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*Published in:*  
Molecular Ecology

*DOI:*  
[10.1111/mec.16397](https://doi.org/10.1111/mec.16397)

**IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.**

*Document Version*  
Publisher's PDF, also known as Version of record

*Publication date:*  
2022

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

Kauzálová, T., Tomášek, O., Mulder, E., Verhulst, S., & Albrecht, T. (2022). Telomere length is highly repeatable and shorter in individuals with more elaborate sexual ornamentation in a short-lived passerine. *Molecular Ecology*, 31(23), 6172-6183. <https://doi.org/10.1111/mec.16397>

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# Telomere length is highly repeatable and shorter in individuals with more elaborate sexual ornamentation in a short-lived passerine

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## Funding information

Grantová Agentura České Republiky, Grant/Award Number: GA21-22160S and GA19-22538S

Handling Editor: Sean Rogers

## Abstract

Quantifying an individual's state as a fitness proxy has proven challenging, but accumulating evidence suggests that telomere length and attrition may indicate individual somatic state and success at self-maintenance, respectively. Sexual ornamentation is also thought to signal phenotypic quality, but links between telomeres and sexual ornamentation have been little explored. To address this issue, we examined whether telomere length and dynamics are predicted by the expression of a sexually selected ornament, the length of the outermost tail feathers (streamers), using longitudinal data from a population of European barn swallows (*Hirundo rustica*). In 139 adult individuals, each measured twice, we further assessed associations of telomere length with age, sex, breeding status and survival. Telomere length showed high individual repeatability ( $R = .97$ ) across years while shortening with age in both sexes. Telomere length and dynamics were not significantly associated with survival to the next year, remaining lifespan or reproduction status (comparing breeding and nonbreeding yearlings). Tail streamer length, a sexually selected trait in barn swallows, was negatively associated with telomere length, independent of sex. Thus, telomere length may reflect the costs of carrying an elaborated sexual ornament, although ornament size did not significantly predict telomere shortening. In conclusion, telomere length in adult barn swallows is a highly consistent trait that shows a negative relationship with sexual ornamentation, suggesting a trade-off between sexual ornamentation and telomere length.

## KEYWORDS

ageing, biomarker, individual quality, life history, repeatability, tail streamer

## 1 | INTRODUCTION

Across eukaryotes, the terminal parts of linear chromosomes are protected by telomeres, highly conserved repetitive noncoding DNA sequences that prevent damage to coding DNA and chromosome

fusion, thus maintaining genome stability (Blackburn, 1991). Telomeres shorten with each cell division due to, amongst other processes, the “end-replication problem” (Blackburn, 1991), especially in cells with high turnover rates, such as haematopoietic cells (Sabharwal et al., 2018). Telomere length (TL) shortens with age in

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most species, both cross-sectionally and longitudinally (Hausmann et al., 2003; Sudyka et al., 2016; Tricola et al., 2018). The importance of the longitudinal approach in telomere biology can be illustrated by the finding that some cross-sectional studies found longer telomeres in older birds (Hausmann et al., 2003; Tricola et al., 2018). This observation, however, can be explained by telomere shortening with age and simultaneous selective disappearance of individuals with short telomeres from a population (Hausmann & Mauck, 2008) rather than longitudinal telomere lengthening, which has not yet been convincingly documented (Fairlie et al., 2016; Hoelzl et al., 2016; Lemaître et al., 2021; Spurgin et al., 2018). The former scenario is also likely because short telomeres have been associated with higher mortality rates in free-living vertebrates (meta-analysis in Wilbourn et al., 2018).

Besides age, heritability and early-life developmental conditions are important sources of individual variation in TL (Atema et al., 2015; Bauch et al., 2019, 2021; Lemaître et al., 2015). The influence of developmental conditions on TL is underpinned by fast telomere shortening during rapid growth in early life (Pauliny et al., 2012; Salomons et al., 2009). Furthermore, TL and its dynamics (i.e., the change in TL with time) are associated with stress-inducing environmental conditions and biological processes, as indicated by a meta-analysis across nonhuman vertebrates (Chatelain et al., 2020). Due to the combination of all the above-mentioned factors influencing telomeres, they are considered more as a biomarker of individual somatic state or allostatic load (also termed collectively as biological age) rather than chronological age (Angelier et al., 2019; Boonekamp et al., 2013; Monaghan, 2010). Observed associations between TL and biological processes should always be carefully interpreted with respect to the level of within-individual repeatability of TL (Kärkkäinen et al., 2021). According to a recent meta-analysis (Kärkkäinen et al., 2021), most variance in TL repeatability across studies was explained by methodological differences. In longitudinal studies, high repeatability signals high precision and reliability of the applied telomere assay (Lindrose et al., 2021). To date, the highest within-individual repeatabilities of telomere measurements have been reported in studies using telomere restriction fragments (TRF) analysis (Kärkkäinen et al., 2021).

