control that was present in all the plates (Cawthon 2002). Briefly, telomere length was calculated as

$$T/S \text{ ratio} = 2^{-(\Delta Ct)} \text{ where } \Delta Ct = (Ct \text{ telomere} - Ct \text{ control gene})$$

using Ct for each primer standardized for the interplate control, i.e. Ct sample – Ct interplate control (Cawthon 2002, Criscuolo et al. 2009). Overall we measured RTL in 427 samples: 57 young nestlings, 287 fledglings and 81 first breeders (see Table 1 in Appendix for number of samples per year, brood, colour morph and storage methods). The coefficient of variation of the RTL of the interplate control sample, corrected by the standard at dilution 2ng μl^{-1}, was 6.8% (mean ± stdev of the RTL is 1.01 ± 0.07). After calculating RTL for all the samples we standardized it (mean 0, SD 1) to improve comparability with other studies, as suggested in Verhulst (2020).

Statistical methods

The RTL of fledglings (2009-2019, n=287, 98 broods) was analysed using a Gaussian linear mixed model (LMM) with brood ID (i.e. a unique numeric code identifying a specific brood/territory in a certain year of breeding) and year as random factor, to take into account for non-independence of offspring within the same brood and for annual variation across years. The RTL of young nestlings (only in 2019, n=57, 15 broods) was analysed using a LMM with brood ID as random factor, to account for non-independence of offspring within the same brood. RTL of both young nestlings and fledglings were compared between colour morphs (grey or brown) and considering as

covariates: sex (determined with PCR method), standardized mass, brood size (determined when sampling and measuring the offspring before fledging), sibling rank (i.e. ranking within the brood as biggest, smallest and intermediate size siblings) and age at sampling (estimated from wing length). Among young nestlings, few offspring were few days older than the average in the group (4 on 57 nestlings); however adding/removing these individuals from the dataset did not affect that statistical results and thus the full dataset is here presented. The interaction between color morph and standardized mass at fledging was also tested but was not statistically significant and was thus removed from the final model to avoid over-parametrization.

For samples from 2019 we calculated the change in RTL between young nestlings and fledglings (n=49, 15 broods, hereafter "telomere shortening") by subtracting RTL at nestling from the RTL at fledging (see methods in Karell et al. 2017). The change in RTL during nestling period was measured using a LMM with brood ID as random factor, to account for non-independence of offspring within the same brood, and included the same variables as in the previously described model. However, "growth", i.e. the mass at fledging minus the mass as young nestlings, was considered as covariate instead of standardized mass and the number of days between the two sampling were included instead of age. RTL as young nestling was also included to the model to control for the initial RTL measurement (as in Karell et al. 2017).

The RTL of first-breeders (i.e. individuals breeding for the first time within the study area, 2013-2019, n=81) was analysed with a LMM with year as random factor. The explanatory variables considered were colour morph (either grey or brown), sex and age (divided in 3 classes: 1-, 2- or 3-year-old and older). The interactions between colour morph and sex, and the one between sex and clutch size, were also tested but, since both

were not statistically significant, they were removed from the final model to avoid over-parametrization.

The probability for a first breeder to return in the following year to reproduce was analysed with a generalized linear mixed model (GLMM) with binomial distribution and year as random factor; explanatory variables included in the models were colour morph, RTL at first breeding, and sex. The interaction between standardized RTL and colour morph was also tested but was not statistically significant and was thus removed from the final model to avoid over-parametrization.

Laying date and storage method were initially tested in all the models but did not affect the RTL at any life stage and were thus removed from all final models (full models including these variables are presented in Appendix in Table 2). In the model for first breeders also clutch size was originally tested but was removed from the final model (see full model in Appendix in Table 2). All the explanatory variables, in all the models, were tested for collinearity (using "olsrr" package). All the analyses were run in R 3.6.1 (R Development team 2019). In all the models the degrees of freedom were calculated with Satterthwaite's method; all models were run as LMMs with normal distribution apart from the probability to return as breeder, which was run in a GLMM with binomial distribution. LMMs and GLMMs were conducted with functions "lmer" and "glmer" respectively, within the "lme4" package (Bates et al., 2015) and least square means were calculated within "emmeans" package (Lenth 2019).

