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Sperm production is negatively associated with muscle and sperm telomere length in a species subjected to strong sperm competition

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Abstract

Life-history theory suggests that ageing is one of the costs of reproduction. Accordingly, a higher reproductive allocation is expected to increase the deterioration of both the somatic and the germinal lines through enhanced telomere attrition. In most species, males' reproductive allocation mainly regards traits that increase mating and fertilization success, that is sexually selected traits. In this study, we tested the hypothesis that a higher investment in sexually selected traits is associated with a reduced relative telomere length (RTL) in the guppy (*Poecilia reticulata*), an ectotherm species characterized by strong pre- and postcopulatory sexual selection. We first measured telomere length in both the soma and the sperm over guppies' lifespan to see whether there was any variation in telomere length associated with age. Second, we investigated whether a greater investment in pre- and postcopulatory sexually selected traits is linked to shorter telomere length in both the somatic and the sperm germinal lines, and in young and old males. We found that telomeres lengthened with age in the somatic tissue, but there was no age-dependent variation in telomere length in the sperm cells. Telomere length in guppies was significantly and negatively correlated with sperm production in both tissues and life stages considered in this study. Our findings indicate that telomere length in male guppies is strongly associated with their reproductive investment (sperm production), suggesting that a trade-off between reproduction and maintenance is occurring at each stage of males' life in this species.

KEYWORDS

reproductive investment, sexual selection, sperm competition, telomere attrition, telomere dynamics, trade-off theory

1 | INTRODUCTION

Telomeres are repetitive sequences of DNA located at the end of the eukaryotic chromosomes (Blackburn, 1991). These noncoding regions assemble with the telomere-binding proteins, and as a single DNA lagging strand complex, form a cap at the end of the chromosome (Armanios & Blackburn, 2012). The telomere complex, on one hand, protects the coding sequence from attrition, on the other hand, sets a limitation for the cell replicative potential, therefore acting as a 'mitotic clock' (Olovnikov, 1996).

Telomeres shorten with each round of somatic cell division because the DNA polymerase is unable to completely replicate the lagging strand, a phenomenon known as the end of replication problem (Watson, 1972). Telomere attrition is not only a by-product of cell division, but it is also affected by environmental stressors (Chatelain et al., 2020). The telomere sequence is in fact enriched with guanine nucleotides, which are particularly vulnerable to oxidative stress in both somatic and germinal lines (Barnes et al., 2019; Bekaert et al., 2004; Friesen et al., 2020). Regardless of the mechanism involved in telomere erosion, cellular senescence is triggered when telomere length (TL) falls below a certain threshold, the so-called Hayflick limit (Hayflick, 1965). Telomere attrition, on the contrary, can be reduced by the action of the reverse transcriptase telomerase that adds repetitive units of DNA to the telomeric region at each round of cell division (Zvereva et al., 2010). Furthermore, telomere lengthening can rarely occur through recombination between sister telomeres, a mechanism known as alternative lengthening of telomeres (ALT) (Kass-Eisler & Greider, 2000; Liu et al., 2007). Maintaining a balanced TL is critical because abnormal telomere shortening or elongation rates are linked to dysfunctional phenotypes (Vaiserman & Krasnienkov, 2021).

As the rate of telomere attrition affects cellular senescence and death, TL is considered a hallmark of aging (López-Otín et al., 2013). Although there is evidence of a negative correlation between TL and individual longevity in several species, this correlation appears to be weak according to a meta-analysis that compares telomere attrition patterns across non-human vertebrates (Remot et al., 2022). In fact, telomere dynamics (i.e. the rate of TL variation across lifespan) are highly variable among taxa, and wide variability exists also within species and between tissues. Such variation suggests that the rate of telomere erosion does not follow a universal pattern but may rather be shaped by species-specific selective pressure according to life-history strategies (Monaghan & Haussmann, 2006; Olsson et al., 2018; Remot et al., 2022). Along the slow-fast continuum of life-history strategies (Dantzer & Fletcher, 2015), fast-living species have a short lifespan, early sexual maturation and high fecundity. In contrast, slow-living species have a long lifespan, late sexual maturation and low fecundity (Stearns, 1992).

The evolution of life-history traits depends on the resource allocation strategy adopted by a population in which a finite budget of resources is expected to be traded-off between growth and reproduction (Kirkwood & Austad, 2000). Evidence that a greater allocation in reproduction is counterbalanced by a shorter longevity has been confirmed by numerous empirical studies that investigated the role of the reproductive load on the ageing rate (Candolin, 1998; Hunt et al., 2004; Lemaître et al., 2020; Pike et al., 2007; Preston et al., 2011; Robinson et al., 2006; van Voorhies, 1992). If an increased reproductive investment results in a decreased somatic maintenance and vice versa, individual phenotypic variability should arise consistently with the allocation strategy adopted (Stearns, 1992), including individual variation in TL (Tarka et al., 2018).

The cost of reproduction is expected to be greater in the sex (usually male) that is under stronger sexual selection. Indeed, male competition for the access to the females led to the evolution of costly traits that contribute respectively to increased mating (precopulatory traits, i.e. ornaments and weapons) and fertilization (postcopulatory traits, i.e. ejaculate features) success (Andersson & Simmons, 2006). The relative investment in pre-versus postcopulatory traits can vary according to the mating system. In promiscuous species, males allocate more resources on traits that ensure fertilization (postcopulatory traits) since they face strong sperm competition. In contrast, when female mating rate is low, males should increase the investment on precopulatory traits in order to secure mating (Parker & Pizzari, 2010; Puniamoorthy et al., 2012; Simmons & Emlen, 2006). Allocation in both pre- and postcopulatory traits can come at the cost of higher production of reactive oxygen species (ROS), a source of telomere erosion (Kawanishi & Oikawa, 2004; Monaghan et al., 2009). As expected, the investment in precopulatory traits, such as head coloration in the Australian painted dragon, or tail length in the European barn swallow, is negatively correlated with somatic telomere attrition in individuals with more prominent ornamentation (Kauzálová et al., 2022; Rollings et al., 2017). Consistently, TL negatively correlates with male gonad size in the Atlantic silversides (Gao & Munch, 2015) and with sperm velocity in lizards (Friesen et al., 2020), suggesting that the postcopulatory investment leads to a heightened telomere erosion as well. Indeed, enhanced allocation on postcopulatory traits, such as sperm production, can occur through faster spermatogenesis (Ramm et al., 2014). This process implies a higher rate of cellular division, and thus greater telomere erosion in the germinal line, with potential repercussion on fertility (Cariati et al., 2016; Thilagavathi et al., 2013).

Consequently, a trade-off between the investment in sexually selected traits and the rate of telomere erosion is expected to occur in both the somatic and the germinal lines, particularly in species subjected to strong sexual selection, where the investment in sexually selected traits is high and trade-offs with maintenance (including TL) are more pronounced.

The guppy (*Poecilia reticulata*) is a classical model species for sexual selection studies. Males exhibit orange-coloured spots that positively affect male mating success (Houde, 1997). These carotenoid-based ornaments are costly to produce, as indicated by the fact that their expression is condition-dependent (Andersson, 1986; Grether et al., 2004; Locatello et al., 2006; Nicoletto & Kodric-Brown, 1999). Furthermore, the high level of polyandry typical of this species foresees strong sperm competition. Increased sperm production is the most common evolutionary response to increased levels of sperm competition (Lüpold et al., 2020). Guppies make no exception, and the number of sperm transferred during copulation is the best predictor of fertilization success (Boschetto et al., 2011). Male guppies indeed evolved large sperm reserves that allow them to successfully inseminate several females consecutively (Magris et al., 2020). Once depleted, sperm reserves are restored in a few days (Kuckuck & Greven, 1997). As a result, the ejaculate production represents a significant component of the reproductive budget of an individual and shows stronger condition dependence than other sexually selected traits (Devigili et al., 2017; Gasparini et al., 2013). Male guppies show senescence for both male orange colours and ejaculate traits, but aging is faster for precopulatory traits (Gasparini et al., 2010, 2019).