Life-history theory predicts that individuals are selected to allocate limited resources between self-maintenance, growth and reproduction in a way that maximizes their fitness (Stearns, 1992). Quantifying an individual's state in this context is challenging, but the line of evidence mentioned above suggests that TL and its dynamics may indicate the individual somatic state and investment in self-maintenance, respectively (Monaghan & Ozanne, 2018; Vedder et al., 2017). Thus, TL or telomere dynamics may be related to reproductive investment, a resource-demanding process (Sudyka, 2019). Because age at first reproduction varies significantly across individuals within populations (e.g., Fay et al., 2016), the individual strategy to reproduce early in life may also be associated with TL. Longitudinal studies across vertebrates have shown that an earlier age at first reproduction is associated with lower survival rates (e.g., Ancona et al., 2015; Blomquist, 2009; Desprez et al., 2014). Delayed

reproduction could then indicate the individual strategy to allocate more resources to survival-sustaining processes.

Investment in sexual signals, to prospective and current mates, can constitute a significant part of reproductive investment and thereby of life history evolution. However, very few studies have investigated the connection of TL and its dynamics with the expression of secondary sexual traits, such as coloration in American painted dragon (Giraudeau et al., 2016) or horn length in Soay sheep (Watson et al., 2017). In birds, Taff and Freeman-Gallant (2017) reported that males of common yellowthroats (*Geothlypis trichas*) with brighter UV-yellow bib coloration had longer telomeres and less telomere attrition. On the other hand, spotless starling (*Sturnus unicolor*) males with longer ornamental throat feathers had shorter telomeres and a lower rate of telomere shortening than males with shorter throat feathers, while females with longer throat feathers had longer TL (Azcarate-García et al., 2020).

The barn swallow (*Hirundo rustica*) is an iconic species in sexual selection research, possessing several traits proposed to play a role in sexual selection (Møller, 1988; Pap et al., 2019; Wilkins et al., 2016) and reflecting individual condition and health (Hund et al., 2020). For example, the length of prolonged outermost tail feathers (hereafter tail streamers), both of natural size or experimentally elongated, positively affected male pairing success and reproductive output in this species (Møller, 1988; Pap et al., 2019; Saino et al., 1997; Vortman et al., 2011). Males with elongated tail ornaments were also preferred in extra-pair copulations (Møller, 1988), although the natural tail streamer length was not significantly associated with extra-pair paternity in another barn swallow population (Micháľková et al., 2019). At the same time, there is evidence indicating that the expression of elongated tail streamers is associated with costs: long tail streamers compromised manoeuvrability in adults of both sexes (Rowe et al., 2001) and were associated with higher oxidative damage in males (Vitousek et al., 2016). This may result in a negative association between tail ornament expression and TL, as increased oxidative stress reduces TL (Reichert & Stier, 2017; von Zglinicki, 2002). Although TL in barn swallow nestlings did not predict tail streamer length measured in the next breeding season (Caprioli et al., 2013), whether there is a link between tail streamer length and TL in adult male and female barn swallows has not been investigated. However, a previous study in adult birds showed that male barn swallows with more intense ventral plumage coloration (another presumed sexually selected trait) had longer telomeres, while an opposite trend was observed in females (Parolini et al., 2017).

Here, we measured TL and its dynamics (representing two different aspects of telomere biology—current state and change in time, respectively) using the TRF approach in a longitudinally studied European barn swallow population where information about age, sex, and individual phenotypic and reproductive traits was available for all individuals (Pap et al., 2019; Petrželková et al., 2015). In this short-lived species (average lifespan of adult birds is ~1.5 years), we aimed to evaluate (i) the rate of telomere shortening with age, (ii) the relationship between investment into a secondary sexual trait (tail streamer length) and TL, and (iii) the associations of TL and its

dynamics with components of individual fitness, namely survival, remaining lifespan and delayed reproduction of individuals in their first breeding season. There are two possible scenarios, not mutually exclusive, regarding the relationship between TL and the measured parameters. TL could indicate individual quality, which would be reflected in a positive relationship between TL and the observed variables (e.g., birds with longer tail streamers would also have longer telomeres), and/or TL could reflect investments in fitness-enhancing processes such as reproduction and sexual ornamentation vs. self-maintenance, which would result in a negative relationship between TL and phenotypic traits. Similarly, we hypothesized that the rate of telomere shortening may be faster in low-quality individuals or those investing highly in sexual ornamentation due to a trade-off between these investments and self-maintenance.

## 2 | MATERIALS AND METHODS

### 2.1 | Study population and data collection

The barn swallow is a socially monogamous and semicolonally breeding small passerine with a deeply forked tail representing a secondary sexual trait in males (Møller, 1988). We studied European barn swallows at three isolated farms near Třeboň, Czech Republic (49.0077N, 14.7590E). Mean adult annual survival in our study area is ~40% and the oldest bird recorded in our population was 8 years old. Mean age in the population is 1.57 years (data 2010–2019) and, on average, 1-year-old individuals represent about 66% and 2-year-old individuals ~21% of the whole population. In each breeding season an attempt was made to systematically catch all adult birds by repeated mist-netting and mark them with a numbered aluminium ring complemented with a unique set of plastic colour rings to distinguish social pairs. When considering all individuals recorded at the localities in multiple breeding seasons, only in <0.5% of cases was an individual missed in one of the seasons, while being recorded in the preceding and the following seasons, demonstrating the sampling efficiency. All localities were searched systematically for active barn swallow nests, and nest-holders were identified by their colour rings using cameras and binoculars. Breeding localities were visited every day from the beginning of May to mid-July and then at least once a week until early September, and nests were checked every 4–5 days to determine the breeding/nonbreeding status of uniquely marked individuals. Among 1-year-old individuals, 71% of birds (86% of female yearlings and 58% of male yearlings) initiated a nesting attempt (data 2010–2018). At every capture, we measured morphological parameters, including the length of the left and right outermost tail feathers (to the nearest 0.5 mm). Only birds with tail streamer feathers intact (unbroken) were considered, with a single maximum value of a tail streamer length for an individual in a given year used in the analyses. More than 99% of adult barn swallows return to the same breeding site (Michálová et al., 2019; Møller et al., 2003; Møller & de Lope, 1999), and hence adult birds that do not return to the locality can reliably be considered dead (Costanzo, Ambrosini, et al.,