Results

The RTL of tawny owl offspring did not differ between the morphs, either as young nestlings (least square means: grey -6.05 ± 0.03, CI: -6.12 and -5.98; brown -6.04 ± 0.04, CI: -6.11 and -5.96; Table 1a, Figure 1) or as fledglings (least square means: grey -5.27 ± 0.2, CI -5.32 and -5.21; brown -5.29 ± 0.02, CI -5.35 and -5.24; Table 1b, Figure 1). Offspring RTL also did not differ between the sexes, and showed no relations with standardized mass, brood size, age at sampling or their rank within the brood (Table 1a,b). Individual RTL as young nestling did not correlate with RTL as fledgling (Pearson's correlation r = 0.03, t_{50}=0.21, P=0.84; see Figure S1 in Appendix for individual variation across the two ages). Telomere shortening during nestling period did not vary according to morph or any of the variables considered apart from initial RTL as young nestling (Table 1c).

Among first-breeders, males had longer RTL than females (Table 1d; Figure 2) but there were no differences between morphs (least square means: grey 0.89 ± 0.04, CI 0.80 and 0.98; brown 0.92 ± 0.04, CI 0.83 and 1.01, Table 1d, Figure 1) or depending on age (Table 1d). RTL overall decreased across the three life stages (young nestlings, fledglings and first breeders), although no differences among morphs were observed (Figure 1). The probability to return to breed in the following breeding season did not vary according to RTL at first breeding (estimate ± SE 1.13 ± 2.41, z=0.47, P=0.639), nor according to sex (estimate ± SE -0.55 ± 0.62, z=-0.89, P=0.376) or colour morph (estimate ± SE 0.15 ± 0.61, z=0.247, P=0.805).

Discussion

We found no morph-specific differences in telomere length among offspring during the developmental period, neither as young nestling nor at fledging, nor during the first breeding attempt. Although our results on relative telomere length varied markedly between individuals during nestling period, we did not find any indications that this would be related to colour morph. These results suggest that during early life, both morphs have similar telomere length and thus the previously documented shorter telomeres in brown adults seem to be due to a morph-specific telomere shortening during adult life (Karell et al. 2017). We did find that females had shorter telomeres than males at their first breeding attempt, which is probably resulting from differential reproductive efforts of the two sexes at the early breeding stage when the sampling occurred (i.e. after egg formation and incubation by the female, but prior to nestling feeding which is performed by the male). Finally, the probability to return to the breeding area the following year did not depend on telomere length at first breeding, suggesting that telomere length may not be an indicator of individual quality in this species.

Telomere dynamics in nestlings and fledglings

In our dataset, tawny owls did not vary in their telomere dynamics (RTL and telomere shortening) according to their colour morph during the growth stage (nestling phase). These results are in line with a previous study on colour dimorphic white-throated sparrow (*Zonotrichia albicollis*), where no morph-sex differences were observed (Grunst et al. 2019). In a previous study in barn swallows (*Hirundo rustica*), where male nestlings are significantly darker than females consistent with adult sexual dichromatism, RTL increased with increasing plumage pheomelanism in male nestlings

(Costanzo et al. 2017). In tawny owls, on the other hand, the pheomelanic colouration is independent of age and sex, and in this system no correlation between colour morph and telomere length was observed during nestling phase. This observed lack of covariation suggests that different energetic requirements and/or life-history strategies adopted as adults could be the main drivers of the differential morph-specific telomere dynamics previously observed in this long-lived species (Karell et al. 2017). Indeed, a direct association between melanogenesis and telomere dynamics should result in morph-specific telomere length at early life stage, since it would be expected to be most pronounced during development and feather growth. A direct effect of melanin production might be particularly evident in tissues directly involved in this process, such as growing feathers, but a strong link of melanic coloration on telomere dynamics should be visible also in other tissues, including blood samples (as in Karell et al. 2017 and in Costanzo et al. 2017).

Morph-specific differences could result from life history trade-offs between investment in reproduction and somatic maintenance across different life history stages (e.g. Stearns 1989, Ricklefs and Wikelski 2002). Melanism could be linked with telomere dynamics via the pleiothropic melanocortin system (Ducrest et al. 2008). Genes regulating melanin expression also regulate e.g. (immunological) stress response and oxidative stress (Almasi et al. 2010, Roulin and Ducrest 2011, Emaresi et al. 2016), which can in turn affect telomere dynamics (Haussmann and Marchetto 2010, Asghar et al. 2015a, Angelier et al. 2018, Chatelain et al. 2020). In tawny owls, blood parasites and induced immune responses have strong effects on somatic maintenance in brown individuals (Gasparini et al. 2009, Karell et al. 2011b), which is also linked to faster telomere shortening (Karell et al. 2017). Tawny owl's colour morphs differ also in life span (Brommer et al. 2005), survival under harsh winter conditions (Karell et al. 2011

a) and in their parental investment (Emaresi et al. 2014). Offspring of the brown morph seem to have different growth/energy requirements: they convert food to growth more efficiently under favourable food conditions (Piault et al. 2009) and have consistently higher mass at fledging, regardless of food conditions, compared to grey offspring (Morosinotto et al. 2020). Thus, if brown parents allocate more resources and reproductive effort than grey parents, they will raise offspring in better condition but potentially suffer higher costs of reproduction. These previous findings, combined with the lack of variation in RTL here observed, suggest that different strategies adopted toward life history trade-offs, might be the main drivers of telomere dynamics in this long-lived polymorphic species.