Here, we aim to test whether any variation in TL is associated to either age (telomere dynamics) or pre- and postcopulatory sexually selected traits investment in male guppies. We did the following predictions: (i) to observe little variation in the TL of somatic and sperm cells as a function of age in guppies according to previous findings on short-lived ectotherm species (Harel et al., 2015; Lund et al., 2009; Olsson et al., 2018; Remot et al., 2022); and (ii) if a higher investment in sexually selected traits is traded-off against maintenance, males with enhanced sexual traits will have shorter telomeres, supporting the trade-off hypothesis (TOH).

We first estimated telomere dynamics in the somatic and in the sperm cells in order to disclose any evidence of somatic or reproductive aging in this species. We thus measured somatic TL at birth (undetermined sex), at 5 ± 1 (full adult males) and at 12 ± 1 months (old males). We further measured sperm TL at 5 ± 1 and 12 ± 1 months. Second, we assessed the relationship between the investment in pre- (relative area of orange spots) and postcopulatory traits (relative sperm production) and TL in the somatic and in the sperm cells. Since a steeper decline in maintenance and reproduction appears with aging (Jones et al., 2014; Nussey et al., 2013), we estimated the relationship between the investment in sexually selected traits and somatic and sperm TL in both young and old males.

2 | MATERIALS AND METHODS

Guppies used in this experiment are descendants of a stock collected from Lower Tacarigua River in Trinidad. They are maintained as a self-sustained population at the Botanical Garden of the University of Padova where the experimental fish have been collected at the fry stage and subsequently acclimatized to the laboratory conditions in stock tanks.

2.1 | Experimental design

Fry were haphazardly collected from monitored stock tanks at 1 ± 1 days old to be subsequently euthanized with an overdose of MS222 (Matthews & Varga, 2012) and stored at −20°C in absolute ethanol until DNA extraction. Experimental males (5 ± 1 and 12 ± 1 months old) were selected haphazardly from the stock tanks. Guppy's sperm can undergo senescence when stored in the male gonads before mating (Gasparini et al., 2014); moreover, variation in TL has been found at different spermatogenesis stages (Fice & Robaire, 2019). To standardize any possible TL variation attributable to these processes, we stripped each male to equalize their initial sperm age (Gasparini et al., 2009). We then placed the males into individual tanks (2.5 L). At the age of 5 ± 1 months, all males are sexually mature full adult (Magurran., 2005), while at age 12 ± 1 months, males show reproductive ageing (Gasparini et al., 2019). After 4 days of isolation to allow replenishment of the ejaculate reserves, males have been photographed to assess the relative orange area (orange area over body area %), and then stripped to assess the relative sperm production (residuals of bundle count over body area). After phenotypic traits capture, males were euthanized with an overdose of MS222 and a sample of muscle and sperm were collected (protocol below) and stored in absolute EtOH at −20°C.

2.2 | Precopulatory trait measurement

Male body and orange area were captured under a ZEISS Stemi 2000-C stereomicroscope through a single sided digital photograph (Canon EOS 450D) after individual anaesthesia in MS222 (0.15 g/L) water-based solution (Chambel et al., 2015). The relative orange area (the orange area over the body area %) was measured with the ImageJ software (http://rsbweb.nih.gov/ij/download.html) (Evans et al., 2003).

2.3 | Postcopulatory trait measurement

Sperm collection and count occurred 4 days after a first strip procedure, a step made with the purpose of flattening sperm age variation (see above). After anaesthesia, each male was placed on a black slide in 800 μL of saline solution (NaCl 0.9%) under a stereomicroscope. His gonopodium was swung over a 180 degrees angle for four times, afterwards, a gentle pressure was applied to the abdomen cavity and sperm were released. Guppy's sperm are packaged in bundles, each carrying around 22.000 sperm cells (Cattelan et al., 2018). Bundles were photographed and counted with the ImageJ software. The relative sperm production was calculated as the standardized residuals of the bundles count (N_{bundles}) over the body area (mm^2).

For each male, 30 sperm bundles were collected and centrifuged at 5000 rpm in a refrigerated Beckman Coulter microfuge 20R at 4°C for 5 min, and the pellet stored in 50 μL of saline solution (NaCl 0.9%) at −20°C until needed.

2.4 | RTL measurement

A total of 115 somatic tissues (whole body 1 ± 1 days old = 27 and muscle 5 ± 1 = 62, 12 ± 1 = 26 months old), and 74 sperm (age: 5 ± 1 = 56, 12 ± 1 = 26) samples were collected. In the 12 ± 1 age class, both muscle and sperm tissues belong to the same males, while in the 5 ± 1 age class, muscle and sperm tissues have been sampled from independent males except for 11 males from which we collected both tissues. We adopted a cross-sectional design because the small size of male guppies makes euthanasia necessary for muscle collection.

Genomic DNA was extracted from muscle, and sperm using the Bio Basic EZ-10 Spin Column Genomic Minipreps kit for animal sample according to the manufacturer's protocol except for sperm samples for which the kit ACL lysis solution was replaced by 300 μL of RTL lysis buffer (Qiagen) and 3 μL of mercaptoethanol. Samples were eluted from the column with either 50 μL (muscle) or 20 μL (sperm) of the kit Elution buffer. DNA quality was checked with a Nanodrop ND-2000 C spectrophotometer (Thermo Scientific, USA) for 260/280 ratio greater than 1.8 and 260/230 ratio greater than 1.9. Quantity was measured with the Qubit (Invitrogen) using the AccuGreen Broad Range dsDNA quantification kit (Biotium). Samples were diluted at the concentration of 2.5 ng/μL. Relative telomere length was measured using real time qPCR (Cawthon, 2002) technique that provides telomere quantity as ratio between the telomeric DNA and a nonvariable copy reference gene, here the melanocortin 1 receptor (Monteforte et al., 2020). We used the telomere primers Tel1b and Tel2b (Criscuolo et al., 2009) and the melanocortin 1 receptor MCR1-F and MC1-R primers (Monteforte et al., 2020). Amplification cocktail and protocol were the same as in Monteforte et al. (2020) and run in an Applied Biosystems™ 7500 Real-Time PCR System. Each plate contained three interpolate calibrators and a negative control, all run in triplicates. Baseline and cycle quantification (Cq) values were corrected using the LinRegPCR software ver. 2017.1 (Ruijter et al., 2009). Removal of between-run variation was obtained with Factor qPCR (Ruijter et al., 2015). Relative telomere length was obtained following the equation proposed by Pfaffl (Pfaffl, 2001) as reported in Monteforte et al. (2020). We set the acceptance threshold for amplifications efficiency of 100 ± 20%. Interassay coefficients of variation (CV) were 7.9% for telomere plates and 1.1% for MC1R plates, while intra-assay CVs were 1.4% for telomere plates and 0.65% for MC1R plates.

