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Contents

Summary

Telomeres, the DNA sequences capping the ends of linear chromosomes, are ancient molecular structures that are shared among most eucaryotes. The telomeres protect chromosomes from degradation and are vital to genome integrity. Telomeres shorten with each cell division, and due to oxidative stress-inducing processes. Short telomeres are predictive of poor health and reduced survival in some animals. Thus, telomere length (TL) may underpin life-history trade-offs between growth, survival, and reproduction, or act as a biomarker of individual quality or pace-of-life. However, the ecological and evolutionary role of TL in shaping variation in life-history traits in wild animals is not well-known. In this thesis, I investigated causes and consequences of TL variation in populations of wild house sparrows.

I found that TL was negatively associated with body size both during an artificial size selection experiment and in natural populations. However, this association was not due to a negative genetic correlation between TL and body size. There was a small heritable component of early-life TL, but variation in TL among individuals was mainly driven by environmental (annual) variance. Inbred sparrows had short telomeres. Furthermore, I identified several novel putative genes underlying variation in TL. Early-life TL was shaped by effects of weather conditions and population density, but there was no strong evidence for associations between TL and fitness components. Individuals with shorter early-life TL tended to have higher reproductive output and higher chances of successful dispersal as expected if TL reflects pace-of-life syndromes. TL generally shortened with age but was apparently elongated within some individuals. There was a moderate heritability of the change in TL within individuals and large cohort effects on the change in TL. Thus, selection on TL or the telomere rate of change may produce evolutionary change in TL over time and annual environmental stochasticity in early-life will generate persistent heterogeneity in TL among cohorts. Across bird species, TL was significantly negatively associated with the fast-slow axis of life-history variation, suggesting that TL may have evolved to mediate trade-offs between physiological requirements underlying the diversity of pace-of-life strategies.

In conclusion, TL in house sparrows is a dynamic, heritable, polygenic trait that is negatively affected by growth, inbreeding, and environmental stressors during early-life, but the evidence that the fate of the telomere reaches beyond itself, the cell, and the organism is equivocal. Indeed, early-life TL may reveal subtle long-term costs of early life-conditions and may act as a biomarker of pace-of-life both within house sparrows and across bird species.

Sammenfatning

Telomerer, DNA-sekvenserne der findes på enderne af lineære kromosomer, er urgamle molekylære strukturer, som er fælles for de fleste eukaryoter. Telomererne beskytter kromosomerne mod at blive ødelagt, og de er livsvigtige for genomets integritet. Telomerer forkortes ved hver celledeling og som respons på oxidativt stress. Korte telomerer kan forudsige dårligt helbred og lavere overlevelse hos nogle dyr. Derfor kan telomerlængden (TL) være med til at underbygge livshistorieafvejninger mellem vækst, overlevelse og reproduktion, eller agere som en biomarkør for individuel kvalitet eller livsrytme. Dog er telomerernes økologiske og evolutionære role i at forme variationen i livshistorieegenskaber i vilde dyr ikke tilstrækkeligt undersøgt. I denne afhandling har jeg undersøgt årsager og konsekvenser af variation i TL i vilde bestande af gråspurve.

Jeg fandt ud af, at TL var negativt associeret med kropsstørrelse både under et selektionseksperiment og i naturlige bestande. Denne sammenhæng skyldtes dog ikke en genetisk korrelation mellem TL og kropsstørrelse. Der var en lille arvelig komponent af TL, men forskellene i TL var primært drevet af årlig miljømæssig variation. Indavlede spurve havde kortere telomerer. Derudover identificerede jeg adskillige nye gener, som kan ligge bag variationen i TL. TL i det tidlige liv var påvirket af vejrforhold og bestandstætheder, men der var ikke stærk evidens for sammenhænge mellem TL og overlevelses- og formeringsevner. Spurveunger med korte telomerer tenderede mod at have højere reproduktiv succes og højere sandsynlighed for at sprede sig til andre bestande som forventet, hvis TL afspejler livsrytmesyndromer. TL blev generelt kortere med alderen, men blev tilsyneladende længere hos nogle individer. Der var en moderat arvelighed af ændringen i TL inden for individer og store kohorteeffekter på denne ændring. Dermed kan selektion på TL eller ændringen i TL skabe evolutionære ændringer i TL over tid, og årlig miljømæssig stokasticitet vil skabe vedvarende heterogenitet i TL mellem kohorter. På tværs af fuglearter havde de ”langsomtlevende” arter korte telomerer, mens de ”hurtigtlevende” arter havde lange telomerer. Dette tyder på, at TL har udviklet sig til at formidle de fysiologiske afvejninger der ligger bag diversiteten i livshistoriestrategier.

TL i gråspurve fremstår som et foranderligt, arveligt, polygent træk, som påvirkes negativt af vækst, indavl og stressfaktorer i miljøet i det tidlige liv, men hvorvidt telomerens skæbne rækker ud over den selv, cellen og organismen, er fortsat til diskussion. Dog afslører TL stilfærdige, men langsigtede, omkostninger ved forhold i det tidlige liv, og TL kan muligvis fungere som en biomarkør for livsrytme både inden for gråspurve og på tværs af fuglearter.

List of most used abbreviations and quantities

AICc	Akaike's information criterion corrected for small sample sizes
ARS	annual reproductive success
β	regression coefficient
b/bp	base pairs
BC	before Christ
CI	confidence interval
Cov	covariance
DNA	deoxyribonucleic acid
ER	evidence ratio
F	inbreeding coefficient
FISH	fluorescence *in situ* hybridization
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
GLMM	generalized linear mixed-effects model
GRM	genomic relationship matrix
GWAS	genome-wide association study
h^2	narrow-sense heritability
HPD	highest posterior density intervals
IBD	identical by descent
λ	Pagel's lambda (phylogenetic signal)
LMM	linear mixed-effects model
LRS	lifetime reproductive success
MAC	maternal age at conception
MCMC	Markov chain Monte Carlo
MLP	Michael Le Pepke
MS	microsatellite
Mya	million years ago
n	sample size
n	haploid number
NAO	North Atlantic Oscillation
PAC	paternal age at conception
PC	principal component
PED	pedigree
POL	pace-of-life
qPCR	quantitative polymerase chain reaction
R^2	coefficient of determination
REML	restricted maximum likelihood
ROH	runs-of-homozygosity
ROS	reactive oxygen species
S	supporting information
SE	standard error
SD	standard deviation
SNP	single nucleotide polymorphism
TA	telomerase activity
TL	telomere length
TRF	telomere restriction fragment
V	variance
w	Akaike weight

List of papers

I. **Pepke, M.L.,**[*] Kvalnes, T., Rønning, B., Jensen, H., Boner, W., Sæther, B.-E., Monaghan, P., & Ringsby, T.H. (2021) Artificial size selection experiment reveals telomere length dynamics and fitness consequences in a wild passerine, *Authorea preprint*, doi.org/10.22541/au.161447476.67562312/v1 [1]

II. **Pepke, M.L.,**[*] Kvalnes, T., Lundregan, S., Boner, W., Monaghan, P., Sæther, B.-E., Jensen, H. [§], & Ringsby, T.H. [§] (2021) Genetic architecture and heritability of early-life telomere length in a wild passerine, *Authorea preprint*, doi.org/10.22541/au.161961744.48479988/v1 [2]

III. **Pepke, M.L.,**[*] Niskanen, A., Kvalnes, T., Boner, W., Sæther, B.-E., Ringsby, T.H. [§], & Jensen, H. [§] (2021) Inbreeding is associated with shorter early-life telomere length in a wild passerine, *bioRxiv preprint*, doi.org/10.1101/2021.10.10.463797 [3]

IV. **Pepke, M.L.,**[*] Kvalnes, T., Ranke, P.S., Araya-Ajoy, Y.G., Wright, J., Sæther, B.-E., Jensen, H., & Ringsby, T.H. (2021) Causes and consequences of variation in early-life telomere length in a bird metapopulation, *EcoEvoRxiv preprint*, doi.org/10.32942/osf.io/9zctr [4]

V. **Pepke, M.L.,**[*] Kvalnes, T., Araya-Ajoy, Y.G., Ranke, P.S., Wright, J., Sæther, B.-E., Jensen, H., & Ringsby, T.H. (manuscript) Longitudinal telomere dynamics in wild house sparrows. [5]

VI. **Pepke, M.L.,**[*] Ringsby, T.H., & Eisenberg, D.T.A. (2021) Early-life telomere length covaries with life-history traits and scales with chromosome length in birds, *bioRxiv preprint*, doi.org/10.1101/2021.08.07.455497 [6]

Declarations of contributions

[1] MLP analyzed the data and wrote the manuscript. MLP and WB measured telomere lengths. MLP and BR constructed pedigrees. THR, HJ, and B-ES designed the artificial selection experiment. THR and PM designed the telomere investigation. THR, HJ, TK, and BR contributed to the fieldwork and TK curated field data. All authors contributed to the intellectual content through input, comments, and edits when writing up the manuscript. This version is slightly different from the preprint.

[2] MLP measured telomeres, analyzed the data, and wrote the manuscript with comments from all authors. WB and PM advised telomere measurements. TK, HJ, THR, and SL advised statistical analyses. B-ES, THR, and HJ established the study system. THR, HJ, and TK contributed to the fieldwork. This version is slightly different from the preprint.

[3] MLP measured telomeres, analyzed data, and wrote the manuscript with contributions from all authors. WB supervised telomere measurements. HJ, AKN, and TK contributed to the genotype data processing, pedigree construction, and in designing statistical analyses. THR, BE-S, and HJ initiated the study system. THR, HJ, and TK contributed to the fieldwork.

[4] MLP measured telomeres, analyzed the data, and wrote the manuscript with contributions from all authors. THR, B-ES, and HJ, initiated the study system. THR, HJ, TK, PSR, YGA-A, and MLP contributed to the fieldwork. This version is slightly different from the preprint.

[5] MLP measured telomeres, analyzed the data, and wrote the manuscript with contributions from all authors. THR, B-ES, and HJ, initiated the study system. THR, HJ, TK, PSR, YGA-A, and MLP contributed to the fieldwork.

[6] MLP and DTAE conceived the ideas. MLP compiled and analyzed data and wrote the manuscript with contributions from all authors. This version is slightly different from the preprint.

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[§] Joint senior authors.

Introduction

Evolution by means of natural selection works through the differential survival and reproductive success of different phenotypes (Darwin, 1859). Thus, understanding the processes generating heritable phenotypic variation in fitness-related traits is fundamental to understanding the perpetuation of varieties by natural means of selection (Darwin & Wallace, 1858). However, only a Darwinian demon may simultaneously maximize all aspects of fitness (e.g. immortality and infinite fecundity; Law, 1979), while all other organisms are bound by cardinal trade-offs such as between survival and reproduction (Williams, 1966) and by evolutionary constraints (Arnold, 1992). How organisms differ in the ability to acquire and the decision to allocate energy into survival, growth, and reproduction shapes the biodiversity of pace-of-life strategies within and across species (Roff, 1992; Stearns, 1989; van Noordwijk & de Jong, 1986). However, our knowledge about the proximate mechanisms that underpin life-history trade-offs is still limited (Flatt & Heyland, 2011; Ricklefs & Wikelski, 2002; Speakman et al., 2015; Zera & Harshman, 2001). A candidate for participating in mediating life-history trade-offs is telomeres (Haussmann & Marchetto, 2010; Monaghan, 2010; Monaghan & Haussmann, 2006; Young, 2018).

The beginning of understanding the end

"Your telomeres, it turns out, are listening to you." (Blackburn & Epel, 2017)

Telomeres (*telos méros*, lit. "end part", Muller, 1938) are short repetitive hexameric DNA sequences, $(TTAGGG)_n$, located at the beginning and at the end of linear chromosomes (Fig. 1a, Blackburn & Gall, 1978; Blackburn & Szostak, 1984). This ancient molecular structure and function is highly conserved across most organisms whose cells have a nucleus (Blackburn, 1991; Meyne et al., 1990; Meyne et al., 1989). A single-stranded telomeric overhang loops back to invade the double-stranded telomere sequence to form the T-loop, which prevents the telomere from being recognized as a DNA breakpoint and avoid chromosomal fusion (Fig. 1b, Griffith et al., 1999). This T-loop is further protected by a complex of shelterin proteins (de Lange, 2005) and the formation of G-quadruplexes (Paeschke et al., 2005). During cell division, DNA replication of the lagging strand cannot be completed beyond the last Okazaki fragment on a linear chromosome (Olovnikov, 1973). Thus, in order to protect the coding DNA, the solution to this "end replication problem" is progressive telomere shortening with subsequent cell divisions (Levy et al., 1992). In absence of telomere maintenance, the telomere length (TL) limits the replicative capacity of cells (Allsopp et al., 1992); for example, cultured normal somatic human cells will stop dividing after 40 to 60 doublings, which is known as the Hayflick limit (Hayflick & Moorhead, 1961; Shay & Wright, 2000). Beyond this limit, telomeres become critically short, which may trigger chromosome instability and apoptosis (Counter et al., 1992). The immortality of germline or cancer cells may then be obtained through the function of telomerase, an enzyme capable of synthesizing telomeres (Greider & Blackburn, 1985; Kim et al., 1994). Telomeres may also be maintained by telomerase-independent mechanisms (Cesare & Reddel, 2010). However, while introduction of telomerase may extend cellular lifespan (Bodnar et al., 1998), telomerase activity (TA) is a key feature of most cancers (Shay et al., 2001).

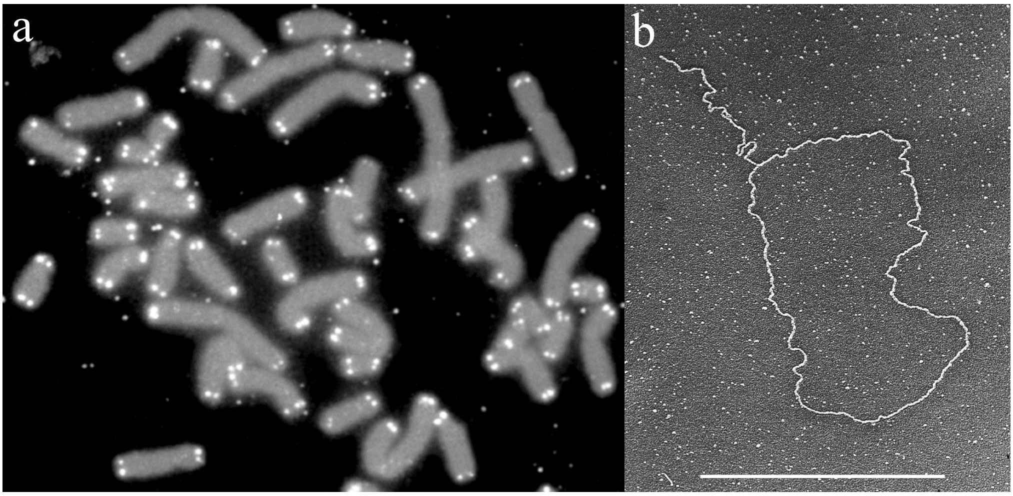

Fig. 1: a) Human metaphase chromosomes (grey) after hybridization with a Q-FISH probe to visualize the telomeres (white) at the chromosome ends (U.S. Department of Energy, Human Genome Program. Public domain license). **b)** An electron microscope image of Henrietta Lacks' telomeric DNA cross-linked with psoralen to reveal the t-loop (reproduced with permission from Jack D. Griffith, see Griffith et al., 1999). The bar equals 5 kb.

Telomeres are highly susceptible to oxidative damage *in vitro* probably because of their high guanine content (Kawanishi & Oikawa, 2004; Wang et al., 2010) and less proficient DNA repair (Fumagalli et al., 2012; von Zglinicki, 2002). In a seminal study, Epel et al. (2004) found that TL responds to psychological stress in humans, through effects of higher oxidative stress and lower TA. This led to a multitude of studies attempting to establish TL as a mediator of long-term negative effects of early-life stress in humans (Blackburn & Epel, 2012; Entringer et al., 2011; Ridout et al., 2018). At the same time, the role of telomeres in (human) aging-related disease (Blackburn et al., 2015) and cancer biology (Wright & Shay, 2005) was being unfolded (Aviv & Shay Jerry, 2018). In a review, Reichert and Stier (2017) found support for a link between oxidative stress and telomere attrition *in vivo* in 10 out of 18 studies. However, it is difficult to measure oxidative stress (Monaghan et al., 2009) and perhaps as a consequence of this, it is still debated whether or how much telomeres shorten due to oxidative stress (Ahmed & Lingner, 2018; Boonekamp et al., 2017; Gil et al., 2019; Zane et al., 2021). Yet, several studies suggest a pivotal role for environmental stressors in shaping telomere dynamics in free-living animals (reviewed in Chatelain et al., 2020). It is a tenet of this thesis that oxidative stress (increased production of reactive oxygen species (ROS) or decreased antioxidant defenses) provides a functional mechanism linking TL changes, energy expenditure and allocation within individuals (Casagrande & Hau, 2019; Chatelain et al., 2020; Selman et al., 2012; von Zglinicki et al., 2003).

Telomere length and life-history trade-offs

Sexual reproduction recapitulates our unicellular ancestry, and the subsequent expansion of the organism occurs mainly through increases in cell number (Conlon & Raff, 1999), but also through increases in cell size (Lloyd, 2013). Thus, the “initial” TL at conception shortens with each additional round of cell divisions required to obtain a certain size (Jennings et al., 1999). Furthermore, TL shortens due to the increased oxidative stress associated with maintaining a certain size (Smith et al., 2016). Consequently, body size negatively correlates with TL within several species (Caprioli et al., 2013; Monaghan & Ozanne, 2018; Ringsby et al., 2015; Scott et al., 2006; Spurgin et al., 2018). Moreover, organisms do not grow at their maximum growth rate (Metcalfe & Monaghan, 2001, 2003), and there is also some evidence that faster growth results in increased telomere shortening in determinate growers (Geiger et al., 2012; Salmón et al., 2021; Vedder et al., 2018). TL therefore reveals a cost of growth and may play a role in current-future trade-offs and ageing.

In another seminal paper, Heidinger et al. (2012) showed that early-life TL predicted lifespan in captive zebra finches (*Taeniopygia guttata*). Indeed, the telomere may be analogized to the thread of fate that is cut short by the Norns of the Norse mythology to determine the lifespan of the mortal. TL, or the change in TL, has been shown to be associated with survival, longevity, senescence, reproductive success, and individual quality in a range of wild animals (Asghar et al., 2015; Chatelain et al., 2020; Eastwood et al., 2019; Froy et al., 2021; Haussmann et al., 2005; Heidinger et al., 2021a; Olsson et al., 2018b; Sudyka, 2019; van Lieshout et al., 2019; Wilbourn et al., 2018). This suggests that TL may act as a mediator of the resource allocation trade-offs between growth, survival (self-maintenance), and reproduction (Monaghan, 2014; Monaghan & Haussmann, 2006). This is often thought to imply a causal involvement of TL in the associations with fitness (Young, 2018). Inferring causation from correlation requires some level of grasping the underlying cause *a priori* (hence the details of telomere functioning above), but relying mainly on correlative evidence, I shall be careful about inferring the causal role of TL in this thesis. The problem with assuming a causal involvement (and adaptive role) of TL, is that we still know little about the costs of TL maintenance (Monaghan & Ozanne, 2018; Power et al., 2021). Such costs may not be limited to the energy required to rebuild telomeres (which may indeed be negligible comparing the bp's of TL attrition to the Gbp's of genome content; Young, 2018), but may include costs associated with cancer risks, the adaptive regulatory role of TL, or non-canonical roles of TA (Eisenberg, 2011; Smith et al., 2021; Young, 2018). TL may also reflect experienced (oxidative) stress, i.e. TL may be a biomarker of individual condition and physiological state (Bateson & Poirier, 2019; Boonekamp et al., 2013; Pepper et al., 2018) without implying a causal role of TL on fitness.

In an evolutionary and ecological context, TL is also an interesting trait because its structure and function have deep evolutionary roots and are consequently shared among many species (Gomes et al., 2010; Meyne et al., 1990). Indeed, insights into the ecological and evolutionary role of TL have been obtained from studies on a diversity of organisms including mammals (Gomes et al., 2011; Power et al., 2021), birds (Heidinger et al., 2012; Tricola et al., 2018) and other reptiles (Olsson et al., 2018a), amphibians (Burraco et al., 2020), insects (Boonekamp et al., 2021; Koubová et al., 2021), echinoderms (Ebert et al., 2008), corals (Rouan et al., 2021), fungi (Kupiec, 2014), protozoans (Blackburn et al., 2006), and plants (Choi et al., 2021). Here, we shall focus on the birds, and in particular the world's most ubiquitous bird, the house sparrow (Anderson, 2006).

Aims

The general aim of this thesis is to advance our understanding of the ecological and evolutionary significance of TL in animals in the wild, and in particular, to explore the role of TL in mediating life-history trade-off both within and across species. This is obtained by investigating the causes and consequences of TL variation within populations of free-living house sparrows (paper I-V) and across bird species (paper VI).

In paper I, the hypothesized correlation between body size and TL is investigated using an artificial size selection experiment to test the effect of deviating from the optimal body size on TL in house sparrows. I further test if variation in early-life TL underpins any fitness consequences of the experimentally induced changes in body size.

In paper II, the relative importance of genetic and environmental variation in TL is quantified using a long-term field study on natural populations of house sparrows. In particular, I test if a genetic correlation underlies the phenotypic correlation between body size and TL that was explored in paper I. Furthermore, I use genome-wide association analysis to identify putative genes underlying variation in TL.

In paper III, I examine the associations between various measures of inbreeding and early-life TL. Specifically, I test if inbreeding is associated with shorter TL in wild house sparrow populations, which is hypothesized as a physiological basis for inbreeding depression.

In paper IV, I disentangle the environmental effects that shape variation in early-life TL, and I investigate if early-life TL has any fitness consequences in the natural unmanipulated house sparrow populations.

In paper V, I explore changes in TL with age within individual house sparrows. I test the hypothesis that TL shortens with age, I estimate heritability of the change in TL (ΔTL) within individuals, and I conduct a preliminary investigation of factors associated with ΔTL.

In paper VI, I examine the evolution of TL in a meta-analysis across bird species and test if TL is associated with the fast-slow axis of life-history variation, that underlies the diversity of pace-of-life (POL) strategies. Furthermore, I test the hypothesis that average chromosome length covaries with average TL.

General methods

Study species and system

“I'd rather be a sparrow than a snail.” (Simon & Garfunkel, 1970)

This thesis relied on field data collected across an average human generation time in house sparrow island populations along the coast of northern Norway (1993-2020). Two of these populations (Leka and Vega) were part of an artificial body size selection experiment (Kvalnes et al., 2017), while two other populations (Træna and Hestmannøy) were left unmanipulated (by researchers, e.g. Araya-Ajoy et al., 2021) and remain a high maintenance asset of the founding fathers[1]. The field work procedures are detailed repetitively through papers I-V. Importantly, birds were blood sampled, and (tarsometa-)tarsus length was measured and used as a proxy for structural body size.

Fig. 2: A pair of house sparrows on the island of Hestmannøy in northern Norway. On the left is a female prior to ringing, while the male on the right was ringed by MLP with a unique combination of color rings for identification. Photo: Peter S. Ranke (August 2021).

With a generic forename referring to its rapidity, a species-specific hominess, and an Egyptian hieroglyph for “little, evil, or bad” (Houlihan & Goodman, 1986), the existence of house sparrows (Fig. 2) precedes their essence (e.g. Sartre, 1946), yet, they have been considered a notoriously dowdy and stiff-feathered (Fordyce, 1990), but transcending (von Goethe, 1833), eroticized (Catullus, 57 BC) attribute of Aphrodite (Sappho, 600 BC). They live closely associated with human habitation, indeed, this anthrodependent commensal

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[1] See «Declarations of contributions», p. 7.

organism (Cauchard & Borderie, 2016) owes its most recent idiosyncratic evolutionary trajectory to the development of human agriculture and anthropogenic change (Ravinet et al., 2018). It is an early taxon cycle stage species (Marzal et al., 2011; Pepke et al., 2019), and from its biblical center-of-origin (Sætre et al., 2012), house sparrows have colonized all continents except Antarctica (Hanson et al., 2020). This pre-adaptive commensalism (Driscoll et al., 2009) is particularly evident in remote, inhospitable places of Earth (Summers-Smith, 1963), such as northern Norway, where the sparrows probably appeared in the Bronze Age (Alström et al., 2015). This undirected evolutionary process, which should not be mistaken for domestication (Diamond, 1997), has provided us with an animal for whom the important tool of long-term bird field studies, the nest box, and the associated researcher, are no less natural presences than the tree hole, and the associated woodpecker, are to other popular hole-nesting passerine study species (e.g. Hogstad, 2017; Mänd et al., 2005; Møller, 1992).

The house sparrow is considered a model organism that has contributed to our understanding of microevolution, local adaptation, sexual selection, and physiology for over a century (Bumpus, 1899; Hanson et al., 2020). The telomere biology of house sparrows, humans, and most other vertebrates is highly conserved (Fulnecková et al., 2013). Considering the last three decades of research on telomere dynamics in humans (Shay & Wright, 2019), we may therefore, for the purpose of this thesis, consider humans a model organism for understanding the ecological and evolutionary role of telomere length in house sparrows.

Do the telomere ends justify the means?

A range of methods to quantify the number of telomeric base pairs in the genome have been developed during the past three decades (Nussey et al., 2014). In the so-called "golden standard" method, the telomere restriction fragment (TRF) assay, gel electrophoresis of digested DNA followed by hybridization and image analysis of the gel smear allows estimating the distribution of TLs (in kb) across all chromosomes (Harley et al., 1990). This may reveal TL dynamics beyond the mean TL (Atema et al., 2019; Atema et al., 2021). The hybridization may be using either denaturing blots (Kimura et al., 2010) or non-denaturing, in-gel hybridization techniques (Haussmann & Mauck, 2008). The latter methods ensures that only terminal telomeres are probed, while denaturing binds all telomeric DNA within the genome, including (presumably non-functional) interstitial telomeres (Foote et al., 2013). TRF offers high resolution, information on the distribution rather than just the mean (e.g. Hemann et al., 2001), high repeatability (Kärkkäinen et al., 2021), and feasibility of comparative studies (e.g.

Gomes et al., 2011) but is technically demanding, time-consuming, and requires relatively large amounts of DNA (Haussmann & Mauck, 2008; Lai et al., 2018). In this thesis, I used data from published TRF studies to avoid the time-consuming step, and to be able to compare absolute TLs across different species (paper VI). Other methods to quantify absolute TL rely for instance on fluorescence *in situ* hybridization (FISH, Fig. 1a, Lansdorp et al., 1996) that are no less technically demanding and labor intensive, requiring cultured cells (but see Aubert et al., 2012). However, FISH may be used to measure TL of single chromosome arms (see also Baird et al., 2003; Poon & Lansdorp, 2001) to reveal telomere-chromosome dynamics (which I discuss in paper VI) or TL dynamics across species (Pepke & Eisenberg, 2020; Whittemore et al., 2019). New and existing methods to measure TL are continuously being developed (Hudon et al., 2021; Lee et al., 2017b; Miga et al., 2020).

The quantitative polymerase chain reaction (qPCR) method of measuring TL has become very popular especially in ecological field studies and in non-model vertebrates. In qPCR, the amount of telomeric DNA (T) is quantified relative to the amount of a non-telomeric non-variable ("housekeeping") reference gene sequence (S, Cawthon, 2002). This accounts for differences in the number of cells in the samples. To account for among-plate variation, this T/S ratio is measured relative to that of a standard sample of the same DNA that is included in all assays (Cawthon, 2002). In this thesis, I utilized two decades of fieldworkers collecting blood samples from several long-term field studies. The sample size (around 4000 samples) and amounts of DNA available from the blood samples made the high-throughput qPCR the preferred method for my study (Nussey et al., 2014). I had to use two different standard samples, one for the experimental (Ringsby et al., 2015; paper I) and another for the unmanipulated populations and these are therefore not combined in the analyses (papers II-V). Short interstitial telomeric repeats are also amplified during qPCR, which may bias the mean TL (Foote et al., 2013; Nussey et al., 2014). However, Ringsby et al. (2015) validated the qPCR method in house sparrows using the standard and the in-gel TRF methods, and neither Ringsby et al. (2015) nor Meyne et al. (1990) detected any substantial interstitial telomeres in this species. Yet, qPCR has lower and more variable within-individual repeatability compared to TRF (Kärkkäinen et al., 2021), which may lead to methodological effects across studies as evidenced in recent meta-analyses (Chatelain et al., 2020; Remot et al., 2021; Wilbourn et al., 2018).

I measured TL in DNA extracted from whole blood, which is therefore primarily measured in (avian nucleated) erythrocytes. Erythrocytes in house sparrows have a relatively short lifespan of one month *in vivo* (Gillooly et al., 2012), and erythrocyte turnover rate increases with increasing metabolic rate (Rodnan et al., 1957). Erythrocytes are produced in the bone marrow and their TL is therefore thought to reflect the replicative (regeneration) potential and stress experienced by hematopoietic stem cells (Sidorov et al., 2009; Vaziri et al., 1994). However, TLs are often positively correlated across somatic tissues of birds and mammals (Benetos et al., 2011; Daniali et al., 2013; Demanelis et al., 2020; Kärkkäinen et al., 2020; Prowse & Greider, 1995; Reichert et al., 2013), and I assume that to be the case in house sparrows as well.

Notes on statistical methodology

I employed a wide range of statistical methods to address very different questions in this thesis. In general, the hierarchical structure of levels of variation, and with several sources of random variability, within the data, suggest the use of (generalized) linear mixed-effects models (LMMs, Bolker et al., 2009; Zuur et al., 2009). In papers II and V, I used a type of mixed-effect model that takes all relationships from a pedigree into account (“animal model”, Wilson et al., 2010) to decompose variance in TL into genetic and environmental sources, and to estimate heritabilities and genetic correlations. Accurate estimates of heritabilities and genetic correlations using animal models require large sample sizes (Kruuk, 2004). Here, I relied on a Bayesian framework using flexible MCMC algorithms that e.g. allow estimating heritabilities, genetic correlations, and associated uncertainties from posterior distributions (Morrissey et al., 2014). With my large sample sizes, the choice of prior did not really influence the estimated posterior distributions (Hadfield, 2019). In papers I and IV, I used survival analysis (proportional hazards regression, Kleinbaum & Klein, 2005) to relate the time to death to TL and other traits. Throughout this thesis, I used an information-theoretic approach to model selection (Burnham & Anderson, 2002) for both data exploration (van de Pol et al., 2016) and hypothesis testing (Tredennick et al., 2021).

Some species are more equal than others (Orwell, 1945) due to shared streams of heredity (Simpson, 1945). Consequently, in paper VI, I applied phylogenetic comparative methods (Felsenstein, 1985) to examine (phylogenetically corrected) correlations between TL and other traits across species. Researchers still tend to overlook phylogenetic non-independence in studies using small numbers of species (Pepke & Eisenberg, 2020), however, I encourage testing alternative evolutionary models in all such cases (paper VI).

Main results and discussion

Telomere length under artificial body size selection (paper I)

"If man goes on selecting, and thus augmenting any peculiarity, he will almost certainly modify unintentionally other parts of the structure, owing to the mysterious laws of correlation." (Darwin, 1859)

Artificial selection by breeders has served as a vivid "experiment on a gigantic scale" (Darwin, 1868) when first exploring the mechanisms of natural selection through analogy (Darwin, 1859). Artificial selection experiments on a smaller scale have since revealed insights into adaptive evolution and quantitative genetics (Conner, 2003; Hill & Caballero, 1992). I investigated the role of TL in shaping trade-offs between growth, survival, and reproduction during a 4-year artificial selection experiment for either larger or smaller tarsus length, a proxy for body size in house sparrows (Kvalnes et al., 2017; Ringsby et al., 2015). A negative association between nestling TL and tarsus length was observed (*n*=566, paper I) as hypothesized due to the additional number of cell divisions and increased oxidative stress associated with acquiring and maintaining larger size (Monaghan & Ozanne, 2018).

Males had longer telomeres than females, but this sex difference was not observed in the unmanipulated populations which included a much larger sample size (see papers II and IV). Analyses of mortality during and after the experiment (11 years) indicated weak disruptive selection on TL because both short and long early-life telomeres tended to be associated with the lowest mortality rates. In addition, there was a tendency for a negative association between TL and annual reproductive success, but only in the population where body size was increased experimentally.

Body size is often found to be under positive selection in wild animals (Kingsolver & Pfennig, 2004), including house sparrows (Jensen et al., 2004). Accordingly, positive selection on body size over time may result in shorter TL which may be associated with shorter lifespan (Wilbourn et al., 2018). We now want to know whether this (albeit small) negative phenotypic association between TL and size is due to a negative genetic correlation (Roff, 1995), or if it is shaped entirely by environmental co-variances.

Low heritability, large cohort effects of early-life telomere length (paper II)

I utilized the long-term monitoring of two unmanipulated house sparrow populations to decompose variation in early-life TL into genetic and environmental sources of variation, and to estimate the genetic correlation between TL and tarsus length. The heritability of TL was low (h^2=0.04, n=2662), which is similar to some other wild animal populations (Foley et al., 2020; Sparks et al., 2021), but TL heritability estimates vary greatly across species (Dugdale & Richardson, 2018; Froy et al., 2021; van Lieshout et al., 2021; Vedder et al., 2021).

Environmental (annual) variance was the main source of variation in TL (paper II). Such year effects are interesting because they may reflect large-scale environmental variation during early ontogenetic stages such as weather conditions. The presence of large year (cohort) effects thus suggests persistent impacts of the early-life environment on TL (Froy et al., 2021; van Lieshout et al., 2019). Consistent cohort effects are known to shape phenotypic and fitness variation in wild animals (Gaillard et al., 2003; Jensen et al., 2006; Metcalfe & Monaghan, 2001; Reid et al., 2003b) and perhaps also in humans (Lummaa, 2003).

I also found some evidence for parental effects playing a role in shaping variation in TL (paper II). There was no association between TL and parental age at conception. Parental and environmental effects documented in other studies (Monaghan & Metcalfe, 2019) suggest that some of the variation in TL may be inherited through epigenetic carry-over effects (Bauch et al., 2019; Eisenberg, 2019) that are not resolved by comparing early-life TLs of parents and offspring. Such effects may be more important in shaping nestling TL loss, rather than early-life TL (Heidinger et al., 2016).

There was also a negative phenotypic association between TL and tarsus length in these populations, but there was no evidence for a genetic correlation between TL and tarsus length. This suggests that TL and body size are determined by different genes and that the correlation between TL and size is caused by non-genetic environmental effects.

A polygenic trait

Understanding the genetic architecture of variation in TL is fundamental in elucidating the processes driving telomere evolution. Previous studies point to a polygenic basis of TL in humans (e.g. Codd et al., 2013; Codd et al., 2021; Li et al., 2020). Several of these studies have identified genetic loci associated with TL in adults, thus it is unclear whether these loci represent the genetic basis of TL or susceptibility to TL shortening during life (Weng et al., 2016). However, there is some evidence that early-life TL is more genetically determined than telomere attrition (Bauch et al., 2021; Wang et al., 2021). I utilized a high-density SNP array (Lundregan et al., 2018) to perform a genome-wide association study that yielded several putative genes underlying variation in early-life TL for a subset of the house sparrows (*n*=383, paper II). These candidate genes (all novel) have been inferred to be involved in processes such as oxidative stress, cellular growth, skeletal development, cell differentiation and tumorigenesis. However, the small heritability and polygenic nature of TL make it difficult to identify causal genes with limited *a priori* information on the identified SNP variants.

Inbreeding erodes telomeres (paper III)

"...it is a great law of nature, that all organic beings profit from an occasional cross with individuals not closely related to them in blood; and that, on the other hand, long-continued close interbreeding is injurious." (Darwin, 1868)

TL may be a biomarker of somatic integrity in several species (Bateson & Poirier, 2019; Haussmann & Marchetto, 2010; Pepper et al., 2018). Inbreeding may compromise the physiological state of an organism, for example by reducing immune response or increasing metabolism and oxidative stress levels (Ketola & Kotiaho, 2009; Pedersen et al., 2008; Reid et al., 2003a) resulting in inbreeding depression (Charlesworth & Willis, 2009). The house sparrows of this thesis are known to be affected by inbreeding depression (Niskanen et al., 2020), so I tested the hypothesis that inbreeding is associated with shorter telomeres (Bebbington et al., 2016). I used genomic (and pedigree-based) estimates of inbreeding to show a negative association between early-life TL and inbreeding levels in house sparrows (*n*=371, paper III). To illustrate this effect, consider Charles Darwin, who married his first-cousin: If the results from house sparrows can be directly generalized to humans, I expect that his 10 children (F=0.0630)[2] may have had 20% shorter telomeres compared to their non-inbred equals. This may not have come as a surprise to Darwin, who was well-aware of the "evil" of inbreeding (his wording; Darwin, 1876).