2017). Similarly, unringed individuals that first appear on the breeding locality can be assumed to be 1-year-old recruits from another locality. Thanks to the intensity of fieldwork, with all birds present at each breeding colony being captured every year, it was possible to estimate the age and lifetime trajectories of measured phenotypic and reproductive traits of all birds included in the analysis with reasonably high precision.

### 2.2 | Blood sampling

TL was measured in blood samples collected from the jugular vein with a sterile insulin syringe from 139 adults in the years 2012–2017 during the respective breeding season (April–July, with the majority of samples obtained in May). The samples were selected from a larger set of samples according to the availability of longitudinal samples and its demographic structure reflects that of the whole study population (mean age of the individuals when first sampled was 1.47 years, with 1-year-old individuals representing 71% and 2-year-old individuals representing 17% of sampled individuals). The whole blood samples ( $n = 119$ ) or separated red blood cells after the centrifugation step ( $n = 159$ ) were directly deep frozen in liquid nitrogen. The type of sample did not affect TL according to the linear mixed model controlled for measurement (first or second) with random effect of bird ID (estimate  $\pm$  SE =  $35.93 \pm 24.17$  bp,  $t = 1.49$ ,  $p = .14$ ), thus excluding any methodological constraint. Our data set consists of 96 males and 43 females, each individual sampled twice during its life (122 individuals in two consecutive years, 16 after 2 years, and one individual 3 years apart). Tail streamer length is sexually selected in males and, based on our previous results, oxidative damage increases with streamer length in males (Vitousek et al., 2016), so we focused preferentially on male samples. Thirty-nine of a total of 98 samples from 1-year-old birds represented nonbreeding individuals (Table S1).

### 2.3 | Telomere measurement assay

We used TRF analysis without the denaturation step to measure the end-chromosome telomeric repeats without the interstitial telomeric sequences (ITS), which are quite abundant in birds (Foote et al., 2013). Genomic DNA was extracted from 5  $\mu$ l of erythrocytes or whole blood using the CHEF Genomic DNA Plug kit (Bio-Rad) followed with Proteinase K overnight digestion at 50°C. Half of each agarose plug was digested for ~18 h in a water bath at 37°C with a mix of enzymes *HindIII* (30 U), *Hinfl* (15 U) and *MspI* (30 U) in NEBuffer 2.1 (New England Biolabs). We used a 0.8% nondenaturing agarose gel (Pulsed Field Certified Agarose, Bio-Rad) for separation of TRF by pulsed-field gel electrophoresis at 14°C for 24 h (3.5 V  $\text{cm}^{-1}$ , initial switch time 0.5 s, final switch time 7.0 s). For size calibration, two different  $^{32}\text{P}$ -labelled size ladders were used (1-kb DNA ladder, New England Biolabs; DNA Molecular Weight Marker XV, Roche Diagnostics). Dried gels (gel dryer, model 538, Bio-Rad) were hybridized overnight at 37°C with a hybridization

probe containing  $^{32}\text{P}$ -endlabelled oligo (5'-CCCTAA-3')<sub>4</sub> that binds only to the telomeres at the ends of chromosomes, preventing the simultaneous detection of the problematic ITS. Each radioactive gel was exposed overnight to the phosphor screen (MS, PerkinElmer) and the transmitted signal was visualized using a phosphor imager (Cyclone Storage Phosphor System, PerkinElmer).

The average TL for each sample was calculated from the sample-specific TL distribution using densitometry in imageJ2 (Rueden et al., 2017) as previously described (Salomons et al., 2009). Male and female samples, as well as different sampling years, were distributed randomly on 13 gels with the two samples of the same individual positioned in adjacent lanes on the same gel to ensure the most precise comparison.

## 2.4 | Statistical analysis

### 2.4.1 | Descriptive analysis of TL variation

We used the statistical software R (version 4.0.5, R Core Team, 2020) running in RSTUDIO IDE (ver. 1.4.1106; RStudioTeam, 2020) to describe the basic information about telomere length in our population (mean TL, SD, range of TL and TL  $\pm$  SD in males and females separately). The repeatability of within-individual TL measurements, as well as control samples measured repeatedly on different gels, was calculated using the package *RPTR* in R (Stoffel et al., 2017) for Gaussian data with 1000 parametric bootstrap iterations for confidence interval estimation and 1000 permutations. Bird ID was used as a random effect. The interassay repeatability of the same samples measured on different gels was  $0.82 \pm 0.10$  (95% confidence interval [CI] = 0.533–0.926,  $p < .001$ ). Neither the gel ID nor the birth year of the individuals had any effect on TL (Table S2), and therefore we do not include them as random effects in the following analysis.