2.5 | Nonvariable copy reference gene assessment

The reference gene serves as a baseline for the quantification of RTL. For a correct esteem of RTL, the reference gene should present nonvariable copy number (non-VCN) in the genome. The melacortin 1 receptor (MC1R) used in this study has been conserved as a single copy gene in divergent fish species (Selz et al., 2007) as well as in *P. reticulata* according to the analysis of its genome (GCF_000633615.1) (Künstner et al., 2016). However, to further validate the MC1R is a non-VCN gene, we followed the method of Smith and colleagues (Smith et al., 2011). We established a panel of four non-VCN candidate genes on four different linkage groups (LG) of the guppy genome: the MC1R itself on LG3, the intermediate filament protein ON3-like on LG2, an uncharacterized protein on LG1 and the β actin on LG 8. The intermediate filament protein and the uncharacterized protein showed similar expression in high and low sperm guppy selected lines (Cattelan, Vidotto, et al., 2020), while the β actin is usually used as a housekeeping gene in gene expression analysis (e.g., Zhang et al., 2023). Locus ID, primers sequences and RT-qPCR profiles are reported in Table S1. We run a real-time qPCR on 15 muscle tissue's samples estimating the RTLs using our putative non-VCN genes as reference. We then estimated covariation in copy numbers through a correlation matrix, which measures the strength of pairwise linear relationships between RTLs of the candidate genes. Pearson's correlation coefficient ranges from 0.88 to 0.98 (Tables S2 and S3, Figure S1) indicating strong collinearity between all the genes we analysed and suggesting that in particular MC1R, the β actin and the uncharacterized protein do not present VCN and can be suitable reference genes for RTL quantification in guppies.

2.6 | Statistical analysis

We investigated telomere dynamics throughout guppy's lifespan in the somatic tissue running a linear regression model (LM) with RTL (log transformed to meet the model assumptions) as the dependent variable and age as the explanatory variable. We tested age classes pairwise comparison running a general contrast between factor levels. To quantify the magnitude of the difference between age classes RTL, we calculated the effect size according to Cohen's d metric. In order to investigate telomere dynamics in the sperm germinal line, we run a LM using sperm RTL (log transformed to meet the model assumptions) as the dependent variable and age as the explanatory variable. To seek any trade-off between sexually selected traits and TL, we correlated by mean of generalized linear mixed model (GLMM) the RTL as dependent variable with pre- (relative orange area) and postcopulatory (relative sperm production) traits, age (5 ± 1 and 12 ± 1 months) and tissue (muscle and sperm) as fixed predictors. Male identity was entered as random factor to account for repeated measures of TL between tissues within males. Starting from the full model, we removed one by one the least significant predictor to confirm the correlation robustness. The reduced models are presented in (Table S6). All analyses were conducted using Matlab version R2018a.

This research complies the ethical requirement and was approved by the Italian health ministry, authorization n. 624/2022-PR.

3 | RESULTS

3.1 | Somatic telomere dynamics

We investigated somatic telomere dynamics throughout guppies' lifespan comparing RTL between newborn, adult and old males. Relative telomeres length significantly increased in the somatic line as a function of age (Table 1, Figure 1). Mean RTL at birth was 1.131 (±0.726 SD). Muscle mean RTL was 1.577 (±0.908 SD) at

TABLE 1 Results from the linear regression model (LM) in which muscle log RTL (Relative Telomeres Length) was the dependent variable and age (1 ± 1 days old, 5 ± 1 and 12 ± 1 months) was the predictor.

Muscle RTL			
Fixed factor	Estimate	*t*	*p*
Age	.013	2.382	**.018**

Note: Significant terms in bold. $R^2 = 0.047$.

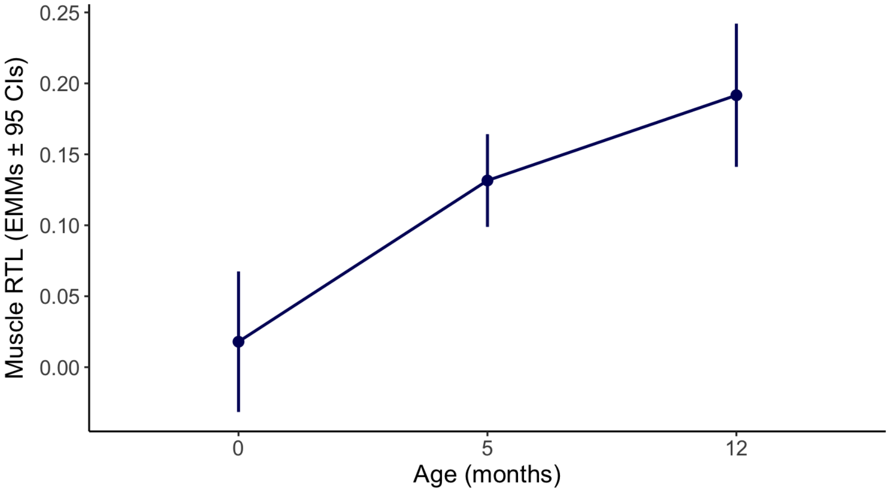

FIGURE 1 Estimated marginal means of muscle log relative telomeres length at different ages (1 ± 1 days old, 5 ± 1 and 12 ± 1 months) from the linear regression model (LM) in Table S1.

TABLE 2 Results from the linear regression model (LM) in which sperm log RTL (Relative Telomeres Length) was the dependent variable and age (5 ± 1 and 12 ± 1 months) was the predictor.

Sperm RTL			
Fixed factor	**Estimate**	***t***	***p***
Age	.003	.284	.776

Note: $R^2 = .001$.

5 ± 1 months and 1.868 (±1.221 SD) at 12 ± 1 months. The pairwise comparison between age classes confirms a significant RTL elongation between 1 ± 1 days old and 12 ± 1 months old ($p = 0.040$), while no significance difference was found between 1 ± 1 and 5 ± 1 ages ($p = 0.139$), and between 5 ± 1 and 12 ± 1 months ($p = 0.578$) (Table S4). We measured the effect size between age classes, and we found that RTL effect size between 1 ± 1 and 5 ± 1 (Cohen's $d = -0.242$) and between 5 ± 1 and 12 ± 1 (Cohen's $d = -0.288$) was small according to Cohen's indexing d, while the effect size tended to be medium when comparing newborn with old males (1 ± 1 and 12 ± 1, Cohen's $d = -0.436$) (Table S4).

3.2 | Sperm telomere dynamics

We further analysed telomere dynamics in sperm of full adult and old males. Sperm mean RTL ranged from 1.549 (±1.1654 SD) at 5 ± 1 months to 1.499 (±1.111 SD) at 12 ± 1 months. No significant difference in sperm RTL was found between adult and old males (Table 2, Figure 2). The effect size was consistently reporting a very small effect (Cohen's $d = 0.044$) (Table S5).

3.3 | Sexually selected traits investment and telomere length trade-off

We examined the relationship between somatic and sperm germinal line RTL with the investment in sexually selected traits (relative orange area, relative sperm production) to test whether the investment in pre- and postcopulatory sexually selected traits leads to enhanced telomere erosion. We analysed this relationship in both adult and old males to consider the putative effect of aging in telomere attrition. Relative telomeres length was significantly shorter and negatively correlated with relative sperm production in the somatic and in the sperm germinal lines independently of the age stage (Table 3, Figure 3). The significant association between relative sperm production and RTL persists when removing other predictors from the model (Table S6). None of the others sexual traits predicted RTL (Table 3). The significant term 'tissue' indicates that sperm RTL was consistently shorter than muscle RTL since no significant interaction of the predictors with the tissues was found (Table S7).

4 | DISCUSSION

4.1 | Somatic telomere dynamics

This study aimed to investigate whether the investment in pre- and postcopulatory sexually selected traits (orange coloration and sperm number) is associated with telomere length in both the somatic and the sperm cells. Since the cost of somatic and reproductive maintenance is expected to be greater with ageing, we first assessed telomere dynamic in the somatic and sperm cells; second, we investigated whether any relationship between telomere length and the investment in sexually selected traits was evident in both full adult and old stage of life in guppy males.