Shorter early-life TL may well be generally symptomatic of inbreeding across species, however, the relationship between TL and inbreeding depression will depend on the relevance of TL in shaping individual fitness within each species.

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[2] This inbreeding coefficient is higher than that resulting from a single first-cousin union because Darwin's (and his wife's) grandparents were third cousins (Álvarez et al., 2015).

Environmental conditions shape early-life telomere length (paper IV)

"It's raining again. Oh no, my love's at an end" (Hodgson et al., 1982).

At the ends of chromosomes, we may not find love, but perhaps a biomarker of individual exposure to environmental stressors such as weather conditions, pathogen infection, and competition (Chatelain et al., 2020). Environmental conditions may be particularly important in early-life and have long-lasting consequences (Lindström, 1999; Metcalfe & Monaghan, 2001). I investigated how environmental conditions influenced individual heterogeneity in early-life TL in the unmanipulated house sparrow (*n*=2462) populations, and the associations between TL, dispersal probability, and fitness. I found a negative effect of population density on TL, but only in one of the populations where the sparrows live in gardens (Træna, compared to the farm-living population on Hestmannøy, paper IV). The differences between the two populations may be related to differences in food availability, but it was beyond the scope of this thesis to directly quantify habitat quality (though this work has commenced, Pepke, 2020). There was also an effect of weather conditions reflected by the North Atlantic Oscillation index on TL. The effects of competition and weather testify to the oxidative stress mediated effects of early-life environmental conditions as drivers of individual variation in TL (Casagrande & Hau, 2019; Chatelain et al., 2020; Metcalfe & Olsson, 2021).

Associations between early-life telomere length and fitness

"There is a special providence in the fall of a sparrow." (Shakespeare, 1603)

Similar to the experimental populations, I found no associations between TL and survival (*n*=2462), but a tendency for a negative association between TL and ARS (*n*=396). Individuals with shorter TL also tended to have higher chances of successful natal dispersal (*n*=455). Individuals with short telomeres may exhibit a faster POL reflected in a higher reproductive output, while individuals with longer telomeres prioritize resources into self-maintenance (Giraudeau et al., 2019; Young, 2018; paper IV). Thus, TL may be a biomarker of POL syndromes across individuals. Natural variation in early-life TL appears to play a very limited role in modifying fitness in these house sparrows. While the life-history of the house sparrow may only be understood backwards; it must be lived forwards (e.g. Kierkegaard, 1843): Thus, the wear and tear on TL during life may reveal the (complex) adaptive significance of TL.

Telomere length as a dynamic trait (paper V)

“Time travels in divers paces with divers persons.” (Shakespeare, 1623)

TL often shortens with age within individuals (Remot et al., 2021), but there is an increasing number of studies showing TL elongation or that TL may both increase or decrease at different times in life (e.g. Bateson & Nettle, 2017; Brown et al., 2021; Hoelzl et al., 2016). Consequently, ΔTL rather than TL may be important in mediating effects of oxidative stress on fitness through life (Boonekamp et al., 2014; Wood & Young, 2019). In this longitudinal study, I measured TLs in a subset of house sparrows (n=226) that were recaptured in the unmanipulated populations at different times throughout their natural lifespan (0-9 years). I observed instances of telomere increases and decreases, but TL generally declined with age within individuals (paper V). I used animal models to show that there was a moderate heritability of ΔTL (h^2=0.21), which was higher than the heritabilities of early- and later-life TL measurements (h^2~0.15) in this subset of surviving individuals. There were also considerable cohort effects on ΔTL. A preliminary investigation of factors affecting ΔTL indicated that the birds on Træna experienced more telomere shortening than birds on Hestmannøy. This study demonstrates the dynamic nature of TL and provides a basis for testing hypotheses on the associations between ΔTL and fitness.

Telomere length as a life-history pace-maker across species (paper VI)

If selective forces are shaping associations between TL dynamics and life-history trade-offs within species (paper IV), this might translate into an evolutionary allometry of TL and life-history traits across species. Several studies have investigated the relationship between the telomere rate of change and lifespan across species (Dantzer & Fletcher, 2015; Tricola et al., 2018). I explored the adaptive significance of (absolute) TL in shaping life-history strategies across species. I hypothesized that TL might be used as a measure to assess individual life-history strategies along a fast-slow life-history continuum. TL was longer in fast-lived bird species (with short generation times and lifespans, large clutch sizes, and a small body mass) compared to slow-lived bird species (paper VI), which is similar to what is found across mammals (Gomes et al., 2011). I proposed functional optimizations underlying this pattern: Slow-lived species have short telomeres as an anti-cancer mechanism, limiting the risk of accumulating oncogenic mutations through replicative cell senescence (Campisi, 2001; Pepke

& Eisenberg, 2021; Risques & Promislow, 2018). Fast-lived species have long telomeres due to increased oxidative stress associated with high rates of reproduction (e.g. Sudyka, 2019).

When the end is near (to the centromere)

I found some evidence for positive covariation between mean TL and mean chromosome length across bird species as hypothesized by Klegarth and Eisenberg (2018). The most compelling explanation behind this observation is the telomere–centromere antagonism, suggesting that TL is adjusted to mitigate interference with the centromere during mitosis (Slijepcevic, 2016), but other mechanisms may be involved (paper VI). The mechanisms determining TL across species are unlikely to be limited by the selective forces entailed by life-history trade-offs (including cancer risk, Tian et al., 2018; Young, 2018), but are integrated with the molecular structures of the whole organism. More data is clearly needed to understand this potential mechanism within and across chromosomes.

Conclusions and future perspectives

The long and short of it

"Better a sparrow, living or dead, than no birdsong at all." (Catullus, 60 BC)

In this thesis, I have attempted to identify salient features of the telomere biology of wild house sparrows: TL is a heritable, polygenic trait (paper II) that is negatively affected by inbreeding (paper III) and shaped in early-life by effects of growth (paper I) and environmental conditions (paper IV). I found no evidence that early-life TL was positively associated with fitness (paper IV), contrary to what has been observed in other studies (Heidinger et al., 2021a; Wilbourn et al., 2018), and indeed, TL may both decrease and increase through life (paper V). Instead, early-life TL may be a biomarker of POL both within (paper IV) and across species (paper VI). Consequently, the evidence that the fate of the telomere reaches beyond itself, the cell, and the organism is equivocal. It should not be forgotten that many other factors than TL may be involved in shaping life-history trade-offs (Flatt & Heyland, 2011) and senescence patterns (López-Otín et al., 2013) in different species.

All's well that ends well

"All men shall die because they cannot join the end to the beginning" (Alcmaeon, 450 BC)

Cancer is as at least as ancient as telomeres (Domazet-Lošo & Tautz, 2010), and the origin of this deep cellular betrayal of the organism is probably linked to the very emergence of metazoan multicellularity (Aktipis et al., 2015). Long telomeres or increased TA may increase the risk of acquiring an oncogenic mutation before cell proliferation ceases due to telomere crisis (Aviv et al., 2017; Pepke & Eisenberg, 2021). However, long telomeres also increase immune function required to combat cancers (Helby et al., 2017) and short telomeres can result in chromosomal instability leading to some types of cancer (Aviv et al., 2017; Ma et al., 2011). This so-called TL paradox is not yet resolved (Eisenberg & Kuzawa, 2018), but TL appears to be balanced by selection acting in opposing directions to minimize both neoplastic and non-neoplastic diseases (Protsenko et al., 2020). Although we may be quick to dispatch cancer as a selective force in the life of the relatively short-lived house sparrows[3], the general absence of cancer (in early-life) may not imply that no fitness-related costs are paid to maintain that status (Thomas et al., 2018). However, the selection pressures incurred by oncogenic phenomena are not well-known in wildlife (Hamede et al., 2020; Thomas et al., 2017).

House sparrows and other birds are relatively long-lived and small-bodied compared to non-flying mammals, but they exhibit a comparable range of mean TLs (Gomes et al., 2011; paper VI). However, there are indications that birds are relatively more cancer resistant than mammals (Effron et al., 1977; Møller et al., 2017). One may wonder if the large-bodied dinosaurs ancestral to our house sparrows have endowed them with strong cancer defenses reminiscent of giants (Erten et al., 2020), and how this may have influenced the evolution of the optimal TL in house sparrows. Empirical data on variation in TL and cancer susceptibility within and across species is needed to start answering such questions. Furthermore, it is unknown whether there is a direction in the evolution of TL and how TL changes during speciation (paper VI): perhaps ontogeny recapitulates phylogeny[4] for telomeres?

A cautionary tail and some loose ends

"Are not two sparrows sold for only a penny?" (Matthew, 70)

The field of telomere eco-evo calls for experimental studies manipulating TL and/or TA (Criscuolo et al., 2018). This would allow testing the causal involvement of TL in shaping individual quality, senescence, and fitness (Reichert et al., 2014). A candidate for such

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[3] House sparrows also develop tumors (Møller et al., 2017).

[4] Not assuming any pagan causality to be implied by this literary epitome (Gould, 1977).

manipulations is TA-65, a putative telomerase activator with roots[5] in Chinese medicine (de Jesus et al., 2011). While TA-65 may be used in the treatment of telomere disorders, it is unfortunately also sold as a dietary supplement for healthy humans (TA Sciences), which obviously warrants caution giving the involvement of TA and TL in natural cancer defense systems (Aviv, 2020). Similarly, the promotion of telomerase therapy by researchers with undisclosed commercial interests may downplay the role of telomeres and telomerase in cancer biology. Indeed, TA may be targeted in cancer therapy (Guterres & Villanueva, 2020). Alternatively, it could be possible to experimentally create house sparrows that lack TA (e.g. TERT/TERC -/- knock-out mutants, Blasco, 2001), or for a start, measure TA in wild house sparrows and examine their life-history traits (Smith et al., 2021).

A plethora of life-style advice for humans on how to specifically maintain TL through diet and behavior (e.g. Blackburn & Epel, 2017), and a corresponding multitude of correlative studies showing for instance that TL is positively correlated with more frequent coitus (Cabeza de Baca et al., 2017) and ball-playing (Hagman et al., 2021), and higher intake of chocolate (Chen et al., 2020), red wine (da Luz et al., 2012), and red meat (Kasielski et al., 2016), warrants some caution in disseminating findings from telomere research. For instance, the discovery of the novel association between TL and Aquaporin-1 (AQP1) gene in this thesis (paper II) does not motivate the use of cosmetics stimulating aquaporin expression with claimed anti-ageing effects (Verkman, 2008; Verkman et al., 2008).

Future studies are needed to investigate whether TL dynamics are coupled with population dynamics, e.g. as a physiological mechanism underlying density-dependent regulation of POL variation within populations (Araya-Ajoy et al., 2021). Such studies may also quantify selection pressures acting on ΔTL and TL in different environments. Which processes drive the plastic response in TL to different environmental conditions (e.g. Brown et al., 2021; Smith et al., 2021; Turbill et al., 2013), what are the costs of TL maintenance (Young, 2018), and to what degree is TL restored in response to oxidative stress (Lee et al., 2017a; Marasco et al., 2021)? Probing the complex regulatory pathways of TL maintenance in wild animals may help answer such questions (Angelier et al., 2018; Heidinger et al., 2021b; Spießberger et al., 2021; Wolf et al., 2021). New molecular mechanism underlying telomere dynamics are still being identified, for instance Li et al. (2017) discovered a telomere-associated protein, TZAP, that regulates the upper limit of TL (telomere trimming) presumably

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[5] It is derived from the roots of Mongolian milkvetch (*Astragalus membranaceus*, Harley et al., 2011).

to maintain an “optimal” TL. The adaptive potential of variation in TA is just being uncovered (Criscuolo et al., 2018; Smith et al., 2021), and the role of interstitial telomere sequences is largely unknown (Aksenova & Mirkin, 2019). Due to the artificial selection being imposed on tarsus length, I have focused on associations between TL and this trait (as an indicator for body size), but future studies should address the relation between TL and the phenome. Furthermore, how TL of the organisms interacts within the holobiont; including the microbiota (i.e. the community of microbes, Velando et al., 2021) and the oncobiota (i.e. the community of cancer cells, discussed above) is largely unknown.

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No bird is an island (but see Koop et al., 2014), but the pandemic peninsularity of engaging in a *sui generis* scientific field (Williams, 1965) has left me with this horcrux (*sensu* Rowling, 2007) of a thesis. While avoiding to perish (Nettle, 2017) and to publish (Medawar, 1963), I almost wished I hadn’t gone down this rabbit-hole (Carroll, 1865) – and yet – now I watch, and am as a sparrow upon the house top (David et al., 900-400 BC). However, I am only a sparrow amongst a great flock of sparrows (Perón, 1951); and I thank the many fieldworkers, my mentors, supervisors and collaborators, both the Norse, the Gaels, and the Nearctic for their presence; and I apologize to my acquaintances, relatives, confidants, and my jovial progeny for my absence. I thank M. Le Moullec, P.S. Ranke, and J.O.P. Pedersen for discussions of this text, and P.S. Ranke and J.D. Griffith for providing the images. I also thank the Research Council of Norway, Forsknings- og undervisningsfondet i Trondheim, and Arbeids- og velferdsforvaltningen (NAV) for funding me through the commodities of the Norwegian continental shelf.

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Papers I-VI

PAPER I

Artificial size selection experiment reveals telomere length dynamics and fitness consequences in a wild passerine

Running title: Telomere dynamics under body size selection

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ABSTRACT

Telomere dynamics could underlie life-history trade-offs among growth, size, and longevity, but our ability to quantify such processes in natural, unmanipulated populations is limited. We investigated how 4 years of artificial selection for either larger or smaller tarsus length, a proxy for body size, affected early-life telomere length (TL) and several components of individual fitness in two insular populations of wild house sparrows over a study period of 11 years. The artificial selection was expected to shift the populations away from their optimal body size and increase the phenotypic variance in body size. The results showed that there was a negative correlation between nestling TL and tarsus length under both selection regimes. Males had longer telomeres than females and there was a negative effect of harsh weather on TL. The mortality analyses indicated disruptive selection on TL because both short and long early-life telomeres tended to be associated with the lowest mortality rates. In addition, there was a tendency for a negative association between TL and annual reproductive success, but only in the population where body size was increased experimentally. Our results suggest that natural selection for optimal body size in the wild may be associated with changes in TL during growth, which is known to be linked to longevity in some bird species.

INTRODUCTION

Telomeres, the nucleoprotein complexes involving tandem repeats of a non-coding DNA sequence, prevent the ends of linear chromosomes from inappropriately activating the DNA repair machinery (Blackburn, 1991). In the absence of restoration, telomeres shorten with each cell division due to incomplete replication of DNA at the chromosome ends, and their eventual dysfunction limits cell replicative potential (Hayflick, 1965). Telomeres may be further eroded by other processes including oxidative damage (Reichert & Stier, 2017; von Zglinicki, 2002). Telomere length (TL) might therefore reflect the cumulative costs associated with acquiring and maintaining a particular body size, since this is linked to cell replication levels (Monaghan & Ozanne, 2018), as outlined in Fig. 1. The functional relationships between body size, growth and telomere dynamics might thus play an important role in shaping the optimal body size under natural selection (Erten & Kokko, 2020; Ringsby et al., 2015).

Body size has been shown to negatively correlate with TL within different tetrapod species (Caprioli et al., 2013; Debes, Visse, Panda, Ilmonen, & Vasemagi, 2016; Pauliny, Wagner, Augustin, Szep, & Blomqvist, 2006; Ringsby et al., 2015; Scott, Haussmann, Elsey, Trosclair, & Vleck, 2006; Spurgin et al., 2018), which is thought to be due to the additional number of cell divisions required for acquiring larger size, and the increased oxidative stress associated with maintaining larger size (Monaghan & Ozanne, 2018). Life-history theory predicts that organisms allocate available energy among different components of fitness, which leads to trade-offs and selection for optimal solutions (Stearns, 1989). Trade-offs may also occur due to antagonistic interactions; for instance, if allocation of energy into developmental growth has negative long-term consequences associated with telomere shortening (Fig. 1, Monaghan, Metcalfe, & Torres, 2009; Wilbourn et al., 2018; Young, 2018). Negative correlations between fitness-related traits may conform to the hypothesized life-history trade-offs (Futuyma, 2010), but they are difficult to observe in the wild, for instance due to variation among individuals in resource acquisition (van Noordwijk & de Jong, 1986) and random environmental variation (Pujol et al., 2018). Nonetheless, physiological or genetic constraints generating life-history trade-offs may be detected by comparing different phenotypes or genotypes (Reznick, 1985), or through experimental manipulations involving for example natural selection in a controlled environment or artificial selection in the traditional breeder's approach (Conner, 2003; Pick et al., 2020; Postma, Visser, & Van Noordwijk, 2007). Correlated responses to artificial selection then suggest additive genetic covariance between a trait and the selected trait (Conner, 2003).

In a large scale experimental study, an artificial directional selection regime on body size, as indicated by tarsus length, was imposed annually and in opposite directions during four consecutive years in two island populations of wild house sparrows (*Passer domesticus*) in northern Norway (Fig. 2). Each winter, adult sparrows with tarsus lengths smaller or larger than given thresholds were removed from each population to produce a significant bidirectional change in mean tarsus length across the adult breeding populations (Kvalnes et al., 2017). Relatively high heritabilities for tarsus length have been found in these populations (h^2=0.3-0.4, Kvalnes et al., 2017), thus we expected, based on the Breeder's equation (Lande, 1979), the artificial selection to result in significant responses in offspring tarsus lengths and growth rates. Here, we initially show how the artificial selection on parental tarsus length affected the size of their offspring measured during the nestling stage. We use tarsus length as a proxy for structural body size, as is commonly done in avian studies (Araya-Ajoy et al., 2019; Rising & Somers, 1989).

In a previous study based on a subsample of chicks from the population undergoing artificial selection for larger adult body size, Ringsby et al. (2015) showed that the selection regime had indeed extended the range of chick body size at its upper end, and that this was associated with a reduction in TL in red blood cells. In this study, we examined whether artificial selection for either smaller or larger body size of adults would lead to longer or shorter early-life TL, respectively, in the offspring. We also examined the results in chicks whose parents were not subjected to the selection regime (as controls). We constructed genetic pedigrees to identify nestlings with parents that were subjected to artificial selection. We then investigated how individual TL in nestling cohorts changed under the different size selection regimes: We expected that increasing body size through artificial selection would lead to shorter TLs through increased number of cell divisions (Falconer, Gauld, & Roberts, 1978) and oxidative stress associated with increased energy expenditure (Geiger et al., 2012; Monaghan & Ozanne, 2018; Pauliny, Devlin, Johnsson, & Blomqvist, 2015; Smith, Nager, & Costantini, 2016). Similarly, we expected that selection for smaller body size (and thus presumably decreased nestling growth) led to a slower rate of TL reduction and therefore longer TL (Vedder, Verhulst, Zuidersma, & Bouwhuis, 2018). Since TL was measured in early-life (average age 11±1.5 SD days in both populations) we also examined environmental factors previously shown to influence telomere loss during this period (Chatelain, Drobniak, & Szulkin, 2020), specifically brood competition (Boonekamp, Mulder, Salomons, Dijkstra, & Verhulst, 2014) and weather conditions (Foley et al., 2020; Graham, Bauer, Heidinger, Ketterson, & Greives, 2019).

We assumed that the populations were close to their local optimal body sizes when the artificial selection was initiated (Kvalnes et al., 2017). Kvalnes et al. (2017) found that artificially selected adult birds had reduced fitness. Thus, the annual artificial selection was expected to shift the populations away from their optimal body size and increase the phenotypic variance across the populations (Kvalnes et al., 2017). Here, we investigated whether changes in TL following the artificial size selection over a period of 5 years might mechanistically underpin fitness effects due to the deviation from the optimal body size. Thus, the survival and reproduction of all individuals on both islands were monitored during and after the selection events. Hypothesizing that shorter TL relative to body size would be associated with lower survival and reduced lifespan (Heidinger et al., 2012), because shorter telomeres have been shown to reflect adverse early-life conditions (Eastwood et al., 2019; Wilbourn et al., 2018), we tested the effect of TL on short-term (first-year) survival under the two selection regimes, and on long-term survival (lifespan) after the artificial selection events. Similarly, we tested if longer early-life TL predicted higher future reproductive success (Bauch, Becker, & Verhulst, 2013; Heidinger et al., 2012; Pauliny et al., 2006).

MATERIALS AND METHODS

Study system and sampling

The study was performed on the islands of Vega (163 km^2, 65°40'N, 11°55'E) and Leka (57 km^2, 65°06'N, 11°38'E) off the coast of northern Norway (Fig. S1.1) in the years 2002-2012. On both islands, the sparrows live closely associated with humans mainly on dairy farms, where they nest in cavities and have access to grain and shelter all year. During the breeding season from 2002-2006 (during and after the artificial selection experiment, Fig. 2) all nests were visited regularly (Appendix S1). Chicks were marked with color rings for identification. Year-round mist-netting ensured that around 90% of the winter population at both islands were marked at all times during the study (Kvalnes et al., 2017). Tarsometatarsus (tarsus) length (a proxy for body size) was measured using calipers to the nearest 0.01 mm. Because tarsus length increases with nestling age, we estimated standardized tarsus length as the residuals of a multiple linear regression of tarsus length on age and age squared (i.e. a quadratic model) separately for each sex. The standardizations were made for each population separately such that we did not have to assume equal growth trajectories between treatments. Age-corrected nestling tarsus length is a good predictor of adult tarsus length (Fig. S1.2, r=0.74, for n=220 birds that were recaptured as adults). Adult tarsus length repeatability is generally high (~0.94) within our study system (Jensen et al., 2003). A small blood sample (25 μL) was collected from all nestlings around 11 days (5-17 days) old by puncturing the brachial vein. Blood was stored in 1 ml absolute ethanol at room temperature in the field and subsequently in the laboratory at -20°C until DNA extraction.

Artificial selection on tarsus length

In February-March each year from 2002-2012 (11 years, Fig. 2) ca. 90% of the house sparrows on Leka (*n*=89-222 per year) and Vega (*n*=102-352 per year) were caught using mist-netting and then held in separate aviaries with *ad libitum* water and food for up to 12 days until most birds had been captured . During 2002-2005, adult sparrows with tarsi shorter (Leka, referred to as the *high* population) or longer (Vega, referred to as the *low* population) than the island population mean ±0.3 SD, within each sex, were translocated to distant localities and thus removed from the breeding populations (see Kvalnes et al., 2017 for details of the selection procedure). Thus, both populations were subject to the same artificial selection pressure, but in opposite directions. In the years following the end of the selection experiment (from 2006) all birds were released back at the original capture location (Rønning et al., 2016).

Weather data

Local weather data for temperature, humidity, precipitation, atmospheric pressure, and wind speed retrieved from The Norwegian Meteorological Institute (2018) were averaged over 30 days prior to sampling in order to reflect average environmental conditions before and during the nestling growth phase (Appendix S1). Due to intercorrelation among weather data variables (Fig. S1.3), we used principal component analysis (detailed in Appendix S1, Fig. S1.4 and Table S1.1) to explore relationships between local weather variables and the regional daily North Atlantic Oscillation (NAO) index (retrieved from the National Oceanic and Atmospheric Administration (NOAA), 2018) averaged over the same 30-day period (NAO_30). Based on these analyses we decided to use only the NAO weather variable in the subsequent analyses (Appendix S1). The

NAO_30 index is positively correlated with temperature, humidity and atmospheric pressure and negatively correlated with wind speed and amount of precipitation in our study area (Fig. S1.3) and therefore might be used as a simple index of overall harshness of weather conditions during the nestling stage; negative and positive NAO_30 indexes may indicate "harsh" and "benign" conditions, respectively (e.g. Stenseth et al., 2003).

Molecular analyses

DNA extraction is described in Appendix S1. Extracted DNA was stored at -20°C. Relative erythrocyte TLs were measured in all nestlings (*n*=566 in total, average age 11.3±1.5 SD days ranging from 5-16 days on Leka [*n*=158] and 10.8±1.5 SD days ranging from 7-17 days on Vega [*n*=408], Table 1) from 2002-2006 (5 cohorts from two populations, Fig. 2) using the qPCR method (Cawthon, 2002; Criscuolo et al., 2009). The qPCR method measures the ratio of the telomere repeat copy number amount of telomere sequence to a control single copy gene number (a non-variable "housekeeping" gene; GAPDH) relative to a reference sample. This ratio is referred to as the relative telomere length (TL). DNA concentration was measured using a FLUOstar Omega scanner (BMG Labtech). DNA samples were diluted with dH_2O to yield 1.67 ng/µL (corresponding to 10 ng of DNA per well in the PCR assay) and subsequently stored at -78°C. All samples were randomized and run in triplicates on 96-well plates, each plate including a 2-fold serial dilution (40-2.5 ng/well) of a "standard sample" of DNA from a single individual used to produce standard curves for each plate, a non-target control sample, and the reference sample (all in triplicates). Following Ringsby et al. (2015), PCR assays were prepared using the Absolute blue qPCR SYBR green Low Rox master mix (ThermoFisher Scientific) and run using a Stratagene Mx3005p system and analyzed using the MxPro qPCR software (Agilent) with a manually adjusted threshold fluorescence. Primers and thermal profiles are specified in Appendix

S1. Relative TLs were calculated taking plate amplification efficiencies (all within 100±10%, mean telomere assay efficiency was 102.2±3.8%, and 101.9±3.4% for GAPDH assays across 2x21 plates) into account using the Pfaffl (2001) method. All telomere analyses were performed at the University of Glasgow and included 507 individuals measured in 2017 by M.L. Pepke (this study) and 60 individuals (a subset of male and female nestlings from both populations from 2002 and 2005) measured in 2014 by W. Boner under identical laboratory conditions and using the same reference sample (Ringsby et al., 2015).

Molecular sexing and microsatellite pedigree reconstruction are described in Appendix S1. Each nestling was classified into one of three selection categories (Table 1): *Selected* (1): Both parents had been subject to the selection regime, i.e. captured during a winter selection event and allowed to stay in the population. *Intermediate* (0.5): one parent had been subject to artificial selection. *Unselected* (0): No parents had been artificially selected (i.e. accidentally not captured during any winter selection event) and these individuals are therefore the unselected controls. Unknown genetic parents were assumed not to have been artificially selected (i.e. not captured or immigrated after the selection event and thus not included in the pedigree).

Statistical analyses

Temporal changes in telomere and tarsus lengths during artificial selection

To analyze how nestling tarsus length and TL were affected by the artificial selection for longer (*high*) and shorter tarsi (*low*) during the study from 2002-2006, we used linear mixed effects models (LMMs, R package *lme4*, Bates, Mächler, Bolker, & Walker, 2015; R Core Team, 2020) including year (birth cohort) as a continuous predictor variable, as well as the quadratic effect of year (year^2). Tarsus length and TL are expected to change during development (Boonekamp et al., 2014; Hall et al., 2004) and there might be sexual differences in morphology (Cordero, Griffith, Aparicio, & Parkin, 2000) and telomere dynamics (Barrett & Richardson, 2011). Thus, nestling age (number of days since hatching) and sex were included as explanatory variables in all models. Individuals in selection category 1 are expected to show the strongest response in tarsus length and those in category 0 are effectively unselected controls. Thus, selection category (0, 0.5, or 1) was included as a factor in some models. We tested for effects of successive selection by including an interaction term between selection category and year. All models assumed a Gaussian error distribution and included a random intercept for brood identity to account for the non-independence of nestlings from the same brood. We structured these analyses into four sections, where we analyzed each selection regime (*high* or *low* population) separately for each response variable (tarsus length and TL). To identify the predictors most supported by the empirical data we constructed and compared alternative candidate models (Burnham & Anderson, 2002) fitted with maximum likelihood within each section using Akaike's information criterion (Akaike, 1973) corrected for small sample sizes (AICc, Hurvich & Tsai, 1989). All models were validated visually by diagnostic plots and model parameters are given from models refitted with restricted maximum likelihood (REML). We only included predictor variables with intercorrelation Pearson's $r<0.5$ for all relevant pairs of explanatory variables.

Effects of tarsus length and weather on telomere length

We investigated factors affecting nestling TL (response variable) by constructing 25 biologically plausible LMMs with combinations of the explanatory variables sex, standardized tarsus length, brood size (number of chicks in the nest at the time of sampling), and the NAO_30 index. Island population identity (i.e. *high* or *low* selection regime as a categorical variable) and age (continuous variable) were included as fixed effects in all models. In addition, an interaction term between population identity and tarsus length was included in some models to test for an effect of the artificial selection regimes on the relationship between TL and tarsus length. Brood identity and year (cohort) effects were accounted for by inclusion as random intercepts in all models. Again, candidate models were compared using AICc.

Effects of tarsus and telomere lengths on survival

The effect of nestling TL on short- and long-term survival was analyzed using two different approaches: First, we analyzed the effect of TL on first-year survival (i.e. before removal of artificially selected individuals) using generalized LMMs (*lme4* package) with a binomial error distribution and a logit link function (logistic regression) using the bobyqa optimizer to improve model convergence (Bates et al., 2014). Birds that were never observed after the season in which they were born (effectively after end of January when field work recommenced) were considered to be dead. We used AICc to compare 26 candidate models including the explanatory variables TL, tarsus length, non-linear effects of TL and tarsus length (TL^2 and tarsus $length^2$), the NAO_30 index, and interactions between tarsus length and population identity and between TL and population identity. The interactions were added to test if the effect of TL and tarsus length was opposite or in other ways differed between populations (treatments). Population identity and sex were included as fixed factors and brood identity and year were included as random intercepts in all models.

Second, to test the effect of nestling TL on survival throughout the life of individuals we used multivariate Cox proportional hazards regression to estimate hazard ratios (HR), which is the relative risk of death or disappearance compared to the total population (Cox, 1972). Thus, a HR>1 indicates an increased mortality given an increase in the trait. 26 candidate models with the same fixed effects as described above were constructed using the *survival* package (Therneau, 2015). We accounted for non-independence of broods by including brood identity as clusters. Individuals that were removed during the artificial selection were right-censored (*n*=60) and the last observation of an individual was used as an estimate of the (minimum) lifespan measured in number of days since hatching. This procedure accounts for the right-censoring caused by the artificial removal of individuals, but the censoring is non-independent with respect to tarsus length. However, when combining both populations, the resulting bias to the hazard function is expected to act in opposite directions. We may underestimate absolute survival probabilities, as recapture rates were not accounted for in this approach. However, since both populations were carefully monitored each year, we can assume that >90% of the individuals that were present in the study populations were recorded from year to year (Kvalnes et al., 2017), and that the total observation interval correlates with lifespan. No other birds were censored as observations continued until 2012; two years after the last record of any sampled individual (in 2010). The proportional hazards assumption was tested using the correlation between scaled Schoenfeld residuals and time. We used the *simPH* package to simulate and plot the effects of the predictor variables on the hazard ratios (Gandrud, 2015).

Effects of tarsus and telomere lengths on reproductive success

Finally, the effect of nestling TL on the total number of recruits produced per individual (LRS, lifetime reproductive success) was analyzed. We included only individuals that survived until breeding and that were not removed during the artificial selection (and thus allowed to reproduce). In the *high* population, 10 out of 22 recruiting individuals produced at least one recruit and 39 out of 80 individuals did so in the *low* population. While reproduction *per se* may accelerate telomere loss (Sudyka, 2019), we test here the predictive value of early-life TL and/or tarsus length on subsequent reproductive output (Eastwood et al., 2019). Since LRS and lifespan (measured in years from first to last observation) were highly correlated (*high* population; Pearson's *r*=0.83,

$p<0.0001$, *low* population; Pearson's $r=0.70$, $p<0.0001$), we controlled LRS for lifespan (which is equivalent to the individual average annual reproductive success, ARS). We fitted a set of generalized LMMs with a Poisson error distribution separately for each population to facilitate model convergence, using the package *glmmTMB* (Brooks et al., 2017). Sex was included as fixed factor and brood identity and year were included as random intercepts in all models. We compared the same 9 candidate models for each population using AICc and models were validated using the DHARMa package (Hartig, 2020).

RESULTS

Temporal changes in tarsus and telomere lengths during artificial selection

In the first year (2002) of the selection experiment, there was no significant difference in average nestling tarsus lengths between the *high* and *low* populations ($n=167$, $\beta_{low}=0.22$, lower and upper 95% confidence interval (CI)=[-0.25, 0.70] accounting for age at sampling and sex). Across the study period (2002-2006), nestling tarsus length increased in the *high* population ($n=158$, model ranked 1: $\beta_{year}=0.70$, CI=[0.01, 1.38], Figs. 3a, S2.1 and S2.2, Tables S2.1 and S2.2) and decreased in the *low* population ($n=408$, model ranked 1: $\beta_{year}=-0.63$, CI=[-1.23, -0.04], Figs. 4a, S2.1 and S2.2, Tables S2.1 and S2.2). In both populations, there was a weak tendency for a curvilinear change over the years, indicating that after the initial divergence the change in tarsus length ceased (*high*, model ranked 1: $\beta_{year^{\wedge}2}=-0.09$, CI=[-0.20, 0.03]; *low*, model ranked 1: $\beta_{year^{\wedge}2}=0.10$, CI=[-0.00, 0.20], Figs. 4a and 5a, Table S2.2). As expected, in the *high* population, the tarsus of nestlings with both parents artificially selected (Fig. S2.1) was on average longer than the tarsus of nestlings produced by unselected individuals (i.e. with parents not subjected to artificial selection; model ranked 1, selected vs. unselected: $\beta_{selected}=0.52$, CI=[0.04, 1.02], Fig. 3b, Tables S2.1 and S2.2), and tended to be shorter than tarsus of nestlings produced by unselected individuals in the *low* population (model ranked 2, ΔAICc=0.6, selected vs. unselected: $\beta_{selected}$=-0.38, CI=[-0.80, 0.03], Fig. 4b, Table S2.1). Intermediate individuals with one artificially selected parent showed a similar weak tendency when compared to the unselected (*high*: $\beta_{intermediate}$=0.25, CI=[-0.21, 0.72]; *low*: $\beta_{intermediate}$=-0.31, CI=[-0.69, 0.08]).

Across the study period, nestling TL decreased linearly in the *high* population (model ranked 2, ΔAICc=0.0: β_{year}=-0.26, CI=[-0.50, -0.01], Figs. 3c and S2.1, Tables S2.1 and S2.2), but there was no evidence for change in the *low* population (model ranked 2, ΔAICc=1.9: β_{year}=0.01, CI=[-0.02, 0.03], Figs. 4c and S2.1, Table S2.1). In the *high* population there was weak evidence for a curvilinear change over the years, indicating that after the initial increase the change in TL ceased (model ranked 2: $\beta_{year^{\wedge}2}$=0.04, CI=[-0.00, 0.08], Fig. 3c, Table S2.2), but there was no evidence for any curvilinear change in the *low* population (model ranked 4, ΔAICc=0.7: $\beta_{year^{\wedge}2}$=0.01, CI=[-0.01, 0.03], Fig. 4c). The model ranked 1 in the *low* population included only sex and age (Tables S2.1 and S2.2). TL of nestlings with both parents artificially selected did not differ from those of unselected individuals in the *high* population (model ranked 3, ΔAICc=0.7, selected vs. unselected: $\beta_{selected}$=0.02, CI=[-0.18, 0.22], Fig. 3d, Tables S2.1) nor in the *low* population (model ranked 3, ΔAICc=3.0, selected vs. unselected: $\beta_{selected}$=0.03, CI=[-0.07, 0.13], Fig. 4d, Table S2.1). However, intermediate individuals with one artificially selected parent showed weak evidence for a tendency towards shorter telomeres when compared to the unselected in the *high* population ($\beta_{intermediate}$=-0.13, CI=[-0.32, 0.07], Fig. 3d), and towards longer telomeres compared to unselected individuals in the *low* population ($\beta_{intermediate}$=0.05, CI=[-0.05, 0.15], Fig. 4d). Overall, our results show some evidence for an inverse association between changes in tarsus length and TL across cohorts in both populations as a consequence of artificial selection away from optimal body size, and this pattern was more evident in the *high* population.

Effects of tarsus length and weather on telomere length

Combining data from both populations (n=566), TL was found to be negatively related to tarsus length (model ranked 1: β_{tarsus}=-0.03, CI=[-0.06, -0.00], Tables 2 and 3, Fig. 5a) and positively related to the NAO_30 index (β_{NAO_30}=0.11, CI=[0.02, 0.20], Fig. 5b). This means that telomeres were shorter in larger individuals and when overall weather conditions were harsh. In addition, TLs were shorter in females than males (β_{female}=-0.08, CI=[-0.14, -0.01]), and TLs were shorter in the *low* population compared to the *high* population (β_{low}=-0.17, CI=[-0.23, -0.09]).