Our results derive from a long-term cross-sectional dataset where samples were collected in multiple years and in different storage methods. Detailed and sensitive molecular level measurements, such as RTL, from samples of free-living individuals can be prone to biases due to methodological issues and non-standardised sampling protocols. Previous studies have, e.g., shown that storage method may impact telomere measurement (e.g. Reichert et al. 2017). Here we did not find any major differences due to storage in our data set (see Appendix). In contrast to our expectations, we also did not find a correlation between repeated RTL measures of individuals as young nestlings and fledglings, which could derive from non-standardized interval between samples and limited sample size (49 offspring in 15 broods). In the larger cross-sectional dataset we do find a general pattern where RTL decreases across life stages as reported in previous studies (e.g. Bize et al. 2009, Salomons et al. 2009, Asghar et al. 2015a).

Telomere length in first-breeders

Adult tawny owls at their first-breeding event did not differ in their RTL according to their colour morph. This lack of morph-specific variation at first breeding attempt,

together with the lack of effect at nestling phase, further suggest the importance of different life-history strategies (i.e. cost of reproduction) shaping telomere dynamics. We also found that first-breeder females had shorter telomeres than first-breeder males. This result is most probably a reflection of our timing of sampling, which occurred after the incubation just a few days after offspring hatched and before the intensive feeding of the offspring starts. Tawny owls have very distinct roles in parental care: females incubate the eggs and brood and defend the nestlings, while males provide food, first to the incubating female and then to both the female and the offspring throughout most of the rearing period. Thus, the parental investment of the males will increase during the rearing period leading to a fairly equal total investment by parents for the breeding event. Our result that female first-breeders have shorter telomeres than first-breeder males are thus probably resulting from the higher investment of females than of males during the early-phase of reproduction, due to egg production and incubation. Thus, sex differences in telomere dynamics are not expected when considering individuals that have bred in multiples years. Accordingly, telomere dynamics in experienced tawny owl breeders were not sex-specific (Karell et al. 2017). Our result that first-breeder females have shorter RTL than first-breeder males due to differential parental effort at the stage of sampling supports previous experimental studies in other systems, which report that parental effort can have costs in terms of reduced telomere length (Reichert et al. 2014, Sudyka et al. 2014). In zebra finches both parents exhibited substantial reduction in telomere length in experimentally enlarged broods persisting up to a year after the breeding event (Reichert et al. 2014).

Finally, we did not find support for our hypothesis that first breeders with shorter telomeres had lower probability to return to breed in the following year. We expected this pattern because in tawny owls, faster telomere shortening as adult is associated with

shorter life span (Karell et al. 2017) and the probability to breed was previously shown to be affected by current food availability (Roulin et al. 2003, Karell et al. 2009, Hoy et al. 2016) but not by previous nest predation events (Karell et al. 2020). Thus, individual condition, at least in terms of telomere length *per se*, does not seem to affect probability to breed.

Conclusion

Understanding how telomere dynamics vary across life-stages is important in order to understand the physiological mechanisms underlying individual fitness. Here we show that colour-morph specific telomere dynamics in tawny owls are not predefined at hatching nor during the developmental stage before fledging. Indeed, morph-specific patterns become apparent only after the first breeding event, most likely as a consequence of the differential life-history strategies adopted (life history syndromes) by the two colour morphs during their breeding career. Thus our data, although mostly cross-sectional, combined with previous studies on morph-specific life history strategies and sensitivity to environmental conditions, suggest that different genotypes (i.e. highly heritable morphs) can exhibit different telomere dynamics and senescence patterns.

Data archive:

All the data will be uploaded in a public data repository before publication

Declarations

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Figure Legends

Figure 1: Estimates ± 95% CI of the LMM on standardized RTL across the three life stages (young nestlings, fledglings and first breeders) and colour morphs (grey vs. brown). The model was build using a full dataset (all datasets per life stages combined) and using as random factor broodID for nestlings measures (both young and old) and ID for the adults.