In order to study telomere dynamics, we measured guppy TL at three different ages, namely at birth (unsexed individuals), 5 (full adult males) and 12 months (senescent males). We found that somatic TL significantly increased with age. There are two possible explanations for this observation. First, since we adopted a cross-sectional design (i.e. sampling of different individuals at different ages), the increased TL as a function of age may be the consequence of the lack of individuals carrying shorter telomeres with ageing; hence, TL increment with age could be overestimated. Therefore,

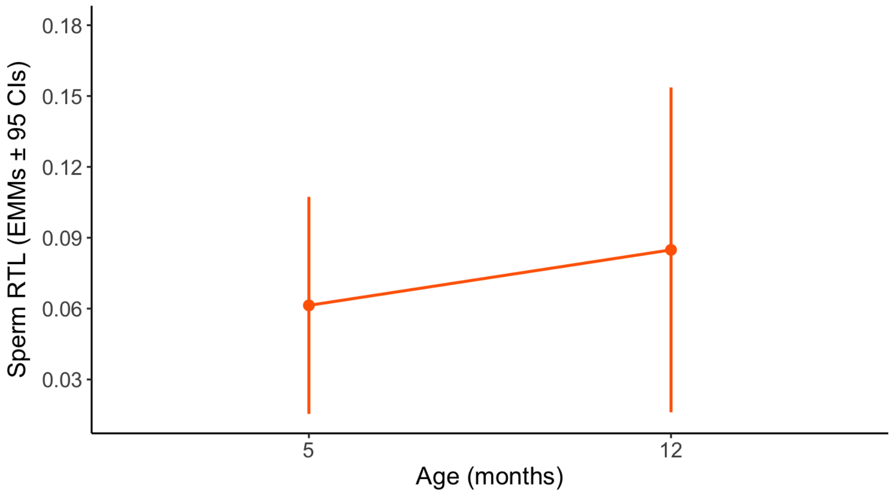

FIGURE 2 Estimated marginal means of sperm log relative telomeres length at different ages (5 ± 1 and 12 ± 1 months) from the linear regression model (LM in Table 2.

TABLE 3 Results from the generalized linear mixed model (GLMM) in which log RTL (Relative Telomeres Length) was the dependent variable and age (5 ± 1 and 12 ± 1 months), relative orange area (orange area on the body area %), relative sperm production (standardized residuals of sperm bundles count on the body area), and tissue (muscle or sperm) were the fixed factors and male ID was the random factor.

Full model	Estimate	*t*	*p*
Fixed factors			
Relative orange area	−.086	−0.133	.893
Age	−.016	−1.787	.076
Sperm production	−.094	−3.259	**.001**
Tissue	−.137	−2.394	**.018**
Random factor			
Male ID	.057		

Note: Significant terms are in bold.

we cannot exclude a bias due to the selective disappearance of males with shorter telomeres in the old class of age. However, no difference was found in the relationship between TL and age while comparing cross-sectional and longitudinal data in a meta-analysis (Remot et al., 2022).

An alternative explanation for the telomere elongation pattern found in guppies relies in the anticancer hypothesis which states that telomerase suppression in senescent stage of life occurs as a mechanism to prevent cancer formation (Gomes et al., 2011). This suppressed activity of telomerase is proposed to be an evolutionary consequence of higher metabolic and cellular division rates typical of endotherms species with determinate growth (Olsson et al., 2018). For this reason, telomeres shortening is less likely observed in small-bodied, short-lived ectotherm species (Olsson et al., 2018; Tian et al., 2018) where the telomerase enzyme is instead consistently expressed throughout lifespan (Anchelin et al., 2013; Lund et al., 2009). However, there is no unique pattern of telomere dynamics among taxa, as telomere elongation over the course of lifespan has been found even in some mammal species (Criscuolo et al., 2018; Hoelzl et al., 2016; Panasiak et al., 2020; Spurgin et al., 2018; Tissier et al., 2022) and no TL variation across ages has been found in fishes (Remot et al., 2022). These results support the idea that telomere dynamics could depend on species-specific life-history strategies rather than following a universal pattern of TL shortening.

4.2 | Sperm telomere dynamics

We measured sperm telomere dynamics from the ejaculate of fully adult and old male guppies. We found no statistically significant difference in sperm TL between these two age classes. This result suggests that in guppies, like in other species (Alibardi, 2015; Anchelin et al., 2013; Autexier & Lue, 2006; Harel et al., 2015), the activity of telomerase does not decline with age in proliferative tissues, including male gonads. Indeed, it has been shown that the telomerase is constitutively expressed throughout the lifespan in the gonads of several fish species, such as the lake trout *Salvelinus namaycush* (Purchase et al., 2022), the zebrafish *Danio rerio* (Anchelin et al., 2013) and the killifish *Nothobranchius furzeri* (Harel et al., 2015).

To date, telomere dynamics in sperm cells have been studied primarily in rodents and humans, where no consistent pattern of positive or negative relationship between TL and age has been found; however, sperm deterioration with age is a common phenomenon (Monaghan & Metcalfe, 2019). In guppies, sperm performance (swimming velocity and competitive fertilization success) does not significantly decline with age according to longitudinal studies (Gasparini et al., 2010, 2019). The absence of TL variation in sperm cells of adult and old males found in this study is therefore coherent with the lacking age-dependent decline observed in guppies' ejaculate traits. The evolutionary reasons why ejaculate traits age at a slower rate than other sexually selected traits remain unclear (Gasparini et al., 2019; Sanghvi et al., 2023).

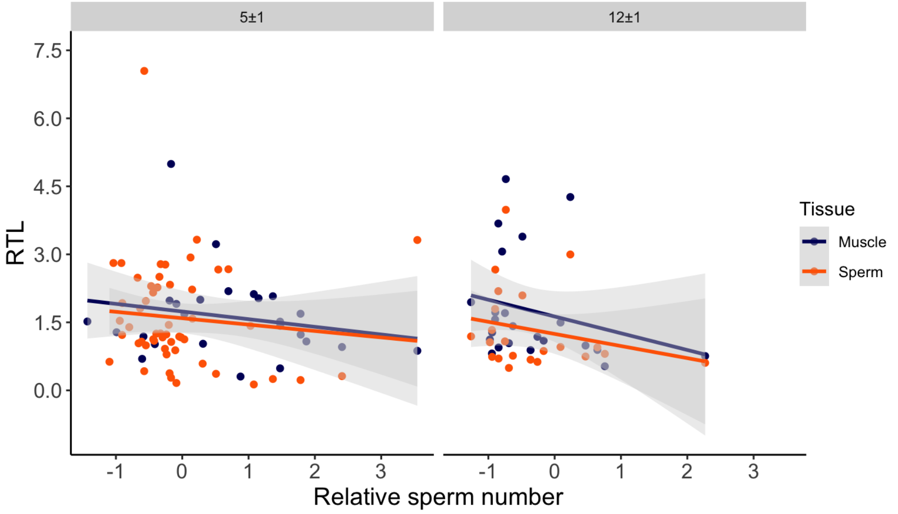

FIGURE 3 Correlation between log relative telomeres length (RTL) and relative sperm production across ages and tissues. Muscle RTL is reported in blue while sperm RTL is in orange.