Effects of telomere length on survival

Average first-year survival from fledging to recruitment was 29%, mean minimum lifespan was 152±11 days (n=566), and maximum recorded lifespan was 5.7 years. There was a positive effect of tarsus length on first-year survival (i.e. recruitment probability, model ranked 1: odds ratio $(OR)_{tarsus}$=2.04, CI=[1.19, 3.48], Table S2.3). This model also suggested that the positive effect of tarsus length on first-year survival was stronger in the *high* population compared to the *low* population ($OR_{low*tarsus}$=0.55, CI=[0.31, 0.97]). We did not find any evidence for a consistent effect of TL on first-year survival (model ranked 2: ΔAICc=1.9, $(OR)_{TL}$=0.89, CI=[0.51, 1.55]).

The Cox proportional hazards regression analyses of long-term survival revealed a negative effect of tarsus length on the risk of disappearance (hazard ratio $(HR)_{tarsus}$=0.90, CI=[0.82, 0.97], Tables S2.4 and S2.5, Fig. 6a). In addition, the second-best model suggested a tendency for a curvilinear effect of TL on mortality ($HR_{TL\text{^}2}$=0.74, CI=[0.52, 1.05]), where nestlings with both short and long TL have lower long-term mortality rates (Fig. 6b, Table S2.5).

Effects of telomere length on reproductive success

In the *high* population, there was weak evidence for a negative effect of TL on LRS while controlling for lifespan (model ranked 2, ΔAICc=0.1, β_{TL}=-1.24, CI=[-2.52, 0.04], Tables 4 and S2.6) suggesting that individuals with short telomeres in early-life produced more recruits annually. In the *low* population, the best model was the simplest model including only the effects of sex and lifespan (Tables 4 and S2.6).

DISCUSSION

Life-history theory suggests that individuals allocate their acquired resources between growth, reproduction, and self-maintenance (Roff & Fairbairn, 2007; Stearns, 1989; Zera & Harshman, 2001). In this experimental study, a negative relationship between nestling TL and tarsus length was found under the artificial selection regimes for both larger and smaller tarsi (Table 3, Fig. 5a). This negative association between TL and structural body size suggests that telomere dynamics might mediate a trade-off between investment in early-life growth and long-term somatic maintenance (Metcalfe & Monaghan, 2003; Monaghan & Ozanne, 2018; Ringsby et al., 2015).

Artificial selection for larger individuals in the *high* population caused TL to decrease as tarsus length increased during the four years of selection (Fig. 3), which confirms the observation by Ringsby et al. (2015). However, effect sizes were small (Table S2.2). There was no strong evidence that TL increased as tarsus length decreased in the *low* population (Fig. 4). It is possible that the artificial selection for smaller body size in adults only caused a small change in offspring size because the proportion of additive genetic variance may be lower for small compared to large individuals (Charmantier, Kruuk, Blondel, & Lambrechts, 2004). Thus, selecting for smaller tarsus length for multiple years, as in our experiment, may accumulate adult individuals that are smaller than their predicted size due to for instance malnutrition or disease caused by e.g. environmental or parental effects (Angelier, Vleck, Holberton, & Marra, 2015).

The artificial selection pressure on larger body size was accompanied by a reduction in TL that was probably not counteracted within the nestling period by increased investment into telomere maintenance (i.e. canalization, Vedder, Verhulst, Bauch, & Bouwhuis, 2017). Early-life changes in TL have been hypothesized to influence long-term somatic state (Boonekamp, Simons, Hemerik, & Verhulst, 2013; Eisenberg, 2011; Vedder et al., 2017). The enzyme telomerase can elongate telomeres (Blackburn, 1991), but its activity is assumed to be a physiologically costly process (Criscuolo et al., 2018; Hatakeyama et al., 2016) or with potential increased cancer risk effects (Pepke & Eisenberg, 2021; Seluanov, Gladyshev, Vijg, & Gorbunova, 2018). Accordingly, somatic telomerase activity is generally assumed to be repressed in birds (Gomes, Shay, & Wright, 2010), though more investigation of this is needed since some somatic telomerase activity has been detected (Haussmann, Winkler, Huntington, Nisbet, & Vleck, 2007). Early-life erythrocyte TL in house sparrows has been estimated to 15-20 kbp (Ringsby et al., 2015). If 50-100 bp of telomeric DNA are lost with each cell division (Lansdorp, 1995), early hematopoietic stem cells would have the potential of 150-400 divisions, many more than is needed for growth and maturation of the adult house sparrow (Sidorov, Kimura, Yashin, & Aviv, 2009). However, increased oxidative stress associated with acquiring and maintaining a larger body size (Alonso-Alvarez, Bertrand, Faivre, & Sorci, 2007) could accelerate the shortening of telomeres significantly (Reichert & Stier, 2017) providing an explanation for the observed negative association between size and TL.

The growth and survival of house sparrow nestlings depend on early-life conditions such as habitat quality and insect food being supplied by the parents (Anderson, 2006). Larger sparrows have higher juvenile and adult survival (Jensen, Steinsland, Ringsby, & Sæther, 2008; Ringsby, Sæther, & Solberg, 1998), and harsh weather during the nestling period increases juvenile mortality (Ringsby, Sæther, Tufto, Jensen, & Solberg, 2002). The associations between TL and both body size and the weather proxy (NAO_30) in nestlings (Table 3 and Fig. 5) suggest that TL is determined by complex and potentially counter-acting effects of growth, nutrition and external factors (Angelier et al., 2015; Nettle et al., 2016). For instance, malnutrition may lead to reduced growth, but also increased oxidative stress and telomere attrition (Nettle et al., 2017). Also, indirect effects of weather conditions may cause foraging stress (Spurgin et al., 2018) or maternal stress effects during breeding that negatively affect offspring TL (Haussmann Mark, Longenecker Andrew, Marchetto Nicole, Juliano Steven, & Bowden Rachel, 2012; Mizutani, Tomita, Niizuma, & Yoda, 2013), and direct effects of weather may cause shortening of telomeres, such as thermal stress observed in other tetrapods (Debes et al., 2016; Foley et al., 2020; Graham et al., 2019). Thus, generally habitat quality is important, with shorter telomeres in low-quality habitats (Angelier, Vleck, Holberton, & Marra, 2013; Watson, Bolton, & Monaghan, 2015; Wilbourn et al., 2017). The regional NAO_30 index must be interpreted with respect to local conditions along the northern Norwegian coast but might be a better single proxy for the overall weather conditions by reducing complexity and avoiding problems of model variable selection (Hallett et al., 2004; Stenseth et al., 2003). Thus, a low NAO_30 index, which in our study area corresponds to a combination of low temperatures, strong winds and rainfall during a 30-day interval before TL sampling, was found to significantly reduce TL in nestlings, when correcting for body size (Fig. 5b). This is consistent with studies reporting shorter telomeres because of poor nutrition, competition, or thermoregulation (reviewed in Chatelain et al., 2020).

Several studies have reported a negative association between TL and mortality risk; mainly in birds (reviewed in Wilbourn et al., 2017) and humans (reviewed in Boonekamp et al., 2013;

Wang, Zhan, Pedersen, Fang, & Hagg, 2018). This association can be attributed to either the biomarker characteristic of TL reflecting cumulative environmental stressors (Angelier, Costantini, Blevin, & Chastel, 2018; Monaghan, 2014; Nettle et al., 2017; Pepper, Bateson, & Nettle, 2018) or the direct effect of having short telomeres leading to cellular senescence and certain diseases (Blackburn, Epel, & Lin, 2015; Young, 2018). However, this correlation is not universal across tetrapods, with some studies finding no correlation in birds (Boonekamp et al., 2014), mammals (Fairlie et al., 2016), and reptiles (Olsson et al., 2011), or that shorter telomeres correlate with higher survival in birds (Wood & Young, 2019), snakes (Ujvari & Madsen, 2009), and fish (McLennan et al., 2017).

Ringsby et al. (2015) suggested that the changes in TL induced by the artificial size selection could underpin a trade-off between body size and lifespan if TL is related to lifespan (Heidinger et al., 2012). In this study, we found little support for an effect of TL on short-term survival (i.e. survival of juveniles until recruitment) after accounting for the positive association between tarsus length and survival (Table S2.3). Body size is likely to be an important component of juvenile mortality if the mortality is mainly due to extrinsic factors (Eastwood et al., 2019; Wood & Young, 2019), as expected in juvenile house sparrows (Ringsby et al., 1998). The artificial selection increased the range of body sizes across the populations, which was predicted to more clearly reveal effects of TL on fitness compared to unmanipulated populations. However, the evidence for individuals with either short or long telomeres to have reduced mortality risk through life, controlling for the negative effect of tarsus length, was weak (Fig. 6b). The observation in humans that short telomeres are associated with age-dependent degenerative diseases and long telomeres with higher cancer incidence rates (Aviv, Anderson, & Shay, 2017), suggests the opposite of our findings (i.e. that both short and long TL is associated with higher mortality). However, there is probably little or no constraints on TL imposed by cancer or age-dependent diseases in free-living, short-lived sparrows (Møller, Erritzøe, & Soler, 2017).

Some correlative studies may have overlooked disruptive selection on TL, and such patterns can be confounded by (unmeasured) telomerase expression in somatic cells with high proliferation rates (Cerchiara et al., 2017; Klapper, Heidorn, Kühne, Parwaresch, & Guido, 1998; Ujvari & Madsen, 2009). However, if TL is inversely related to telomere loss later in life (Bauch, Becker, & Verhulst, 2014; Verhulst, Aviv, Benetos, Berenson, & Kark, 2013), measuring TL at a later age may generate the expected positive correlation between survival probability and TL (Wood & Young, 2019). Alternatively, in individuals with short telomeres, TL may be traded off against some unmeasured component of individual quality (Wilson & Nussey, 2010). Yet, when controlling for lifespan, short telomeres were weakly associated with higher recruit production in the *high* population (Table 4). This might suggest that there are additional negative impacts on TL associated with acquiring an artificially increased body size that deviates from the optimal body size under the prevailing conditions. Such impacts may act through increased competition when siblings are larger (Nettle et al., 2016) and increased oxidative stress during growth (Geiger et al., 2012). This indicates that in the *high* population, high fitness birds were bigger and had shorter telomeres.

Telomeres were longer in male than in female house sparrows, also when correcting for size (Table 3). We also note that males tended to have higher LRS (Table 4), but only in the *high* population, where just 6 males managed to reproduce at least one recruit. There were no sex-differences in survival in our study (Table S2.5), which has been suggested to underlie sex-specific telomere dynamics in humans, mice, and sand lizards (reviewed in Barrett & Richardson, 2011). In similar Norwegian house sparrow populations, males had longer individual generation times

(Araya-Ajoy et al., 2021), but there was no sex-biased mortality or senescence patterns (Holand et al., 2016). In birds and mammals, adult mortality appears to be biased towards the heterogametic sex (Liker & Szekely, 2005), which may be caused by the potentially unmasked expression of deleterious sex-linked alleles (Trivers, 1985). In birds, females are the heterogametic sex, but sexual differences in telomere dynamics have only rarely been observed among bird species (Remot et al., 2020). Unmeasured sex-specific differences in growth dynamics (in house sparrows, Cleasby, Burke, Schroeder, & Nakagawa, 2011) or differential telomere loss under parasite infection (Sudyka, 2019) could also generate the observed TL sex differences.

The evolutionary significance of the observed changes in TL induced by the artificial size selection will depend on the heritability of TL, which vary considerably among populations (Dugdale & Richardson, 2018). Future studies may show whether the relationship between size and TL is underpinned by genetic correlations (Monaghan & Ozanne, 2018). We found that during the important nestling phase, TL was influenced by tarsus length and weather conditions and varied between sexes and populations. Body size, as indicated by tarsus length, was an important determinant of survival, but both short and long telomeres tended to predict lower mortality across the populations after the range of body sizes had been artificially increased. In individuals larger than their optimal size in the wild, TL was reduced, which may have been associated with an increased reproductive output. When selecting for smaller adult body size, we observed a smaller response in nestling size and TL, and no relationship between TL and reproductive success. Thus, this experiment revealed complex relationships between TL and fitness that were unexpected based on current theory and empirical knowledge. The fitness effects captured by the interaction between TL and body size suggest that the evolution of TL will depend on other phenotypic parameters and their genetic covariances.

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DATA ACCESSIBILITY

All data will be made available on the Dryad Digital Repository or another open access channel upon acceptance of the manuscript.

AUTHOR CONTRIBUTIONS

MLP analyzed the data and wrote the manuscript. MLP and WB measured telomere lengths. MLP and BR constructed pedigrees. THR, HJ, and B-ES designed the artificial selection experiment. THR and PM designed the telomere investigation. THR, HJ, TK, and BR contributed to the fieldwork and TK curated field data. All authors contributed to the intellectual content through input, comments, and edits when writing up the manuscript.

FIGURES AND TABLES

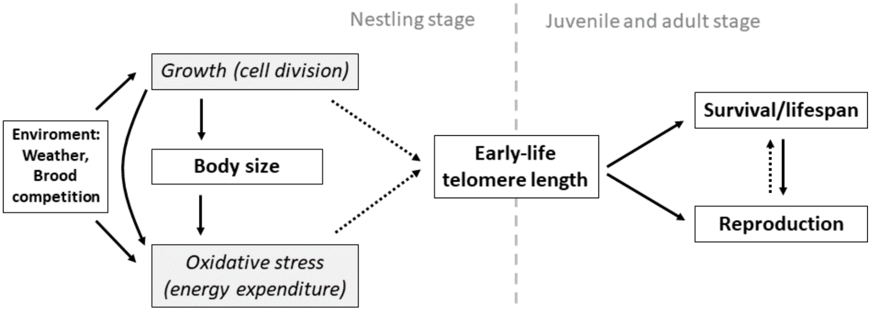

Fig. 1: Hypotheses of expected positive (solid lines) and negative (dotted lines) effects shaping variation in early-life telomere length during the nestling stage, and later-life fitness consequences.

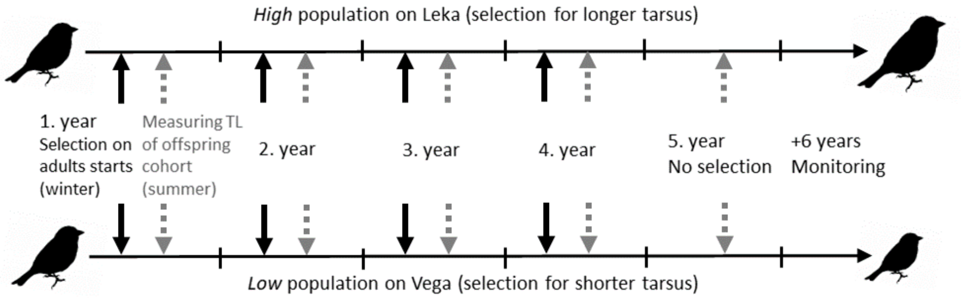

Fig. 2: Timeline of the artificial size selection experiment on free-living house sparrows conducted in parallel on two islands in Norway starting in year 2002 (1st year). Adult birds were captured each winter for 4 years and selected for either longer or shorter tarsus length (each selection event showed with a solid black arrow). Telomere lengths (TL) were measured on nestling cohorts produced in the subsequent summer breeding season (showed as dotted grey arrows). Birds that were not removed during the artificially selection stayed in their populations, and survival and recruit production were monitored until all sampled birds were assumed to had died.

Table 1: Number of sampled offspring (*n*=566) in each artificial selection category (1: both parents artificially selected; 0.5: one parent subject to artificial selection; 0: no parents artificially selected, i.e. unselected controls) from year 2002-2006 in two island populations selected for larger (*high*) and smaller (*low*) tarsus length, respectively. Unknown genetic parents were assumed not to have been artificially selected.

Population:		***High* (Leka)**						***Low* (Vega)**					
Selection category:	**Description:**	**2002**	**2003**	**2004**	**2005**	**2006**	**Sum:**	**2002**	**2003**	**2004**	**2005**	**2006**	**Sum:**
***Selected* (1)**	Both parents artificially selected.	5	18	16	21	14	**75**	45	48	25	40	14	**172**
***Intermediate* (0.5)**	One parent artificially selected.	26	5	3	12	13	**59**	54	33	16	19	41	**163**
***Unselected* (0)**	No parent artificially selected.	16	3	3	2	1	**25**	21	8	18	5	21	**73**
Sum:		**47**	**26**	**22**	**35**	**28**	**158**	**120**	**89**	**59**	**64**	**76**	**408**

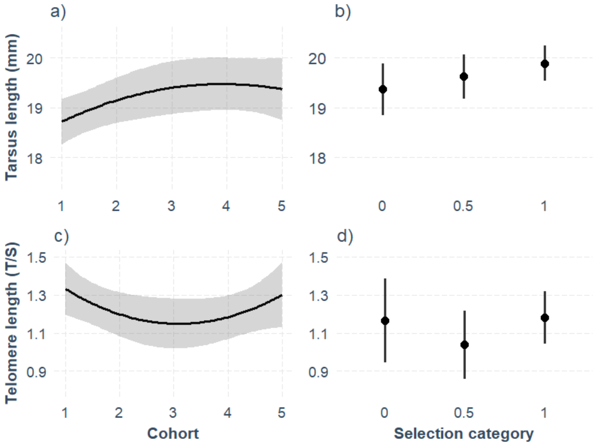

Fig. 3: *High* population; effect plots of trends in nestling tarsus (a) and telomere lengths (c) during artificial selection for larger tarsus length in parents. The artificial selection ended after cohort 4 was born. The effect of selection category (*0*: no parents selected (unselected), *0.5*: one parent selected, and *1*: both parents selected) on tarsus (b) and telomere length (d) is shown.

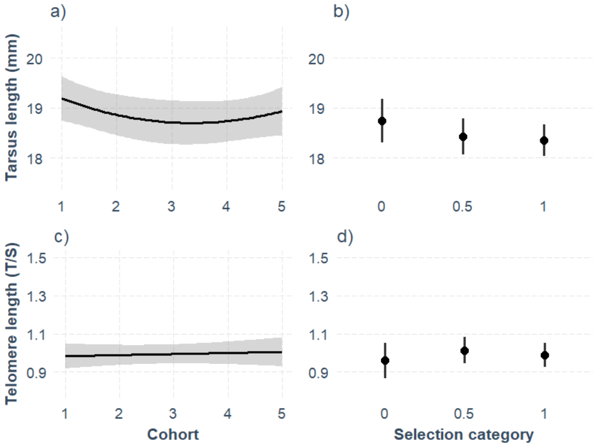

Fig. 4: *Low* population; effect plots of trends in nestling tarsus (a) and telomere lengths (c) during artificial selection for smaller tarsus length in parents. The artificial selection ended after cohort 4 was born. The effect of selection category (*0*: no parents selected (unselected), *0.5*: one parent selected, and *1*: both parents selected) on tarsus (b) and telomere length (d) is shown.

Table 2: Linear mixed effects models with ΔAICc<5 of variation in early-life telomere length in house sparrow nestlings (*n*=566) in two island populations. All models included random intercepts for brood identity and year. The models are ranked by AICc, and number of degrees of freedom (df) and model weights (w) are shown.

Model		**ΔAICc**	**df**	**w**
1	***TL = sex + age + population + tarsus + NAO_30***	**0.0**	**9**	**0.288**
2	*TL = sex + age + population + tarsus + NAO_30 + population*tarsus*	1.3	10	0.147
3	*TL = sex + age + population + age + tarsus + NAO_30 + brood size*	2.1	10	0.102
4	*TL = sex + age + population + age + NAO_30*	2.3	8	0.091
5	*TL = sex + age + population + tarsus*	3.0	8	0.065
6	*TL = sex + age + population + tarsus + NAO_30 + population*tarsus + brood size*	3.4	11	0.052
7	*TL = age + population + tarsus + NAO_30*	4.1	8	0.038
8	*TL = sex + age + population + NAO_30 + brood size*	4.4	9	0.032
9	*TL = sex + age + population + tarsus + population*tarsus*	4.5	9	0.031
10	*TL = sex + age + population*	4.6	7	0.029

Table 3: Estimates (*β*) with standard errors (SE) and lower and upper 95% confidence intervals (CI) from a linear mixed effects model of variation in telomere length (TL, *n*=566). Random intercepts for brood identity and year were included in the model.

Response variable: TL	***β***	**SE**	**Lower CI**	**Upper CI**
intercept	1.086	0.125	0.850	1.334
tarsus	*-0.029*	*0.014*	*-0.056*	*-0.002*
sex (female)	*-0.077*	*0.031*	*-0.137*	*-0.016*
population (low)	*-0.164*	*0.036*	*-0.233*	*-0.093*
NAO_30	*0.103*	*0.047*	*0.015*	*0.194*
age	0.009	0.011	-0.013	0.029
$\sigma^2_{\text{brood ID}}$ (*n*=205)	0.003		0.000	0.014
σ^2_{year} (*n*=5)	0.001		0.000	0.006

Marginal R^2 / Conditional R^2: 0.070 / 0.096

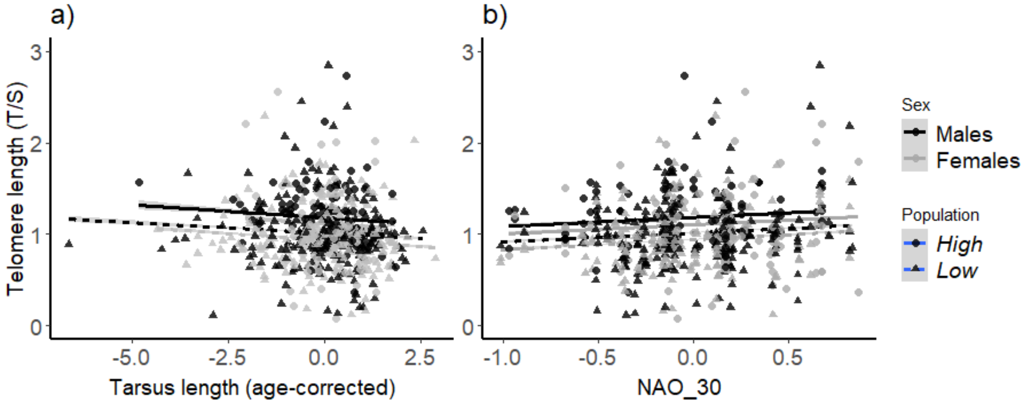

Fig. 5: The relationship between nestling telomere length (TL) and (a) age-corrected tarsus length and (b) the NAO_30 index (the average NAO index across 30 days prior to TL measurement) with regression lines based on the highest ranked model according to the AICc (Tables 2 and 3). Although there is considerable variation in TLs among individuals of similar size, tarsus length and the NAO_30 index significantly correlate with TL. Males (black) have longer telomeres than females (grey) and individuals in the *high* population (circles, full line) have longer telomeres than those in the *low* population (triangles, dotted line).

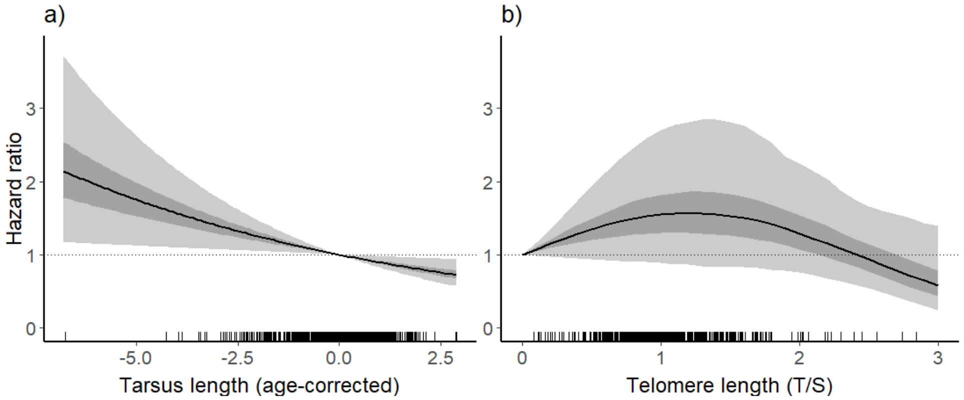

Fig. 6: Cox proportional hazards regression models showing simulated values of hazard ratios (i.e. mortality risk) with 95% and 50% confidence intervals in light grey and dark grey respectively. Hazard ratios are shown as a function of a) tarsus length, and b) telomere length. Larger individuals had lower long-term mortality, and model selection indicated a tendency for both short and long telomeres to be associated with lower mortality risk.

Table 4: Estimates with 95% confidence intervals (CI) and standard errors (SE) from the highest ranked generalized linear models (see Table S2.5) fitted using a Poisson distribution of variation in lifetime reproductive success (LRS) corrected for lifespan for each population (*high*/*low*). Random intercepts for brood identity and year were included as random factors in all models. Only individuals that were not removed during the artificial selection and that survived until breeding were included in these analyses.

***High* (*n*=22): LRS**	***β***	**SE**	**Lower CI**	**Upper CI**
intercept	0.029	0.724	-1.390	1.447
lifespan	*0.718*	*0.146*	*0.432*	*1.003*
sex (female)	-1.113	0.582	-2.253	0.027
TL	-1.244	0.653	-2.524	0.036
$\sigma^2_{brood\ ID}$ (*n*=15)	0.000		0.000	0.000
σ^2_{year} (*n*=5)	0.000		0.000	0.000
***Low* (*n*=80): LRS**				
intercept	-2.012	0.434	-2.862	-1.162
lifespan	*0.929*	*0.137*	*0.660*	*1.198*
sex (female)	-0.029	0.346	-0.708	0.649
$\sigma^2_{brood\ ID}$ (*n*=59)	0.562		0.447	1.257
σ^2_{year} (*n*=5)	0.052		0.013	4.119

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

Appendix S1: Notes on methods.

Appendix S2: Notes on results.

Supporting information

Artificial size selection experiment reveals telomere length dynamics and fitness consequences in a wild passerine

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APPENDIX S1: Notes on methods

Fieldwork

Both study islands are dominated by cultivated land (silage production), heathland, mountains, and sparse forest. The house sparrow is a small sedentary passerine (Anderson, 2006), thus the geographical separation of the islands (52 km, Fig. S1.1) ensured no dispersal between populations (Kvalnes et al., 2017). The house sparrows show strong site fidelity and generally do not display adult dispersal (Anderson, 2006; Pärn et al., 2009), such that any emigrants do not return to the natal population. The average generation time in similar house sparrow populations is about 2 years (Jensen et al., 2008), and average lifespan is about 2 years (Jensen et al., 2004), but the maximum recorded lifespan is 13 years in the wild (Klimkiewicz & Futcher, 1987).

The sparrows breed from mid-May to mid-August and may lay up to 3 broods per season (with on average 4.6±0.06 eggs in this study). All accessible and active nests were visited at least every 9th day. Laying and hatching date were thus recorded for each brood (207 broods from 158 nests). Mist-netting took place during the breeding season, in autumn (September-November), and in late winter (February-March). On average, 56.4% (*high* population) and 62.9% (*low* population) of the captured adults were removed each year, whereas the selected birds were released back at their capture location. Thus, because ca. 10% of the individuals present at the time of artificial selection were not captured, the artificially selected individuals constituted ca. 78% of the breeding populations (see Kvalnes et al., 2017).

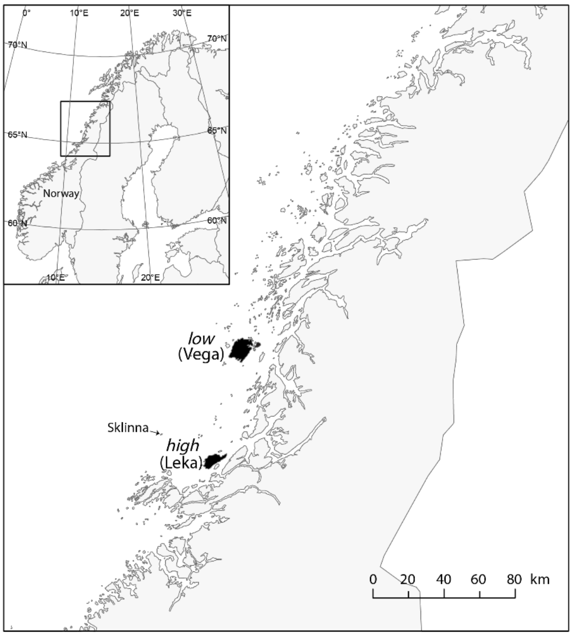

Fig. S1.1: Map of the two house sparrow study populations and the weather station at Sklinna (see below) in northern Norway.

Nestling vs. adult tarsus lengths

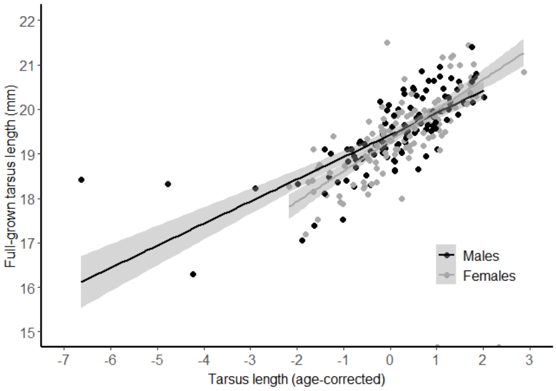

Fig. S1.2: Age-corrected nestling tarsus length was highly correlated with full-grown tarsus length (*r*=0.74, for *n*=220 birds that were recaptured in the autumn [full-grown juveniles] or later as adults [maximum length used in case of multiple measurements]). Measurements of adult birds were done by different fieldworkers and calibrated with an experienced fieldworker: Adult measurements were adjusted if mean differences differed significantly (compared across 30 individual birds, $p<0.05$, paired t-test), see Kvalnes et al., 2017 for details.

Weather data

Daily mean air temperature (K), precipitation (mm), average wind speed (m/s), average atmospheric pressure at sea level (hPa) and mean relative humidity (%) were measured at Vega (65°42'06.1'N, 11°51'22.3'E) and at Sklinna (65°12'08.3'N, 10°59'47.4'E, 29 km northwest of Leka, Fig. S1.1) from 2002-2006 and compiled from The Norwegian Meteorological Institute online database (2018). Due to missing data, precipitation measurements for Vega were used for both islands in the period 4/2004-12/2006, however, the correlation between precipitation on the two islands (1/2001-3/2004) was high (r_P=0.67, p<0.05). The North Atlantic Oscillation (NAO) index is the difference in atmospheric pressure at sea level between the Subtropical (Azores) High and the Subpolar (Iceland) Low (National Oceanic and Atmospheric Administration, 2019). This teleconnection has been associated with population fluctuations, demography, and phenotypic variation in several species in the northern hemisphere (Stenseth et al., 2002; 2003; Hallett et al., 2004). In our system, the averaged NAO index one month before chick sampling (NAO_30) is positively correlated with temperature and atmospheric pressure and negatively correlated with wind speed and amount of precipitation during the nestling stage in our study site (Fig. S1.3-4 and Table S1.1). The NAO_30 may therefore be used as an index of overall harshness of weather conditions (Ringsby et al., 2002) leading up to the sampling point; e.g. a low NAO_30 index represents a combination of e.g. high rainfall, high winds and low temperatures. Local weather variables may not capture such complex associations between weather and nestling conditions, and e.g. insect abundance, which may be better represented by large-scale climate indices (Hallett et al., 2004).

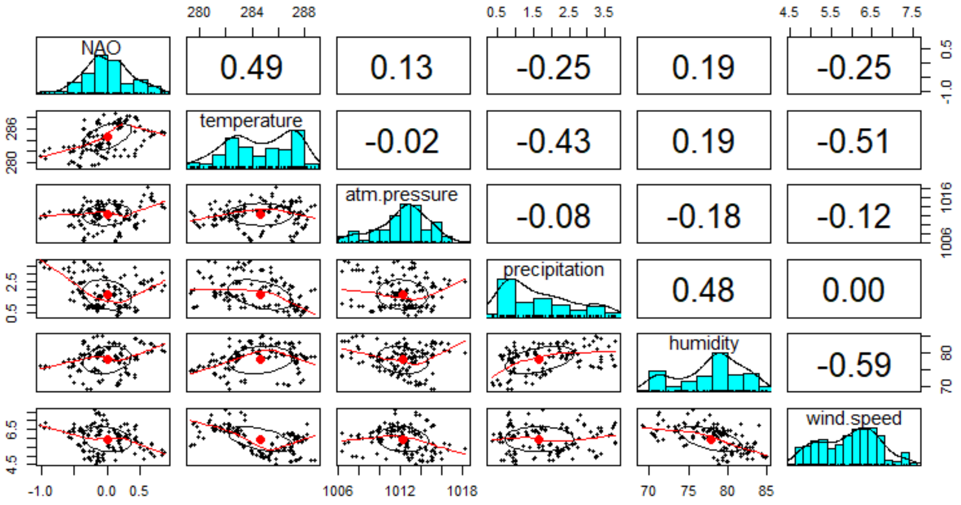

Fig. S1.3: Pearson's correlations coefficients between weather variables: the North Atlantic Oscillation index (NAO_30), temperature, atmospheric (atm.) pressure, precipitation, humidity and wind speed.

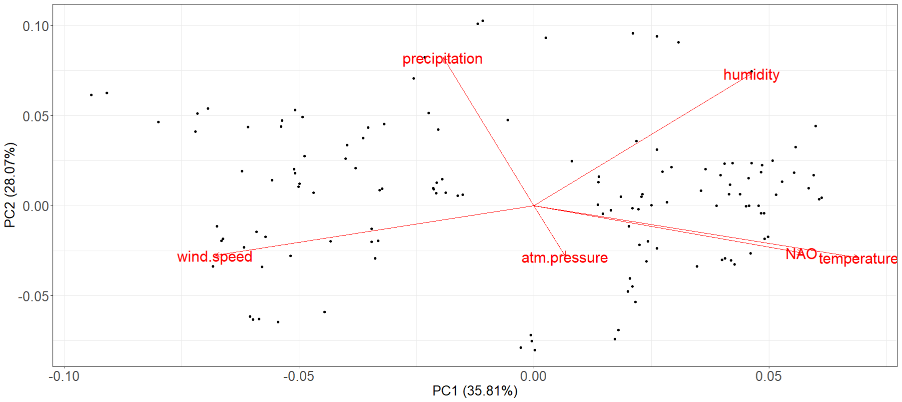

Fig. S1.4: Principal component analysis of NAO_30, temperature, atmospheric pressure, wind speed, precipitation and humidity averaged over 30 days prior to sampling date (*n*=566).

Table S1.1: Principal component loadings.

Variable:	**PC1**	**PC2**
NAO_30	0.4621	-0.2133
temperature	0.5610	-0.2350
precipitation	-0.1563	0.6663
humidity	0.3765	0.5941
wind speed	-0.5501	-0.2240
atm. pressure	0.0544	-0.2285

DNA extraction

A small amount of the collected whole blood was lysed in 60 µl Lairds buffer, with 90 µg proteinase K (Sigma Aldrich), and incubated at 50°C for 3 hours, and DNA was extracted using the ReliaPrep Large Volume HT gDNA Isolation System (Promega) following the manufacturers protocol but with elution of DNA in 25 mM Tris HCl (pH 8). All samples had DNA concentrations >15 ng/µL and an acceptable 260/280 absorbance ratio between 1.8-2.2. All DNA extractions were done in Trondheim, Norway, and subsequently shipped to Glasgow, UK, on dry ice (-78°C) within 24 h.

Telomere length measurements

TL measured from blood is representative of other somatic cells (Reichert et al., 2013; Daniali et al., 2013). Since qPCR measures both terminal and interstitial telomeric sequences, Ringsby et al. (2015) validated the qPCR method for house sparrows using both the Southern blot Telomere Restriction Fragment method (TRF), which does include interstitial repeats, and the in-gel TRF method, which does not (see Nussey et al., 2014 for details of these methods).

PCR assays were prepared with telomere primers Tel1b (5'-CGG TTT GTT TGG GTT TGG GTT TGG GTT TGG GTT TGG GTT-3') and Tel2b (5'-GGC TTG CCT TAC CCT TAC CCT TAC CCT TAC CCT TAC CCT-3') at a final concentration of 500 nM and GAPDH (glyceraldehyde-3-phosphate dehydrogenase) primers at 200 nM (forward primer 5'-GAG GTG CTG CTC AGA ACA TTA T-3' and reverse primer 5'-ACG GAA AGC CAT TCC AGT AAG-3'), which were stored at -20°C. The telomere thermal profile was 15 min at 95°C, followed by 27 cycles of 15 s at 95°C, 30 s at 58°C, and 30 s at 72°C. GAPDH thermal profile was 15 min at 95°C, followed by 40 cycles of 15 s at 95°C, 15 s at 60°C. Both assays were followed by melt curve analysis (58-95°C 1°C/5 s ramp) and checked for a single peak dissociation curve. The mean of cycle thresholds (Ct) of the sample triplicates were used, but samples were excluded in the rare case of fluorescent signal thresholds (Ct values) differing >0.5 cycles within equally spread triplicates. Samples with very large Ct values (>16 for telomere and >24 for GAPDH assays) were excluded because they fell outside the standard curves. The average of the reference sample cycle thresholds (Ct) across all plates were 9.42±0.13 SD ($CV_{interplate}$=1.39%, $CV_{intraplate}$=0.72%) for telomere assays and 20.58±0.09 SD ($CV_{interplate}$=0.46%, $CV_{intraplate}$=0.40%) for GAPDH assays.