### 2.4.2 | Effect of age

We analysed the data using linear mixed effects models using the R packages *LME4* (Bates et al., 2015) and *LMERTEST* (Kuznetsova et al., 2017). We ran a model with TL as a dependent variable and age and sex as explanatory variables including bird ID as a random effect. To distinguish within- and between-individual effects of age on telomere shortening, we followed the within-subject centring approach (van de Pol & Wright, 2009) and partitioned the “age” variable into two components – mean age and delta age. Mean age (calculated from the two measurements of each individual) represented the cross-sectional effect and delta age (i.e., the difference between the actual age and mean age) represented the within-individual effect based on longitudinal data. To test for the presence of selective disappearance, we estimated the difference between the within- and between-individual slopes using an unpaired t-test based on their

means and standard errors from the model (Salomons et al., 2009). We also implemented the alternative model to test for selective disappearance, as suggested by van de Pol and Wright (2009), with both actual age and average age as explanatory variables with the very same result as the t-test we performed. Since both approaches provided similar results, we decided to not include the latter to reduce the redundant models.

### 2.4.3 | Regression to the mean

Not only TL but also telomere dynamics (defined as the difference in TL within individual per year) may be related to individual survival. To correct for the statistical dependency of a difference between successive measurements on the baseline measurement due to the regression to the mean (RTM; Verhulst et al., 2013), we adjusted the values of annual change in TL according to Berry et al. (1984) before fitting the models. To test whether the rate of telomere shortening depends on the initial TL, we ran a linear model with RTM-corrected telomere shortening as the dependent variable and initial TL as the explanatory variable. Because the interval between the two sampling events varied among individuals in this study, we also ran a linear model with telomere shortening corrected for RTM as the dependent variable and the number of days between the two measurements as the explanatory variable. The rate of telomere shortening was independent of the exact time interval between sampling events in days (estimate  $\pm$  SE =  $-0.17 \pm 0.53$ ,  $t = -0.32$ ,  $p = .75$ ), therefore we consider only the age difference in years within individuals in the models.

### 2.4.4 | Effect on survival

To analyse the effects of TL and its shortening rate on survival, we ran a linear regression model with a binary dependent variable “mortality” encoded as follows: 0 when the bird survived to the consecutive breeding season and 1 when the bird presumably died (see above). The model also included initial TL, rate of telomere shortening and sex as covariates. To correct for the possible differences between males and females, we further added interactions of sex with telomere variables. As an additional way to determine the effects of TL and telomere shortening on mortality in our population, we used the “coxph” function implemented in the R package *SURVIVAL* (Therneau, 2021) to run Cox proportional-hazards models. Unlike the binomial model above, which only evaluates short-term survival to the following season, the Cox model uses available data over individual lifespans, thereby incorporating the effect on long-term survival. Individuals that did not return after the second sampling year were assigned a zero survival interval. Sex and all first-order interactions were added as covariates. Because all individuals were followed until they disappeared from the population (died), the Cox model was performed without censoring.



## 2.4.5 | Subsets of individuals

Throughout the paper, we distinguish between the data set comprising birds of all ages ( $n = 139$ ), and a subset of birds sampled in two consecutive years ( $n = 122$ ), further subsetted to 1-year-old birds sampled in their first and second breeding season (hereafter called “young” birds,  $n = 90$ ). Many analyses focused predominantly on the “young” bird subset to avoid potential bias resulting from an unequal number of individuals in each age category originating in the species demography (see Table S1 for specific sample sizes). Also, ornament expression (tail streamer length) increases nonlinearly with age in barn swallows, with the largest difference being detectable between the first and second breeding season (Pap et al., 2019), simplifying interpretation of the analyses restricted to young birds. However, analyses were also performed with the entire data set including older individuals; these yielded similar results and are therefore presented in the Supporting information only. In linear model analyses involving a single measurement of all individuals (see Tables S5, S6b and S8), the variable age and its respective interactions were included among predictors compared to the same analyses using a subset of “young” birds only (see Tables 2 and 5; Table S6a).

## 2.4.6 | TL and fitness-related traits

To test the hypothesis that telomeres are biomarkers of individual quality in “young” birds, we ran a generalized linear model with the binary dependent variable “breeding” encoded as 0 when the bird did not breed in its first breeding season and 1 when it started to breed. TL in the first breeding season, sex and their interaction were the explanatory variables.

To test the potential association of investment into the length of tail streamers (i.e., a secondary sexual trait) with TL, we ran a linear mixed effects model with TL as the dependent variable, sex, age, mean tail streamer length (calculated from the two measurements per individual), delta tail streamer length (i.e., the deviation of actual tail length from the individual mean) and all the interactions with sex as explanatory variables, and bird ID as a random effect. We partitioned the individual tail streamer length values into mean and delta to distinguish the within- and between-individual effects of tail streamer length on TL (van de Pol & Verhulst, 2006). Tail streamer length was also highly repeatable within individuals across years ( $0.901 \pm 0.016$ , 95% CI = 0.863–0.928,  $p < .001$ ). For six individuals in which streamer length was measured in only one of the two years for which we measured telomere length, we took that one measurement as the mean value and set the delta streamer length variable to zero. Due to significant differences between tail streamer length in males and females, we used the within-sex centred values of the mean tail length (package *MISTY* in R; Yanagida, 2020) in the model to enable the main effect estimates of streamer length to be properly interpreted without the need to remove the interaction term from the model (Schielzeth, 2010). We evaluated potential effects of the costs of growing the tail streamers on telomere dynamics (difference

in TL between the first and second sample year) by running a linear model with the rate of telomere shortening corrected for RTM as the dependent variable, and sex (as a dummy variable), initial tail length and the within-individual difference in tail length between sampling events as the explanatory variables.