4.3 | Sexually selected traits investment and telomere length trade-off

In order to reveal a trade-off between TL and male investment in sexually selected traits, we assessed whether males' orange coloration and sperm production were associated with TL variation in both the somatic and the sperm cells, and in full adult and senescent males. Our results indicate a potential trade-off between sperm production and TL is occurring in both somatic and sperm cells, and in both full adult and old males. Intriguingly, the area of orange (carotenoid) coloration did not predict TL in guppy, unlike what has been found in the Australian painted dragon (Giraudeau et al., 2016; Rollings et al., 2017). Guppies promiscuous mating system implies intense sperm competition and competitive fertilization success is indeed strongly predicted by the number of sperm transferred during mating (Boschetto et al., 2011); hence, selection for sperm production is expected to be stronger with respect to other sexual traits. Moreover, diet restriction has a stronger negative effect on sperm production than orange coloration (Cattelan, Evans, et al., 2020) indirectly suggesting that the physiological cost associated to sperm production is larger. The negative association between TL and sperm production, paralleled by the lack of association with orange coloration suggest that, in guppy, sperm production may have a larger cost even for TL maintenance. In this study, sperm production was negatively associated with TL in both adult and senescent males and in both muscle and sperm cells. The current literature suggests that males with high sperm production may pay two costs: One is a reduced longevity, as shorter somatic TL is expected to be associated with an increased ageing rate (reviewed in Lemaître et al., 2020; López-Otín et al., 2013). The second cost consists of a reduced sperm competition success, if sperm production is negatively associated with sperm quality (Friesen et al., 2020) and reproductive success (Pauliny et al., 2018). However, to confirm these predictions, a longitudinal study testing for both longevity and fertility is necessary. Both sperm production and TL have a high sire heritability (Chik et al., 2022; Eisenberg & Kuzawa, 2018; Gasparini et al., 2013; Vedder et al., 2022); hence, the investigation on the interplay between the two could reveal potential costs also in the offspring (Eisenberg & Kuzawa, 2018).

Finally, we find that TL is shorter in sperm than in the muscle tissue. The opposite pattern has been found in humans (reviewed in Eisenberg, 2011) and mice (Ramos-Ibeas et al., 2019), while there is no difference between blood and sperm TL in an ectothermic species (Friesen et al., 2020). It has been proposed that the evolution of long telomeres in sperm results from the selective pressure on the fitness cost of premature aging in species with an increased reproductive lifespan (Eisenberg, 2011). At the same time, in such species, telomerase suppression may have evolved in the soma for contrasting cancer formation (Eisenberg, 2011) leading to opposite telomere dynamics in the soma and in the sperm. On the contrary, in our study species, TL is shorter in sperm tissues with respect to the muscle. We can speculate that, on one hand, the intense sperm production typical of guppies could provoke enhanced telomeres attrition in sperm cell. On the contrary, selection on telomerase activity in the soma may be more relaxed in short-lived ectotherm species. If the diversity of telomere dynamics in somatic and gametic cells across different taxa is the result of selective pressures deriving from diversified life-history strategies would only be clarified be widening telomere dynamics investigation.

In conclusion, our findings suggest that male investment in sexually selected traits should be taken into account in the investigation of telomere dynamics since there is increasing evidence that telomere erosion may mediate the cost of reproduction in males (Friesen et al., 2020; Kauzálová et al., 2022; but see Taff & Freeman-Gallant, 2017). Especially in polyandrous species, in which postcopulatory sexual selection can be predominant, the association between sperm investment and TL can reveal hidden costs of the male reproductive output.

AUTHOR CONTRIBUTIONS

EM and AG designed the study. EM and SC collected the samples and the phenotypic data, EM and AG performed the DNA analysis, EM and AP performed the statistical analyses and EM wrote the manuscript with contribution from all authors.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available at http://doi.org/10.6084/m9.figshare.24183882.

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REFERENCES

Alibardi, L. (2015). Immunolocalization of the telomerase-1 component in cells of the regenerating tail, testis, and intestine of lizards. *Journal of Morphology*, *276*(7), 748–758. https://doi.org/10.1002/jmor.20375

Anchelin, M., Alcaraz-Pérez, F., Martínez, C. M., Bernabé-García, M., Mulero, V., & Cayuela, M. L. (2013). Premature aging in telomerase-deficient zebrafish. *Disease Models & Mechanisms*, *6*(5), 1101–1112. https://doi.org/10.1242/dmm.011635

Andersson, M. (1986). Evolution of condition-dependent sex ornaments and mating preferences: Sexual selection based on viability differences. *Evolution*, *40*(4), 804–816. https://doi.org/10.1111/j.1558-5646.1986.tb00540.x

Andersson, M., & Simmons, L. W. (2006). Sexual selection and mate choice. *Trends in Ecology & Evolution*, *21*(6), 296–302. https://doi.org/10.1016/j.tree.2006.03.015

Armanios, M., & Blackburn, E. H. (2012). The telomere syndromes. *Nature Reviews Genetics*, *13*, 693–704. https://doi.org/10.1038/nrg3246

Autexier, C., & Lue, N. F. (2006). The structure and function of telomerase reverse transcriptase. *Annual Review of Biochemistry*, *75*(1), 493–517. https://doi.org/10.1146/annurev.biochem.75.103004.142412

Barnes, R. P., Fouquerel, E., & Opresko, P. L. (2019). The impact of oxidative DNA damage and stress on telomere homeostasis. *Mechanisms of Ageing and Development*, *177*, 37–45. https://doi.org/10.1016/j.mad.2018.03.013

Bekaert, S., Derradji, H., & Baatout, S. (2004). Telomere biology in mammalian germ cells and during development. *Developmental Biology*, *274*(1), 15–30. https://doi.org/10.1016/j.ydbio.2004.06.023

Blackburn, E. H. (1991). Structure and function of telomeres. *Nature*, *350*(6319), 569–573. https://doi.org/10.1038/350569a0

Boschetto, C., Gasparini, C., & Pilastro, A. (2011). Sperm number and velocity affect sperm competition success in the guppy (*Poecilia reticulata*). *Behavioral Ecology and Sociobiology*, *65*(4), 813–821. https://doi.org/10.1007/s00265-010-1085-y

Candolin, U. (1998). Reproduction under predation risk and the trade-off between current and future reproduction in the threespine stickleback. *Proceedings of the Royal Society of London Series B: Biological Sciences*, *265*(1402), 1171–1175. https://doi.org/10.1098/rspb.1998.0415

Cariati, F., Jaroudi, S., Alfarawati, S., Raberi, A., Alviggi, C., Pivonello, R., & Wells, D. (2016). Investigation of sperm telomere length as a potential marker of paternal genome integrity and semen quality. *Reproductive Biomedicine Online*, *33*(3), 404–411. https://doi.org/10.1016/j.rbmo.2016.06.006

Cattelan, S., Di Nisio, A., & Pilastro, A. (2018). Stabilizing selection on sperm number revealed by artificial selection and experimental evolution. *Evolution*, *72*(3), 698–706. https://doi.org/10.1111/evo.13425

Cattelan, S., Evans, J. P., Garcia-Gonzalez, F., Morbiato, E., & Pilastro, A. (2020). Dietary stress increases the total opportunity for sexual selection and modifies selection on condition-dependent traits. *Ecology Letters*, *23*(3), 447–456. https://doi.org/10.1111/ele.13443

Cattelan, S., Vidotto, M., Devigili, A., Pilastro, A., & Grapputo, A. (2020). Differential gene regulation in selected lines for high and low sperm production in male guppies. *Molecular Reproduction and Development*, *87*(4), 430–441. https://doi.org/10.1002/mrd.23332

Cawthon, R. M. (2002). Telomere measurement by quantitative PCR. *Nucleic Acids Research*, *30*(10), e47. https://doi.org/10.1093/nar/30.10.e47

Chambel, J., Pinho, R., Sousa, R., Ferreira, T., Baptista, T., Severiano, V., Mendes, S., & Pedrosa, R. (2015). The efficacy of MS-222 as anaesthetic agent in four freshwater aquarium fish species. *Aquaculture Research*, *46*(7), 1582–1589. https://doi.org/10.1111/are.12308