Molecular sexing and pedigree reconstruction

Sex of 534 fledglings was determined using amplification of the CHD-gene located on the avian sex chromosomes following Griffiths et al. (1998) as described in Ringsby et al. (2015). 32 individuals were sexed based on adult plumage. We used 13 polymorphic microsatellite markers scored using the GeneMapper 4.0 software (Applied Biosystems) to assign parentage in CERVUS 3.0 (Kalinowski et al., 2007). Genotyping procedures are detailed in Rønning et al. (2016). For each nestling, CERVUS calculates a LOD-score (log-likelihood ratio) for all putative parents, which is compared to the critical values generated by the simulated parentage analyses. All sampled adults present on an island after the winter field season were included as putative parents, and we used a 95% parentage assignment confidence threshold. 526 (93%) of 566 nestlings could be assigned to at least one parent with 95% confidence. Nestlings within the same clutch were assumed to have the same mother.

APPENDIX S2: Notes on results

Changes in tarsus and telomere lengths across cohorts

The comparisons shown in Fig. S2.1 including all individuals (i.e. all selection categories) revealed that mean nestling tarsus length increased significantly at Leka (*high*) between the first and all following cohorts (Fig. S2.1a). In contrast, at Vega (*low*) the mean tarsus was significantly shorter only in the third (2004) and fourth (2005) cohort when compared to the first cohort (2002, Fig. S2.1c). Mean TL decreased significantly in the *high* population between the first and fourth cohorts, and between the second and fourth cohorts, respectively. After the artificial selection was stopped, we observed a significant increase in TL (2006, Fig. S2.1b). There were no significant changes in mean TL across cohorts in the *low* population.

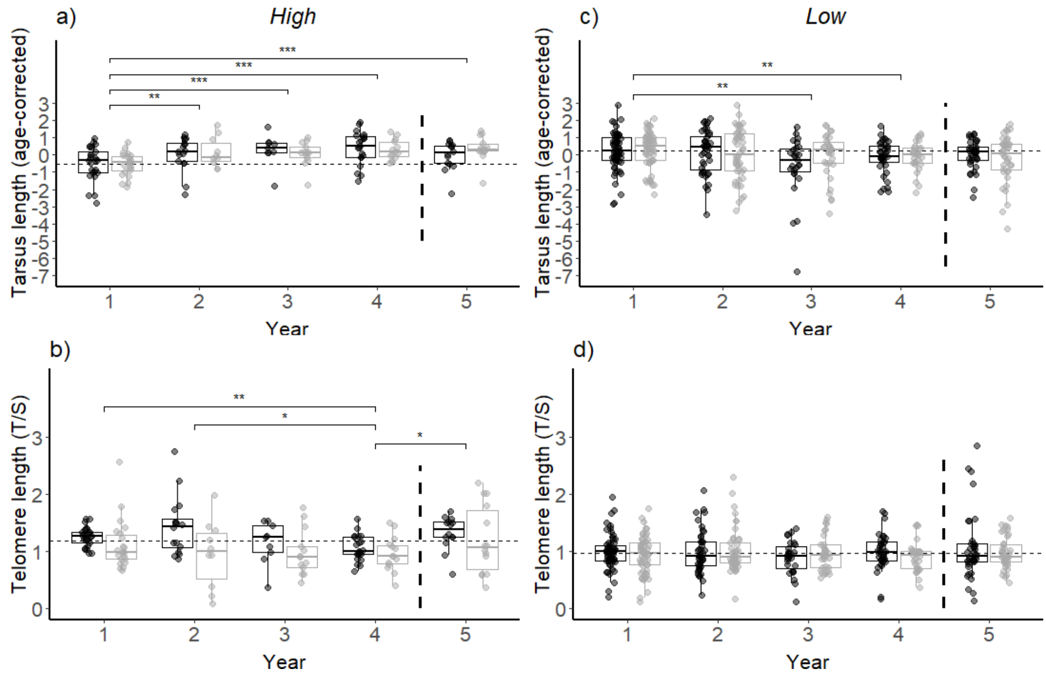

Fig. S2.1: Changes in nestling tarsus length (a and c) and TL (b and d) in two populations of house sparrows under artificial selection for longer (*high*, a and b) or shorter (*low*, c and d) adult tarsi. The last selection event was year 4, and the end of the artificial selection is indicated with a vertical dotted line. Males (black) and females (grey) are shown for each cohort and significantly different cohorts are indicated (Wilcoxon signed-rank test: ***<0.001; **<0.01; *<0.05). The dotted horizontal line marks the average of the first cohort across sexes (i.e. in the first year of artificial size selection, see Fig. 2 in the main text).

The comparisons below (Fig. S2.2) included only individuals with both parents having been subjected to the artificial size selection (i.e. selection category 1) for which we expected the strongest directional changes in tarsus length. For the *high* population, there was a significant increase in mean tarsus length between the first and fourth cohort (2005, Fig. S2.2a), and a significant decrease in mean TL between the second and the fourth cohort (Fig. S2.2b). After the selection stopped, we observed a significant increase in TL (2006, Fig. S2.2b). In the *low* population, there was a significant decrease in tarsus length between the first and the second and third cohorts (2003 and 2004, Fig. S2.2c), but there were no significant changes in TL the *low* population (Fig. S2.2d).

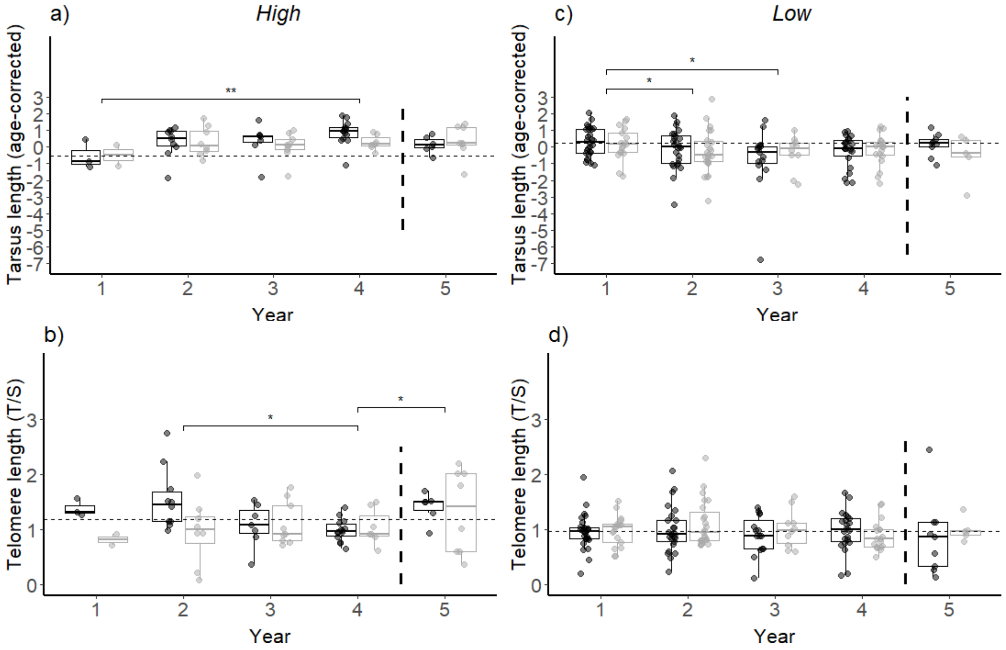

Figure S2.2: Changes in nestling tarsus length (a and c) and telomere length (b and d) of nestlings with both parents artificially selected (selection category 1) for either longer (*high*, a and b) or shorter (*low*, c and d) adult tarsi. The last selection event was year 4, and the of the artificial selection is indicated with a vertical dotted line. Males (black) and females (grey) are shown for each cohort and significantly different cohorts are indicated (Wilcoxon signed-rank test: ***<0.001; **<0.01; *<0.05). The dotted horizontal line marks the average of the first cohort across sexes (i.e. in the first year of artificial size selection).

AICc table of models of changes in tarsus and telomere lengths

Table S2.1: Candidate models explaining the variation in early-life tarsus length or TL (response variables) including the effects of sex, age, selection category, year, year^2 and an interaction term between year and selection category (cat.) in offspring of house sparrows under artificial selection for either longer (*high*, Leka) or shorter (*low*, Vega) tarsi in the years 2002-2006. All models included brood identity as random factor. The models are ranked by AICc and shows the number of degrees of freedom (df) and model weight (w). The chosen top model is marked in bold (see main text).

***High* (Leka, n=158): tarsus length**	**ΔAICc**	**df**	**w**
1 ***Tarsus = sex + age + year + year***^{2} ***+ selection cat.***	**0.0**	**9**	**0.262**
2 *Tarsus = sex + age + year + selection cat.*	0.1	8	0.251
3 *Tarsus = sex + age + year + year*^{2}	0.3	7	0.23
4 *Tarsus = sex + age + year*	1.9	6	0.101
5 *Tarsus = sex + age + year + selection cat. + year*selection cat.*	2.4	10	0.077
6 *Tarsus = sex + age + year + year*^{2} *+ selection cat. + year*selection cat.*	3.2	11	0.053
7 *Tarsus = sex + age + selection cat.*	4.7	7	0.024
8 *Tarsus = sex + age*	11	5	0.001
***High* (Leka, n=158): telomere length**			
1 *TL = sex + age*	0.0	5	0.2717
2 ***TL = sex + age + year + year***^{2}	**0.0**	**7**	**0.269**
3 *TL = sex + age + year + year*^{2} *+ selection cat.*	0.7	9	0.1957
4 *TL = sex + age + year*	1.6	6	0.1197
5 *TL = sex + age + selection cat.*	2.3	7	0.0851
6 *TL = sex + age + year + selection cat.*	4.2	8	0.0336
7 *TL = sex + age + year + year*^{2} *+ selection cat. + year*selection cat.*	5.2	11	0.0203
8 *TL = sex + age + year + selection cat. + year*selection cat.*	8.1	10	0.0048
***Low* (Vega, n=408): tarsus length**			
1 ***Tarsus = sex + age + year + year***^{2}	**0.0**	**7**	**0.247**
2 *Tarsus = sex + age + year + year*^{2} *+ selection cat.*	0.6	9	0.178
3 *Tarsus = sex + age*	0.8	5	0.163
4 *Tarsus = sex + age + selection cat.*	1.2	7	0.134
5 *Tarsus = sex + age + year*	1.6	6	0.113
6 *Tarsus = sex + age + year + selection cat.*	1.6	8	0.111
7 *Tarsus = sex + age + year + year*^{2} *+ selection cat. + year*selection cat.*	4.1	11	0.032
8 *Tarsus = sex + age + year + selection cat. + year*:selection cat.*	4.9	10	0.021
***Low* (Vega, n=408): telomere length**			
1 ***TL = sex + age***	**0.0**	**5**	**0.479**

2	*TL = sex + age + year*	1.9	6	0.189
3	*TL = sex + age + selection cat.*	3.0	7	0.107
4	*TL = sex + age + year + year*^{2}	3.5	7	0.085
5	*TL = sex + age + year + selection cat. + year*selection cat.*	4.2	10	0.059
6	*TL = sex + age + year + selection cat.*	4.9	8	0.041
7	*TL = sex + age + year + year*^{2} *+ selection cat. + year*selection cat.*	6.1	11	0.022
8	*TL = sex + age + year + year*^{2} *+ selection cat.*	6.6	9	0.018

Model estimates of changes in tarsus and telomere lengths

Table S2.2: Model estimates (β) with standard errors (SE) and lower and upper 95% confidence intervals (CI) for predictor variables included in the top candidate linear mixed model marked in bold in Table S2.1. All models included brood identity as random factor.

High **(Leka, n=158): tarsus length**	***β***	***SE***	***Lower CI***	***Upper CI***
intercept	*14.52*	*0.80*	*13.02*	*16.07*
year	*0.70*	*0.37*	*0.01*	*1.38*
year^2	-0.09	0.06	-0.20	0.03
sex (female)	-0.07	0.13	-0.31	0.21
age	*0.32*	*0.06*	*0.20*	*0.43*
intermediate (0.5)	0.25	0.24	-0.21	0.72
selected (1)	*0.52*	*0.27*	*0.04*	*1.02*
$\sigma^2_{\text{brood ID}}$	0.28±0.53 SD		0.25	0.70
Marginal R^2 / Conditional R^2: 0.336 / 0.566				
High **(Leka, n=158): telomere length**	***β***	***SE***	***Lower CI***	***Upper CI***
intercept	*1.23*	*0.30*	*0.66*	*1.80*
year	*-0.26*	*0.13*	*-0.50*	*-0.01*
year^2	*0.04*	*0.02*	*-0.00*	*0.08*
sex (female)	*-0.20*	*0.06*	*-0.32*	*-0.07*
age	0.03	0.02	-0.02	0.07
$\sigma^2_{\text{brood ID}}$	0.02±0.13 SD		0.00	0.22
Marginal R^2 / Conditional R^2: 0.088 / 0.186				
Low **(Vega, n=408): tarsus length**	***β***	***SE***	***Lower CI***	***Upper CI***
intercept	*17.35*	*0.64*	*16.08*	*18.62*
year	*-0.63*	*0.30*	*-1.23*	*-0.04*
year^2	0.10	0.05	-0.00	0.20
sex (female)	-0.11	0.11	-0.32	0.10
age	*0.19*	*0.05*	*0.09*	*0.30*
$\sigma^2_{\text{brood ID}}$	0.66±0.81 SD		0.64	0.96
Marginal R^2 / Conditional R^2: 0.061 / 0.454				
Low **(Vega, n=408): telomere length**	***β***	***SE***	***Lower CI***	***Upper CI***
intercept	*0.98*	*0.13*	*0.72*	*1.23*
sex (female)	-0.03	0.04	-0.10	0.04
age	0.00	0.01	-0.02	0.02
$\sigma^2_{\text{brood ID}}$	0.00±0.06 SD		0.00	0.13
Marginal R^2 / Conditional R^2: 0.002 / 0.028				

AICc table of first-year survival analyses

Table S2.3: Binomial generalized linear mixed models with ΔAICc<5 of variation in first-year survival in house sparrows in two island populations. All models included random intercepts for brood identity and year. The models are ranked by AICc, and number of degrees of freedom (df) and model weights (w) are shown. The second-ranked model included TL, but the effect of TL on survival was uncertain (OR_{TL}=0.89, CI=[0.51, 1.55])

	Model	**ΔAICc**	**df**	**w**
1	***Survival = sex + population + tarsus + population*tarsus***	**0.0**	**7**	**0.282**
2	*Survival = sex + population + tarsus + population*tarsus + TL*	1.9	8	0.110
3	*Survival = sex + population + tarsus + population*tarsus + NAO_30*	2.0	8	0.102
4	*Survival = sex + population + tarsus + tarsus*^{2}	2.4	7	0.084
5	*Survival = sex + population + tarsus*	2.5	6	0.082
6	*Survival = sex + population + tarsus + population*tarsus + TL + population*TL*	4.0	9	0.039
7	*Survival = sex + population + tarsus + tarsus*^{2} *+ TL*	4.3	8	0.032
8	*Survival = sex + population + tarsus + TL*	4.3	7	0.032
9	*Survival = sex + population + tarsus + tarsus*^{2} *+ NAO_30*	4.5	8	0.030
10	*Survival = sex + population + tarsus + NAO_30*	4.5	7	0.030
11	*Survival = sex + population*	4.7	5	0.027
12	*Survival = sex + population + tarsus + tarsus*^{2} *+ TL + TL*^{2} *+ NAO_30*	4.9	9	0.024

AICc table for Cox proportional hazards regression analyses

Table S2.4: AICc table of candidate models with ΔAICc<5 including the effects of NAO_30, TL, tarsus length, and non-linear effects of TL and tarsus length, and interaction terms between population identity and TL or tarsus length, respectively. All models included sex and population identity as fixed effects and brood identity as clusters (random effect). The models are ranked by AICc and shows the number of degrees of freedom (df) and model weight (w). The curvilinear effect of tarsus length present in two out of five models with ΔAICc<2, was uncertain and weak, i.e. close to 1 ($HR_{tarsus\text{^}2}$=0.98, CI=[0.96, 1.01]).

	Model (*n*=566)	**ΔAICc**	**df**	**w**
1	***Mortality = sex + population + tarsus***	**0.0**	**3**	**0.172**
2	***Mortality = sex + population + tarsus + TL + TL***^{2}	**0.7**	**5**	**0.120**
3	*Mortality = sex + population + tarsus + tarsus*^{2}	0.9	4	0.112
4	*Mortality = sex + population + tarsus + population*tarsus*	1.2	4	0.094
5	*Mortality = sex + population + tarsus + tarsus*^{2} *+ TL + TL*^{2}	1.4	6	0.084
6	*Mortality = sex + population + tarsus + NAO_30*	2.0	4	0.063
7	*Mortality = sex + population + tarsus + TL*	2.0	4	0.062
8	*Mortality = sex + population + tarsus + tarsus*^{2} *+ TL*	2.9	5	0.041
9	*Mortality = sex + population + tarsus + tarsus*^{2} *+ NAO_30*	2.9	5	0.041
10	*Mortality = sex + population + tarsus + population*tarsus + TL*	3.2	5	0.034

11	*Mortality = sex + population + tarsus + population*tarsus + NAO_30*	3.2	5	0.034
12	*Mortality = sex + population + tarsus + tarsus*^{2} *+ TL + TL*^{2} *+ NAO_30*	3.4	7	0.031
13	*Mortality = sex + population + TL + population*TL + tarsus*	3.8	5	0.025
14	*Mortality = sex + population + tarsus + TL + NAO_30*	4.0	5	0.023
15	*Mortality = sex + population + tarsus + tarsus*^{2} *+ TL + NAO_30*	4.9	6	0.015

Model estimates for Cox proportional hazards regression analyses

Table S2.5: Estimates with standard errors (SE), hazard ratios (HR), the 95% confidence intervals (CI) of the hazard ratios, for predictor variables included in the first and second-best models marked in bold in Table S2.4. All models included brood identity as cluster.

Response variable: survival over time (ΔAICc = 0.0)	***β***	***SE***	***HR***	***Lower CI***	***Upper CI***
sex (female)	-0.11	0.09	0.90	0.76	1.07
population (low)	0.01	0.10	1.01	0.80	1.26
tarsus	*-0.11*	*0.04*	*0.90*	*0.82*	*0.97*
Concordance= 0.544 (SE = 0.017)					
Response variable: survival over time (ΔAICc = 0.7)					
sex (female)	-0.11	0.09	0.90	0.76	1.07
population (low)	0.01	0.10	1.01	0.80	1.28
tarsus	*-0.11*	*0.04*	*0.90*	*0.82*	*0.97*
TL	0.73	0.43	2.07	0.86	4.97
TL^2	-0.30	0.17	0.74	0.52	1.05
Concordance= 0.551 (SE = 0.017)					

AICc tables for analyses of lifetime reproductive success

Table S2.6: AICc table of candidate models with ΔAICc<5 of lifetime reproductive success (LRS) including the effects of lifespan, sex, tarsus length, and TL analyzed separately for each population. Brood identity and year were included as random intercepts in all models.

***High* population (*n*=22)**		**ΔAICc**	**df**	**w**
1	***LRS = sex + lifespan***	0.0	5	0.423
2	***LRS = sex + lifespan + TL***	0.1	6	0.401
3	*LRS = sex + lifespan + tarsus*	3.8	6	0.063
4	*LRS = sex + lifespan + TL + tarsus*	4.3	7	0.050
5	*LRS = sex + lifespan + TL + TL*^{2}	4.5	7	0.046
***Low* population (*n*=80)**		**ΔAICc**	**df**	**w**
1	***LRS = sex + lifespan***	0.0	5	0.326
2	*LRS = sex + lifespan+ tarsus*	1.8	6	0.135
3	*LRS = sex + lifespan + tarsus + tarsus*^{2}	2.0	7	0.122

4	$LRS = sex + lifespan + TL + TL^2$	2.1	7	0.117
5	$LRS = sex + lifespan + TL$	2.3	6	0.101
6	$LRS = sex + lifespan + tarsus + tarsus^2 + TL + TL^2$	3.1	9	0.070
7	$LRS = sex + lifespan + tarsus + TL + TL^2$	3.7	8	0.052
8	$LRS = sex + lifespan + tarsus + TL$	4.2	7	0.041
9	$LRS = sex + lifespan + tarsus + tarsus^2 + TL$	4.4	8	0.036

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PAPER II

Genetic architecture and heritability of early-life telomere length in a wild passerine

Running title: Genetic architecture of telomere length

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ABSTRACT

Early-life telomere length (TL) is associated with fitness in a range of organisms. Little is known about the genetic basis of variation in TL in wild animal populations, but to understand the evolutionary and ecological significance of TL it is important to quantify the relative importance of genetic and environmental variation in TL. In this study, we measured TL in 2746 house sparrow nestlings sampled across 20 years and used an animal model to show that there is a small heritable component of early-life TL (h^2=0.04). Variation in TL among individuals was mainly driven by environmental (annual) variance, but also brood and parental effects. Parent-offspring regressions showed a large maternal inheritance component in TL ($h^2_{maternal}$=0.44), but no paternal inheritance. We did not find evidence for a negative genetic correlation underlying the observed negative phenotypic correlation between TL and structural body size. Thus, TL may evolve independently of body size and the negative phenotypic correlation is likely to be caused by non-genetic environmental effects. We further used genome-wide association analysis to identify genomic regions associated with TL variation. We identified several putative genes underlying TL variation; these have been inferred to be involved in oxidative stress, cellular growth, skeletal development, cell differentiation and tumorigenesis in other species. Together, our results show that TL has a low heritability and is a polygenic trait strongly affected by environmental conditions in a free-living bird.

INTRODUCTION

Telomeres are nucleoprotein structures that cap the ends of linear chromosomes in most eukaryotes (Blackburn, 1991). Understanding the causes of individual variation in telomere length (TL) is important because this trait has been shown to predict variation in survival or lifespan within and among species, particularly in birds (Bize, Criscuolo, Metcalfe, Nasir, & Monaghan, 2009; Froy et al., 2021; Heidinger et al., 2012; Joeng, Song, Lee, & Lee, 2004; Monaghan, 2010; Pepke & Eisenberg, 2021; Tricola et al., 2018; Wilbourn et al., 2018) and individual fitness in wild animals (Eastwood et al., 2019, but see Wood & Young, 2019). Telomeres shorten through life in many organisms (Dantzer & Fletcher, 2015; Remot et al., 2021) due to cell division, oxidative stress, and other factors (Jennings, Ozanne, & Hales, 2000; Reichert & Stier, 2017). This can result in telomere dysfunction, genome instability, cell death (Nassour et al., 2019), and organismal senescence (Herbig, Ferreira, Condel, Carey, & Sedivy, 2006). Individual TL or telomere loss may act as biomarkers or sensors of exposure to intrinsic and extrinsic stressors (Bateson, 2016; Houben, Moonen, van Schooten, & Hageman, 2008), and hence reflect individual condition (Rollings et al., 2017), but the physiological mechanisms underlying the ontogenetic variation in TL are not well understood (Erten & Kokko, 2020; Monaghan, 2014). Several studies have investigated the potential of telomere dynamics (i.e. individual differences in TL and telomere loss rate) in mediating life-history trade-offs both across (Dantzer & Fletcher, 2015; Pepke & Eisenberg, 2020) and within relatively long-lived species (Monaghan, 2010; Spurgin et al., 2018). However, despite being an ecologically important trait in many species (Wilbourn et al., 2018), knowledge about the genetic architecture of TL and its adaptive potential in wild populations remains scarce (Dugdale & Richardson, 2018).

Quantifying the additive genetic variance of a trait is required to understand mechanisms driving adaptive evolution, i.e. the response to selection on a trait (Ellegren & Sheldon, 2008; Kruuk, Slate, & Wilson, 2008; Lande, 1979). However, the magnitude of the heritability and mode of inheritance of TL is not well-known in populations of wild animals, and few general patterns have been described (Bauch, Boonekamp, Korsten, Mulder, & Verhulst, 2019; Dugdale &

Richardson, 2018; Horn et al., 2011). Utilizing long-term pedigree data, individual variation in early-life TL can be decomposed into various genetic and environmental sources of variation through a type of mixed-effect model ('animal model'), which takes all relationships from the pedigree into account (Kruuk, 2004; Wilson et al., 2010). Estimates of TL heritabilities from studies using animal models (reviewed in Dugdale & Richardson, 2018) have varied considerably across wild bird populations, from h^2=0 (n=177, in white-throated dippers, *Cinclus cinclus*, Becker et al., 2015) to h^2=0.74 (n=715, in western jackdaws, *Coloeus monedula*, Bauch, Boonekamp, Korsten, Mulder, & Verhulst, 2021). While most studies are characterized by relatively small sample sizes, recent long-term studies on Seychelles warblers (*Acrocephalus sechellensis*, n=1317, h^2=0.03-0.08, Sparks et al., 2021) and common terns (*Sterna hirundo*, n=387, h^2=0.46-0.63, Vedder et al., 2021) also revealed contrasting estimates of TL heritabilities. Epidemiological studies of humans have documented consistently high TL heritabilities, ranging from h^2=0.34-0.82 (Broer et al., 2013). In humans, some studies reported strong paternal inheritance (e.g. Njajou et al., 2007) or maternal inheritance (e.g. Broer et al., 2013) or that there were no differences in parental mode of inheritance (e.g. Eisenberg, 2014). In birds, several studies have documented maternal effects on offspring telomere dynamics (Asghar, Bensch, Tarka, Hansson, & Hasselquist, 2015; Heidinger et al., 2016; Horn et al., 2011; Reichert et al., 2015), or effects of parental age at conception on offspring TL (Eisenberg & Kuzawa, 2018; Marasco, Boner, Griffiths, Heidinger, & Monaghan, 2019; Noguera José, Metcalfe Neil, & Monaghan, 2018). Reichert et al. (2015) found a significant correlation between mother-offspring TL measured at 10 days of age in king penguins (*Aptenodytes patagonicus*), but not when TL was measured at later ages (>70 days). This may be because post-natal telomere loss rate is strongly influenced by individual environmental circumstances (Chatelain, Drobniak, & Szulkin, 2020; Wilbourn et al., 2018) and does not always correlate strongly with chronological age (Boonekamp, Mulder, Salomons, Dijkstra, & Verhulst, 2014; Boonekamp, Simons, Hemerik, & Verhulst, 2013).

Faster growth in early life is associated with reduced longevity (Metcalfe & Monaghan, 2003) and TL may be involved in mediating the trade-off between growth rate and lifespan (Salmón, Millet, Selman, & Monaghan, 2021; Young, 2018). Accordingly, a negative phenotypic correlation between TL and body size or growth rate has been documented within several species (Monaghan & Ozanne, 2018, but see Boonekamp et al., 2021). Telomeres are known to shorten during growth (Ringsby et al., 2015), but a negative phenotypic correlation may also indicate the existence of a negative genetic correlation (Roff, 1995; Roff & Fairbairn, 2012). Froy et al. (2021) reported a modest negative genetic correlation (r_A=-0.2) between body weight and TL in feral Soay sheep (*Ovis aries*). Furthermore, we have previously shown that artificial directional selection on body size in wild house sparrows (*Passer domesticus*) affected TL in the opposite direction (Pepke et al., *submitted* 2021). This suggests that there is a genetic correlation between the two traits. Thus, quantifying the genetic correlation between TL and body size enables us to determine whether the two traits can evolve independently of each other or if the pattern of selection on both traits is needed for predicting evolutionary responses (Kruuk et al., 2008).

TL is a complex phenotypic trait (Aviv, 2012; Hansen et al., 2016) expected to be polygenic, i.e. affected by small effects of many genes (Dugdale & Richardson, 2018; Hill, 2010). Accordingly, numerous genome-wide association studies (GWAS), which tests correlative associations of single-nucleotide polymorphisms (SNPs) with specific traits, have identified several loci correlated with TL in humans that map to genes involved in telomere and telomerase maintenance, DNA damage repair, cancer biology, and several nucleotide metabolism pathways (e.g. Andrew et al., 2006; Codd et al., 2010; Codd et al., 2013; Coutts et al., 2019; Deelen et al., 2013; Delgado et al., 2018; Jones et al., 2012; Levy et al., 2010; Li et al., 2020; Liu et al., 2014; Mangino et al., 2015; Mangino et al., 2012; Mirabello et al., 2010; Nersisyan et al., 2019; Ojha et al., 2016; Soerensen et al., 2012; Vasa-Nicotera et al., 2005; Zeiger et al., 2018). None of the GWA studies in humans specifically tested the marker associations of early-life TL, which pose a challenge to the interpretation of the results, as TL shortens through life in humans (Blackburn, Epel, & Lin, 2015) and genes may have different impacts at various life stages (Weng et al., 2016). Furthermore, large sample sizes and dense sampling of genetic loci is needed to ensure high power in GWA studies (Mackay, Stone, & Ayroles, 2009) and resolve any pleiotropic effects (Prescott et al., 2011). The genes influencing TL in humans that were identified through GWAS only explain a small proportion of the inter-individual variation in TL (<2 %, Aviv, 2012; Codd et al., 2013; Fyhrquist, Saijonmaa, & Strandberg, 2013). One GWAS on TL of a non-human species (dairy cattle, *Bos taurus*) was recently performed (Ilska-Warner et al., 2019) supporting the polygenic nature of early-life TL. However, domesticated species in captivity may display TL dynamics that are not representative of natural populations (Eisenberg, 2011; Pepke & Eisenberg, 2021). There are to the best of our knowledge no previous GWAS on TL performed in natural populations.

In this study, we aim to provide novel insights into the genetic architecture of TL and the evolutionary mechanisms by which natural selection can alter telomere dynamics using data from a passerine bird. We obtained a single measure of TL in individuals (*n*=2746) born within 20 cohorts in two natural insular populations of wild house sparrows at a similar age (ca. 10 days), in addition to individuals at the same age in two insular populations that underwent artificial selection on body size for 4 consecutive years (*n*=569, Kvalnes et al., 2017; Pepke et al., *submitted* 2021). First, we estimate the phenotypic correlations between TL and tarsus length (as a proxy for body size, Araya-Ajoy et al., 2019; Senar & Pascual, 1997) in house sparrow nestlings. Second, we test for effects of parental age on offspring TL. Third, we estimate heritability, environmental variances, and parental effects on early-life TL, and test for genetic correlations between TL, tarsus length, and body condition in the natural populations (primary analyses). Nestling body condition (body mass corrected for structural body size, Schulte-Hostedde, Zinner, Millar, & Hickling, 2005) is included here to account for the component of body size that is not explained by tarsus length, which could be due to variation in the mass of other tissues or fat reserves (Peig & Green, 2010). We then use similar analyses in the artificially selected populations to validate our results from the primary analyses. Finally, we use high-density genome-wide Single Nucleotide Polymorphism (SNP) genotype data (Lundregan et al., 2018) in a GWAS to identify genetic regions and potential candidate genes underlying variation in early-life TL within wild house sparrows (up to *n*=383).

MATERIALS AND METHODS

Study populations and data collection

The study was performed in four insular house sparrow populations off the coast of northern Norway (Fig. S1.1 in Appendix S1). The study periods differed between the populations with data from Hestmannøy (66°33’N, 12°50‘E) in the years 1994-2013, Træna (Husøy island, 66°30’N, 12°05‘E) in the years 2004-2013, and Leka (65°06’N, 11°38’E) and Vega (65°40’N, 11°55’E) both in the years 2002-2006. Hestmannøy and Træna were unmanipulated natural populations and are included in the primary analyses. The populations of Leka and Vega underwent artificial size selection (see Kvalnes et al., 2017; Pepke et al., *submitted* 2021) and were analyzed separately in a set of secondary analyses as replications of the primary analyses. All four islands are characterized by heathland, mountains, and sparse forest. The sparrows live closely associated with humans and within the study area they are found mainly on dairy farms

(Hestmannøy, Vega and Leka), where they have access to food and shelter all year, or in gardens and residential areas (Træna), where they may be more exposed to weather conditions (Araya-Ajoy et al., 2019). Natural nests inside barns or artificial nest boxes were visited at least every 9th day during the breeding season (May-August) to sample fledglings (5-14 days old, with a median of 10 days). All individuals were ringed using a unique combination of a metal ring and three plastic color rings. Fledged juvenile sparrows and unmarked adults were captured using mist nets from May to October. These procedures ensured that approximately 90% of all adult birds were marked on all islands during the study period (Jensen, Steinsland, Ringsby, & Sæther, 2008; Kvalnes et al., 2017). We measured tarsometatarsus (tarsus) length using digital slide calipers to nearest 0.01 mm and body mass to nearest 0.1 g with a Pesola spring balance (see Appendix S1). Morphological measurements were taken by different fieldworkers. All fieldworkers were carefully trained to consistently use the same measurement technique of THR or, in some cases, another experienced fieldworker (Kvalnes et al., 2017). For 234 out of 2746 nestlings, no nestling morphological measurements were made. Following Schulte-Hostedde et al. (2005) nestling body condition was calculated as the residuals of a linear regression of mass on tarsus length (both $\log_{10}$-transformed). To avoid collinearity in models where both nestling age and tarsus length were included as covariates, we age-corrected tarsus length by using the residuals from a regression of tarsus length on age and age squared (to account for the diminishing increase in tarsus length with age). One blood sample (25 μL) was collected from each fledgling, which was stored in 96% ethanol at room temperature in the field and subsequently at -20°C in the laboratory until DNA extraction.

Molecular sexing and pedigree construction

DNA extraction is described in Appendix S1. Sex of most fledglings (*n*=2641) was determined using amplification of the CHD-gene located on the avian sex chromosomes as described in Griffiths, Double, Orr, and Dawson (1998). 21 individuals were sexed exclusively based on their phenotype as adults and 84 nestlings could not be sexed. The pedigree construction is detailed in previous studies (Billing et al., 2012; Jensen et al., 2008; Jensen et al., 2003; Rønning et al., 2016). Briefly, we used individual genotypes on 13 polymorphic microsatellite markers scored using the GeneMapper 4.0 software (Applied Biosystems) to assign parentage in CERVUS 3.0 (Kalinowski, Taper, & Marshall, 2007). Nestlings within the same clutch were assumed to have the same mother. Nestlings with missing parents (unassigned: *n*=662 with missing mother and *n*=700 with missing father) were assigned dummy parents, assuming that nestlings within the same clutch were full siblings and thus had the same (unassigned) parents. The dummy parents were included in the pedigree as founders. We calculated individual inbreeding coefficients (*F*) based on the microsatellite pedigree using the R package 'pedigree' (Coster, 2012). Pedigrees were ordered using the R package 'MasterBayes' (Hadfield, Richardson, & Burke, 2006) and pruned to only contain informative individuals. The pruned pedigrees included 4118 individuals (3093 maternities and 3130 paternities) in the natural populations, and 1057 individuals in artificially selected populations. Maximum pedigree depth was 13 generations, the number of equivalent complete generations (the sum of the proportion of known ancestors across all generations, Wellmann, 2021) was 1.510, and mean pairwise relatedness was 0.003.

Telomere length measurements

Relative erythrocyte telomere lengths (TL) of 2746 nestlings from Hestmannøy and Træna (sample sizes are detailed in Table S1.1) were successfully measured using the real-time quantitative polymerase chain reaction (qPCR) amplification method by Cawthon (2002) with modifications by Criscuolo et al. (2009). Primer sequences, PCR assay setup and thermal profiles followed Pepke et al. (*submitted* 2021) and are detailed in Appendix S1. Briefly, this method measures the ratio of telomere sequence relative to the amount of a non-variable gene (GAPDH) and a reference sample. The reference sample consisted of pooled DNA from 6 individuals, which was also included as a 2-fold serial dilution (40-2.5 ng/well) on all plates to produce a standard curve, in addition to a non-target control sample (all in triplicates). Samples were randomized and run on 2x125 96-well plates (telomere and GAPDH assays, respectively). The qPCR data was analyzed using the qBASE software (Hellemans, Mortier, De Paepe, Speleman, & Vandesompele, 2007), which computes relative TL as the ratio (T/S) of the telomere repeat copy number (T) to a single copy gene number (S) similar to Cawthon (2002). In qBASE the T/S ratio is calculated as calibrated normalized relative quantities (CNRQ) that control for differences in amplification efficiency between plates and for inter-run variation by including three inter-run calibrators from the standard curve. All individual plate efficiencies were within 100±10% (mean telomere assay efficiency was 97.5±3.9%, and 97.6±4.2% for GAPDH assays). The average of the reference sample cycle thresholds (Ct) across all plates were 10.54±0.03 S.D. and 21.53±0.02 S.D. for telomere and GAPDH assays, respectively. Thus, while reproducibility of TL measurements within the reference sample of the same DNA sample extract is high, we performed DNA re-extraction of the same blood samples for 25 individuals to test TL consistency across DNA extractions (Appendix S1). The re-extractions were run on different plates and the TL estimates of these samples remained highly correlated (R^2=0.75, Fig. S1.3). For these individuals, the average of the TL measurements was used in subsequent analyses. All reactions for the primary analyses (from the populations on Hestmannøy and Træna) were performed by the same person (MLP). MLP and WB generated the secondary dataset (*n*=569 on 2x21 plates, from the populations on

Leka and Vega) as described in Pepke et al. (*submitted* 2021). The primary and secondary datasets used different reference samples and are therefore not combined in the analyses.