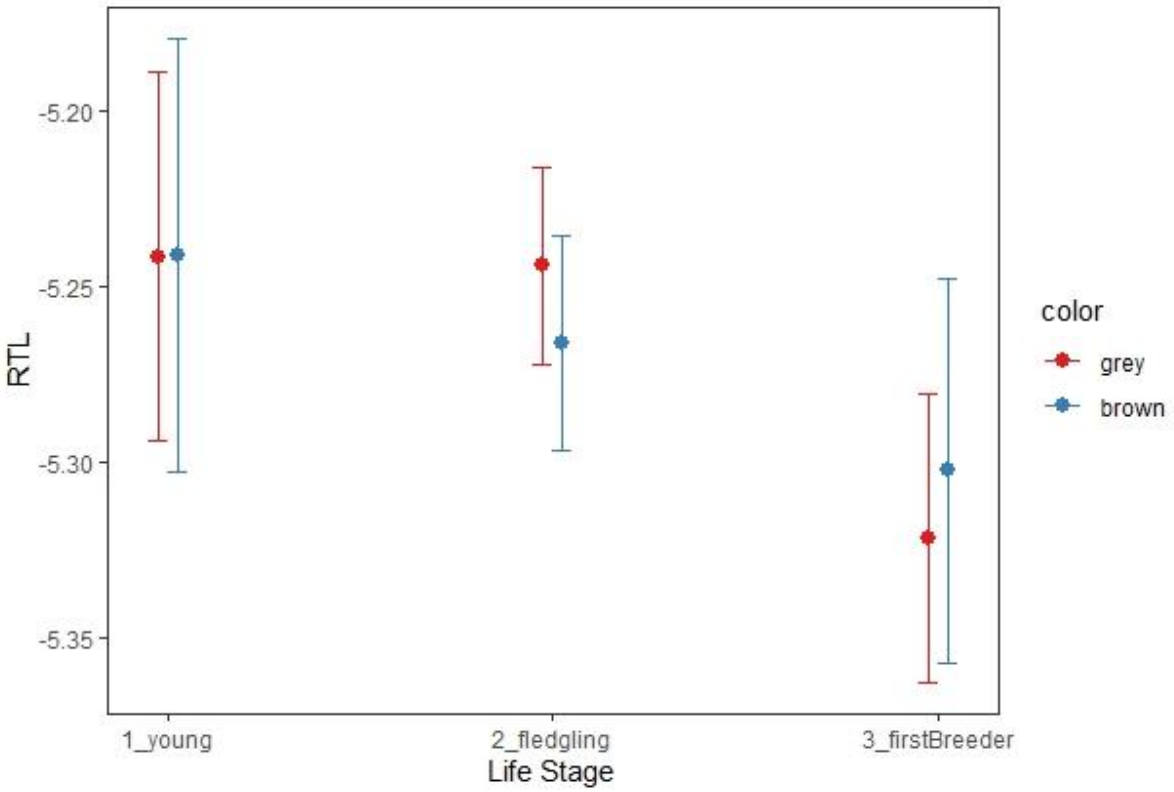

Figure 2: Estimates ± 95% CI of the LMM on standardized RTL at first breeding attempt according to sex. Sampling occurred just after the incubation period and before the offspring feeding period, when parental effort differ the most between sexes (females incubate the eggs, whereas males are the main food providers to the offspring).