Chatelain, M., Drobniak, S. M., & Szulkin, M. (2020). The association between stressors and telomeres in non-human vertebrates: A meta-analysis. *Ecology Letters*, *23*(2), 381–398. https://doi.org/10.1111/ele.13426

Chik, H. Y. J., Sparks, A. M., Schroeder, J., & Dugdale, H. L. (2022). A meta-analysis on the heritability of vertebrate telomere length. *Journal of Evolutionary Biology*, *35*(10), 1283–1295. https://doi.org/10.1111/jeb.14071

Criscuolo, F., Bize, P., Nasir, L., Metcalfe, N. B., Foote, C. G., Griffiths, K., Gault, E. A., & Monaghan, P. (2009). Real-time quantitative PCR assay for measurement of avian telomeres. *Journal of Avian Biology*, *40*(3), 342–347. https://doi.org/10.1111/j.1600-048X.2008.04623.x

Criscuolo, F., Smith, S., Zahn, S., Heidinger, B. J., & Haussmann, M. F. (2018). Experimental manipulation of telomere length: Does it reveal a corner-stone role for telomerase in the natural variability of individual fitness? *Philosophical Transactions of the Royal Society B: Biological Sciences*, *373*(1741), 20160440. https://doi.org/10.1098/rstb.2016.0440

Dantzer, B., & Fletcher, Q. E. (2015). Telomeres shorten more slowly in slow-aging wild animals than in fast-aging ones. *Experimental Gerontology*, *71*, 38–47. https://doi.org/10.1016/j.exger.2015.08.012

Devigili, A., Belluomo, V., Locatello, L., Rasotto, M. B., & Pilastro, A. (2017). Postcopulatory cost of immune system activation in *Poecilia reticulata*. *Ethology Ecology & Evolution*, *29*(3), 266–279. https://doi.org/10.1080/03949370.2016.1152305

Eisenberg, D. T. A. (2011). An evolutionary review of human telomere biology: The thrifty telomere hypothesis and notes on potential adaptive paternal effects. *American Journal of Human Biology*, *23*(2), 149–167. https://doi.org/10.1002/ajhb.21127

Eisenberg, D. T. A., & Kuzawa, C. W. (2018). The paternal age at conception effect on offspring telomere length: Mechanistic, comparative and adaptive perspectives. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *373*(1741), 20160442. https://doi.org/10.1098/rstb.2016.0442

Evans, J. P., Zane, L., Francescato, S., & Pilastro, A. (2003). Directional postcopulatory sexual selection revealed by artificial insemination. *Nature*, *421*(6921), 360–363. https://doi.org/10.1038/nature01367

Fice, H. E., & Robaire, B. (2019). Telomere dynamics throughout spermatogenesis. *Genes*, *10*(7), 525. https://doi.org/10.3390/genes10070525

Friesen, C. R., Rollings, N., Wilson, M., Whittington, C. M., Shine, R., & Olsson, M. (2020). Covariation in superoxide, sperm telomere length and sperm velocity in a polymorphic reptile. *Behavioral Ecology and Sociobiology*, *74*(6), 74. https://doi.org/10.1007/s00265-020-02855-8

Gao, J., & Munch, S. B. (2015). Does reproductive investment decrease telomere length in *Menidia menidia*? *PLoS ONE*, *10*(5), e0125674. https://doi.org/10.1371/journal.pone.0125674

Gasparini, C., Devigili, A., Dosselli, R., & Pilastro, A. (2013). Pattern of inbreeding depression, condition dependence, and additive genetic variance in Trinidadian guppy ejaculate traits. *Ecology and Evolution*, *3*(15), 4940–4953. https://doi.org/10.1002/ece3.870

Gasparini, C., Devigili, A., & Pilastro, A. (2019). Sexual selection and ageing: Interplay between pre- and post-copulatory traits senescence in the guppy. *Proceedings of the Royal Society B: Biological Sciences*, *286*(1897), 20182873. https://doi.org/10.1098/rspb.2018.2873

Gasparini, C., Kelley, J. L., & Evans, J. P. (2014). Male sperm storage compromises sperm motility in guppies. *Biology Letters*, *10*(11), 20140681. https://doi.org/10.1098/rsbl.2014.0681

Gasparini, C., Marino, I. A. M., Boschetto, C., & Pilastro, A. (2010). Effect of male age on sperm traits and sperm competition success in the guppy (*Poecilia reticulata*). *Journal of Evolutionary Biology*, *23*(1), 124–135. https://doi.org/10.1111/j.1420-9101.2009.01889.x

Gasparini, C., Peretti, A. V., & Pilastro, A. (2009). Female presence influences sperm velocity in the guppy. *Biology Letters*, *5*(6), 792–794. https://doi.org/10.1098/rsbl.2009.0413

Giraudeau, M., Friesen, C. R., Sudyka, J., Rollings, N., Whittington, C. M., Wilson, M. R., & Olsson, M. (2016). Ageing and the cost of maintaining coloration in the Australian painted dragon. *Biology Letters*, *12*(7), 20160077. https://doi.org/10.1098/rsbl.2016.0077

Gomes, N. M. V., Ryder, O. A., Houck, M. L., Charter, S. J., Walker, W., Forsyth, N. R., Austad, S. N., Venditti, C., Pagel, M., Shay, J. W., & Wright, W. E. (2011). Comparative biology of mammalian telomeres: Hypotheses on ancestral states and the roles of telomeres in longevity determination. *Aging Cell*, *10*(5), 761–768. https://doi.org/10.1111/j.1474-9726.2011.00718.x

Grether, G. F., Kasahara, S., Kolluru, G. R., & Cooper, E. L. (2004). Sex–specific effects of carotenoid intake on the immunological response to allografts in guppies (*Poecilia reticulata*). *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *271*(1534), 45–49. https://doi.org/10.1098/rspb.2003.2526

Harel, I., Benayoun, B. A., Machado, B., Singh, P. P., Hu, C.-K., Pech, M. F., Valenzano, D. R., Zhang, E., Sharp, S. C., Artandi, S. E., & Brunet, A. (2015). A platform for rapid exploration of aging and diseases in a naturally short-lived vertebrate. *Cell*, *160*(5), 1013–1026. https://doi.org/10.1016/j.cell.2015.01.038

Hayflick, L. (1965). The limited in vitro lifetime of human diploid cell strains. *Experimental Cell Research*, *37*(3), 614–636. https://doi.org/10.1016/0014-4827(65)90211-9

Hoelzl, F., Smith, S., Cornils, J. S., Aydinonat, D., Bieber, C., & Ruf, T. (2016). Telomeres are elongated in older individuals in a hibernating rodent, the edible dormouse (*Glis glis*). *Scientific Reports*, *6*(1), 36856. https://doi.org/10.1038/srep36856

Houde, A. E. (1997). *Sex, color, and mate choice in guppies*. Princeton University Press. https://press.princeton.edu/titles/6156.html

Hunt, J., Brooks, R., Jennions, M. D., Smith, M. J., Bentsen, C. L., & Bussière, L. F. (2004). High-quality male field crickets invest heavily in sexual display but die young. *Nature*, *432*(7020), 1024–1027. https://doi.org/10.1038/nature03084

Jones, O. R., Scheuerlein, A., Salguero-Gómez, R., Camarda, C. G., Schaible, R., Casper, B. B., Dahlgren, J. P., Ehrlén, J., García, M. B., Menges, E. S., Quintana-Ascencio, P. F., Caswell, H., Baudisch, A., & Vaupel, J. W. (2014). Diversity of ageing across the tree of life. *Nature*, *505*(7482), 169–173. https://doi.org/10.1038/nature12789

Kass-Eisler, A., & Greider, C. W. (2000). Recombination in telomere-length maintenance. *Trends in Biochemical Sciences*, *25*(4), 200–204. https://doi.org/10.1016/S0968-0004(00)01557-7

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

Kawanishi, S., & Oikawa, S. (2004). Mechanism of telomere shortening by oxidative stress. *Annals of the new York Academy of Sciences*, *1019*(1), 278–284. https://doi.org/10.1196/annals.1297.047

Kirkwood, T. B. L., & Austad, S. N. (2000). Why do we age? *Nature*, *408*(6809), 233–238. https://doi.org/10.1038/35041682

Kuckuck, C., & Greven, H. (1997). Notes on the mechanically stimulated discharge of spermiozeugmata in the guppy, *Poecilia reticulata*: A quantitative approach. *Zeitschrift Fur Fischkunde*, *4*, 73–88.