Statistical analyses

The correlation between tarsus length and telomere length

We first tested the phenotypic correlation between TL and tarsus length (as a proxy for body size) within 2462 house sparrow nestlings from Hestmannøy and Træna. TL (response variable) was $\log_{10}$-transformed and linear mixed-effects models (LMMs) were fitted with a Gaussian error distribution (R package '*lme4*', Bates, Mächler, Bolker, & Walker, 2015). Sex differences in TL are known for house sparrows (Pepke et al., *submitted* 2021). Thus, models included sex, (continuous) fledgling age at sampling, hatch day (numbered day of year mean centered across years), and island identity as fixed effects. We fitted random intercepts for brood identity, year, and qPCR plate identity to account for the non-independence of nestlings from the same brood, year and plate. Because our study populations are known to be affected by inbreeding depression (Niskanen et al., 2020), we included the inbreeding coefficient (*F*, continuous) as a fixed effect (Reid & Keller, 2010). We then compared models with and without (age-standardized) tarsus length using Akaike's information criterion corrected for small sample sizes (*AICc*, Akaike, 1973; Hurvich & Tsai, 1989), and Akaike weights (*w*) and evidence ratios (*ER*) to determine the relative fit of models given the data (Burnham & Anderson, 2002). Models were validated visually by diagnostic plots and model parameters are from models refitted with restricted maximum likelihood (REML). Estimates and 95% confidence intervals (CI) are reported.

Parental age effects on offspring telomere length

We tested whether maternal age at conception (MAC [mean 1.8±1.1 S.D. years, range 1-7 years], *n*=373 mothers with *n*=1967 offspring) or paternal age at conception (PAC [mean 2.1±1.2

S.D. years, range 1-8 years], *n*=388 fathers with *n*=1927 offspring) predicted TL in offspring from Hestmannøy and Træna. We applied within-subject centering (van de Pol & Wright, 2009) to separate within-parental age effects (e.g. senescence) from between-parental age effects (e.g. selective disappearance), by including both the mean parental age at conception and the deviation from the mean parental age for each parent as fixed effects in two LMMs (for fathers and mothers, respectively) explaining variation in offspring TL ($\log_{10}$-transformed). Both models included island identity and sampling age as fixed effects, and random intercepts for year, qPCR plate identity, and either maternal identity or paternal identity.

Heritabilities and genetic correlation of telomere length, tarsus length, and body condition

We used a multivariate Bayesian animal model (Hadfield, 2019; Kruuk, 2004) fitted with Markov chain Monte Carlo (MCMC) to estimate heritability and genetic correlations of early-life TL, age-standardized tarsus length and body condition in the two natural island populations (Hestmannøy and Træna, *n*=2662) and the two manipulated island populations (Leka & Vega, *n*=569) that underwent artificial size selection. TL was $\log_{10}$-transformed and all traits were fitted with a Gaussian error distribution using the R package 'MCMCglmm' (Hadfield, 2010). Models included sex, fledgling age at sampling (associated only with TL and condition), island identity, and inbreeding coefficient (F) as fixed effects (Wilson, 2008), which were fitted such that different regression slopes were estimated for each trait (Hadfield, 2019). To estimate variance components, random intercepts were included for individual identity linked to the pedigree ('animal', V_A), brood identity (V_B) nested under mother identity, father (V_F) and mother identity (V_M), and birth year (cohort effects, V_Y). Parental effects include those influences on offspring TL that are repeatable across the lifetime of the mother or father (Kruuk & Hadfield, 2007), while brood identity accounts for other common environmental effects (McAdam, Garant, & Wilson, 2014). House sparrows are multi-brooded laying up to 3 clutches in a season and may breed in multiple years, with an average of 3.6±1.3 S.D. fledglings per brood in this study. Furthermore, to account for variance associated with measurement error we included qPCR plate identity (V_O, associated only with TL, see e.g. Froy et al., 2021; Sparks et al., 2021). Random effects were generally specified with 3x3 covariance matrices to estimate the variances and covariances between the effects for each trait. We used inverse-Wishart priors for random effects and residual variances in the multivariate model ($V=I_3$ and nu=3, Hadfield, 2019). We re-ran analyses with other relevant priors (parameter expanded) to verify that results were not too sensitive to the choice of prior. The MCMC chain was run for 2,000,000 iterations, sampling every 500 iterations after a burn-in of 5% (100,000 iterations). Mixing and stationarity of the MCMC chain was checked visually and using Heidelberger and Welch's convergence test (Heidelberger & Welch, 1983) implemented in the 'coda' package (Plummer, Best, Cowles, & Vines, 2006). All autocorrelation values were <0.1 and effective sample sizes were >3,000. The narrow-sense heritability was calculated as the posterior mode of the proportion of phenotypic variance (V_P) explained by additive genetic variance (Wilson et al., 2010): $h^2 = \frac{V_A}{V_A+V_B+V_F+V_M+V_Y+V_O+V_R}$, where V_R is the residual variance. We also estimated heritabilities excluding V_O from the total phenotypic variance since it does not represent biological variance (de Villemereuil, Morrissey, Nakagawa, & Schielzeth, 2018). Estimates are provided as their posterior mode with 95% highest posterior density intervals (HPD). All analyses were performed in R version 3.6.3 (R Core Team, 2020).

We also ran univariate models of TL, tarsus length and body condition including the same fixed and random effects as in the multivariate model (Appendix S2). For comparison with previous studies (e.g. Asghar et al., 2015), we tested whether maternal TL and/or paternal TL predicted offspring TL using two LMMs (parent-offspring regressions, Appendix S2). Parental heritabilities ($h^2_{maternal}$ and $h^2_{paternal}$) can be estimated from parent-offspring regressions as the slope multiplied by two (one sex contributes half of the genes to their offspring). We used the R package 'pedantics' (Morrissey & Wilson, 2010) to show that, based on parent-offspring regression, the pruned pedigree of the natural populations had ≥80% power to detect heritabilities ≥0.21 (see Fig. S1.2 and Appendix S1). Furthermore, we estimated maternal (V_{DAM}) and paternal (V_{SIRE}) genetic effects (e.g. Wolf & Wade, 2016) in a multivariate animal model by fitting random intercepts for maternal and paternal identity linked to the pedigree to quantify these effects while accounting for the environmental variances specified above (Appendix S2). Maternal and paternal heritabilities were calculated as: $h^2_{maternal} = \frac{V_{DAM}}{V_P}$ and $h^2_{paternal} = \frac{V_{SIRE}}{V_P}$, respectively (Wilson et al., 2005). To test for sex-specific heritabilities (e.g. Jensen et al., 2003; Olsson et al., 2011), we ran a bivariate animal model of TL in females and males as two different phenotypic traits with a genetic correlation between them (Appendix S2).

SNP genotype data and association analyses

Nestlings that survived to adulthood (recruited) on Hestmannøy and Træna were genotyped on a high-density 200K SNP array (detailed in Lundregan et al., 2018) with median distances between SNPs shorter than 5,000 bp. SNPs were originally identified from whole-genome re-sequencing of 33 individual house sparrows which were mapped to the house sparrow reference genome (Elgvin et al., 2017). DNA was extracted as described in Hagen et al. (2013), separately from telomere analyses. Data preparation and quality checks were performed using the 'GenABEL' package (GenABEL project developers, 2013). We removed SNPs or individuals for which there was more than 5% missing data, the minor allele frequency (MAF) was less than 1%, or pairwise identity-by-state (IBS) was more than 95%. After quality control, the genomic relationship matrix (GRM) was computed based on 180,650 [180,666] autosomal markers in 373

[383] individuals (142 [145] males and 137 [142] females from Hestmannøy and 47 [48] males and 47 [48] females from Træna) with numbers in brackets showing sample sizes when individuals with missing tarsus length measurements are included. We then performed two GWA analyses by fitting LMMs for the variation in TL using the package 'RepeatABEL' (Rönnegård et al., 2016): The first model included age-standardized tarsus length as a covariate, and the second model did not. Both models included sex, age, hatch day (mean centered), *F*, and island identity as fixed effects, and brood identity, year, qPCR plate, and the GRM fitted as random effects. We estimated the proportion of phenotypic variance explained by each SNP as: $h^2_{SNP} = \frac{2pq\beta^2}{V_P}$, where p and q are the allele frequencies and β is the estimated allele substitution effect (Falconer & Mackay, 1996). Finally, we determined if SNPs significantly associated with TL were within 100 kb of any gene within the annotated house sparrow genome, because this is the distance that linkage disequilibrium decays to background levels in this species (Elgvin et al., 2017; Hagen et al., 2020). Gene ontology (GO) searches were performed using the Gene Ontology Annotation (GOA) database (Binns et al., 2009; Huntley et al., 2015) to obtain an overview of biological processes and molecular functions known to be influenced by the genes.

RESULTS

The correlation between tarsus length and telomere length

The model explaining variation in TL that included tarsus length was ranked higher than the model without tarsus length ($\Delta AICc$=2.5, w_1=0.78, $ER_1=w_1/w_2$=3.55). There was a negative association between tarsus length and TL ($\beta_{tarsus\ length}$=-0.004±0.002, CI=[-0.007, -0.000], n=2462, Fig. 1 and Table 1), such that larger nestlings generally had slightly shorter early-life telomeres.

Thus, an increase in (age-corrected) tarsus length of 1 mm was associated with a decrease in TL of 0.8%.

Parental age effects on offspring telomere length

There was no evidence for associations between offspring TL and MAC ($\beta_{\Delta MAC}$=0.001±0.004, CI=[-0.007, 0.009], $\beta_{mean\ MAC}$=0.001±0.005, CI=[-0.008, 0.010], Fig. S2.1a,c) or PAC ($\beta_{\Delta PAC}$=0.005±0.003, CI=[-0.002, 0.011], $\beta_{mean\ PAC}$=-0.001±0.003, CI=[-0.008, 0.005], Fig. S2.1b,d).

Heritabilities and genetic correlations of telomere length, tarsus length, and body condition

We found non-zero additive genetic variances (V_A) for TL (V_A=0.009, HPD=[0.008, 0.010]), tarsus length (V_A=0.201, HPD=[0.111, 0.314]) and body condition (V_A=0.006, HPD=[0.005, 0.006]) in the natural populations (Table 2, Fig. 2). The main component contributing to variance in TL was between-year differences (V_Y, explaining 68% of the total variance), while maternal (V_M, 7%), paternal (V_F, 7%), brood (V_B, 6%), and qPCR plate variances (V_O, 5%) also explained considerable proportions of the total phenotypic variance (Fig. 2). Combined, the environmental effects captured 87% of the phenotypic variance in TL. Variation in TL measurements across years is shown in Fig. S2.2. For tarsus length and condition, the main variance components were among different broods (38%) and among years (76%), respectively (Table 2, Fig. 2). The heritabilities were h^2=0.039 for TL (HPD=[0.022, 0.057]), h^2=0.080 (HPD=[0.045, 0.124]) for tarsus length, and h^2=0.027 (HPD=[0.015, 0.043]) for body condition. Heritability for TL increased slightly to h^2=0.041 (HPD=[0.023, 0.061]) when excluding qPCR plate variance (V_O) from the total phenotypic variance (Table 2). The heritability estimates were of the same magnitude in the univariate animal models (Table S2.1). There was no evidence for a genetic correlation between TL and tarsus length (r_A=-0.029, HPD=[-0.120, 0.078]) or between TL and condition (r_A=-0.011, HPD=[-0.080, 0.055]).

Parent-offspring regressions showed a large maternal inheritance component in TL ($h^2_{maternal}$=0.435±0.156, CI=[0.127, 0.741]), but no paternal inheritance (Fig. S2.3). Including parental genetic effects in a multivariate animal model confirmed slightly higher maternal ($h^2_{maternal}$=0.078, HPD=[0.048, 0.099]) than paternal heritability of TL ($h^2_{paternal}$=0.072, HPD=[0.043, 0.089], Table S2.2). We found no evidence of differences in sex-specific heritabilities of TL (Table S2.3).

In the analyses of the artificially selected populations (Leka and Vega, Table S2.4) we found comparable heritability estimates for TL (h^2=0.031, HPD=[0.005, 0.061]) and body condition (h^2=0.018, HPD=[0.004, 0.049]), and a slightly higher estimate for tarsus length (h^2=0.126, HPD=[0.040, 0.237]). Similarly, there was no evidence for genetic correlations between TL and tarsus (r_A=-0.036, HPD=[-0.224, 0.149]) or between TL and body condition (r_A=-0.008, HPD=[-0.129, 0.140], Table S2.4).

GWA analyses

When controlling for the phenotypic effect of tarsus length on TL, nine SNPs showed evidence for an association with early-life TL (Table 3, Fig. 3), with a Bonferroni (1935) corrected threshold of $P \leq 2.77 \times 10^{-7}$ at the genome-wide P-value threshold (i.e. the nominal P=0.05 divided by 180,650 markers) and a genomic inflation factor of λ=1.0489±0.0002 (Fig. S2.3). Using the annotated house sparrow genome, a total of 22 genes on five chromosomes were found to be located within proximity (±100 kb) of six of the top SNPs (Table 4). Four SNPs that showed weak evidence for an association with TL (nominal $0.05<P<0.10$) are also shown in Table 3. Among three of these SNPs we identified three genes within ±100 kb on three chromosomes (Table S2.5).

SNPa429690 is located on chromosome 2 within the Aquaporin-1 (AQP1) gene, which encodes the AQP1 water channel membrane protein. The AQP1 protein is abundant in erythrocytes (where TL is measured) and important in regulating body water transport and balance (Nielsen et al., 2002), but also in a range of other physiological functions including cell migration, wound healing, fat metabolism and oxidative stress (Saadoun, Papadopoulos, Hara-Chikuma, & Verkman, 2005; Verkman, Anderson, & Papadopoulos, 2014). The same SNP is located 39 kb from the growth hormone-releasing hormone receptor (GHRHR), which controls body growth (Mullis, 2005), and has been associated with telomerase activity (Banks et al., 2010), lifespan (Soerensen et al., 2012) and the progression of several types of cancer (Chu et al., 2016; Schally et al., 2018; Villanova et al., 2019). Humans with over-expression of growth hormones and consequently insulin-like growth factor 1 (IGF-1) have shorter telomeres (Aulinas et al., 2013; Deelen et al., 2013; Matsumoto et al., 2015; Monaghan & Ozanne, 2018). SNPa17235 was close (11 kb) to FRMD4B (FERM domain-containing protein 4B), which is involved in epithelial cell polarity that is important in tissue morphogenesis (Ikenouchi & Umeda, 2010). This SNP was also near other genes related to cell proliferation (UBA3 and TMF1), skeletal muscle organization (LMOD3) and oxidative stress (ARL6IP5, see Table 4). SNPa450086 was 76 kb from OXR1 (oxidation resistance protein 1) that regulates expression of several antioxidant enzymes (Volkert, Elliott, & Housman, 2000). SNPa108592 was in the vicinity (43-84 kb) of several genes on chromosome 15 linked to cell proliferation, ubiquitination and immune response (Table 4). SNPi16410 was closest to SHCBP1 (70 kb) and CDCA4 (76 kb), which are both involved in cell proliferation and probably apoptosis (Asano et al., 2014; Wang et al., 2008; Xu, Wu, Li, Huang, & Zhu, 2018; Zou et al., 2019). SHCBP1 is upregulated by growth factor stimulation (Schmandt, Liu, & McGlade, 1999). CDCA4 is likely involved in the regulation of hematopoietic stem cells from where erythrocytes (reflecting TL) are derived (Abdullah, Jing, Spassov, Nachtman, & Jurecic, 2001). Expression of the SCN4A gene (68 kb from SNPa491204) has previously been correlated with TL in human stem cells (Wang et al., 2017). SNPa491204 was 49 kb from the growth hormone gene GH (which is linked to TL as mentioned above, see also Pauliny, Devlin, Johnsson, & Blomqvist, 2015) and WNT9B (40 kb) of the Wnt/β-catenin signaling pathway, which is modulated by telomerase (Park et al., 2009). In Appendix S2 we mention interesting genes found beyond the ±100 kb limits of the top SNPs.

When not controlling for the effect of tarsus length on TL, the same top SNPs were identified as in the analysis above where tarsus length was included (Table S2.6). In addition, SNPa208275 was associated with TL and found 47 kb from FGFR2 encoding a tyrosine-protein kinase that is a receptor for fibroblast growth factors that regulates several aspects of cell proliferation and bone morphogenesis (Table S2.7, Katoh, 2009).

DISCUSSION

The evolutionary response to selection on telomere length depends on the additive genetic variance of TL and the strength and sign of any genetic correlations with other traits under selection (Lande & Arnold, 1983). Dugdale and Richardson (2018) criticized past quantitative genetic studies of TL on the main grounds that 1) they applied basic regression analyses that did not consider environmental effects impacting TL and as a consequence of that, additive genetic effects may have been overestimated in previous studies; 2) TL changes with age, complicating the fact that parents and offspring are often sampled at different ages; and 3) sample sizes were too small to provide enough power to separate genetic and environmental effects using animal models. Here, we have accommodated this critique by 1) using mixed-effect animal models to partition genetic and environmental effects; 2) measuring early-life TL in both offspring and parents at the same time point in life (as fledglings); and 3) collect TL data from more than 3300 individuals across 4 populations, which represent a considerably larger sample size than those of previous wild animal studies.

We found that around 4% of the variation in early-life TL in house sparrows at the end of the nestling growth period was determined by additive genetic variation. The relatively small additive genetic variance and large year variance in early-life TL appears to be in accordance with the effects of relative growth and weather conditions on TL in similar sparrow populations (Pepke et al., *submitted* 2021). The lack of repeated individual TL sampling in this study may prevent us from fully separating between permanent environmental effects and the common environmental effects (brood effects and parental effects, Wilson et al., 2010). However, with several offspring measures for each brood, mother, and father, most of any permanent environmental variance would be included in the residual variance (Kruuk & Hadfield, 2007). In addition, recent longitudinal studies have found negligible permanent environmental effects on TL (Froy et al., 2021; Seeker et al., 2018; Sparks et al., 2021; van Lieshout et al., 2021; Vedder et al., 2021).

Similarly small but significant heritabilities of TL have been reported using animal models for e.g. nestling collared flycatchers, *Ficedula albicollis* (h^2=0.09, Voillemot et al., 2012), Seychelles warblers (h^2=0.03-0.08, Sparks et al., 2021) and adult greater mouse-eared bats, *Myotis myotis* (h^2=0.01-0.06, Foley et al., 2020), in which TL correlates with several weather variables. These studies also documented considerable year or cohort effects on TL (Foley et al., 2020; Sparks et al., 2021) similar to studies finding no heritability of TL in white-throated dippers (Becker et al., 2015) and European badgers (*Meles meles*, van Lieshout et al., 2021). In comparison, studies based on parent-offspring regression have often found higher TL heritabilities in e.g. king penguins (h^2=0.2, Reichert et al., 2015), jackdaws (*Coloeus monedula*, h^2=0.72, Bauch et al., 2019), and sand lizards (*Lacerta agilis*, h^2=0.5-1.2, Olsson et al., 2011). The heritability of TL in house sparrows is comparable to that of many life-history traits and considerably lower than many morphological traits (e.g. Mousseau & Roff, 1987; Visscher, Hill, & Wray, 2008), which may suggest that TL is under strong selection in the wild (Voillemot et al., 2012) or that there are considerable non-additive genetic or environmental influences on early-life TL. However, recent animal model studies on common terns (h^2=0.5, Vedder et al., 2021) and Soay sheep (h^2=0.2, Froy et al., 2021) found high TL heritabilities and a modest positive genetic correlation (i.e. with CIs overlapping zero) between TL and lifespan (r_A=0.4 and r_A=0.3, respectively). The causes of the variable TL heritability estimates found across wild animal populations, particularly in birds, remain unknown. Curiously, Pepke et al. (*submitted* 2021) reported indications of weak non-linear or negative associations between TL and various measures of fitness (survival and reproductive success) in house sparrows, suggesting that the environmentally pliant TL dynamics of these relatively fast-lived birds may be very different from several other bird species (reviewed in Wilbourn et al., 2018). In other species, positive associations between early-life TL and survival have been documented (Wilbourn et al., 2018), which may translate into an increased lifetime reproductive success (Bichet et al., 2020; Eastwood et al., 2019; Sudyka, 2019).

A considerable proportion of the phenotypic variance in TL could be attributed to brood and parental effects (Fig. 2). However, we did not find evidence that parental effects were transmitted through a parental age at conception effect (Fig. S2.1). Paternal age effects, which has been observed in several other species (Eisenberg & Kuzawa, 2018), may not manifest in these house sparrows because the mean age at reproduction in this study was low (around 2 years). Parent-offspring regressions (Fig. S2.2) suggested a stronger component of maternal heritability

($h^2_{maternal}$=0.44) rather than paternal heritability of TL. Maternal heritability estimates from parent-offspring regressions includes both direct additive genetic, maternal genetic and maternal environmental effects (Wilson et al., 2005), and we found a lower maternal heritability ($h^2_{maternal}$=0.078) when using the animal models (Table S2.2). Maternal inheritance of TL has been found in several bird species (Asghar et al., 2015; Becker et al., 2015; Horn et al., 2011; Reichert et al., 2015) and in some studies on humans (Broer et al., 2013), where this has been attributed to an X-linked gene (Nawrot, Staessen, Gardner, & Aviv, 2004) or implied genomic imprinting (Reichert et al., 2015). In our study, we did not detect sex differences in TL heritability (Table S2.3). However, we would expect higher heritability for the sex in which TL is less strongly associated with fitness given similar genetic architectures (Merilä & Sheldon, 1999; Roff, 2012). Such an association with fitness was found by Heidinger, Kucera, Kittilson, and Westneat (2021), where early-life TL was positively related to lifetime reproductive success in house sparrows, but only in females. Maternal effects on offspring TL are expected to be strongest in early-life (Wolf, Brodie Iii, Cheverud, Moore, & Wade, 1998) and could act through e.g. yolk-deposited components in the egg (Criscuolo, Torres, Zahn, & Williams, 2020; Noguera, da Silva, & Velando, 2020; Stier et al., 2020a) or post-laying through maternal care behavior (e.g., incubation and feeding rate, Stier, Metcalfe, & Monaghan, 2020b; Viblanc et al., 2020). Our results suggest that such effects may have a genetic basis that will respond to selection: For heritable traits like TL, maternal inheritance of offspring TL may be expected to increase the expected rate of adaptive evolution of TL above what would be expected from the heritability alone (Hadfield, 2012; Lande & Kirkpatrick, 1990; Räsänen & Kruuk, 2007; Wolf et al., 1998).

There was evidence for additive genetic variance in the tarsus length of sparrow nestlings, but the heritability estimate (h^2=0.080, Table 2) was considerably smaller than those of adult house sparrows in a larger sample of populations in the same area (Araya-Ajoy et al., 2019; Jensen et al., 2008) and other bird species (Merilä & Sheldon, 2001). However, there was a large brood effect on nestling tarsus length suggesting common environmental effects within broods (e.g. Potti & Merino, 1994). For instance, variation in clutch size, seasonal differences in food availability, weather conditions (Ringsby, Sæther, Tufto, Jensen, & Solberg, 2002), and provisioning rates by parents (Ringsby, Berge, Sæther, & Jensen, 2009) may induce intra-clutch competition and variation in the degree to which nestlings are able to achieve their adult tarsus lengths at fledging (Metcalfe & Monaghan, 2001; Naef-Daenzer & Keller, 1999). Furthermore, measurement error is probably higher for the incompletely ossified nestling tarsi, which are covered by a soft fleshy skin tissue that contributes to the measured length.

Individuals with shorter tarsi (a proxy for structural size, Araya-Ajoy et al., 2019) were found to have longer telomeres, although the effect of tarsus length on TL was small and there was considerable variation in TL for a given size (Fig. 1). This confirms previous observations of a prevailing negative phenotypic correlation between body size and TL within house sparrows (Ringsby et al., 2015; Pepke et al., *submitted* 2021) and other species (Monaghan & Ozanne, 2018). We did not find evidence for a significant negative genetic correlation between TL and tarsus length (Table 2). Instead, the negative phenotypic association between TL and tarsus length may have no genetic basis but is shaped by common environmental effects that affect both traits in opposite directions (e.g. Kruuk et al., 2008) including processes related to the incomplete replication of chromosome ends during cell division and increased oxidative stress during growth (e.g. Monaghan & Ozanne, 2018). The lack of a genetic correlation between TL, tarsus length or body condition could also be attributed to selection acting simultaneously on some correlated, unmeasured trait (Merilä, Sheldon, & Kruuk, 2001). Both with and without controlling for the effect of tarsus length on TL, our GWAS on TL identified several genes involved in skeletal development, cellular growth and differentiation that may regulate body growth or size (Table 4, S2.5, and S2.7), which could, however, suggest some genetic basis of the negative correlation between TL and size. For instance, several growth factors were downregulated in telomerase deficient mouse bone marrow stromal stem cells (Saeed & Iqtedar, 2015) suggesting that short telomeres or telomere loss could also be a constraint on proliferation potential. Thus, because several of the genes that may regulate TL during early development appear to also be involved in cell proliferation or morphogenesis, such genes may have co-evolved.

None of the genes highlighted in our analysis have previously been linked to TL in GWA studies (reviewed in the introduction). Table 4 does not provide an exhaustive list of potential biological processes or molecular functions associated with variation in TL, and with little a priori information on the identified SNPs, we are cautious in interpreting these apparent associations as causal (Pavlidis, Jensen, Stephan, & Stamatakis, 2012). Furthermore, the low heritability and polygenic nature of TL make it challenging to identify putative causal genes, which consequently only explain a small part of the total phenotypic variance in TL, and our limited sample size (n=383) is likely to upwardly bias effect sizes and SNP heritabilities due to the Beavis effect (Slate, 2013). Our GWAS on TL was limited to a subset of recruiting individuals, which may affect power to detect associations between SNPs and TL if the genotype or phenotype affects recruitment probability. Pepke et al. (*submitted* 2021) found no association between TL and first-year survival in house sparrows, but that recruits had longer tarsi.

Several of the identified candidate genes (Table 4, S2.5 and S2.7) are involved in cell proliferation and apoptosis during which TL and telomerase activity invariably play an important role (Greider, 1998; Masutomi et al., 2003). Telomerase activity has not been investigated in house sparrows. However, for example the RHOF gene product functions cooperatively with CDC42 and Rac to organize the actin cytoskeleton (Ellis & Mellor, 2000), and the latter complex participates in the control of telomerase activity in human cancer cells (Yeh, Pan, & Wang, 2005). CDC42 is activated by FGD4 (Chen et al., 2004), which was found within a major locus affecting TL in humans (Vasa-Nicotera et al., 2005). SNPa108592 was found near several genes involved in cell proliferation, differentiation, immune response, and ubiquitination (Table 4). Ubiquitination regulates several shelterin components and telomerase activity (Peuscher & Jacobs, 2012; Yalçin, Selenz, & Jacobs, 2017). The closest gene, ORAI1 (43 kb), the keeper of the gates of calcium ions (Homer, 1924), is crucial for lymphocyte activation and immune response (Feske et al., 2006). Although not linked to ORAI1 mutations, calcium ion levels can modulate telomerase activity (reviewed in Farfariello, Iamshanova, Germain, Fliniaux, & Prevarskaya, 2015).

We identified a particularly interesting gene associated with TL, AQP1. The AQP1 channel not only conducts water across cell membranes, but also hydrogen peroxide, a major reactive oxygen species (ROS, Tamma et al., 2018), and nitric oxide (Herrera, Hong Nancy, & Garvin Jeffrey, 2006), which is an important regulator of oxidative stress (Pierini & Bryan, 2015) and a weak oxidant itself (Radi, 2018). Furthermore, increased availability of nitric oxide may activate telomerase and thereby prevent replicative senescence (in endothelial cells, Vasa, Breitschopf, Zeiher Andreas, & Dimmeler, 2000). Enhanced oxidative stress associated with endothelial cell senescence may also be mediated by AQP1-regulated nitric oxide flow (Chen et al., 2020; Tamma et al., 2018). In AQP1 knocked-out erythrocytes (where TL was measured) cell lifespan was shortened (Mathai et al., 1996) and angiogenesis is inhibited in AQP1 knocked-out chicken embryos (Camerino et al., 2006) and mice (Saadoun et al., 2005). Telomeres are particularly sensitive to ROS and shorten due to oxidative stress during growth (Reichert & Stier, 2017; von

Zglinicki, 2002). For instance, Kim, Noguera, Morales, and Velando (2011) found a negative genetic correlation between growth and resistance to oxidative stress in yellow-legged gull (*Larus michahellis*) chicks, which could be mediated by TL (see also Smith, Nager, & Costantini, 2016). Another candidate gene, OXR1, 76 kb from SNPa450086, has a well-described antioxidant function (Oliver et al., 2011; Volkert et al., 2000) and is upregulated in senescent human cells (Zhang et al., 2018). Knockdown of OXR1 increases ROS production and ultimately induces apoptosis (Oliver et al., 2011; Zhang et al., 2018), which could be due to telomere crisis.

Over-expression of AQP1 has been associated with several types of cancer (Verkman, Hara-Chikuma, & Papadopoulos, 2008), suppression of apoptosis (Yamazato et al., 2018) and may play an important role in tumor biology (Saadoun et al., 2005; Tomita et al., 2017). Other candidate genes including GHRHR, SHCBP1 (Tao et al., 2013), GH (Boguszewski & Boguszewski, 2019), and OXR1 (Yang et al., 2015) are also involved in tumorigenesis. Cancer prevalence is not well-studied in wildlife (Pesavento, Agnew, Keel, & Woolard, 2018), but tumors have been documented in house sparrows (Møller, Erritzøe, & Soler, 2017). Genes affecting both TL and cancer risk (Jones et al., 2012; Tacutu, Budovsky, Yanai, & Fraifeld, 2011) could underlie the antagonistic pleiotropy of trade-offs between long telomeres in early-life (with potential benefits to growth, reproduction, and other oxidative stress inducing processes) and later-life cancer mortality (Tian et al., 2018). Cancer is often viewed as a senescence-related pathology (Lemaître et al., 2020). However, the absence of cancer in early-life should not lead us to conclude that a somatic and potentially fitness-related cost is not paid to maintain that status (Thomas et al., 2018).

We have shown that TL is a heritable, polygenic trait with considerable environmental variation and a maternal inheritance component in a wild passerine. It is, however, important that future studies attempt to confirm the putative candidate genes identified here as associated with

TL in other wild populations. Even though the additive genetic component was small, selection on variation in TL may produce evolutionary change in TL over time in wild populations. The large component of variation in early-life TL caused by annual environmental stochasticity suggests that this will generate heterogeneity in TL among cohorts. Although we did not find a negative genetic correlation underlying the negative phenotypic correlation between TL and body size, we may hypothesize that selection for larger nestling size, which may enhance survival until recruitment (Ringsby, Sæther, & Solberg, 1998), will be associated with selection for shorter early-life TL due to non-genetic mechanisms, which can ultimately influence lifespan or reproductive success.

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DATA ACCESSIBILITY

All data will be made available on Dryad or another open access channel upon acceptance of the manuscript. SNP genotype data is available on Dryad (https://doi.org/10.5061/dryad.hp758sn).

AUTHOR CONTRIBUTIONS

MLP measured telomeres, analyzed the data, and wrote the manuscript with comments from all authors. WB and PM advised telomere measurements. TK, HJ, THR, and SL advised statistical analyses. B-ES, THR, and HJ established the study system. THR, HJ, and TK contributed to the fieldwork.

TABLES AND FIGURES

Figure 1: The negative association between age-corrected tarsus length and telomere length ($\log_{10}$-transformed) in 2462 house sparrow nestlings with a regression line from a LMM shown in Table 1. The 95% confidence interval (grey) reflects only the fixed effects.

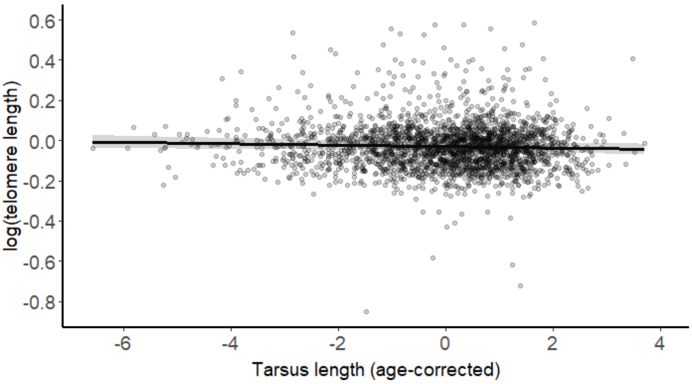

Table 1: Estimates, standard errors (SE), lower and upper 95% confidence intervals (CI) from a LMM of variation in telomere length (TL, *n*=2462). The model included random intercepts for brood identity, qPCR plate identity, and year. Italics indicate parameters with CIs not overlapping zero.

Response variable: $\log_{10}$(TL)	**Estimate**	**SE**	**Lower CI**	**Upper CI**	**10^{estimate}**
intercept	-0.0089	0.0202	-0.0484	0.0305	0.9797
tarsus length	*-0.0035*	*0.0016*	*-0.0066*	*-0.0003*	*0.9920*
sex [female]	-0.0042	0.0039	-0.0119	0.0035	0.9904
island identity [Hestmannøy]	-0.0080	0.0085	-0.0250	0.0089	0.9817
age	-0.0013	0.0015	-0.0043	0.0016	0.9970
inbreeding coefficient (*F*)	-0.1796	0.0941	-0.3638	0.0049	0.6613
hatch day	-0.0001	0.0001	-0.0004	0.0001	0.9998
$\sigma^2_{\text{brood ID}}$ (*n*=948)	0.0038		0.0030	0.0045	1.0088
$\sigma^2_{\text{qPCR plate ID}}$ (*n*=125)	0.0010		0.0007	0.0015	1.0023
σ^2_{year} (*n*=20)	0.0020		0.0010	0.0040	1.0046
$\sigma^2_{\text{residual}}$	0.0071		0.0066	0.0077	1.0165

Marginal R^2 / Conditional R^2: 0.006 / 0.486

Table 2: Posterior modes and lower and upper 95% highest posterior density intervals (HPD) for fixed effects, variance components, and heritability estimates from a multivariate animal model of the co-variation of early-life telomere length, age-corrected tarsus length, and body condition (n=2662). Abbreviations refer to: heritability h^2, additive genetic variance V_A, brood variance V_B, maternal variance V_M, paternal variance V_F, year variance V_Y, qPCR plate variance V_O, residual variance V_R, and with identical subscripts for the co-variances (Cov) including the additive genetic correlation r_A.

Variable	**$\log_{10}$(telomere length)**			**tarsus length**			**body condition**		
		HPD			**HPD**			**HPD**	
Fixed effects	**Esti-mate**	**Lower**	**Upper**	**Esti-mate**	**Lower**	**Upper**	**Esti-mate**	**Lower**	**Upper**
intercept	0.0217	-0.1498	0.2351	-0.0119	-0.3388	0.2948	0.0034	-0.1759	0.2005
sex [female]	-0.0039	-0.0129	0.0075	-0.0662	-0.1525	0.0315	0.0012	-0.0069	0.0105
island identity [Hestmannøy]	-0.0206	-0.0722	0.0291	-0.0009	-0.2705	0.2213	0.0014	-0.0434	0.0375
inbreeding coefficient (F)	-0.2067	-0.5747	0.1478	-0.9762	-3.6177	1.4944	-0.0246	-0.3485	0.2867
age	-0.0046	-0.0093	-0.0003	-	-	-	-0.0007	-0.0063	0.0039
Variance components									
h^2	0.0387	0.0220	0.0565	0.0797	0.0454	0.1239	0.0274	0.0151	0.0427
h^2 (excl. V_O)	0.0407	0.0227	0.0606	-	-	-	-	-	-
V_A	0.0087	0.0078	0.0097	0.2013	0.1114	0.3138	0.0057	0.0052	0.0062
V_B	0.0117	0.0103	0.0134	0.9292	0.7895	1.0830	0.0094	0.0084	0.0108
V_M	0.0145	0.0126	0.0169	0.1495	0.0957	0.2315	0.0126	0.0109	0.0147
V_F	0.0134	0.0115	0.0153	0.1367	0.0934	0.2121	0.0108	0.0094	0.0123
V_Y	0.1406	0.0747	0.2840	0.2132	0.1182	0.4777	0.1387	0.0821	0.2930
V_O	0.0103	0.0082	0.0137	-	-	-	-	-	-
V_R	0.0068	0.0062	0.0075	0.8174	0.7329	0.9050	0.0043	0.0039	0.0047
Co-variances between TL and tarsus					**Co-variances between TL and condition**				
r_A	-0.0293	-0.1204	0.0779		r_A		-0.0113	-0.0802	0.0554
Cov_A	-0.0012	-0.0055	0.0031		Cov_A		-0.0001	-0.0005	0.0004
Cov_B	-0.0033	-0.0145	0.0086		Cov_B		0.0000	-0.0010	0.0009
Cov_M	-0.0005	-0.0070	0.0060		Cov_M		0.0000	-0.0014	0.0015
Cov_F	0.0003	-0.0055	0.0055		Cov_F		-0.0002	-0.0013	0.0011
Cov_Y	-0.0031	-0.1084	0.1015		Cov_Y		-0.0019	-0.0804	0.0819
Cov_R	-0.0049	-0.0098	0.0008		Cov_R		-0.0001	-0.0004	0.0002

Figure 2: Variance components for TL, tarsus length and body condition visualized as relative proportions of the total phenotypic variance. Abbreviations refer to: additive genetic variance V_A, brood variance V_B, paternal variance V_F, maternal variance V_M, qPCR plate variance V_O, residual variance V_R, and year variance V_Y.