## 2.4.7 | Evaluation of models

All full models were first tested against their respective null models using a likelihood ratio test (Forstmeier & Schielzeth, 2011), and only when they were significantly better were full models simplified to obtain the minimal adequate model following Crawley (2014). In short, minimal adequate models were identified through backward elimination of nonsignificant fixed effects ( $p > .05$ ) based on the drop1 function in R and associated changes in deviance expressed as  $\chi^2$ .

## 3 | RESULTS

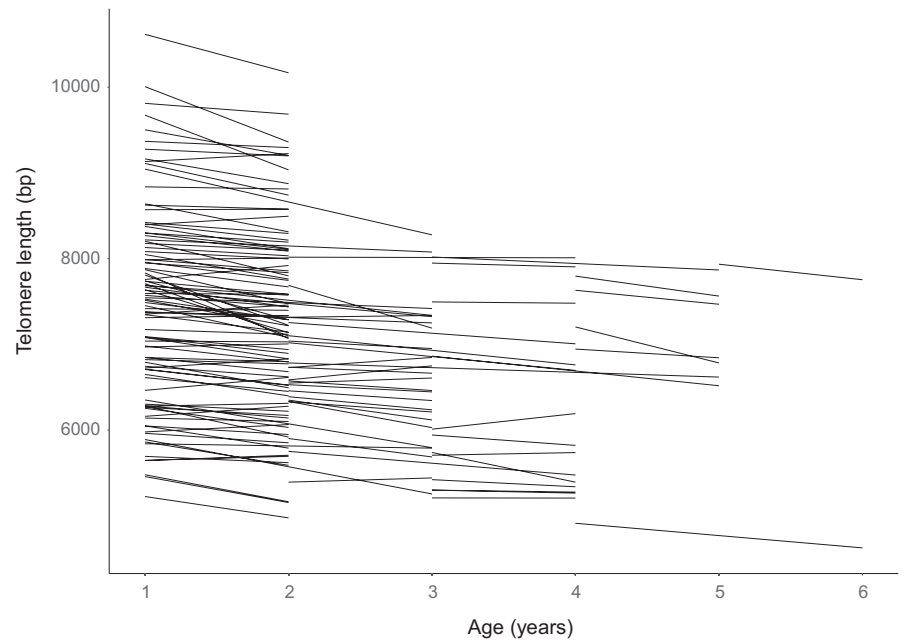
### 3.1 | Repeatability and age-related TL dynamics

Mean TL was 7126 bp (all individuals:  $SD = 1110$  bp, range 4625–10617 bp,  $n = 278$ ; males: mean  $\pm SD = 7250 \pm 1156$  bp, range 4625–10617 bp,  $n = 192$ ; females: mean  $\pm SD = 6848 \pm 947$  bp, range 4975–9369 bp,  $n = 86$ ; Table S3). Intra-individual repeatability of TL across years without correction for age effects was extremely high ( $0.974 \pm 0.004$ , 95% CI = 0.964–0.982,  $p < .001$ ; Figure 1). Telomeres of males were  $328 \pm 193$  bp longer than telomeres of females, but this difference did not reach statistical significance ( $p = .092$ ). Similar to many other species, TL declined significantly with age (Table 1), and interactions of sex with either age component (within and between variation in age) showed no difference in telomere dynamics between sexes (Table 1).

### 3.2 | TL and individual fitness components

The observed difference between the slopes of cross-sectional and longitudinal age effects (Table 1) indicates a counterintuitive tendency towards selective disappearance of individuals with longer telomeres from the population. However, this difference did not reach statistical significance ( $t = -1.91$ ,  $p = .058$ ). There was no relationship between the individual rate of telomere shortening (corrected for “regression-to-the-mean”) and their initial TL, either using the subset of “young” birds ( $0 \pm 0.02$ ,  $t = -0.10$ ,  $p = .93$ ) or all sampled individuals ( $0 \pm 0.01$ ,  $t = -0.32$ ,  $p = .75$ ). Moreover, neither initial TL nor the rate of telomere shortening of individuals between their first and second breeding season predicted the probability of survival to their third breeding season (Table 2), or their remaining lifespan (Table S4). There was also no effect of telomeres on the survival probability after second sampling using

**FIGURE 1** High repeatability of within-individual telomere length as illustrated by the lines connecting the two TL measurements of the same individual. The majority of samples were obtained from “young” birds (1-year-old and 2-year-old birds) covering also the widest range of measured TL