Künstner, A., Hoffmann, M., Fraser, B. A., Kottler, V. A., Sharma, E., Weigel, D., & Dreyer, C. (2016). The genome of the Trinidadian guppy, *Poecilia reticulata*, and variation in the Guanapo population. *PLoS ONE*, *11*(12), e0169087. https://doi.org/10.1371/journal.pone.0169087

Lemaître, J.-F., Gaillard, J.-M., & Ramm, S. A. (2020). The hidden ageing costs of sperm competition. *Ecology Letters*, *23*(11), 1573–1588. https://doi.org/10.1111/ele.13593

Liu, L., Bailey, S. M., Okuka, M., Muñoz, P., Li, C., Zhou, L., Wu, C., Czerwiec, E., Sandler, L., Seyfang, A., Blasco, M. A., & Keefe, D. L. (2007). Telomere lengthening early in development. *Nature Cell Biology*, *9*(12), 1436–1441. https://doi.org/10.1038/ncb1664

Locatello, L., Rasotto, M. B., Evans, J. P., & Pilastro, A. (2006). Colourful male guppies produce faster and more viable sperm. *Journal of Evolutionary Biology*, *19*(5), 1595–1602. https://doi.org/10.1111/j.1420-9101.2006.01117.x

López-Otín, C., Blasco, M. A., Partridge, L., Serrano, M., & Kroemer, G. (2013). The hallmarks of aging. *Cell*, *153*(6), 1194–1217. https://doi.org/10.1016/j.cell.2013.05.039

Lund, T. C., Glass, T. J., Tolar, J., & Blazar, B. R. (2009). Expression of telomerase and telomere length are unaffected by either age or limb regeneration in *Danio rerio*. *PLoS ONE*, *4*(11), e7688. https://doi.org/10.1371/journal.pone.0007688

Lüpold, S., de Boer, R. A., Evans, J. P., Tomkins, J. L., & Fitzpatrick, J. L. (2020). How sperm competition shapes the evolution of testes and sperm: A meta-analysis. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *375*(1813), 20200064. https://doi.org/10.1098/rstb.2020.0064

Magris, M., Zanata, I., Rizzi, S., Cattelan, S., & Pilastro, A. (2020). Trade-offs of strategic sperm adjustments and their consequences under phenotype–environment mismatches in guppies. *Animal Behaviour*, *166*, 171–181. https://doi.org/10.1016/j.anbehav.2020.06.016

Magurran, A. E. (2005). *Evolutionary ecology: The Trinidadian guppy*. Oxford University Press.

Matthews, M., & Varga, Z. M. (2012). Anesthesia and euthanasia in zebrafish. *ILAR Journal*, *53*(2), 192–204. https://doi.org/10.1093/ilar.53.2.192

Monaghan, P., & Haussmann, M. F. (2006). Do telomere dynamics link lifestyle and lifespan? *Trends in Ecology & Evolution*, *21*(1), 47–53. https://doi.org/10.1016/j.tree.2005.11.007

Monaghan, P., & Metcalfe, N. B. (2019). The deteriorating soma and the indispensable germline: Gamete senescence and offspring fitness. *Proceedings of the Royal Society B: Biological Sciences*, *286*(1917), 20192187. https://doi.org/10.1098/rspb.2019.2187

Monaghan, P., Metcalfe, N. B., & Torres, R. (2009). Oxidative stress as a mediator of life history trade-offs: Mechanisms, measurements and interpretation. *Ecology Letters*, *12*(1), 75–92. https://doi.org/10.1111/j.1461-0248.2008.01258.x

Monteforte, S., Cattelan, S., Morosinotto, C., Pilastro, A., & Grapputo, A. (2020). Maternal predator-exposure affects offspring size at birth but not telomere length in a live-bearing fish. *Ecology and Evolution*, *10*(4), 2030–2039. https://doi.org/10.1002/ece3.6035

Nicoletto, P. F., & Kodric-Brown, A. (1999). The relationship among swimming performance, courtship behavior, and carotenoid pigmentation of guppies in four rivers of Trinidad. *Environmental Biology of Fishes*, *55*(3), 227–235. https://doi.org/10.1023/A:1007587809618

Nussey, D. H., Froy, H., Lemaitre, J.-F., Gaillard, J.-M., & Austad, S. N. (2013). Senescence in natural populations of animals: Widespread evidence and its implications for bio-gerontology. *Ageing Research Reviews*, *12*(1), 214–225. https://doi.org/10.1016/j.arr.2012.07.004

Olovnikov, A. M. (1996). Telomeres, telomerase, and aging: Origin of the theory. *Experimental Gerontology*, *31*(4), 443–448. https://doi.org/10.1016/0531-5565(96)00005-8

Olsson, M., Wapstra, E., & Friesen, C. (2018). Ectothermic telomeres: It's time they came in from the cold. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *373*(1741), 20160449. https://doi.org/10.1098/rstb.2016.0449

Panasiak, L., Dobosz, S., & Ocalewicz, K. (2020). Telomere dynamics in the diploid and triploid rainbow trout (*Oncorhynchus mykiss*) assessed by Q-FISH analysis. *Genes*, *11*(7), 786. https://doi.org/10.3390/genes11070786

Parker, G. A., & Pizzari, T. (2010). Sperm competition and ejaculate economics. *Biological Reviews*, *85*(4), 897–934. https://doi.org/10.1111/j.1469-185X.2010.00140.x

Pauliny, A., Miller, E., Rollings, N., Wapstra, E., Blomqvist, D., Friesen, C. R., & Olsson, M. (2018). Effects of male telomeres on probability of paternity in sand lizards. *Biology Letters*, *14*(8), 20180033. https://doi.org/10.1098/rsbl.2018.0033

Pfaffl, M. W. (2001). A new mathematical model for relative quantification in real-time RT–PCR. *Nucleic Acids Research*, *29*(9), e45.