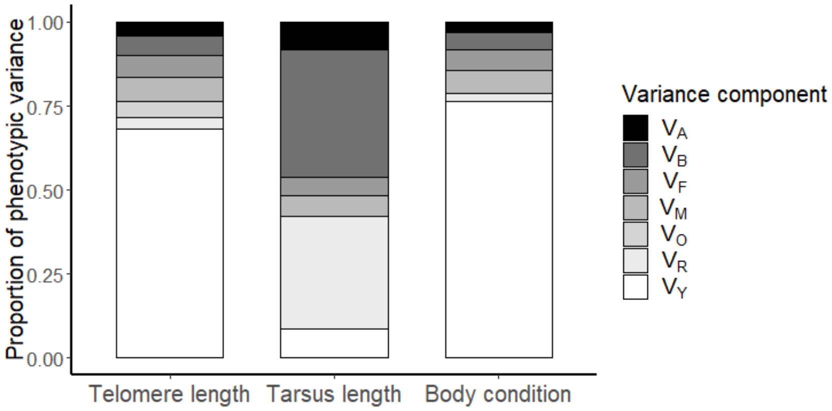

Figure 3: Manhattan plot showing genomic location plotted against -log₁₀(P-value) of the GWA analysis results for early-life telomere length in house sparrows (*n*=373). The dotted line indicates the genome-wide significance threshold (corresponding to $p<0.05$ divided by the number of tests *n*=180,650 SNPs) used to determine the top SNPs listed in Table 3.

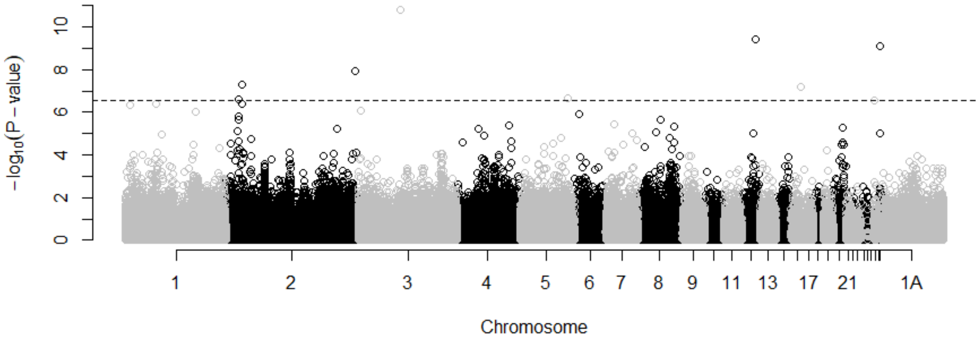

Table 3: Single nucleotide polymorphisms (SNPs) with evidence (italics, above the dashed line) or weak evidence for an association with early-life telomere length in house sparrows (*n*=373). Chromosome number, SNP position, reference allele A1, effect allele A2, estimated effect size (β) with standard error (SE), p-value, and Bonferroni adjusted p-value are shown.

SNP	**Chromosome**	**Position**	**A1**	**A2**	β	**SE**	**p-value**	**adjusted p-value**	h^2_{SNP}
SNPa223513	*3*	*46984591*	*T*	*C*	*0.5770*	*0.0855*	*1.46E-11*	*2.63E-06*	*0.0438*
SNPa17235	*12*	*14959355*	*G*	*A*	*0.3045*	*0.0486*	*3.62E-10*	*6.55E-05*	*0.0413*
SNPa500415	*30*	*133629*	*C*	*T*	*0.2919*	*0.0475*	*8.20E-10*	*0.0001*	*0.0471*
SNPa429690	*2*	*145079103*	*G*	*A*	*0.3627*	*0.0636*	*1.15E-08*	*0.0021*	*0.0391*
SNPa450086	*2*	*17261563*	*G*	*T*	*0.3553*	*0.0651*	*4.82E-08*	*0.0087*	*0.0296*
SNPa108592	*15*	*11173875*	*G*	*T*	*0.3409*	*0.0632*	*6.73E-08*	*0.0122*	*0.0302*
SNPi16410	*5*	*53016672*	*G*	*A*	*0.2242*	*0.0433*	*2.22E-07*	*0.0401*	*0.0312*
SNPa392732	*2*	*13674493*	*A*	*G*	*0.5017*	*0.0971*	*2.40E-07*	*0.0433*	*0.0266*
SNPa491204	*27*	*1191908*	*T*	*C*	*0.1387*	*0.0269*	*2.64E-07*	*0.0478*	*0.0205*
SNPa374949	1	33502667	C	T	0.2175	0.0428	3.84E-07	0.0694	0.0267
SNPa374964	1	33523052	G	A	0.2175	0.0428	3.84E-07	0.0694	0.0267
SNPa450065	2	17288071	C	T	0.2138	0.0422	4.01E-07	0.0724	0.0300
SNPa8679	1	5482366	T	C	0.2624	0.0520	4.59E-07	0.0829	0.0280

Table 4: Genes found within ±100 kb of SNPs in Table 3 with evidence for an association with early-life telomere length house sparrows. Chromosome number, distance (in bp) between SNP and gene, general molecular or biological function or relevance to telomere biology are indicated with references. The list is sorted first by SNP p-value and then by gene distance.

Chr.	**Gene**	**SNP**	**Distance**	**Function**	**Reference**
12	FRMD4B: FERM domain-containing protein 4B (*Homo sapiens*)	SNPa17235	11287	Epithelial cell polarity, scaffolding protein	Ikenouchi & Umeda, 2010
12	LMOD3: Leiomodin-3 (*Homo sapiens*)	SNPa17235	34383	Skeletal muscle filaments organization	Yuen et al., 2014
12	ARL6IP5: PRA1 family protein 3 (*Gallus gallus*)	SNPa17235	42339	Regulates taurine and glutamate transport, apoptosis, oxidative stress	Akiduki & Ikemoto, 2008; Zhou, Ye, Zhao, Li, & Zhou, 2008
12	UBA3: NEDD8-activating enzyme E1 catalytic subunit (*Homo sapiens*)	SNPa17235	54117	Cell proliferation, protein neddylation	Gong & Yeh, 1999; Osaka et al., 1998
12	TMF1: TATA element modulatory factor (*Homo sapiens*)	SNPa17235	67507	Cell growth, immune response, androgen receptor coactivator	Garcia et al., 1992; Perry et al., 2004

12	EOGT: EGF domain-specific O-linked N-acetylglucosamine transferase (*Gallus gallus*)	SNPa17235	86629	Cell metabolism, developmental signaling	Müller, Jenny, & Stanley, 2013
2	AQP1: Aquaporin-1 (*Sus scrofa*)	SNPa429690	0	Water transport, oxidative stress, cell migration, wound healing, fat metabolism, apoptosis	Monzani, Bazzotti, Perego, & La Porta, 2009; Saadoun et al., 2005; Tomita et al., 2017; Verkman et al., 2014
2	GHRHR: Growth hormone-releasing hormone receptor (*Homo sapiens*)	SNPa429690	38572	Regulation of growth hormone	Mullis, 2005; Soerensen et al., 2012; Villanova et al., 2019
2	OXR1: Oxidation resistance protein 1 (*Homo sapiens*)	SNPa450086	75676	Oxidative stress protection	Volkert et al., 2000; Zhang et al., 2018
15	ORAI1: Calcium release-activated calcium channel protein 1 (*Gallus gallus*)	SNPa108592	42546	Immune response, calcium transport	Feske et al., 2006
15	morn3: MORN repeat-containing protein 3 (*Xenopus laevis*)	SNPa108592	53962	Spermatogenesis in *Mus musculus* otherwise uncharacterized	Zhang et al., 2015
15	Kdm2b: Lysine-specific demethylase 2B (*Mus musculus*)	SNPa108592	61359	Ubiquitination, hematopoietic cell differentiation	Vargas-Ayala et al., 2019
15	RNF34: E3 ubiquitin-protein ligase RNF34 (*Bos taurus*)	SNPa108592	71094	Ubiquitination, apoptosis, immune response	Konishi, Sasaki, Watanabe, Kitayama, & Nagawa, 2005; Sasaki et al., 2002; Zhang et al., 2014
15	Tmem120b: Transmembrane protein 120B (*Mus musculus*)	SNPa108592	71684	Fat cell differentiation, obesity	Batrakou, de las Heras, Czapiewski, Mouras, & Schirmer, 2015; Byerly et al., 2010
15	RHOF: Rho-related GTP-binding protein RhoF (*Homo sapiens*)	SNPa108592	82475	Cell proliferation, migration and polarity, cytoskeleton organization	Ellis & Mellor, 2000

15	ANAPC5: Anaphase-promoting complex subunit 5 (*Gallus gallus*)	SNPa108592	83811	Cell proliferation, ubiquitination	Jin, Williamson, Banerjee, Philipp, & Rape, 2008
5	SHCBP1: SHC SH2 domain-binding protein 1 (*Homo sapiens*)	SNPi16410	69671	Cell proliferation, apoptosis, regulator of fibroblast growth factor, immune response	Asano et al., 2014; Buckley et al., 2014; Schmandt et al., 1999; Zou et al., 2019
5	CDCA4: Cell division cycle-associated protein 4 (*Homo sapiens*)	SNPi16410	76340	Cell proliferation, apoptosis, haematopoiesis	Abdullah et al., 2001; Wang et al., 2008; Xu et al., 2018
27	SCN4a: Sodium channel protein type 4 subunit alpha (*Mus musculus*)	SNPa491204	67843	Ion channel activity, muscle contraction, response to oxidative stress	Kassmann et al., 2008; Tsujino et al., 2003. See also Wang et al., 2017
27	GH: Somatotropin (*Anas platyrhynchos*)	SNPa491204	48702	Regulation of growth hormone, bone maturation	Gómez-García, Sánchez, Vallejo-Cremades, de Segura, & del Campo Ede, 2005; van Gool et al., 2010; VanderKuur et al., 1994
27	GOSR2: Golgi SNAP receptor complex member 2 (*Rattus norvegicus*)	SNPa491204	2555	Intra-Golgi transport of proteins	Hay, Chao, Kuo, & Scheller, 1997
27	WNT9B: Protein Wnt-9b (*Homo sapiens*)	SNPa491204	39887	Wnt/β-catenin signaling pathway, cranofacial and kidney development	Bergstein et al., 1997; Bourhis et al., 2010. See also Park et al., 2009

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

Appendix S1: Notes on methods.

Appendix S2: Notes on results.

Supporting information

Genetic architecture and heritability of early-life telomere length in a wild passerine

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Contents

APPENDIX S1: Notes on methods

Map of the study area

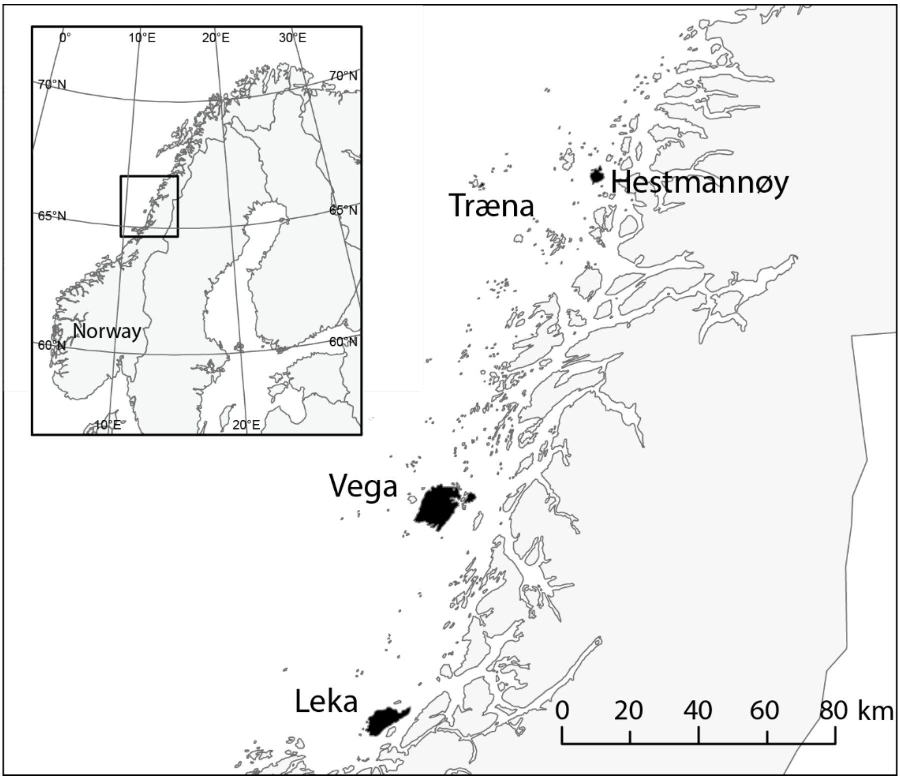

Figure S1.1 Map of the four insular house sparrow study populations in northern Norway. Træna and Hestmannøy refer to the primary analyses in this study, while Vega and Leka constitutes replicates subjected to experimental manipulations.

Morphological measurements

For a subset of the nestlings (189 out of 2746), blood sampling and morphological measurements were not made at the same age (up to ±6 days). In order to include these individuals in the analyses, we predicted age-specific morphological values based on the following procedure: Nestling body size increases with age, so we fitted linear regressions of tarsus length and mass, respectively, on age (including a squared effect of age to account for diminishing growth towards time of fledging) separately for each sex and population. For individuals with unknown sex (*n*=84, see the main text), tarsus length or mass and age were fitted within each population. We then adjusted the measured tarsus length or mass (*n*=189) to the predicted length at the age of blood (TL) sampling using the fitted values from the regressions.

Table S1.1: Number of TL sampled nestlings in each cohort (year) for each island (primary analyses: Hestmannøy and Træna, and secondary analyses: Leka, and Vega).

Cohort (year)	**Hestmannøy**	**Træna**	**Total (Hestmannøy + Træna)**	**Leka**	**Vega**	**Total (Leka + Vega)**
1994	103	-	103	-	-	-
1995	90	-	90	-	-	-
1996	48	-	48	-	-	-
1997	81	-	81	-	-	-
1998	100	-	100	-	-	-
1999	90	-	90	-	-	-
2000	73	-	73	-	-	-
2001	41	-	41	-	-	-
2002	97	-	97	47	120	167
2003	106	-	106	26	89	115
2004	106	67	173	22	59	81
2005	95	90	185	35	67	102
2006	123	59	182	28	76	104
2007	152	127	279	-	-	-
2008	83	38	121	-	-	-
2009	182	35	217	-	-	-
2010	129	44	173	-	-	-
2011	248	65	313	-	-	-
2012	70	24	94	-	-	-
2013	93	87	180	-	-	-
Sum:	2110	636	2746	158	411	569

Parent-offspring heritability power analysis

We used the function rpederr in the R package *pedantics* (Morrissey & Wilson, 2010) to permute the pedigree for the natural populations (Hestmannøy and Træna) to create a plausible complete pedigree and the function *phensim* to simulate phenotypic data across the pedigree 1000 times for heritabilities ranging from 0-0.5.

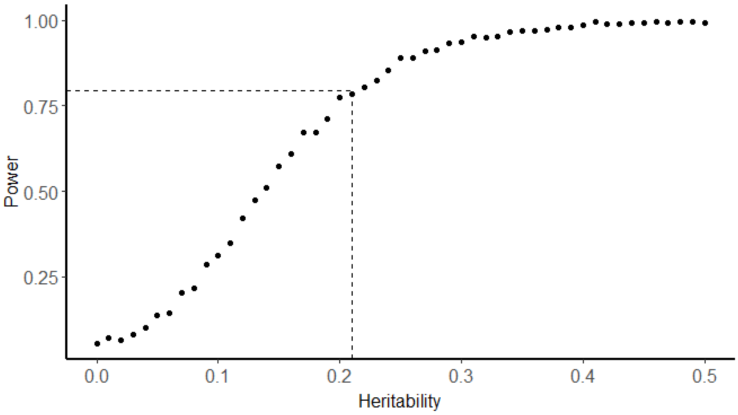

Figure S1.2: Power analysis of the sensitivity of parent-offspring regression to detect heritability. The dotted lines indicate the power of ≥80% to detect a heritability of $h^2 \geq 0.21$. Power to detect a low heritability of 0.1 was 25%, while power to detect high heritabilities >0.4 was close to 100%.

Telomere length measurements

The qPCR method has been validated for house sparrows using both the Southern blot and the in-gel TRF methods (Ringsby et al., 2015). In birds, TL measured in whole blood is primarily derived from (nucleated) erythrocytes. However, correlations between TL measured in different tissues suggest that blood TL is a good proxy of other tissues (Reichert, Criscuolo, Verinaud, Zahn, & Massemin, 2013). DNA was extracted from whole blood using the ReliaPrep Large Volume HT gDNA Isolation System (Promega) following the manufacturers protocol, but with elution of DNA in 25 mM Tris HCl (pH 8), and automated on a Beckman Coulter Biomek NX^P liquid handling system. Extracted DNA was stored at -20 °C. DNA concentration was then measured using a FLUROostar Omega scanner (BMG Labtech) and diluted with dH2O to yield 1.67 ng/mL corresponding to 10 ng of DNA per well in the PCR assay. Diluted DNA was subsequently stored at -78 °C. All DNA extractions were performed at the Norwegian University of Science and Technology (Trondheim, Norway) and DNA was shipped on dry ice (-78°C) to the University of Glasgow (UK), where telomere measurements were performed. PCR assays were prepared with the Absolute blue qPCR SYBR green Low Rox master mix (ThermoFisher scientific), which was stored at -20 °C and during use at 5 °C. Telomere primers were Tel1b (5'-CGG TTT GTT TGG GTT TGG GTT TGG GTT TGG GTT TGG GTT-3') and Tel2b (5'-GGC TTG CCT TAC CCT TAC CCT TAC CCT TAC CCT TAC CCT-3') at a final concentration of 500 nM and GAPDH (glyceraldehyde-3-phosphate dehydrogenase) primers were (forward primer 5'-GAG GTG CTG CTC AGA ACA TTA T-3' and reverse primer 5'-ACG GAA AGC CAT TCC AGT AAG-3') at a final concentration of 200 nM. Primers were stored at -20°C. Each reaction thus contained 12.5 µL Absolute blue qPCR SYBR green Low Rox master mix, forward and reverse primers, and DNA or water (for non-target control wells) with a total volume of 25µL following Ringsby et al. (2015). PCR reactions were run using a Stratagene Mx3005 system and analyzed using the MxPro qPCR software (Agilent). The telomere thermal profile was 15 min at 95 °C, followed by 27 cycles of 15 s at 95°C, 30 s at 58°C, and 30 s at 72°C. GAPDH thermal profile was 15 min at 95°C, followed by 40 cycles of 15 s at 95°C, 15 s at 60°C. Both assays were followed by melt curve analysis (58-95°C 1 °C/5 s ramp) and checked for a single peak dissociation curve. Single samples were excluded in the rare case of fluorescent signal thresholds (Ct values) differing >0.5 cycles within otherwise equally spread triplicates. Samples with very large Ct values (>16 for telomere and >24 for GAPDH assays) were excluded as being outside the standard curves.

Telomere measurement consistency across DNA extractions

We performed DNA re-extraction of the same blood samples for 25 individuals (14 males, 10 females and 1 of undetermined sex) to test TL consistency across different DNA extractions. Extractions and qPCR assays were performed on different plates in separate runs. Intraindividual TL measurements differed by 0.00-0.41 (mean 0.20). TL measurement from first and second extractions were highly correlated (R^2=0.75), demonstrating reasonable consistency in our measurement technique.

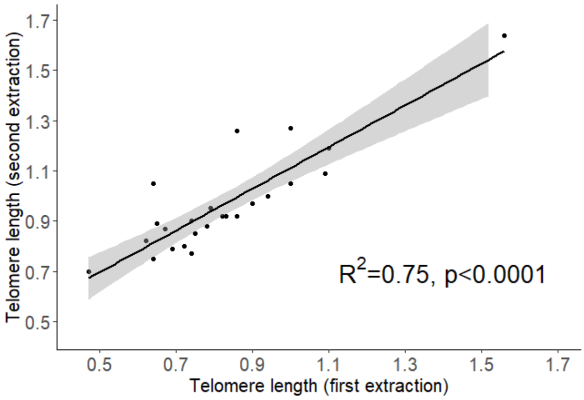

Fig. S1.3: Telomere length (T/S ratios) measured independently after two separate DNA extractions of the same blood sample and subsequent different qPCR runs to estimate TL measurement consistency.

APPENDIX S2: Notes on results

Parental age effects on telomere length

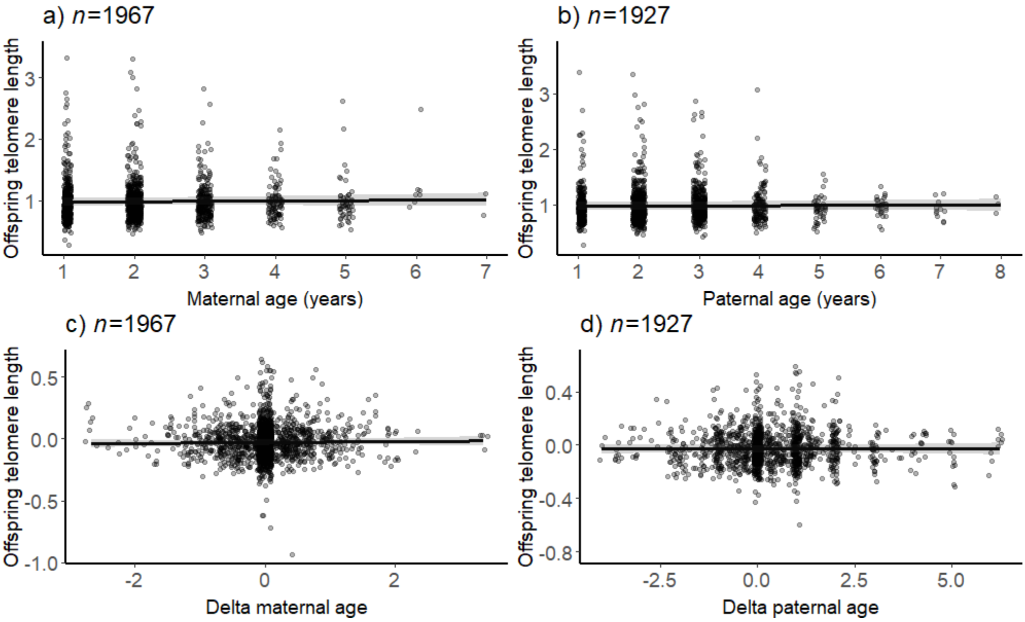

Fig. S2.1: Scatterplots of log$_{10}$-transformed offspring telomere length and a) MAC and b) PAC (shown here for illustrative purposes with jitter around each age class), and c) the deviation in MAC from mean MAC, d) the deviation in PAC from mean PAC. Regressions lines are from LMMs including island identity and offspring age at sampling as fixed effects, and random intercepts for year, qPCR plate identity, and mother or father identity.

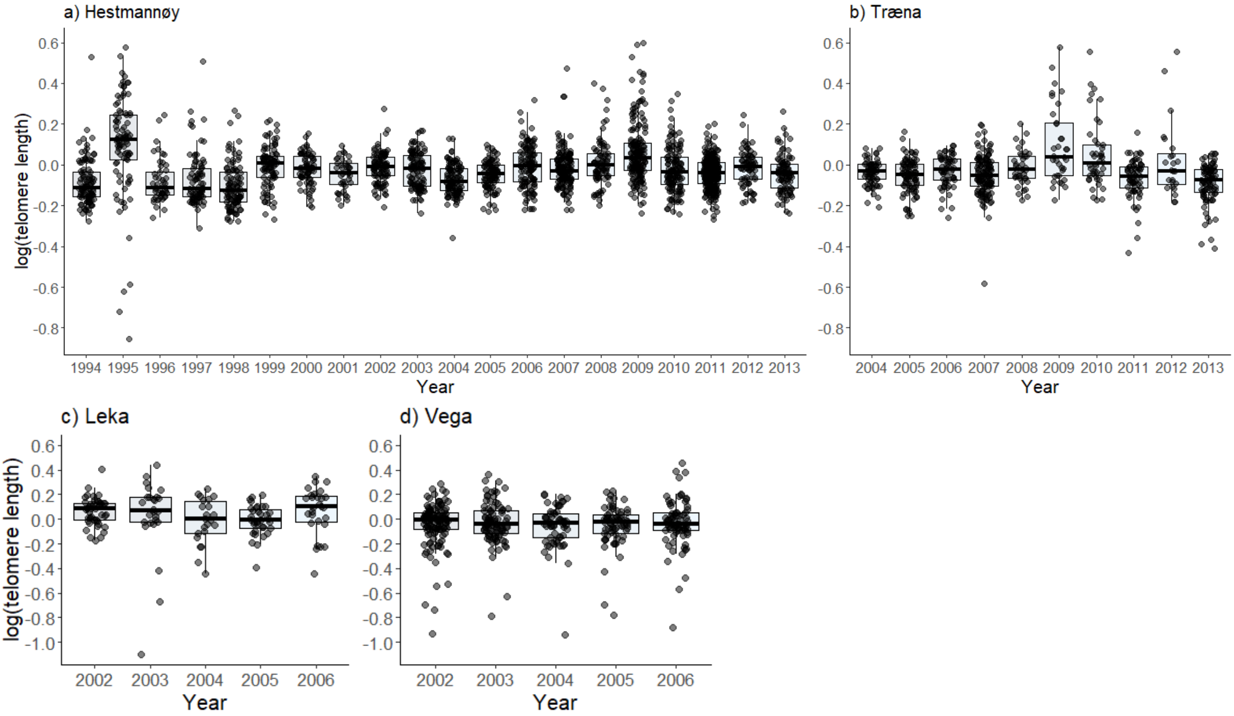

Fig. S2.2: Variation in early-life telomere length across years and islands included in this study: a) Hestmannøy (*n*=2110), b) Træna (*n*=636), c) Leka (*n*=158), and d) Vega (*n*=411). Boxplots show the median, the first, and the third quartiles, and whiskers extend from the hinges to the largest/smallest value no further than 1.5 times the distance between the first and third quartiles (IQR) from the hinges. Overlayed datapoints are $\log_{10}$-transformed telomere lengths. Sample sizes per year are shown in Table S1.1.

Univariate animal models

For the univariate models, we used inverse-Wishart priors with the variance at the limit set to 1 (V=1) and degree of belief parameter (nu=1) for both random effects and residual variances (Hadfield, 2019). The MCMC chain was run for 2,000,000 iterations, sampling every 500 iterations after a burn-in of 5% (100,000 iterations).

Table S2.1: Posterior modes and lower and upper 95% highest posterior density intervals (HPD) for fixed effects, variance components, and heritability estimates from univariate animal models of the variation in early-life telomere length (TL, *n*=2662), age-corrected tarsus length (*n*=2462), or body condition (*n*=2456). Abbreviations refer to: heritability h^2, additive genetic variance V_A, brood variance V_B, maternal variance V_M, paternal variance V_F, year variance V_Y, qPCR plate variance (V_O), and residual variance V_R.

Variable	**$\log_{10}$(telomere length)**			**tarsus length**			**body condition**		
		HPD			**HPD**			**HPD**	
Fixed effects	**Estimate**	**Lower**	**Upper**	**Estimate**	**Lower**	**Upper**	**Estimate**	**Lower**	**Upper**
intercept	0.0297	-0.0865	0.1472	0.0288	-0.2576	0.2660	0.0163	-0.0905	0.1343
sex [female]	-0.0025	-0.0110	0.0058	-0.0633	-0.1491	0.0362	0.0006	-0.0051	0.0068
population [Hestmannøy]	-0.0258	-0.0616	0.0137	-0.0149	-0.2381	0.2333	-0.0020	-0.0305	0.0201
inbreeding coefficient (*F*)	-0.2520	-0.4822	0.0512	-0.9456	-3.4167	1.4628	-0.0166	-0.2359	0.1708
age	-0.0044	-0.0075	-0.0006	-	-	-	-0.0012	-0.0048	0.0019
Variance components									
h^2	0.0570	0.0372	0.0818	0.0527	0.0241	0.1046	0.0343	0.0189	0.0519
h^2 (excl. V_O)	0.0646	0.0384	0.0942	-	-	-	-	-	-
V_A	0.0056	0.0048	0.0064	0.1251	0.0560	0.2435	0.0024	0.0022	0.0027
V_B	0.0060	0.0051	0.0069	0.9783	0.8298	1.1252	0.0038	0.0033	0.0043
V_M	0.0066	0.0055	0.0077	0.0888	0.0507	0.1647	0.0048	0.0041	0.0056
V_F	0.0063	0.0054	0.0075	0.0924	0.0507	0.1566	0.0042	0.0036	0.0048
V_Y	0.0476	0.0268	0.1000	0.1120	0.0520	0.2556	0.0442	0.0269	0.0973
V_O	0.0101	0.0082	0.0136	-	-	-	-	-	-
V_R	0.0048	0.0044	0.0054	0.8636	0.7657	0.9386	0.0020	0.0019	0.0022

Associations between parental and offspring telomere lengths

We tested whether early-life maternal TL (*n*=76 mothers with *n*=423 offspring) or early-life paternal TL (*n*=94 fathers with *n*=351 offspring) predicted TL (response variable) in offspring from Hestmannøy and Træna using LMMs (Bates, Mächler, Bolker, & Walker, 2015) with island identity and sampling age as fixed effects, and random intercepts for year, qPCR plate, and either maternal identity (in the early-life maternal TL model) or paternal identity (in the early-life paternal TL model). Models with and without parental TL were compared using *AICc*, *w*, and *ER*, as described in the main text. Including maternal TL improved the fit of the model explaining variation in offspring TL (w_1=0.94, $ER_1=w_1/w_2$=15.67, model without maternal TL: $\Delta_2 AICc$=5.5). Parent-offspring regression thus revealed clear evidence for a positive association between maternal TL and offspring TL ($\beta_{maternal\ TL}$=0.217±0.078, CI=[0.063, 0.370], *n*=423, Fig. S2.2a). Thus, there was strong evidence for a large maternal inheritance component in early-life TL ($h^2_{maternal}$=0.435±0.156, CI=[0.127, 0.741]). Including paternal TL did not significantly improve the fit of the model explaining variation in offspring TL (w_1=0.70, $ER_1=w_1/w_2$=2.33, model with paternal TL: $\Delta_2 AICc$=1.7). There was no evidence for an association between paternal TL and offspring TL in the second ranked model ($\beta_{paternal\ TL}$=-0.058±0.097, CI=[-0.247, 0.130], *n*=351, Fig. S2.2b).

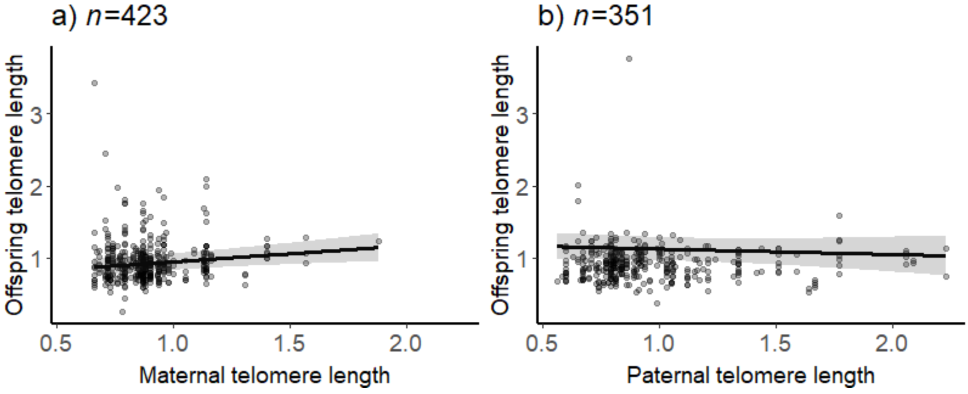

Figure S2.3: Associations between parental and offspring telomere lengths (TL) with regression lines from LMMs described above. The 95% confidence intervals (grey) reflect only the fixed effects. Maternal TL (a) significantly predicted offspring TL, while there was no association with paternal TL (b).

Multivariate animal model including parental genetic effects

Table S2.2: Posterior modes and lower and upper 95% highest posterior density intervals (HPD) for fixed effects, variance components, and heritability estimates from an animal model of the co-variation of early-life telomere length, age-corrected tarsus length, and body condition (*n*=2662). Abbreviations refer to: heritability h^2, additive genetic variance V_A, maternal genetic effect V_{DAM}, paternal genetic effect V_{SIRE}, brood variance V_B, maternal variance V_M, paternal variance V_F, year variance V_Y, qPCR plate variance (V_O), residual variance V_R, and with identical subscripts for the co-variances (*Cov*) including the additive genetic correlation r_A. The MCMC chain was run for 500,000 iterations, sampling every 300 iterations after a burn-in of 5% (25,000 iterations).

Variable	**$\log_{10}$(telomere length)**			**tarsus length**			**body condition**		
		HPD			**HPD**			**HPD**	
Fixed effects	**Esti-mate**	**Lower**	**Upper**	**Esti-mate**	**Lower**	**Upper**	**Esti-mate**	**Lower**	**Upper**
intercept	0.0293	-0.1410	0.2342	-0.0750	-0.2912	0.2213	-0.0057	-0.2398	0.1460
sex [female]	-0.0032	-0.0137	0.0057	-0.0566	-0.1448	0.0298	0.0022	-0.0071	0.0084
island identity [Hestmannøy]	-0.0177	-0.1012	0.0451	0.0028	-0.2050	0.2212	0.0102	-0.0591	0.0651
inbreeding coefficient (*F*)	-0.2392	-0.5976	0.1544	-1.1381	-4.1891	1.1895	0.0330	-0.3058	0.3738
age	-0.0061	-0.0102	0.0002	-	-	-	-0.0014	-0.0062	0.0059
Variance components									
h^2	0.0338	0.0233	0.0461	0.0724	0.0327	0.1030	0.0212	0.0135	0.0331
h^2 (excl. V_O)	0.0352	0.0240	0.0481	-	-	-	-	-	-
$h^2_{maternal}$	0.0780	0.0481	0.0988	0.0523	0.0346	0.0846	0.0649	0.0418	0.1104
$h^2_{paternal}$	0.0717	0.0433	0.0886	0.0462	0.0317	0.0695	0.0636	0.0359	0.0948
V_A	0.0093	0.0085	0.0105	0.2008	0.1022	0.3036	0.0059	0.0056	0.0067
V_{DAM}	0.0201	0.0176	0.0231	0.1426	0.1011	0.2387	0.0187	0.0153	0.0213
V_{SIRE}	0.0173	0.0155	0.0210	0.1190	0.0860	0.1939	0.0166	0.0134	0.0185
V_B	0.0134	0.0123	0.0161	0.8428	0.7232	1.0017	0.0115	0.0102	0.0136
V_M	0.0194	0.0166	0.0220	0.1619	0.0972	0.2041	0.0178	0.0153	0.0212
V_F	0.0180	0.0150	0.0204	0.1463	0.0880	0.2071	0.0151	0.0127	0.0177
V_Y	0.1557	0.0837	0.2629	0.2329	0.1360	0.4371	0.1424	0.0807	0.3245
V_O	0.0109	0.0086	0.0129	-	-	-	-	-	-
V_R	0.0070	0.0065	0.0077	0.8184	0.7455	0.9051	0.0045	0.0042	0.0049
Co-variances between TL and tarsus					**Co-variances between TL and condition**				
r_A	0.0009	-0.1387	0.0644		r_A		-0.0137	-0.0691	0.0472
Cov_A	0.0000	-0.0058	0.0031		Cov_A		-0.0001	-0.0005	0.0004
Cov_{DAM}	0.0007	-0.0080	0.0088		Cov_{DAM}		0.0003	-0.0023	0.0025
Cov_{SIRE}	0.0004	-0.0051	0.0070		Cov_{SIRE}		-0.0003	-0.0023	0.0016
Cov_B	-0.0016	-0.0170	0.0076		Cov_B		0.0000	-0.0012	0.0014
Cov_M	0.0007	-0.0096	0.0055		Cov_M		-0.0003	-0.0023	0.0020
Cov_F	0.0003	-0.0079	0.0054		Cov_F		0.0001	-0.0018	0.0016
Cov_Y	-0.0169	-0.0770	0.0905		Cov_Y		-0.0020	-0.0802	0.1008
Cov_R	-0.0039	-0.0092	0.0016		Cov_R		-0.0001	-0.0004	0.0004

Bivariate animal model testing for sex-specific telomere length heritabilities

Table S2.3: Posterior modes and lower and upper 95% highest posterior density intervals (HPD) for fixed effects, variance components, and heritability estimates from a bivariate animal model for the Hestmannøy and Træna populations (*n*=2462). Here, TL in females and males are specified as two different phenotypic traits (with female TL missing in males and vice versa) with a genetic correlation between them. We used inverse-Wishart priors ($V=I_2$, nu=2) for both random effects and residual variances. The MCMC chain was run for 2,000,000 iterations, sampling every 500 iterations after a burn-in of 5% (100,000 iterations). Abbreviations refer to: heritability h^2, additive genetic variance V_A, brood variance V_B, maternal variance V_M, paternal variance V_F, year variance V_Y, qPCR plate variance (V_O), residual variance V_R, and identical subscripts for the co-variances (*Cov*) including the additive genetic correlation r_A.