**TABLE 1** Telomere length in relation to age and sex

Parameter	Estimate $\pm$ SE	t Value	p-Value	Estimate $\pm$ SE	t Value	p-Value
Intercept	7307.64 $\pm$ 409.21	17.86	<.001	7820.82 $\pm$ 223.90	34.93	<.001
Sex (male)	686.86 $\pm$ 487.44	1.41	.16	—	—	—
Mean age	-209.05 $\pm$ 171.52	-1.22	.23	-340.90 $\pm$ 100.58	-3.39	.001
Delta age	-125.09 $\pm$ 24.59	-5.09	<.001	-147.43 $\pm$ 13.11	-11.24	<.001
Mean age:sex	-169.25 $\pm$ 211.15	-0.80	.42	—	—	—
Delta age:sex	-31.11 $\pm$ 29.02	-1.07	.29	—	—	—
Random: bird ID	var. = 1,082,242 (SD = 1040.3)			var. = 1,109,888 (SD = 1053.5)		

Note: Mixed effects model to test the cross-sectional (mean age) and longitudinal (delta age) changes in telomere length with age ( $n = 278$  measurements in 139 individuals). The difference between slopes of the cross-sectional and longitudinal effect on telomere shortening is nonsignificant ( $t = -1.91$ ,  $p = .058$ ). The full model (on the left) was significantly different from the null model ( $\chi^2 = 105.58$ ,  $p < .001$ ). The simplified minimal adequate model is on the right side.

SE, standard error; SD, standard deviation; var., variance.

the whole data set (Table S5). In accordance with this result, we found no effect of TL or telomere shortening on survival using the Cox proportional-hazards model, neither in the “young” birds nor across all age cohorts (Table S6). Furthermore, TL was not associated with breeding/nonbreeding status of “young” birds in their first breeding season (Table 3).

### 3.3 | TL and tail streamer length

TL in the first and second breeding season showed a negative relationship with individual mean tail streamer length, and this pattern did not differ between sexes (Table 4, Figure 2). As can be seen in Figure 2, there is one outlier that combines very long tail streamers with very long telomeres. When this individual is removed from the analysis the  $p$ -value of streamer length decreases from .042

to .004. When we included individuals more than 2 years old, the result was similar ( $p = .050$ , Table S7;  $p = .011$  when the one outlying individual is removed). Telomere attrition was associated neither with initial tail streamer length nor with the interannual change in tail streamer length in either sex in the “young birds” (Table 5) or when including the older birds (Table S8).

## 4 | DISCUSSION

TL and its dynamics are linked to survival probability, reproduction and growth, and thus potentially play a role in life history variation and mediating life history trade-offs (Hausmann & Marchetto, 2010). Using high-precision TL data, we demonstrated extremely high within-individual repeatability of TL in adult barn swallows and its significant shortening with age. Furthermore, we found

that individuals with longer tail streamers had shorter telomeres, independent of sex, while the rate of telomere shortening was independent of tail streamer length or its interannual change.

**TABLE 2** Mortality in relation to initial telomere length and RTM-corrected change in telomere length per year of “young” individuals after their second breeding season ( $n = 90$ ; the dependent variable is “mortality” encoded 0 when the bird returned to the locality, or 1 when the bird disappeared)

Parameter	Estimate $\pm$ SE	z Value	p-Value
Intercept	1.46 $\pm$ 3.68	0.40	.69
Sex (male)	-2.60 $\pm$ 4.07	-0.64	.52
Initial TL (kb)	-0.05 $\pm$ 0.50	-0.11	.92
Change in TL (kb year <sup>-1</sup> )	-2.35 $\pm$ 3.13	-0.75	.45
Initial TL:sex	0.26 $\pm$ 0.55	0.48	.63
Change in TL:sex	2.58 $\pm$ 3.47	0.74	.46

Note: The full model was not significantly different from the null model ( $p = .42$ ).

Abbreviations: kb, kilobases; SE, standard error; TL, telomere length.

Parameter	Estimate $\pm$ SE	z Value	p-Value	Estimate $\pm$ SE	z Value	p-Value
Intercept	1.41 $\pm$ 4.39	0.32	.75	1.99 $\pm$ 0.62	3.24	.001
Sex (male)	-0.57 $\pm$ 4.66	-0.12	.90	-1.97 $\pm$ 0.66	-2.98	.003
TL	0.08 $\pm$ 0.62	0.13	.89	—	—	—
TL:sex	-0.19 $\pm$ 0.65	-0.30	.77	—	—	—

Note: The dependent variable “breeding” was encoded as 0 when the bird did not breed and 1 when it had a nest (full model on the left, minimal model on the right). The full model was significantly different from the null model ( $\chi^2 = -12.88$ ,  $p = .005$ ).

Abbreviation: SE, standard error.