Pike, T. W., Blount, J. D., Bjerkeng, B., Lindström, J., & Metcalfe, N. B. (2007). Carotenoids, oxidative stress and female mating preference for longer lived males. *Proceedings of the Royal Society B: Biological Sciences*, *274*(1618), 1591–1596. https://doi.org/10.1098/rspb.2007.0317

Preston, B. T., Jalme, M. S., Hingrat, Y., Lacroix, F., & Sorci, G. (2011). Sexually extravagant males age more rapidly. *Ecology Letters*, *14*(10), 1017–1024. https://doi.org/10.1111/j.1461-0248.2011.01668.x

Puniamoorthy, N., Blanckenhorn, W. U., & Schäfer, M. A. (2012). Differential investment in pre- vs. post-copulatory sexual selection reinforces a cross-continental reversal of sexual size dimorphism in *Sepsis punctum* (Diptera: Sepsidae). *Journal of Evolutionary Biology*, *25*(11), 2253–2263. https://doi.org/10.1111/j.1420-9101.2012.02605.x

Purchase, C. F., Rooke, A. C., Gaudry, M. J., Treberg, J. R., Mittell, E. A., Morrissey, M. B., & Rennie, M. D. (2022). A synthesis of senescence predictions for indeterminate growth, and support from multiple tests in wild lake trout. *Proceedings of the Royal Society B: Biological Sciences*, *289*(1966), 20212146. https://doi.org/10.1098/rspb.2021.2146

Ramm, S. A., Schärer, L., Ehmcke, J., & Wistuba, J. (2014). Sperm competition and the evolution of spermatogenesis. *MHR: Basic Science of Reproductive Medicine*, *20*(12), 1169–1179. https://doi.org/10.1093/molehr/gau070

Ramos-Ibeas, P., Pericuesta, E., Peral-Sanchez, I., Heras, S., Laguna-Barraza, R., Pérez-Cerezales, S., & Gutiérrez-Adán, A. (2019). Longitudinal analysis of somatic and germ-cell telomere dynamics in outbred mice. *Molecular Reproduction and Development*, *86*(8), 1033–1043. https://doi.org/10.1002/mrd.23218

Remot, F., Ronget, V., Froy, H., Rey, B., Gaillard, J.-M., Nussey, D. H., & Lemaitre, J.-F. (2022). Decline in telomere length with increasing age across nonhuman vertebrates: A meta-analysis. *Molecular Ecology*, *31*(23), 5917–5932. https://doi.org/10.1111/mec.16145

Robinson, M. R., Pilkington, J. G., Clutton-Brock, T. H., Pemberton, J. M., & Kruuk, L. E. B. (2006). Live fast, die young: Trade-offs between fitness components and sexually antagonistic selection on weaponry in Soay sheep. *Evolution*, *60*(10), 2168–2181. https://doi.org/10.1111/j.0014-3820.2006.tb01854.x

Rollings, N., Friesen, C. R., Sudyka, J., Whittington, C., Giraudeau, M., Wilson, M., & Olsson, M. (2017). Telomere dynamics in a lizard with morph-specific reproductive investment and self-maintenance. *Ecology and Evolution*, *7*(14), 5163–5169. https://doi.org/10.1002/ece3.2712

Ruijter, J. M., Ramakers, C., Hoogaars, W. M. H., Karlen, Y., Bakker, O., Hoff, V. D., van den Hoff, M. J., & Moorman, A. F. (2009). Amplification efficiency: Linking baseline and bias in the analysis of quantitative PCR data. *Nucleic Acids Research*, *37*(6), e45. https://doi.org/10.1093/nar/gkp045

Ruijter, J. M., Ruiz Villalba, A., Hellemans, J., Untergasser, A., & van den Hoff, M. J. B. (2015). Removal of between-run variation in a multi-plate qPCR experiment. *Biomolecular Detection and Quantification*, *5*, 10–14. https://doi.org/10.1016/j.bdq.2015.07.001

Sanghvi, K., Vega-Trejo, R., Nakagawa, S., Gascoigne, S. J. L., Johnson, S., Salguero-Gómez, R., Pizzari, T., & Sepil, I. (2023). No general effects of advancing male age on ejaculates: A meta-analysis across the animal kingdom. *bioRxiv*. https://doi.org/10.1101/2023.04.14.536443

Selz, Y., Braasch, I., Hoffmann, C., Schmidt, C., Schultheis, C., Schartl, M., & Volff, J.-N. (2007). Evolution of melanocortin receptors in teleost fish: The melanocortin type 1 receptor. *Gene*, *401*(1), 114–122. https://doi.org/10.1016/j.gene.2007.07.005

Simmons, L. W., & Emlen, D. J. (2006). Evolutionary trade-off between weapons and testes. *Proceedings of the National Academy of Sciences*, *103*(44), 16346–16351. https://doi.org/10.1073/pnas.0603474103

Smith, S., Turbill, C., & Penn, D. J. (2011). Chasing telomeres, not red herrings, in evolutionary ecology. *Heredity*, *107*(4), 372–373. https://doi.org/10.1038/hdy.2011.14

Spurgin, L. G., Bebbington, K., Fairfield, E. A., Hammers, M., Komdeur, J., Burke, T., Dugdale, H. L., & Richardson, D. S. (2018). Spatio-temporal variation in lifelong telomere dynamics in a long-term ecological study. *Journal of Animal Ecology*, *87*(1), 187–198. https://doi.org/10.1111/1365-2656.12741

Stearns, S. C. (1992). *The evolution of life histories*. Oxford University Press.

Taff, C. C., & Freeman-Gallant, C. R. (2017). Sexual signals reflect telomere dynamics in a wild bird. *Ecology and Evolution*, *00*, 1–7. https://doi.org/10.1002/ece3.2948

Tarka, M., Guenther, A., Niemelä, P. T., Nakagawa, S., & Noble, D. W. A. (2018). Sex differences in life history, behavior, and physiology along a slow-fast continuum: A meta-analysis. *Behavioral Ecology and Sociobiology*, *72*(8), 132. https://doi.org/10.1007/s00265-018-2534-2

Thilagavathi, J., Kumar, M., Mishra, S. S., Venkatesh, S., Kumar, R., & Dada, R. (2013). Analysis of sperm telomere length in men with idiopathic infertility. *Archives of Gynecology and Obstetrics*, *287*(4), 803–807. https://doi.org/10.1007/s00404-012-2632-8

Tian, X., Doerig, K., Park, R., Qin, A. C. R., Hwang, C., Neary, A., Gilbert, M., Seluanov, A., & Gorbunova, V. (2018). Evolution of telomere maintenance and tumour suppressor mechanisms across mammals. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *373*(1741), 20160443. https://doi.org/10.1098/rstb.2016.0443

Tissier, M. L., Bergeron, P., Garant, D., Zahn, S., Criscuolo, F., & Réale, D. (2022). Telomere length positively correlates with pace-of-life in a sex- and cohort-specific way and elongates with age in a wild mammal. *Molecular Ecology*, *31*(14), 3812–3826. https://doi.org/10.1111/mec.16533

Vaiserman, A., & Krasnienkov, D. (2021). Telomere length as a marker of biological age: State-of-the-art, open issues, and future perspectives. *Frontiers in Genetics*, *11*, 630186. https://doi.org/10.3389/fgene.2020.630186 van Voorhies, W. A. (1992). Production of sperm reduces nematode lifespan. *Nature*, *360*(6403), 456–458. https://doi.org/10.1038/360456a0

Vedder, O., Moiron, M., Bichet, C., Bauch, C., Verhulst, S., Becker, P. H., & Bouwhuis, S. (2022). Telomere length is heritable and genetically correlated with lifespan in a wild bird. *Molecular Ecology*, *31*(23), 6297–6307. https://doi.org/10.1111/mec.15807

Watson, J. D. (1972). Origin of concatemeric T7DNA. *Nature New Biology*, *239*(94), 197–201. https://doi.org/10.1038/newbio239197a0

Zhang, C., Ma, J., Qi, Q., Xu, M., & Xu, R. (2023). Effects of ammonia exposure on anxiety behavior, oxidative stress and inflammation in guppy (*Poecilia reticulate*). *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *265*, 109539. https://doi.org/10.1016/j.cbpc.2022.109539

Zvereva, M. I., Shcherbakova, D. M., & Dontsova, O. A. (2010). Telomerase: Structure, functions, and activity regulation. *Biochemistry. Biokhimiia*, *75*(13), 1563–1583. https://doi.org/10.1134/s0006297910130055

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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