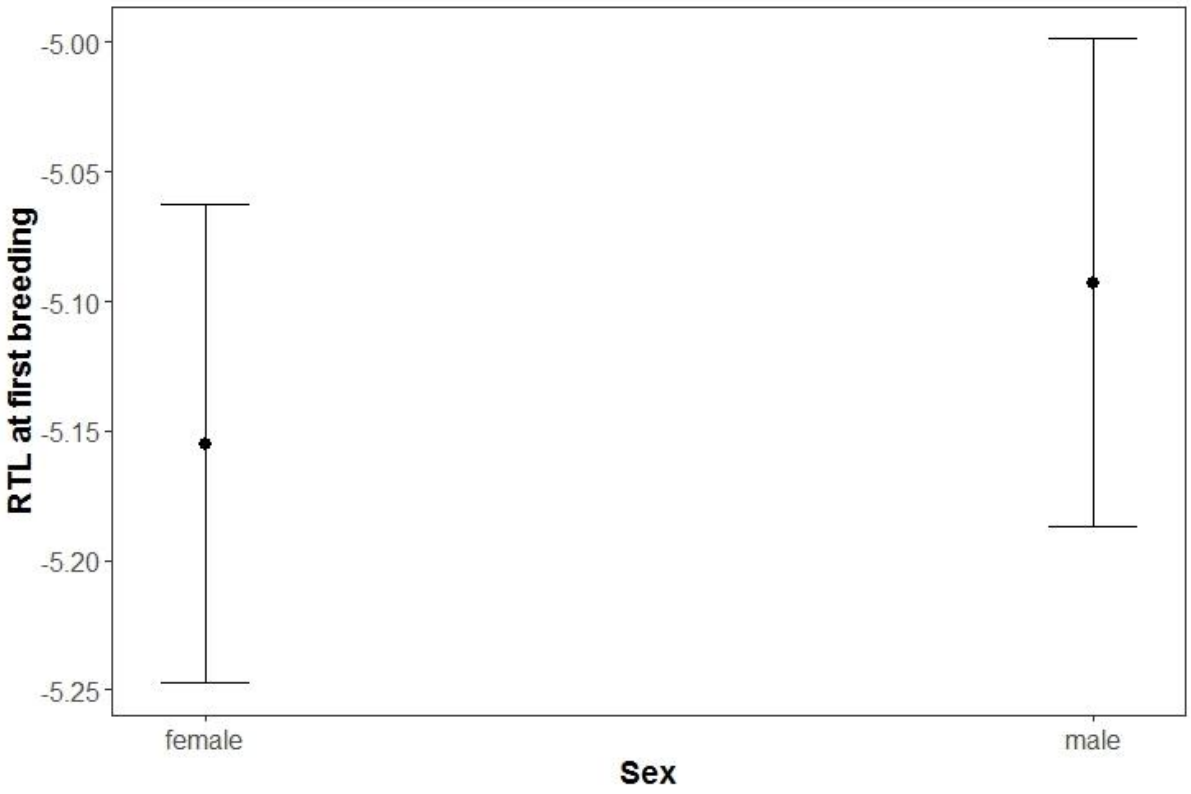

Table Legend

Table 1: LMMs for RTL *a*) of nestlings and *b*) of fledglings, *c*) telomere shortening during nestling period, and *d*) RTL of first-breeders. In all the models RTL was standardized to the mean.

	Variables	Slope ± SE	DenDF	F	P
(a) RTL of nestlings	Morph (brown)	0.01 ± 0.04	49.00	0.09	0.771
(2019, n=57, 15 broods)	Brood size	-0.03 ± 0.04	12.45	0.68	0.426
	Sibling (medium)	0.08 ± 0.04	44.31	1.86	0.167
	(smallest)	-0.06 ± 0.06			
	Standardized mass	-0.0009 ± 0.001	43.26	0.72	0.400
	Sex (male)	-0.04 ± 0.04	42.66	1.14	0.291
	Age at sampling	0.02 ± 0.02	47.22	1.36	0.249
(b) RTL of fledglings	Morph (brown)	-0.03 ± 0.02	271.76	2.07	0.152
(2009-2019, n=287,	Brood size	-0.01 ± 0.01	96.30	0.89	0.348
98 broods)	Sibling (medium)	0.001 ± 0.02	245.46	1.63	0.197
	(smallest)	-0.04 ± 0.03			
	Standardized mass	-0.00005 ± 0.0003	272.24	0.03	0.867
	Sex (male)	0.02 ± 0.02	256.99	0.84	0.361
	Age at sampling	-0.003 ± 0.005	213.16	0.55	0.458
(c) Telomere shortening	Morph (brown)	-0.03± 0.07	26.99	0.17	0.682
(2019, n=49, 15 broods)	Brood size	-0.03 ± 0.05	8.68	0.52	0.491
	Sibling (medium)	0.006 ± 0.06	34.81	0.09	0.918
	(smallest)	-0.03 ± 0.09			
	Growth	-0.02 ± 0.04	20.40	0.20	0.660

	Standardized initial RTL	-1.03 ± 0.23	34.64	20.27	**<.001**
	Sex (male)	0.01 ± 0.06	38.96	0.05	0.817
	Days between sampling	-0.03 ± 0.02	7.46	3.16	0.116
(d) RTL of first-breeders (2013-2019, n=81)	Morph (brown)	0.03 ± 0.03	71.53	0.94	0.334
	Age class (2 y old)	0.01 ± 0.04	72.81	0.46	0.632
	(older)	0.04 ± 0.04			
	Sex (male)	0.06 ±0.03	70.83	4.64	**0.035**

For the class variables morph, sibling, sex and age: 'grey', the 'biggest nestling in the brood', 'female' and '1 year old' are used as reference level respectively. The models 'RTL of nestlings' a) and 'telomere shortening' c) included 'brood ID' as random factor, while the model 'RTL of fledglings' b) included both 'brood ID' and 'year' as random factor. First-breeders model d) include 'year' as random factor. Numerator DF is always equal to 1, apart for the variables 'Sibling' and 'Age class' where it is always 2, and thus only denominator DFs are presented.