**TABLE 4** Telomere length (bp) in relation to age, sex and tail streamer length variables in “young” birds in their first and second breeding season ( $n = 179$ )

Parameter	Estimate $\pm$ SE	t Value	p-Value	Estimate $\pm$ SE	t Value	p-Value
Intercept	7316.90 $\pm$ 226.59	32.29	<.001	7642.80 $\pm$ 118.08	64.72	<.001
Sex	463.90 $\pm$ 269.68	1.72	.088	—	—	—
Age	-186.58 $\pm$ 52.67	-3.54	<.001	-169.00 $\pm$ 19.78	-8.54	<.001
Mean tail length	-44.56 $\pm$ 45.10	-0.99	.33	-33.83 $\pm$ 16.36	-2.07	.042
Delta tail length	15.48 $\pm$ 16.13	0.96	.34	—	—	—
Sex:age	15.84 $\pm$ 67.71	0.23	.82	—	—	—
Mean tail length:sex	12.12 $\pm$ 48.25	0.25	.80	—	—	—
Delta tail length:sex	-16.46 $\pm$ 17.39	-0.95	.35	—	—	—
Random: bird ID	var. = 1,118,988 (SD = 1058)			var. = 1,167,387 (SD = 1080)		

Note: Tail length was partitioned into mean and delta tail length to reduce collinearity with age, with the former being centred within sex. Sex was encoded as a dummy variable (0 for females and 1 for males). The full model with interactions is on the left, and the simplified minimal adequate model on the right. The full model was significantly different from the null model ( $\chi^2 = 62.57$ ,  $p < .001$ ).

Abbreviations: SD, standard deviation; SE, standard error; var., variance.

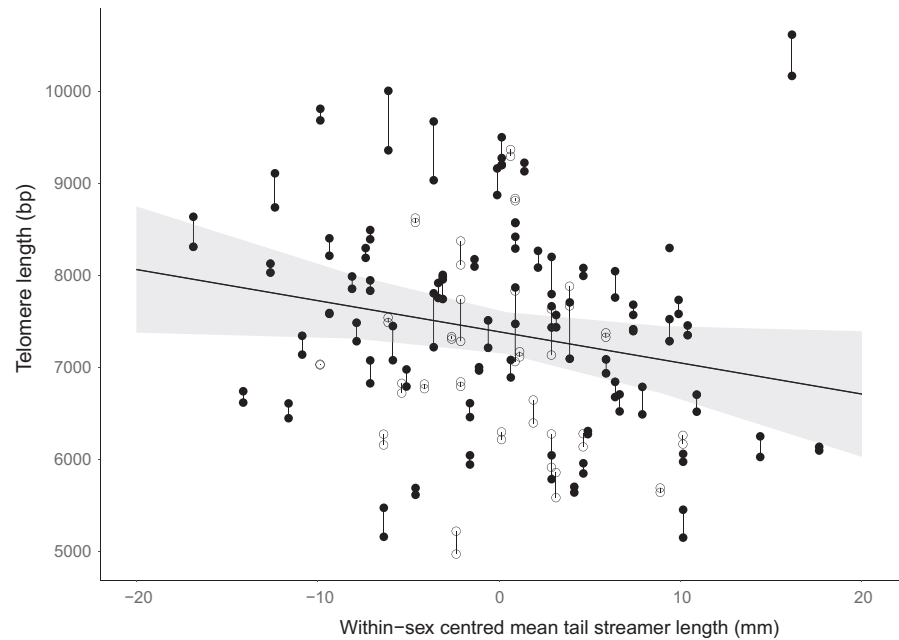
The within-individual consistency in TL we observed (97%) is among the highest values reported in birds (Kärkkäinen et al., 2021), and is within the range of values reported for jackdaws (*Corvus monedula*; Boonekamp et al., 2017), Cory's shearwater (*Calonectris borealis*; Bauch et al., 2020) and Japanese quail (*Coturnix japonica*; Stier et al., 2020). It appears that almost all between-individual variation in TL in our study population arises before the first breeding season with relatively subtle changes in TL later in life. Indeed, individual variation may largely be caused by parental inheritance as both paternal and maternal (epi)genetic effects on offspring TL have been reported in other species (Bauch et al., 2019, 2021; Reichert et al., 2015; Sparks et al., 2020; Vedder et al., 2021). Additionally, adult TL may reflect early-life conditions during the period of rapid nestling growth, mediated, amongst other things, by brood size (Costanzo, Parolini, et al., 2017).

Even with high individual repeatability, TL declined significantly with age, both cross-sectionally and longitudinally, which contrasts with the nonsignificant age effect in another barn swallow population using cross-sectional TL data (Khorauli et al., 2017). Considering the differences in the study design, it appears that it is essential to use a high-precision method for measuring TL to be able to detect the relatively small age effects (Lindrose et al., 2021).

**TABLE 3** Binary linear model of telomere length associations with the breeding/non-breeding status of “young” birds in their first breeding season ( $n = 98$ )



**FIGURE 2** The association of telomere length and tail streamer length in “young” birds in their first and second breeding season. Individual mean tail streamer length was centred within each sex. The two measurements of telomere length from a single individual are connected with a vertical line showing also the age effect on TL. The slope was similar in both sexes (open circles: females; filled circles: males). The shaded area shows 95% CI; for statistics see [Table 4](#)



**TABLE 5** Telomere dynamics (RTM-corrected change in TL between ages 1 and 2 years) in relation to tail streamer length in the first breeding season and its change to the second breeding season in “young” birds ( $n = 89$ )

Parameter	Estimate $\pm$ SE	t Value	p-Value
Intercept	$-154.79 \pm 36.31$	-4.26	<.001
Sex	$-4.68 \pm 42.82$	-0.11	.91
Initial tail length	$-12.87 \pm 7.80$	-1.65	.10
Change in tail length	$16.48 \pm 15.88$	1.04	.30
Initial tail length:sex	$13.09 \pm 8.36$	1.57	.12
Change in tail length:sex	$-19.12 \pm 17.11$	-1.12	.27

Note: Sex was encoded as a dummy variable (0 for females and 1 for males) and tail variables were mean centred within sex. The full model did not differ significantly from the null model ( $F = 0.88$ ,  $p = .50$ ).