Variable	**$\log_{10}$(male telomere length)**			**$\log_{10}$(female telomere length)**		
		HPD			**HPD**	
Fixed effects	**Estimate**	**Lower**	**Upper**	**Estimate**	**Lower**	**Upper**
intercept	-0.0309	-0.2131	0.1550	-0.0092	-0.1659	0.1945
tarsus length	-0.0071	-0.0158	0.0014	-0.0031	-0.0127	0.0042
population [Hestmannøy]	-0.0115	-0.0719	0.0627	-0.0358	-0.0930	0.0343
inbreeding coefficient (*F*)	-0.1474	-0.6572	0.3934	-0.4480	-1.1591	0.3026
age	0.0002	-0.0098	0.0089	-0.0025	-0.0102	0.0063
Variance components						
h^2	0.0577	0.0359	0.0795	0.0561	0.0358	0.0793
h^2 (excl. V_O)	0.0656	0.0383	0.0924	0.0633	0.0364	0.0901
V_A	0.0114	0.0097	0.0132	0.0104	0.0092	0.0124
V_B	0.0133	0.0113	0.0159	0.0127	0.0106	0.0149
V_M	0.0156	0.0129	0.0187	0.0148	0.0122	0.0178
V_F	0.0146	0.0125	0.0179	0.0140	0.0116	0.0167
V_Y	0.0988	0.0537	0.1957	0.0935	0.0523	0.1966
V_O	0.0212	0.0168	0.0281	0.0212	0.0164	0.0274
V_R	0.0092	0.0081	0.0107	0.0088	0.0077	0.0101
Co-variances between male and female TL						
r_A	0.0074	-0.0937	0.1117			
Cov_A	0.0001	-0.0011	0.0012			
Cov_B	0.0004	-0.0011	0.0019			
Cov_M	0.0002	-0.0017	0.0023			
Cov_F	0.0002	-0.0017	0.0020			
Cov_Y	-0.0012	-0.0579	0.0600			
Cov_Q	0.0007	-0.0030	0.0049			
Cov_R	0.0000	-0.0009	0.0008			

Multivariate animal model of artificially selected populations

Table S2.4: Posterior modes and lower and upper 95% highest posterior density intervals (HPD) for fixed effects, variance components, and heritability estimates from a multivariate animal model of the co-variation of early-life telomere length, age-corrected tarsus length, and body condition in two populations (Leka and Vega) under artificial body size selection (*n*=569). Abbreviations refer to: heritability h^2, additive genetic variance V_A, brood variance V_B, maternal variance V_M, paternal variance V_F, year variance V_Y, qPCR plate variance (V_O), residual variance V_R, and similarly for the co-variances (*Cov*) including the additive genetic correlation r_A.

Variable	**$\log_{10}$(telomere length)**			**tarsus length**			**body condition**		
		HPD			**HPD**			**HPD**	
Fixed effects	**Estimate**	**Lower**	**Upper**	**Estimate**	**Lower**	**Upper**	**Estimate**	**Lower**	**Upper**
intercept	-0.0478	-0.9259	0.9717	-0.1257	-1.1153	0.8858	-0.0779	-0.9644	0.8824
sex [female]	-0.0209	-0.0637	0.0251	-0.0266	-0.1796	0.1605	-0.0001	-0.0339	0.0356
population [Vega]	-0.0738	-0.2281	0.0807	0.0845	-0.3204	0.5035	0.0019	-0.1345	0.1403
inbreeding coefficient (*F*)	0.0561	-1.2596	1.6372	-1.0803	-5.4892	3.1063	-0.0343	-1.2966	1.1647
age	0.0076	-0.0244	0.0316	-	-	-	0.0058	-0.0221	0.0278
Variance components									
h^2	0.0313	0.0052	0.0605	0.1264	0.0404	0.2369	0.0175	0.0041	0.0493
h^2 (excl. V_O)	0.0286	0.0059	0.0711	-	-	-	-	-	-
V_A	0.0309	0.0251	0.0382	0.2942	0.1560	0.5643	0.0208	0.0174	0.0251
V_B	0.0444	0.0338	0.0583	0.2659	0.1534	0.4347	0.0378	0.0292	0.0498
V_M	0.0539	0.0389	0.0709	0.2594	0.1439	0.4461	0.0458	0.0347	0.0611
V_F	0.0493	0.0371	0.0657	0.1910	0.1140	0.3351	0.0414	0.0319	0.0550
V_Y	0.4819	0.1261	2.6293	0.5070	0.1609	2.8691	0.4711	0.1613	2.7387
V_O	0.0547	0.0320	0.1074	-	-	-	-	-	-
V_R	0.0237	0.0202	0.0293	0.6072	0.4330	0.7477	0.0154	0.0131	0.0184
Co-variances between TL and tarsus					**Co-variances between TL and condition**				
r_A	-0.0356	-0.2239	0.1487		r_A		-0.0076	-0.1290	0.1399
Cov_A	-0.0031	-0.0236	0.0157		Cov_A		-0.0002	-0.0036	0.0035
Cov_B	-0.0030	-0.0307	0.0212		Cov_B		-0.0006	-0.0079	0.0079
Cov_M	0.0010	-0.0313	0.0299		Cov_M		0.0005	-0.0099	0.0106
Cov_F	0.0011	-0.0251	0.0269		Cov_F		-0.0001	-0.0092	0.0091
Cov_Y	0.0400	-1.3796	1.1906		Cov_Y		0.0117	-1.2634	1.2746
Cov_R	-0.0058	-0.0241	0.0110		Cov_R		0.0001	-0.0026	0.0025

Q-Q plot of the GWA analysis

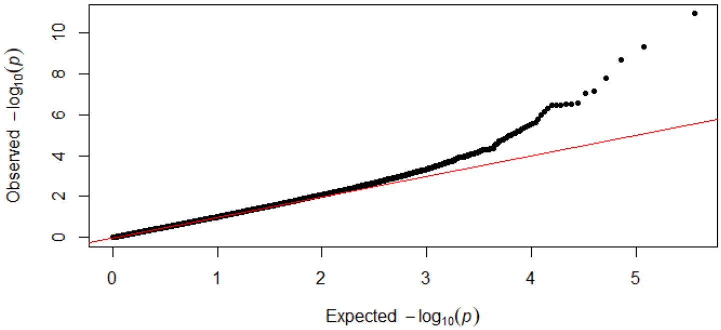

Figure S2.3: Q-Q plot of the observed p-values are plotted against the expected p-values from the GWAS on TL controlling for tarsus length (presented in the main text).

Genes near SNPs with weak evidence for an association with telomere length

Table S2.5: Genes found within ±100 kb of the SNPs with weak evidence for an association with early-life telomere length house sparrows (Table 3, accounting for the effect of tarsus length on telomere length). Chromosome number, distance (in bp) between SNP and gene, general molecular or biological function or relevance to telomere biology are indicated with references. The list is sorted first by SNP P-value (Table 3) and then by gene distance.

Chr.	Gene	SNP	Distance	Function	Reference
1	CTSC: Dipeptidyl peptidase 1 (*Pongo abelii*)	SNPa374964/ SNPa374949	49538/ 69923	Immune response, proteolysis etc.	Popovic, Puizdar, Ritonja, & Brzin, 1996; Rao, Rao, & Hoidal, 1997
1	POU1F1: Pituitary-specific positive transcription factor 1 (*Gallus gallus*)	SNPa8679	28887	Transcription factor, activates growth hormone and prolactin genes	Sobrier et al., 2016; Turton, Strom, Langham, Dattani, & Le Tissier, 2012
1	CHMP2B: Charged multivesicular body protein 2b (*Gallus gallus*)	SNPa8679	39769	Endosomal sorting required for transport complex III, cognition, nucleus organization	Morita et al., 2010; Skibinski et al., 2005

GWA analysis not controlling for the effect of tarsus length

Table S2.6: Single nucleotide polymorphisms (SNPs) with evidence (italics) or weak evidence for an association with early-life telomere length in house sparrows when excluding the effect of tarsus length on TL (*n*=383). Chromosome number, SNP position, reference allele A1, effect allele A2, estimated effect size (*β*) with standard error (SE), p-value, and Bonferroni adjusted p-value are shown. SNPs not identified in the GWA controlling for tarsus length (Table 3) are shown in bold.

SNP	Chro-mosome	Position	A1	A2	*β*	SE	*p*-value	adjusted *p*-value	h^2_{SNP}
SNPa223513	*3*	*46984591*	*T*	*C*	*0.5548*	*0.0848*	*5.98E-11*	*1.08E-05*	*0.0438*
SNPa500415	*30*	*133629*	*C*	*T*	*0.3030*	*0.0466*	*7.75E-11*	*1.40E-05*	*0.0471*
SNPa450086	*2*	*17261563*	*G*	*T*	*0.3822*	*0.0634*	*1.67E-09*	*0.0003*	*0.0296*
SNPa17235	*12*	*14959355*	*G*	*A*	*0.2817*	*0.0480*	*4.57E-09*	*0.0008*	*0.0413*
SNPa429690	*2*	*1.45E+08*	*G*	*A*	*0.3626*	*0.0642*	*1.59E-08*	*0.0029*	*0.0391*
SNPi16410	*5*	*53016672*	*G*	*A*	*0.2401*	*0.0429*	*2.25E-08*	*0.0041*	*0.0312*
SNPa450065	*2*	*17288071*	*C*	*T*	*0.2292*	*0.0418*	*4.20E-08*	*0.0076*	*0.0300*
SNPa208275	***6***	***4778559***	***C***	***T***	***0.2632***	***0.0493***	***9.11E-08***	***0.0165***	*0.0283*
SNPa108592	*15*	*11173875*	*G*	*T*	*0.3393*	*0.0638*	*1.06E-07*	*0.0191*	*0.0302*
SNPa392732	*2*	*13674493*	*A*	*G*	*0.5092*	*0.0979*	*1.98E-07*	*0.0358*	*0.0266*
SNPa392704	***2***	***13625127***	***G***	***A***	***0.4197***	***0.0812***	***2.36E-07***	***0.0427***	*0.0255*
SNPa491204	27	1191908	T	C	0.1369	0.0271	4.51E-07	0.0815	0.0205

Genes near SNPs associated with telomere length not controlling for tarsus length

Table S2.7: Genes found within ±100 kb of the *additional* SNPs (in bold in Table S2.5) with evidence for an association with early-life telomere length identified in a GWA not controlling for tarsus length. Chromosome number, distance (in bp) between SNP and gene, general molecular or biological function or relevance to telomere biology are indicated with references.

Chr.	Gene	SNP	Distance	Function	Reference
6	FGFR2: Fibroblast growth factor receptor 2 (*Gallus gallus*)	SNPa208275	47187	Cell proliferation, apoptosis, embryonic and skeletal development, cancers	Hunter et al., 2007; Katoh, 2009; Zhu et al., 2018

Interesting genes found beyond the ±100 kb limits of SNPs associated with TL

Inferring associations between TL and genes located beyond the ±100 kb limits may be highly speculative. However, we note that the top marker, SNPa223513 (Table 3), was found closest (106 kb) to the SAMD5 (sterile alpha motif domain-containing protein 5) gene, which function is unknown, but may play a role in tumorigenesis (Sa, Lee, Hong, Kong, & Nam, 2017), cancer cell proliferation (Matsuo et al., 2014) or tumor suppression in the cytoplasm (Yagai et al., 2017). SNPa108592 is 263 kb from LRRC43 (leucine-rich repeat-containing protein 43) that belongs to a class of poorly known proteins often associated with innate immunity (Ng & Xavier, 2011). Members of the LRRC superfamily have previously been associated with TL variation in humans (Codd et al., 2010). The same SNP is 363 kb from ZCCHC8 (zinc finger CCHC domain-containing protein 8) that is required for telomerase functioning (Gable et al., 2019). This gene was also found to be associated with TL variation in a recent GWA study of 472,174 humans (Codd et al., 2021).

Among SNPs with weak evidence for an association with TL (Table S2.5), SNPi16410 was found 164 kb from BMP4 (bone morphogenetic protein 4), which is known to induce telomere shortening and replicative senescence *in vitro* (Buckley et al., 2004).

Among additional SNPs associated with telomere length when not controlling for tarsus length (Table S2.7), SNPa208275 was 134 kb from NSMCE4A (non-structural maintenance of chromosomes element 4 homolog A), which is a component of the SMC5-SMC6 complex that is required for telomere maintenance via alternative lengthening of telomeres (ALT, Taylor, Copsey, Hudson, Vidot, & Lehmann, 2008; Cesare & Reddel, 2010 Cesare & Reddel, 2010). The same SNP was found 220 kb from TACC2 (transforming acidic coiled-coil-containing protein 2) that may also regulate ALT (Henson, 2006).

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PAPER III

Inbreeding is associated with shorter early-life telomere length in a wild passerine

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ABSTRACT

Inbreeding can have negative effects on survival and reproduction, which may be of conservation concern in small and isolated populations. However, the physiological mechanisms underlying inbreeding depression are not well-known. The length of telomeres, the DNA sequences protecting chromosome ends, has been associated with health or fitness in several species. We investigated effects of inbreeding on early-life telomere length in two small island populations of wild house sparrows (*Passer domesticus*) known to be affected by inbreeding depression. Using genomic and pedigree-based measures of inbreeding we found that inbred nestling house sparrows have shorter telomeres. This negative effect of inbreeding on telomere length may have been complemented by a heterosis effect resulting in longer telomeres in individuals that were less inbred than the population average. Furthermore, we found some evidence of stronger effects of inbreeding on telomere length in males than females. Thus, telomere length may reveal subtle costs of inbreeding in the wild and demonstrate a route by which inbreeding negatively impacts the physiological state of an organism already at early life-history stages.

INTRODUCTION

Inbreeding may have significant detrimental effects on survival, reproduction, and resistance to disease and other stressors in wild populations (Keller & Waller, 2002). Such decline in fitness resulting from an increase in genome-wide homozygosity is known as inbreeding depression (Charlesworth & Willis, 2009) and is of major concern in small and isolated populations, in particular of endangered species (Bozzuto, Biebach, Muff, Ives, & Keller, 2019; Harrisson et al., 2019; Hedrick & Kalinowski, 2000). Increased homozygosity can lead to reduced fitness due to expression of deleterious recessive alleles ("dominance hypothesis") or increased homozygosity at loci with heterozygote advantage ("overdominance hypothesis", Charlesworth & Willis, 2009). Regardless of the genetic basis for inbreeding depression, it is difficult to identify and quantify the physiological mechanisms underlying the fitness costs of inbreeding (Fox & Reed, 2011; Kristensen, Pedersen, Vermeulen, & Loeschcke, 2010; Losdat, Arcese, Sampson, Villar, & Reid, 2016).

Telomeres are short DNA tandem repeats that are found at the tips of most eukaryotic chromosomes (Blackburn & Gall, 1978; Červenák, Sepšiová, Nosek, & Tomáška, 2021). Telomeres shorten during cell division (Harley, Futcher, & Greider, 1990), but may also shorten due to several other reasons including physiological processes generating oxidative stress (Barnes, Fouquerel, & Opresko, 2019; Monaghan & Ozanne, 2018; Reichert & Stier, 2017; von Zglinicki, 2002). The high guanine content of telomeres (50%) makes them particularly vulnerable to oxidative stress (Kawanishi & Oikawa, 2004). Short telomeres can trigger apoptosis and telomere attrition is considered a hallmark of aging (López-Otín, Blasco, Partridge, Serrano, & Kroemer, 2013), although the causal involvement of telomere shortening in organismal senescence is not well understood (Simons, 2015). However, telomere length (TL) may reflect the cumulative stress experienced by an individual (Bateson, 2016; Monaghan, 2014), and TL or TL shortening are associated with health or fitness in several species (Barrett, Burke, Hammers, Komdeur, & Richardson, 2013; Chatelain, Drobniak, & Szulkin, 2020; Froy et al., 2021; Heidinger, Kucera, Kittilson, & Westneat, 2021; Wilbourn et al., 2018). Thus, TL is increasingly used as a biomarker of somatic integrity in studies of physiological or evolutionary ecology (Bateson & Poirier, 2019; Haussmann & Marchetto, 2010; Pepper, Bateson, & Nettle, 2018; Young, 2018).

Inbreeding depression can be caused by reduced immune response (Charpentier, Williams, & Drea, 2008; Reid, Arcese, & Keller, 2003) and higher maintenance metabolism (Ketola & Kotiaho, 2009), which increases oxidative stress (de Boer et al., 2018a; Okada, Blount, Sharma, Snook, & Hosken, 2011). Thus, inbred individuals may experience higher levels of oxidative stress (Kristensen, Sørensen, Kruhøffer, Pedersen, & Loeschcke, 2005; Pedersen et al., 2008) and thus have shorter telomeres (von Zglinicki, 2002). We therefore hypothesize that TL could provide an integrative measure of the somatic costs associated with inbreeding depression in wild populations, with inbred individuals having shorter telomeres than outbred individuals. However, the few studies investigating associations between inbreeding and TL have found equivocal results. In line with our expectations, Bebbington et al. (2016) found that homozygosity was negatively associated with TL in wild Seychelles warblers (*Acrocephalus sechellensis*) and Seluanov et al. (2008) reported that telomeres were shorter in inbred laboratory strains of Norway rats (*Rattus norvegicus*) in captivity compared to a single wild-caught rat. Many domesticated species are generally assumed to be more inbred than their wild counterparts (Bosse, Megens, Derks, de Cara, & Groenen, 2018; Moyers, Morrell, & McKay, 2018; Wiener & Wilkinson, 2011). However, several studies have found that telomeres were longer in inbred domesticated strains of laboratory mice (*Mus* spp. and *Peromyscus* spp., Hemann & Greider, 2000; Manning, Crossland, Dewey, & Van Zant, 2002; Seluanov et al., 2008), in domesticated strains of pearl millet (*Pennisetum glaucum*, Sridevi, Uma, Sivaramakrishnan, & Isola, 2002), in domesticated inbred chicken (*Gallus gallus*, O'Hare & Delany, 2009), and across several species of domesticated mammals (Pepke & Eisenberg, 2021) compared to non-domesticated species. However, there were no clear differences in TL between inbred and wild leporid strains (Forsyth, Elder, Shay, & Wright, 2005). Other studies found no association between pedigree-based inbreeding coefficients and TL or telomere attrition in humans (*Homo sapiens*, Mansour et al., 2011), wild sand lizards (*Lacerta agilis*, Olsson, Wapstra, & Friesen, 2018), or wild natterjack toads (*Epidalea calamita*, Sánchez-Montes et al., 2020). Becker et al. (2015) reported a weak non-significant but positive association between inbreeding and TL in wild white-throated dippers (*Cinclus cinclus*).

These contrasting results suggest that the telomere dynamics of captive, domesticated species living in a controlled environment may not be representative of wild, free-living populations (Chatelain et al., 2020; Pepke & Eisenberg, 2021; Weinstein & Ciszek, 2002). For instance, captive populations may be less vulnerable to inbreeding because inbreeding depression is greater under stressful environmental conditions (Fox & Reed, 2011; Reed, Briscoe, & Frankham, 2002). Furthermore, captivity may in itself provide conditions that change the telomere dynamics of the populations (Eisenberg, 2011), e.g. Hemann and Greider (2000) attributed the longer telomeres of inbred mice to effects of captive breeding and not inbreeding *per se*. For instance, TL shortening rates may increase during metabolically costly processes such as reproduction (Sudyka, Arct, Drobniak, Gustafsson, & Cichoń, 2019; Wood et al., 2021) and inbreeding may reduce fecundity (Keller & Waller, 2002). Such effects have been suggested to explain the observation of longer adult TL in some inbred domesticated species (Eisenberg, 2011), which could be resolved by measuring TL in early-life. Furthermore, most of the studies of domesticated animals compared TLs of different populations or species and their results may not be extrapolated to natural variation in TL and inbreeding levels within wild populations. Indeed, TL can vary considerably within species (Tricola et al., 2018) and across closely related species (Pepke, Ringsby, & Eisenberg, 2021c) in the wild. Finally, it is not known if outbreeding could be accompanied by a heterosis effect (hybrid vigor, e.g. Charlesworth & Willis, 2009) acting on TL. For instance, the observed fitness benefits of outcrossing inbred populations (Frankham, 2015) could be reflected in TL restoration (Nuzhdin & Reiwitch, 2002; Ozawa et al., 2019).

In this study, we utilized a long-term metapopulation study to examine how inbreeding affects early-life TL in wild house sparrows (*Passer domesticus*). Inbreeding has been shown to reduce fitness components such as recruitment probability, adult lifespan, and both annual and lifetime reproductive success in this metapopulation (Billing et al., 2012; Jensen, Bremset, Ringsby, & Sæther, 2007; Niskanen et al., 2020), but the physiological effects underlying these phenomena remain unknown. We expect that inbred individuals will have shorter telomeres if TL is a general biomarker of somatic integrity and health (e.g. Bebbington et al., 2016; Boonekamp, Simons, Hemerik, & Verhulst, 2013; Wilbourn et al., 2018). The effects of inbreeding on TL might be sex-specific (Benton et al., 2018; Billing et al., 2012; de Boer et al., 2018a; de Boer, Eens, & Müller, 2018b) or depend on environmental conditions (Armbruster & Reed, 2005; Szulkin & Sheldon, 2007). However, TL is negatively associated with body size or growth rate within many species (Monaghan & Ozanne, 2018; Ringsby et al., 2015) and may change with age (Hall et al., 2004; Remot et al., 2021) or vary between sexes (Barrett & Richardson, 2011; Remot et al., 2020) and habitat quality (Angelier, Vleck, Holberton, & Marra, 2013; McLennan et al., 2021; Wilbourn et al., 2017). We therefore account for body size (measured as tarsus length), age, sex, and habitat type, and test for an interaction between inbreeding levels and sex or habitat type, when investigating the association between TL and inbreeding. We use three different measures of inbreeding; marker-based estimates (n=371) which are better at capturing homozygosity and inbreeding caused by distant ancestors not included in a pedigree, and pedigree-based estimates (Kardos, Taylor, Ellegren, Luikart, & Allendorf, 2016) for which larger samples size may be obtained from long-term field studies (n=1195). Finally, to investigate a potential heterosis effect on TL, we test if the association between TL and inbreeding is different among outbred and inbred individuals.

MATERIAL AND METHODS

Study system

This study was conducted in two natural populations of house sparrows in northern Norway. On the island of Hestmannøy (66°33’N, 12°50‘E), the sparrows live around dairy farms, where they nest inside barns in cavities or nest boxes. The island is characterized by cultivated grassland, mountains, forest, and heathland. On the island of Træna (66°30’N, 12°05‘E), 34 km further from the mainland, the sparrows live in gardens of a small human settlement and nest in nest boxes. This island is dominated by heathland, sparse forest, and gardens. The natural breeding environment for house sparrows is human habitation (Hanson, Mathews, Hauber, & Martin, 2020) and they have evolved their commensal relationship with humans for millennia (Ravinet et al., 2018). While human presence or farming provide the natural basis of existence for house sparrows (Ringsby, Sæther, Jensen, & Engen, 2006), demographic characteristics, breeding densities, and inbreeding rates are comparable to other small isolated wild animal populations (Araya-Ajoy et al., 2021; Jensen et al., 2007; Niskanen et al., 2020). In the years 1994-2013 (on Hestmannøy) and 2004-2013 (on Træna), nestlings at the age of 5-14 days were ringed with a unique combination of color rings for identification. Nestlings were also blood sampled by brachial venipuncture, and tarsometatarsus (tarsus) was measured with slide calipers to the nearest 0.01 mm. Tarsus length is here used as an index of body size (Rising & Somers, 1989; Senar & Pascual, 1997). Blood samples (25 µL) were stored in 96% ethanol at room temperature in the field and at -20°C in the laboratory until DNA extraction (described in Pepke et al., 2021b). Birds that were resighted or recaptured in the year following hatching (i.e. from 1995-2014 on Hestmannøy and from 2005-2014 on Træna) were categorized as first-year survivors.

Telomere length measurements

Relative erythrocyte telomere length (TL) was measured in DNA derived from whole blood samples (*n*=2746 nestlings) using the qPCR method (Cawthon, 2002) as described in Pepke et al. (2021a). For this study, we included only individuals with two known parents and at least two known grandparents, or for which genomic inbreeding coefficients could be estimated (described below), resulting in a sample size of *n*=1370 individuals (*n*=1161 from Hestmannøy and *n*=209 from Træna). TL was determined relative to the amount of a non-variable gene (GAPDH) and a reference sample (Criscuolo et al., 2009). All samples were randomized and run in triplicates on 96-well plates. All samples were processed within a few months by the same researcher (MLP) to reduce technical effects. Relative TL was computed using qBASE (Hellemans, Mortier, De Paepe, Speleman, & Vandesompele, 2007) while controlling for inter-run variation. All individual plate efficiencies were within 100±10% (see Pepke et al., 2021a). Sex was determined by amplification of the CHD-gene as described in Jensen et al. (2007).

Microsatellite pedigree construction

Microsatellite (MS) pedigrees (*n*=1857 individuals from Hestmannøy and *n*=342 from Træna including non-phenotyped ancestors) were constructed based on 13 polymorphic microsatellite markers using CERVUS 3.0 (Kalinowski, Taper, & Marshall, 2007) as described in Billing et al. (2012). Maximum pedigree depth was 13 generations. We calculated inbreeding coefficients (F_{PED}), which estimate the expected proportion of an individual's genome that is identical by descent (IBD), based on the MS pedigree for individuals with two known parents and at least two known grandparents (*n*=1057 from Hestmannøy and *n*=138 from Træna, Table 1) using the R package *pedigree* (Coster, 2012). We also selected a subset of individuals with at least two full ancestral generations (i.e. four known grandparents) to only include the most robust estimates of F_{PED} (*n*=313 from Hestmannøy and *n*=7 from Træna).

Genomic inbreeding estimation

Starting from year 1997 (Hestmannøy) or 2004 (Træna), birds that survived until recruitment (*n*=275 from Hestmannøy and *n*=96 from Træna) were genotyped for 200,000 Single Nucleotide Polymorphisms (SNPs) as described in Lundregan et al. (2018). Two genomic inbreeding coefficients were then estimated using 118,810 autosomal SNPs not in strong linkage disequilibrium, as described in Niskanen et al. (2020). The weighted average homozygosity over all loci from the genomic relationship matrix (F_{GRM}) was estimated for the whole metapopulation simultaneously using the GCTA software (Yang, Lee, Goddard, & Visscher, 2011). F_{GRM} gives more weight to homozygotes of the minor allele than of the major allele, and it is an estimate of the correlation between homologous genes of the two gametes of an individual relative to the current population (Yang et al., 2011). F_{GRM} can be negative if the probability that the two homologous genes of an individual are IBD is smaller than that of two homologous genes being drawn at random from the reference population (Wang, 2014; Yang et al., 2011). Thus, the individuals with the smallest estimates of F_{GRM} are expected to be outbred (hybrids) because of e.g. mating involving immigrants (Wang, 2014). The proportion of the genome within runs-of-homozygosity (F_{ROH} ranging from 0 to 1, McQuillan et al., 2008) was estimated using the PLINK software (Purcell et al., 2007). ROH arise through mating of individuals that are IBD, and may therefore be used to estimate inbreeding (Curik, Ferenčaković, & Sölkner, 2014).

Statistical analyses

To test whether TL was affected by inbreeding, we fitted linear mixed models (LMMs) using the package *lme4* (Bates, Mächler, Bolker, & Walker, 2015) in R v. 3.6.3 (R Core Team, 2020). TL (response variable) was $\log_{10}$-transformed to conform to the assumption of normally distributed residuals and the models were fitted with a (continuous) fixed effect of one of the inbreeding coefficients (F_{PED} [*n*=1195], F_{PED} with at least two full generations known [*n*=320], F_{GRM} [*n*=371], or F_{ROH} [*n*=371], see Table 1 for sample size details). Since genomic estimators of inbreeding (F_{GRM} and F_{ROH}) were only available for recruits (first-year survivors), we tested whether the relationship between TL and F_{PED} varied between survivors ("1", *n*=206) and non-survivors ("0", *n*=989) by including an interaction effect between F_{PED} and first-year survival. Tarsus length increases with nestling age, so tarsus length was age-corrected by taking the residuals from a regression of tarsus length on age and age squared. This allowed us to include both tarsus length and age in the models describing variation in TL. Thus, age-standardized tarsus length, fledgling age at sampling (in number of days), hatch day (ordinal date mean centered across years), population identity (categorical: Hestmannøy or Træna), and sex (categorical: male or female) were included as fixed effects in all models. We tested whether the effect of inbreeding on TL varied between sexes and populations by including two-way interaction terms between the inbreeding coefficient and sex or population identity. Random intercepts were fitted for year and brood identity to account for the non-independence of nestlings from the same year and brood. This also controls for within-brood effects of inbreeding levels (Olsson et al., 2018). We then tested whether the inclusion of the inbreeding coefficient and interaction terms improved the baseline model (without the inbreeding coefficient) by comparing the resulting 5 candidate models using Akaike's information criterion corrected for small sample sizes (*AICc*, Akaike, 1973; Hurvich & Tsai, 1989). Akaike weights (*w*) and evidence ratios (*ER*) were calculated to determine the relative fit of models to the data (Burnham & Anderson, 2002). To investigate heterosis effects on TL, we tested if the slopes of the regression between F_{GRM} and TL differed between individuals that were more inbred than on average ($F_{GRM} >$ mean F_{GRM}) and individuals that were less inbred than average ($F_{GRM} <$ mean F_{GRM}). We did this by testing if the inclusion of a regression break point at the mean F_{GRM} improved the models by comparing the resulting 9 candidate models using AICc. Models were validated visually using diagnostic plots of residuals, and model parameters are from models refitted with restricted maximum likelihood (REML). Estimates are reported with standard errors (SE) and 95% confidence intervals (CI). Regression lines were visualized using *ggplot2* (Wickham, 2016).

RESULTS

Individual MS pedigree-based inbreeding coefficients (F_{PED}) varied from 0.000-0.250 (mean 0.007, 16.9% non-zero values). The highest ranked model explaining variation in TL included a negative effect of F_{PED}, but only slightly improved the fit of the baseline model ($\Delta_{2:1}AICc=0.8$ [subscripts denote which ranked models are compared], $w_1=0.36$, $ER_1=w_1/w_2=1.49$, Table S1 in the supporting information). Thus, there was a tendency for TL to be shorter in more inbred sparrows ($\beta_{F\_PED}=-0.169\pm0.101$, CI=[-0.366, 0.028], $n=1195$, Fig. 1a and Table 2). The model ranked third ($\Delta_{3:1}AICc=1.3$) indicated that TL was less associated with F_{PED} in males than in females ($\beta_{F\_PED*sex[female]}=-0.167\pm0.196$, CI=[-0.549, 0.216]), while the model ranked fourth ($\Delta_{4}AICc=1.9$) indicated that TL was less associated with F_{PED} in the Hestmannøy population than in the Træna population ($\beta_{F\_PED*island[Hestmannøy]}=0.115\pm0.314$, CI=[-0.498, 0.728]). However, due to high uncertainty in these parameter estimates, these effects are not deemed reliable.

When only including individuals with at least 2 full ancestral generations known (33.8% non-zero values), the model with F_{PED} was ranked second ($\Delta_{2:1}AICc=1.1$, $\beta_{F\_PED}=-0.205\pm0.198$, CI=[-0.588, 0.189], $n=320$, Fig. 1b, Table S2-3) and the baseline model was highest ranked.

There was a tendency for the negative effect of F_{PED} on TL to be weaker in first-year survivors ($n=206$, mean TL=0.95±0.02, mean $F_{PED}=0.010\pm0.003$) than in non-survivors ($n=989$, mean TL=0.97±0.01, mean $F_{PED}=0.007\pm0.001$, $\beta_{F\_PED*first\text{-}year\ survival}=0.304\pm0.201$, CI=[-0.089, 0.697], $n=1195$, Fig. 1c, Table S4). This effect was uncertain with a CI overlapping zero. This suggests that the following analyses using genomic estimators of inbreeding in recruits were not biased towards stronger inbreeding effects in recruits.

Genomic inbreeding coefficient (F_{GRM}) estimates varied from -0.200 to 0.300 (mean 0.016). The highest ranked model ($\Delta_{2:1}AICc=2.1$, Table S5) showed that TL was shorter in more inbred sparrows ($\beta_{F\_GRM}=-1.517\pm0.293$, CI=[-2.150, -0.920], $n=371$, Fig. 1d, and Table 3). In addition, the effect of F_{GRM} on TL was stronger in the Træna population

($\beta_{F\_GRM*island[Hestmannøy]}$=0.824±0.339, CI=[0.142, 1.529], Table 3) and in males ($\beta_{F\_GRM*sex[female]}$=0.644±0.314, CI=[0.034, 1.262], Table 3).

Including a break point at the mean F_{GRM} improved the model compared to a model with no break point (comparing models without interaction terms which were ranked 8 and 5: $\Delta_{8:5}AICc$=4.5, see Table S6). The highest ranked model ($\Delta_{2:1}AICc$=3.1, Table S6) revealed a strong negative association between TL and F_{GRM} among individuals with F_{GRM}<0.016 but no significant association among inbred individuals with F_{GRM}>0.016 (Fig. 1e and Table 4). This indicates that a heterosis effect resulting in longer telomeres in outbred individuals may explain the negative association found between inbreeding and TL. This model also included an interaction term suggesting that this heterosis effect was stronger in the Træna population (Table 4).

The runs-of-homozygosity inbreeding coefficient (F_{ROH}) estimates varied from 0.000-0.240 (mean 0.010, 73% non-zero values). The best model provided evidence for a negative effect of F_{ROH} on TL (β_{F_ROH}=-1.148±0.512, CI=[-2.144, -0.153], n=371, Fig. 1f, Table S7 and 5). This model also indicated that the negative effect of F_{ROH} tended to be stronger in males ($\beta_{F\_ROH*sex\ [female]}$=0.915±0.610, CI=[-0.270, 2.102]).

Overall, F_{PED} was not a good predictor of genomic estimators of inbreeding (Fig. S1a,c; Pearson's r_P=0.05, n=371), but its relationships with F_{GRM} and F_{ROH} were improved when including only individuals with at least two generations known (Fig. S1b,d; r_P>0.30, n=59). F_{GRM} and F_{ROH} were strongly correlated (Fig. S1e,f; r_P=0.7, n=371).

DISCUSSION

We found evidence across multiple complementary measures of inbreeding that more inbred house sparrow nestlings had shorter telomeres (Fig. 1). Individual differences in TL are established early in life (Entringer, de Punder, Buss, & Wadhwa, 2018), are heritable (Dugdale

& Richardson, 2018; Pepke et al., 2021a), and are positively associated with fitness in some species (Heidinger et al., 2012; Wilbourn et al., 2018). Thus, short telomeres in more inbred individuals may therefore underpin a physiological basis of inbreeding depression in fitness components that has been found in this species (Billing et al., 2012; Jensen et al., 2007; Niskanen et al., 2020) and in other wild animal populations (Keller & Waller, 2002).