Abbreviations: SE, standard error.

Despite the observed tendency of selective disappearance of individuals with longer telomeres from the population suggested by the age-centring approach, neither TL nor telomere attrition predicted survival probability and remaining lifespan. No apparent link between telomeres and survival was reported also in another barn swallow population (Caprioli et al., 2013). As in many other small-bodied passerine species, mortality in barn swallow might mainly be caused by extrinsic factors (e.g., predation, poor weather, food shortage; Wasser & Sherman, 2010), which would mask the effects of individual variation in condition and state, including TL, on survival. Early-life rather than adult TL may predict individual lifespan, as has been shown in a captive population of zebra finches (Heidinger et al., 2012), but no such pattern was detected in free-living barn swallows (Caprioli et al., 2013), further supporting the need to eliminate the extrinsic mortality factors in this type of analysis.

In total, 29% of yearlings did not initiate a nesting attempt in their first breeding season, but variation in breeding activity was not reflected in TL. There are several potential explanations why we did not detect any association between TL and delayed reproduction. First, due to the longitudinal sampling study design, we do not have information about TL in individuals that did not survive their first breeding season (our data set contained about 37% of nonbreeding individuals compared to 29% in the whole population). Second, the majority (79%) of nonbreeding yearlings are males, suggesting that the sex ratio is slightly biased in the population, and thus for at least part of the nonbreeding males the delayed reproduction may reflect the inability to find a mate rather than a reproductive strategy.

Secondary sexual traits (ornaments) are thought to be honest signals reflecting individual quality, with their honesty partly rooted in the fitness costs associated with carrying more elaborate ornaments (see Vitousek et al., 2016). In line with these assumed costs, we found that TL was negatively associated with tail streamer length, a secondary sexual ornament in European populations of barn swallows (Møller, 1988, see Pap et al., 2019 and Wilkins et al., 2016 for our study population). Because individuals with longer tail streamers had shorter telomeres, our findings are in agreement with elongated tail streamers being costly. Although there was no clear association between tail streamer length and telomere dynamics itself, this effect may have been undetectable due to the very high within-individual year to year repeatability of tail streamer length. An experimental study manipulating the length of tail streamers in barn swallows showed that in both sexes, tail streamers exceed the aerodynamic optimum by  $\sim 12$  mm (Rowe et al., 2001). Tail streamer elongation through sexual selection thus seems to have evolved into a costly handicap in barn swallows (Rowe et al., 2001). Moreover, males with long tail streamers in our population had greater levels of oxidative damage than males with shorter streamers (Vitousek et al., 2016). Balancing these costs, the assortative mating based on tail

streamer length or simply a preference for longer-streamered partners may confer a selective advantage to the long-tailed individuals to both sexes, but particularly males (Møller, 1993). Although we did not find evidence for assortative mating with regard to tail streamer length in our barn swallow population (Wang et al., 2019), males with long tail streamers seem to pair earlier (Pap et al., 2019; Wilkins et al., 2016), possibly resulting in their greater reproductive output (Møller, 1988; Saino et al., 1997; Vortman et al., 2011).

In conclusion, our longitudinal study revealed that TL is a highly consistent individual trait in adult barn swallows. TL declined with age but was not associated with delayed reproduction, survival or remaining lifespan. However, we revealed a negative association between TL and tail streamer length, the consequences of which remain to be identified, given that we found no link between TL and some major fitness components (survival and delayed reproduction). As our results suggest that most individual variation in TL is probably determined before the first breeding season, future studies should attempt to link the development of secondary ornamentation, such as tail streamer length, with telomere dynamics over the crucial first year of life. Genetic correlation between TL and sexual ornamentation is another direction worth exploring. In addition, further investigation of fitness-related traits, including individual performance later in life, in association with telomeres is needed to evaluate the importance of TL in connection with life history traits in the barn swallow and other short-lived species.

#### ACKNOWLEDGEMENTS

We are grateful to all students who helped with sample collection in the field. This study was funded by the Czech Science Foundation through projects GA19-22538S and GA21-22160S. T.K. was financially supported by the Traineeship Mobility Programme Erasmus+.

#### CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

#### AUTHOR CONTRIBUTIONS

T.A. and S.V. designed the study. T.K., O.T. and T.A. collected the samples and data in the field, T.K. and E.M. performed the laboratory analysis, T.K. performed data analyses and wrote the manuscript together with T.A., O.T. and S.V.; all authors commented on the manuscript.

#### DATA AVAILABILITY STATEMENT

Additional supporting information may be found online in the Supporting Information section at the end of the article. The data that support the findings of this study are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.0vt4b8h1h>.

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**How to cite this article:** Kauzálová, T., Tomášek, O., Mulder, E., Verhulst, S., & Albrecht, T. (2022). Telomere length is highly repeatable and shorter in individuals with more elaborate sexual ornamentation in a short-lived passerine. *Molecular Ecology*, 31, 6172–6183. <https://doi.org/10.1111/mec.16397>