The effect of inbreeding on TL in house sparrows was negative across all measures of inbreeding, but strongest when using genomic levels of inbreeding (Fig. 1d-f), probably because they are better at capturing homozygosity causing inbreeding depression compared to using a pedigree-based estimator (Fig. 1a-c, Alemu et al., 2021; Huisman, Kruuk, Ellis, Clutton-Brock, & Pemberton, 2016; Kardos et al., 2016). Mating between full siblings or between parent and offspring (F=0.25) resulted in a severe reduction in (relative) TL of 58% (F_{GRM}), 48% (F_{ROH}) or 11% (F_{PED}) compared to breeding between unrelated individuals (Tables 2, 3, and 5). TL may be under strong selection in natural populations (Voillemot et al., 2012). Consequently, strong inbreeding depression is expected for fitness components or traits that are under strong selection (Bérénos, Ellis, Pilkington, & Pemberton, 2016; DeRose & Roff, 1999), The analyses using genomic estimators of inbreeding were limited to recruited individuals, but the negative effect of inbreeding on TL may be even stronger if very inbred individuals, presumably with short telomeres, do not survive their first year and were thus excluded from our analyses (Jensen et al., 2007; Wilbourn et al., 2018). There was a tendency for such an effect when using pedigree-based levels of inbreeding (Fig. 1c and Table S4). We also found some evidence that inbreeding had stronger negative effects on TL in males than females (Tables 3 and 5). Such sex-specific effects of inbreeding are known from other species (de Boer et al., 2018a; de Boer et al., 2018b; Janicke, Vellnow, Sarda, & David, 2013), but have rarely been observed early in life. There was a weak tendency for longer TL in males than females (Tables 2-5), which has been observed in similar house sparrow populations (Pepke et al., 2021b). Thus, males may be better buffered against the effects of inbreeding on TL. However, no sex-specific differences in inbreeding depression were observed in adult sparrows across this study metapopulation (Niskanen et al., 2020).

Increased inbreeding may be accompanied by population decline in small populations (Bozzuto et al., 2019; Chen, Cosgrove, Bowman, Fitzpatrick, & Clark, 2016; Feng et al., 2019), which can drive populations to extinction (O'Grady et al., 2006; Saccheri et al., 1998; Wright, Tregenza, & Hosken, 2007). Niskanen et al. (2020) showed that inbreeding depression in adult sparrows in our study system varied little across years or across the different island environments inhabited by these house sparrows. Hence, the strength of inbreeding depression is similar between populations, but due to harboring more inbred individuals, the relative effect is stronger in smaller populations (Niskanen et al., 2020). Small declining populations may be characterized by gradual population-wide and trans-generational telomere erosion. For instance, Dupoué et al. (2017) observed shorter TL along an extinction risk gradient in populations of common lizards (*Zootoca vivipara*) that are disappearing from low altitudes at their southern range limit, presumably due to climate warming (Sinervo et al., 2010). Combined, these results suggest that TL may represent a potential physiological biomarker or molecular tool in conservation genetics addressing the viability of some small animal populations (Bebbington et al., 2016; Bergman et al., 2019; Dupoué et al., 2017; Madliger, Franklin, Love, & Cooke, 2020).

The negative effect of F_{GRM} on TL (Fig. 1d) was stronger among individuals that were less related than the average population (Fig. 1e). This suggests that longer telomeres in outbred individuals may partly be attributed to a general heterosis effect (Charlesworth & Willis, 2009) involving mating between immigrants and native individuals (Dickel et al., 2021; Ebert et al., 2002). In our study metapopulation, the proportion of dispersers among recruits can be high among the island populations (0.2 on average ranging from 0.0-1.0 across years and islands, Ranke et al., 2021; Saatoglu et al., 2021), and hence most islands are not strongly differentiated (Niskanen et al., 2020). We found that the negative effect of F_{GRM} on TL was stronger in the Træna population (Table 3-4). Træna is known to have a higher proportion of immigrants than Hestmannøy (Ranke et al., 2021), which may contribute to a stronger effect of heterosis in this population (Table 4). Furthermore, the gardens of Træna expose the sparrows to a different environment than the farms on Hestmannøy (Araya-Ajoy et al., 2019; Pärn, Ringsby, Jensen, & Sæther, 2012). Inbreeding depression is expected to have more severe consequences under environmental stress (Armbruster & Reed, 2005; Reed et al., 2002), such as harsh weather or competition (de Boer et al., 2018a; Fox & Reed, 2011; Marr, Arcese, Hochachka, Reid, & Keller, 2006). Telomeres shorten due to environmental stressors such as harsh abiotic conditions (Chatelain et al., 2020). We speculate that environmental differences between the habitats of the two sparrow populations may explain the exacerbated effects of inbreeding on TL in the Træna population. For instance, in juvenile Seychelles warblers a negative relationship between homozygosity and TL was found only in poor seasons, i.e. when food availability was low (Bebbington et al., 2016). In adult Seychelles warblers, the effect of homozygosity on TL was consistently negative across seasons, suggesting that the negative effects of inbreeding accumulate through life and are reflected in telomere erosion (Bebbington et al., 2016). Here, we showed that inbreeding manifests in TL already at the nestling stage in a similar wild passerine.

We measured TL in blood, thus it is possible that inbreeding or heterosis only affected telomeres in erythrocytes (Manning et al., 2002; Olsson, Geraghty, Wapstra, & Wilson, 2020). However, this is unlikely because TLs often correlate well across tissues within the organism (Daniali et al., 2013; Demanelis et al., 2020; Reichert, Criscuolo, Verinaud, Zahn, & Massemin, 2013), especially in early-life (Prowse & Greider, 1995). Although genomic inbreeding estimates were only available for first-year survivors, we may have avoided confounding effects of selective mortality of inbred individuals at much older ages by measuring TL already at the nestling stage (Hemmings, Slate, & Birkhead, 2012; Sánchez-Montes et al., 2020). Furthermore, since the mutation accumulation theory of senescence predicts that deleterious effects of inbreeding increase with age (Charlesworth & Hughes, 1996; Keller, Reid, & Arcese, 2008), we may expect that the effect on TL is persistent and potentially stronger in adult sparrows. Thus, future studies are required to investigate if inbreeding leads to persistently eroded TL throughout life, and if there are combined fitness consequences of any interaction between TL and inbreeding in wild populations. Even in the absence of a mechanism directly linking inbreeding and TL via the effects of oxidative stress (cf. the introduction), we may find inbred individuals to have short telomeres, because inbreeding impairs other physiological processes that affects both fitness and TL (Bebbington et al., 2016). Thus, the conflicting evidence in the literature of an effect of inbreeding on TL (reviewed in the introduction) suggests that an experimental procedure is needed to further elucidate the mechanisms underlying the correlation reported here (Manning et al., 2002), especially in wild populations.

In conclusion, the negative associations between inbreeding levels and TL found in this study suggest that TL may reveal subtle somatic costs of inbreeding in wild populations, and thereby demonstrates a potential route by which inbreeding negatively impacts the physiological state of an organism in early life. The observation of a potential heterosis effect on TL suggests that maintenance of dispersal within this metapopulation is important for mitigating the negative effects of inbreeding.

DECLARATIONS

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Code availability: Not applicable.

Authors' contributions: MLP measured telomeres, analyzed data, and wrote the manuscript with contributions from all authors. WB supervised telomere measurements. HJ, AKN, and TK contributed to the genotype data processing, pedigree construction, and in designing statistical analyses. THR, BE-S, and HJ initiated the study system. THR, HJ, and TK contributed to the fieldwork.

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TABLES AND FIGURES

Table 1: Number of nestling house sparrows of each sex and in total with early-life telomere length and inbreeding coefficient measurements within each population (Hestmannøy and Træna) for each measure of inbreeding (microsatellite pedigree-based inbreeding coefficient [F_{PED}], genomic inbreeding coefficient [F_{GRM}], and runs-of-homozygosity [F_{ROH}]). Number of individuals with at least two known full ancestral generations (gen.) are shown. Number of individuals with F_{GRM} values above and below the mean F_{GRM}, which is used as a break point to differentiate individuals that were more and less inbred than average, respectively, are also shown.

Island population:	**Hestmannøy**			**Træna**			
	Males	**Females**	**Sum:**	**Males**	**Females**	**Sum:**	**Sum:**
F_{PED} (≥1.5 gen.)	511	546	1057	78	60	138	1195
F_{PED} (≥2 full gen.)	148	165	313	4	3	7	320
F_{GRM}	140	135	275	49	47	96	371
$F_{GRM} > 0.016$	43	63	106	26	32	58	164
$F_{GRM} < 0.016$	97	72	169	23	15	38	207
F_{ROH}	140	135	275	49	47	96	371

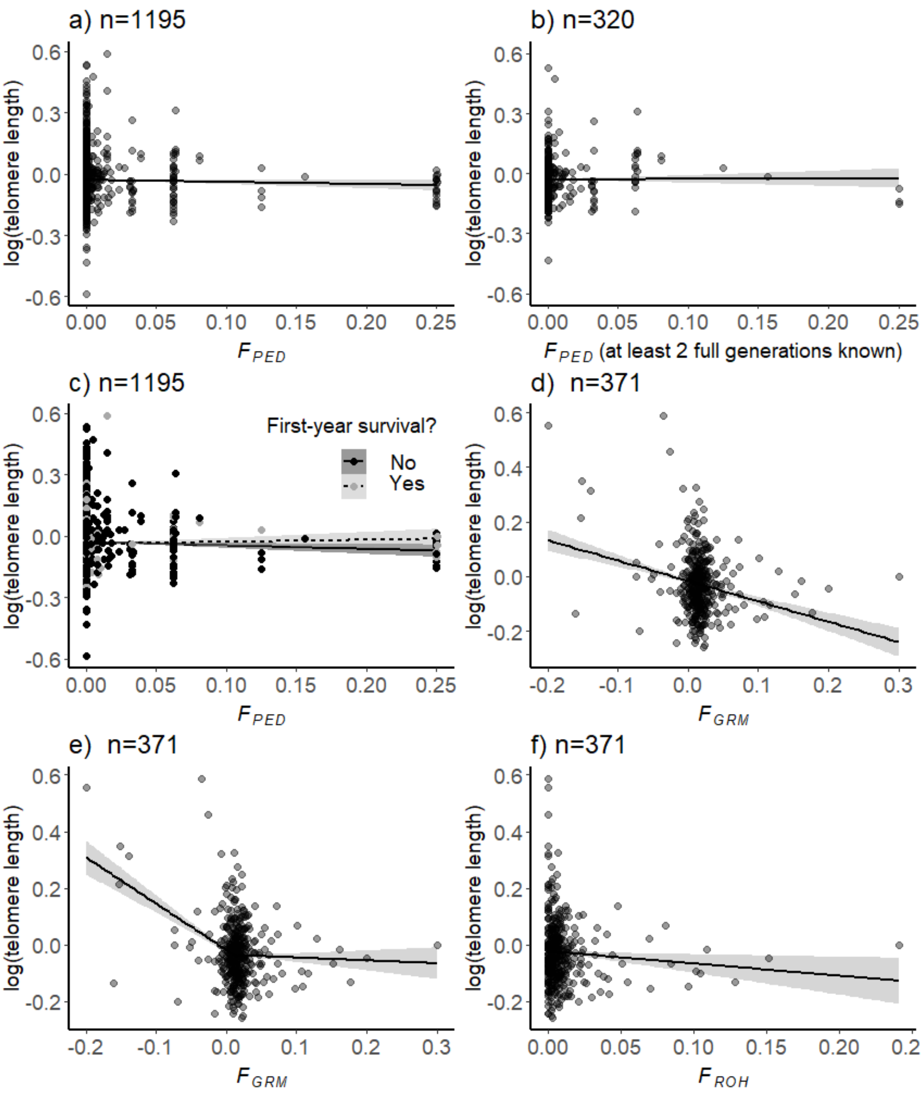

Fig. 1: Associations between early-life telomere length ($\log_{10}$-transformed) and various individual measures of inbreeding in wild house sparrows: a) microsatellite pedigree-based inbreeding coefficient (F_{PED}), b) F_{PED} for individuals with at least two full ancestral generations known, c) testing for an interaction effect between F_{PED} and first-year survival (survivors: *n*=206 in grey, dotted regression line; non-survivors: *n*=989 in black, solid regression line), d) genomic inbreeding coefficient F_{GRM}, e) regression with a break point at the mean F_{GRM} (0.016), and f) runs-of-homozygosity F_{ROH}. Black lines show the predicted effect of the inbreeding coefficient on TL from LMMs described in the text and the grey area shows 95% confidence intervals. Note that the y-axis is not scaled equally across panels and that color of points are graduated for visibility.

Table 2: Estimates, standard errors (SE), lower and upper 95% confidence intervals (CI) from the highest ranked model of F_{PED} predicting variation in early-life TL (*n*=1195, see Table S2 and Fig. 1a). The model included random intercepts for brood identity (ID) and year. Estimates with CIs not overlapping 0 are shown in italics.

Response variable: $\log_{10}$(TL)	***Estimate***	**SE**	**Lower CI**	**Upper CI**
intercept	-3.1E-4	0.037	-0.072	0.071
inbreeding coefficient (F_{PED})	-0.169	0.101	-0.366	0.028
tarsus length	-0.003	0.002	-0.008	0.001
sex [female]	-0.006	0.006	-0.017	0.005
island identity [Hestmannøy]	*0.025*	*0.012*	*0.001*	*0.049*
age	-0.003	0.002	-0.007	0.001
hatch day	-1.4E-4	1.5E-4	-4.4E-4	1.7E-4
$\sigma^2_{\text{brood ID}}$ (*n*=500)	0.002		0.001	0.003
σ^2_{year} (*n*=20)	0.003		0.001	0.006

Marginal R^2 / Conditional R^2: 0.014 / 0.393

Table 3: Estimates, standard errors (SE), lower and upper 95% confidence intervals (CI) from the highest ranked model of F_{GRM} predicting variation in early-life TL (*n*=371, see Table S6 and Fig. 1d).

Response variable: $\log_{10}$(TL)	***Estimate***	**SE**	**Lower CI**	**Upper CI**
intercept	0.069	0.038	-0.004	0.145
inbreeding coefficient (F_{GRM})	*-1.517*	*0.293*	*-2.150*	*-0.920*
tarsus length	-0.001	0.005	-0.011	0.009
sex [female]	-0.016	0.011	-0.039	0.006
island identity [Hestmannøy]	*-0.036*	*0.016*	*-0.068*	*-0.004*
age	-0.006	0.003	-0.012	4.6E-4
hatch day	-3.3E-4	2.8E-4	-0.001	2.1E-4
*F_{GRM} * island [Hestmannøy]*	*0.824*	*0.339*	*0.142*	*1.529*
*F_{GRM} * sex [female]*	*0.644*	*0.314*	*0.034*	*1.262*
$\sigma^2_{\text{brood ID}}$ (*n*=273)	0.004		0.002	0.006
σ^2_{year} (*n*=17)	0.001		0.000	0.003

Marginal R^2 / Conditional R^2: 0.085 / 0.512

Table 4: Estimates, standard errors (SE), lower and upper 95% confidence intervals (CI) from the highest ranked model from Table S7 including a break point at F_{GRM}=0.016 (n=371, see also Table S1). These effects of F_{GRM} are shown in Fig. 1e.

Response variable: $\log_{10}$(TL)	**Estimate**	**SE**	**Lower CI**	**Upper CI**
intercept	0.021	0.037	-0.051	0.095
inbreeding coefficient (F_{GRM})<0.016	*-2.177*	*0.372*	*-3.051*	*-1.379*
inbreeding coefficient (F_{GRM})>0.016	0.189	0.498	-0.780	1.153
tarsus length	-0.001	0.005	-0.011	0.008
sex [female]	-0.006	0.010	-0.027	0.014
island identity [Hestmannøy]	-0.009	0.016	-0.041	0.024
age	-0.005	0.003	-0.011	0.001
hatch day	-3.7E-4	2.7E-4	-0.001	1.5E-4
*F_{GRM}<0.016 * island [Hestmannøy]*	*1.562*	*0.465*	*0.610*	*2.576*
F_{GRM}>0.016 * island [Hestmannøy]	-0.026	0.561	-1.114	1.061
$\sigma^2_{\text{brood ID}}$ (n=273)	0.003		0.001	0.005
σ^2_{year} (n=17)	0.001		0.000	0.003

Marginal R^2 / Conditional R^2: 0.106 / 0.458

Table 5: Estimates, standard errors (SE), lower and upper 95% confidence intervals (CI) from the highest ranked model from of F_{ROH} predicting variation in early-life TL (n=371, see Table S8 and Fig. 1f).

Response variable: $\log_{10}$(TL)	***Estimate***	**SE**	**Lower CI**	**Upper CI**
intercept	0.051	0.040	-0.027	0.130
inbreeding coefficient (F_{ROH})	*-1.148*	*0.512*	*-2.144*	*-0.153*
tarsus length	-0.001	0.005	-0.011	0.010
sex [female]	-0.018	0.012	-0.041	0.005
island identity [Hestmannøy]	-0.020	0.016	-0.052	0.012
age	-0.005	0.003	-0.012	0.001
hatch day	-2.9E-4	3.0E-4	-0.001	2.9E-4
F_{ROH} * sex [female]	0.915	0.610	-0.270	2.102
$\sigma^2_{\text{brood ID}}$ (n=273)	0.006		0.004	0.008
σ^2_{year} (n=17)	0.002		4.6E-4	0.004

Marginal R^2 / Conditional R^2: 0.029 / 0.579

Supporting information

Inbreeding is associated with shorter early-life telomere length in a wild passerine

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Contents

AICc tables and model results of effects of F_{PED} on telomere length

Table S1: Linear mixed effects models (LMMs) of variation in early-life telomere length (*n*=1195) in two island populations of house sparrows. All models included random intercepts for brood identity and year. All models below are ranked by AICc, and number of degrees of freedom (df) and model weights (w) are shown.

Model	**ΔAICc**	**df**	**w**
1 log(TL) = tarsus + sex + island + age + hatch day + F_{PED}	0.0	10	0.36
2 log(TL) = tarsus + sex + island + age + hatch day	0.8	9	0.24
3 log(TL) = tarsus + sex + island + age + hatch day + F_{PED} + F_{PED}*sex	1.3	11	0.19
4 log(TL) = tarsus + sex + island + age + hatch day + F_{PED} + F_{PED}*island	1.9	11	0.14
5 log(TL) = tarsus + sex + island + age + hatch day + F_{PED} + F_{PED}*sex + F_{PED}*island	3.2	12	0.07

Table S2: LMMs of variation in early-life telomere length (*n*=320 limited to individuals with at least 2 full ancestral generations known).

Model	**ΔAICc**	**df**	**w**
1 log(TL) = tarsus + sex + island + age + hatch day	0.0	9	0.47
2 log(TL) = tarsus + sex + island + age + hatch day + F_{PED}	1.1	10	0.27
3 log(TL) = tarsus + sex + island + age + hatch day + F_{PED} + F_{PED} * island	2.8	11	0.12
4 log(TL) = tarsus + sex + island + age + hatch day + F_{PED} + F_{PED} * sex	3.1	11	0.10
5 log(TL) = tarsus + sex + island + age + hatch day + F_{PED} + F_{PED} * sex + F_{PED}*island	4.8	12	0.04

Table S3: Estimates, standard errors (SE), lower and upper 95% confidence intervals (CI) from the second highest ranked model in Table S2 ($\Delta_2 AICc$=1.1, n=320), which included the effect of F_{PED}. The model included random intercepts for brood identity (ID) and year. The effect of F_{PED} is shown in Fig. 1b.

Response variable: $\log_{10}$(TL)	**Estimate**	**SE**	**Lower CI**	**Upper CI**
intercept	0.020	0.072	-0.119	0.161
inbreeding coefficient (F_{PED})	-0.205	0.198	-0.588	0.189
tarsus length	*-0.008*	*0.004*	*-0.016*	*-0.001*
sex [female]	*-0.024*	*0.011*	*-0.045*	*-0.004*
island identity [Hestmannøy]	0.025	0.045	-0.063	0.112
age	*-0.010*	*0.003*	*-0.016*	*-0.003*
hatch day	-1.7E-4	3.0E-4	-4.2E-4	0.001
$\sigma^2_{\text{brood ID}}$ (n=147)	0.002		0.001	0.004
σ^2_{year} (n=20)	0.001		0.5E-4	0.003

Marginal R^2 / Conditional R^2: 0.069 / 0.363

Table S4: Estimates, standard errors (SE), lower and upper 95% confidence intervals (CI) from a model including an interaction term between F_{PED} and first-year survival (n=1195). The model included random intercepts for brood identity (ID) and year. The effect of F_{PED} is shown in Fig. 1c.

Response variable: $\log_{10}$(TL)	**Estimate**	**SE**	**Lower CI**	**Upper CI**
intercept	-0.003	0.037	-0.074	0.069
inbreeding coefficient (F_{PED})	*-0.251*	*0.114*	*-0.474*	*-0.028*
tarsus length	-0.003	0.002	-0.008	0.001
sex [female]	-0.006	0.006	-0.017	0.005
island identity [Hestmannøy]	*0.026*	*0.012*	*0.002*	*0.049*
age	-0.003	0.002	-0.007	0.001
hatch day	-1.3E-4	1.6E-4	-4.3E-4	1.7E-4
first-year survival [1]	-0.002	0.008	-0.017	0.014
F_{PED} * first-year survival [1]	0.304	0.201	-0.089	0.697
$\sigma^2_{\text{brood ID}}$ (n=500)	0.002		0.001	0.003
σ^2_{year} (n=20)	0.003		0.001	0.006

Marginal R^2 / Conditional R^2: 0.015 / 0.395

AICc tables and model results of effects of F_{GRM} on telomere length

Table S5: LMMs of variation in early-life telomere length predicted by F_{GRM} (*n*=371). All models included random intercepts for brood identity and year.

Model		ΔAICc	df	w
1	log(TL) = tarsus + sex + island + age + hatch day + F_{GRM} + F_{GRM} * sex + F_{GRM} * island	0	12	0.65
2	log(TL) = tarsus + sex + island + age + hatch day + F_{GRM} + F_{GRM} * island	2.1	11	0.22
3	log(TL) = tarsus + sex + island + age + hatch day + F_{GRM} + F_{GRM} * sex	3.4	11	0.12
4	log(TL) = tarsus + sex + island + age + hatch day + F_{GRM}	7.5	10	0.06
5	log(TL) = tarsus + sex + island + age + hatch day	23	9	0.00

Table S6: LMMs of variation in early-life telomere length predicted by F_{GRM} (*n*=371) with or without a break point at the mean F_{GRM}=0.016. All models included random intercepts for brood identity and year.

Model		ΔAICc	df	w
1	log(TL) = tarsus + sex + island + age + hatch day + F_{GRM}<0.016 + F_{GRM}>0.016 + F_{GRM}<0.016 * island + F_{GRM}>0.016 * island	0	13	0.62
2	log(TL) = tarsus + sex + island + age + hatch day + F_{GRM}<0.016 + F_{GRM}>0.016 + F_{GRM}<0.016 * island + F_{GRM}>0.016 * island + F_{GRM}<0.016 * sex + F_{GRM}>0.016 * sex	3.1	15	0.13
3	log(TL) = tarsus + sex + island + age + hatch day + F_{GRM} + F_{GRM} * sex + F_{GRM} * island	3.1	12	0.13
4	log(TL) = tarsus + sex + island + age + hatch day + F_{GRM} + F_{GRM} * island	5.3	11	0.05
5	log(TL) = tarsus + sex + island + age + hatch day + F_{GRM}<0.016 + F_{GRM}>0.016	6.1	11	0.03
6	log(TL) = tarsus + sex + island + age + hatch day + F_{GRM} + F_{GRM} * sex	6.6	11	0.02
7	log(TL) = tarsus + sex + island + age + hatch day + F_{GRM}<0.016 + F_{GRM}>0.016 + F_{GRM}<0.016 * sex + F_{GRM}>0.016 * sex	8.9	13	0.01
8	log(TL) = tarsus + sex + island + age + hatch day + F_{GRM}	10.6	10	0.00
9	log(TL) = tarsus + sex + island + age + hatch day	26.1	9	<0.001

AICc tables and model results of effects of F_{ROH} on telomere length

Table S7: LMMs of variation in early-life telomere length predicted by F_{ROH} (*n*=371). All models included random intercepts for brood identity and year.

	Model	ΔAICc	df	w
1	log(TL) = tarsus + sex + island + age + hatch day + F_{ROH} + F_{ROH} * sex	0	11	0.31
2	log(TL) = tarsus + sex + island + age + hatch day + F_{ROH}	0.2	10	0.29
3	log(TL) = tarsus + sex + island + age + hatch day	1.3	9	0.16
4	log(TL) = tarsus + sex + island + age + hatch day + F_{ROH} + F_{ROH} * sex + F_{ROH} * island	1.8	12	0.12
5	log(TL) = tarsus + sex + island + age + hatch day + F_{ROH} + F_{ROH} * island	2.1	11	0.11

Correlations between different measures of inbreeding

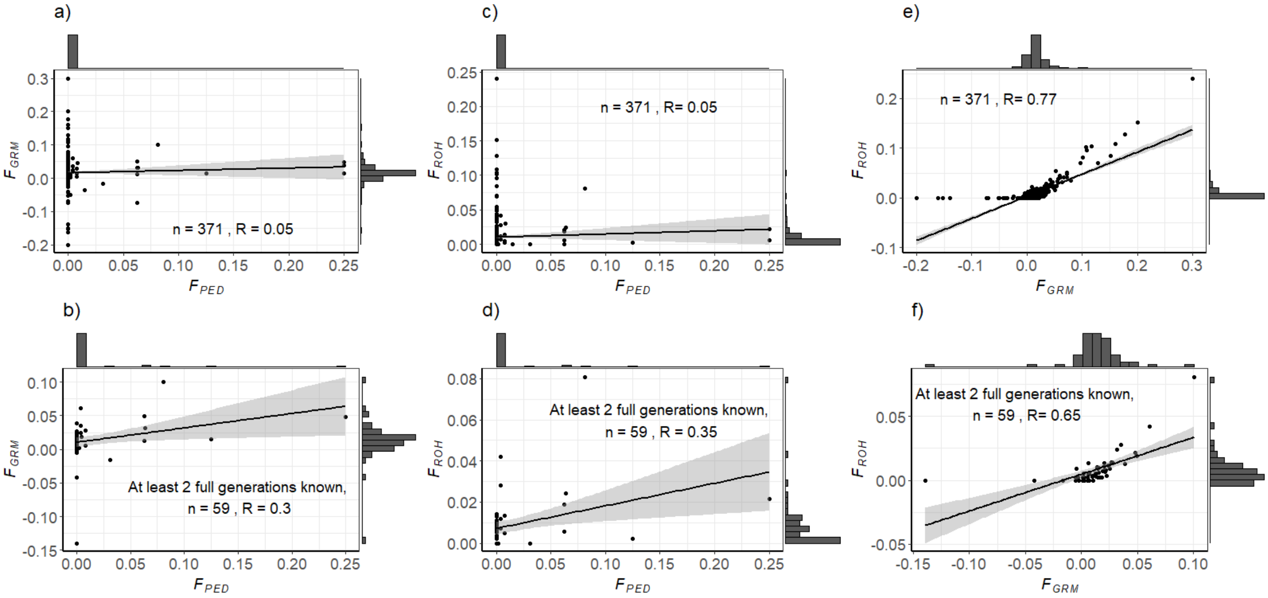

Figure S1: Sample sizes (n) and Pearson's correlation coefficients (R) between different estimators of inbreeding. Lower panels show the same correlations restricted to individuals with at least two full ancestral generations known. Black lines are linear regression lines with 95% confidence intervals shown in grey.

PAPER IV

Causes and consequences of variation in early-life telomere length in a bird metapopulation

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Key words: demography, early-life, individual heterogeneity, pace-of-life, stress, telomere dynamics

ABSTRACT

1. Environmental conditions during early-life development can have lasting effects on individual quality and fitness. Telomere length (TL) may correlate with early-life conditions and may be an important mediator or biomarker of individual quality or pace-of-life, as periods of increased energy demands can increase telomere attrition due to oxidative stress. Thus, knowledge of the mechanisms that generate variation in TL, and the relation between TL and fitness, is important in understanding the role of telomeres in ecology and life-history evolution.

2. Here, we investigate how environmental conditions and morphological traits are associated with early-life TL and if TL predicts natal dispersal probability or components of fitness in two populations of wild house sparrows (*Passer domesticus*).
3. We measured morphological traits and blood TL in 2746 nestlings from 20 cohorts (1994-2013) and retrieved data on weather conditions. We monitored population fluctuations, and individual survival and reproductive output using field observations and genetic pedigrees. We then used generalized linear mixed-effects models to test which factors affected TL in early-life, and if TL predicted dispersal propensity, or was associated with recruitment probability, mortality risk, or reproductive success.
4. We found a negative effect of population density on TL, but only in one of the populations. There was a curvilinear association between TL and the maximum daily North Atlantic Oscillation (NAO) index during incubation, suggesting that there are optimal weather conditions that result in the longest TL. Dispersers tended to have shorter telomeres than non-dispersers. TL did not predict survival, but we found a tendency for individuals with short telomeres to have higher annual reproductive success.
5. Our study showed how early-life TL is shaped by effects of growth, weather conditions and population density, supporting that environmental stressors negatively affect TL in wild populations. In addition, TL may be a mediator or biomarker of individual pace-of-life, with higher dispersal rates and annual reproduction tending to be associated with shorter early-life TL in this study. However, clear associations between early-life TL and individual fitness seems difficult to establish and may differ between different populations in the wild.

INTRODUCTION

Telomeres are short repetitive nucleotide sequences capping the ends of linear chromosomes (Blackburn & Szostak, 1984). Recent studies have shown that individual variation in telomere dynamics might play an important role shaping the life-history of many species, including wild birds (Eastwood et al., 2019; Spurgin et al., 2018; Vedder et al., 2021), reptiles (Olsson et al., 2018a), mammals (Foley et al., 2020; van Lieshout et al., 2019) and fish (McLennan et al., 2016). Telomeres shorten during growth due to cell divisions and oxidative damage (Jennings et al., 1999; von Zglinicki, 2002). Individual differences in telomere length (TL) are established early in life (Entringer et al., 2018; Martens et al., 2021) and may reflect cumulative effects of physiological stress incurred during early life (Chatelain et al., 2020; Nettle et al., 2017; Ridout et al., 2018).

From an eco-evolutionary perspective, individual telomere dynamics are interesting because they have been shown to be associated with survival and reproductive success in some free-living animal populations (Chatelain et al., 2020; Fairlie et al., 2016; Froy et al., 2021; Haussmann et al., 2005; Heidinger et al., 2021; Olsson et al., 2018b; Sudyka, 2019). Furthermore, TL has been shown to predict individual health, quality, or lifespan within several species (Asghar et al., 2015; Eastwood et al., 2019; Fairlie et al., 2016; Heidinger et al., 2012; van Lieshout et al., 2019; Wilbourn et al., 2018). Long telomeres are expected to infer better immune competency (Blackburn et al., 2015), resistance to oxidative damage and metabolic aging (Muñoz-Lorente et al., 2019) and hence higher survival probability (Wilbourn et al., 2018). Covariation between TL dynamics and fitness therefore suggests that TL could act as mediator of the life-history trade-offs between growth, survival, and reproduction (Heidinger et al., 2021; Monaghan, 2014; Monaghan & Haussmann, 2006). Alternatively, TL may be a transient, environmentally pliant trait reflecting experienced stress (i.e. a biomarker, Bateson & Poirier, 2019; Boonekamp et al., 2013), but with few direct fitness consequences.

Whether telomere dynamics underpin constraints in individual variation in life-history strategies remains debated (Monaghan, 2010; Vedder et al., 2017). Giraudeau et al. (2019a) speculated that TL could act as an important physiological mediator of the individual variation in suites of life-history traits (pace-of-life syndromes, e.g. Reale et al., 2010) within species. It has also been suggested that telomere dynamics may underlie behavioral patterns or individual animal personalities (Adriaenssens et al., 2016; Bateson & Nettle, 2018; Espigares et al., 2021). However, studies have yet to identify the mechanisms underlying TL dynamics in natural populations and the potential of using TL as a biomarker of physiological costs of individual experiences, or somatic redundancy, in the wild (Bateson & Poirier, 2019; Boonekamp et al., 2013; Pepke et al., 2021c). To understand the ecological and evolutionary significance of TL it is therefore important to identify causes and consequences of individual variation in TL.

Several environmental stressors may induce oxidative stress-mediated effects on TL, in particular harsh abiotic conditions, poor nutrition, or pathogen infection has been identified (Chatelain et al., 2020; Pepper et al., 2018). Harsh weather conditions are expected to negatively affect TL through an increased stress response elevating glucocorticoid hormone concentrations (Lemaître et al., 2021; Quirici et al., 2016) and metabolic rate resulting in increased production of reactive oxygen species (ROS, e.g. Casagrande et al., 2020; Metcalfe & Olsson, 2021). Weather conditions may thus have direct effects on TL, e.g. through thermoregulation and metabolic activity (Angelier et al., 2018), or indirect effects, e.g. changes in food availability (Criscuolo et al., 2020; Spurgin et al., 2018) or pathogen prevalence (Asghar et al., 2015; Giraudeau et al., 2019b). Depending on the species-specific optima and the range of weather conditions experienced there could be linear or non-linear associations between environmental conditions and TL (Axelsson et al., 2020).

Local demography such as population density may influence the competitive regimes experienced by parents during breeding (Dhondt, 2010). In populations of house sparrows

(*Passer domesticus*), density regulation affected recruit production, which generated variation in pace of life-history strategies across populations (Araya-Ajoy et al., 2021). However, physiological mechanisms mediating such demographic and evolutionary processes remain largely unknown (e.g. Edwards et al., 2021). Changes in TL dynamics may underpin physiological stress responses to changes in demography (Bergman et al., 2019; Gangoso et al., 2021). For instance, Spurgin et al. (2018) found weak evidence for a negative effect of population density on early-life TL and telomere attrition in an island population of Seychelles warblers (*Acrocephalus sechellensis*). They also found that TL was positively associated with abundance of insects, the main food resource for the warblers, indicating that increased food availability may have masked negative effects of increased density on TL (Brown et al., 2021).

Short telomeres may predispose individuals to opt for a faster pace-of-life (Giraudeau et al., 2019a), which could involve an increased probability of dispersal (Cote et al., 2010; Dingemanse et al., 2020). Alternatively, TL may act as a cue that tracks features of the surroundings, such as changes in the environment (e.g. weather conditions and food availability) or demographic changes (e.g. population density) that could influence dispersal. Thus, individuals with long telomeres may be less likely to disperse, if long telomeres are an internal state indicator of an overall benign habitat (mild weather conditions, abundant food resources, or low competition, Wilbourn et al., 2017), which may not induce emigration (Lin & Batzli, 2001). However, if dispersal is condition-dependent (Ims & Hjermann, 2001), and the telomere–survival relationship is causal (Wilbourn et al., 2018) even in early life (Monaghan & Ozanne, 2018), short telomeres may have physiological consequences that prevent dispersal, rendering individuals with long telomeres more likely to become successful dispersers. However, little is known about the physiological mechanisms that could mediate suites of traits associated with dispersal (Clobert et al., 2012). Investigating spatiotemporal variation in traits such as TL that may be involved in producing individual variation in life-history traits therefore seems to be fundamental to a proper understanding of population ecology and life-history evolution.

In this study, we investigate causes and consequences of spatiotemporal variation in early-life TL across two decades in two populations of wild house sparrows located within a large island metapopulation study system (see Fig. S1.1 in Appendix S1 in the Supporting Information). The two populations in our study occupy contrasting habitats: One farm-living population with access to shelter and food throughout the year, and one garden-living population that may be more exposed to weather conditions (Pärn et al., 2009). We have previously showed that there is a low heritability of early-life TL (h^2=0.04) in this metapopulation, and that individual variation in TL is mainly driven by environmental (among year) variance resulting in consistent cohort effects in early-life TL (Pepke et al., 2021a). This long-term study allows us to disentangle the effects of weather conditions during pre- and post-natal stages on variation in TL. First, we investigate functional relationships between early-life TL, fledgling body size and condition, local population density fluctuations, weather variables and habitat type. Second, we test if early-life TL is associated with natal dispersal within the metapopulation. We hypothesize that short TL may be associated with exploratory behavior (Adriaenssens et al., 2016) that increases the chance of dispersal (Dingemanse et al., 2003). Finally, we quantify consequences on recruitment probability, mortality risk, and reproductive success of variation in early-life TL and whether these differ between habitat types.

MATERIALS AND METHODS

Study system and field data collection

We monitored two insular house sparrow populations; one on Hestmannøy (66°33'N, 12°50'E, 12.9 km^2) in the years 1994-2020, and one on Træna (i.e. Husøy island, 66°30'N, 12°05'E, 1.5 km^2) from 2004-2020, both located in an archipelago in northern Norway (Fig.

S1.1). These islands are characterized by heathland, mountains, and sparse forest. On Hestmannøy ("farm island"), close to the mainland, cultivated grassland (silage production and grazing) dominates the landscape, and the sparrows live closely associated with humans on dairy farms, where they have access to food (grain) and shelter (barns) all year. Most nests are found in cavities inside sheltered or heated barns. In contrast, on Træna ("non-farm island"), ca. 34 km further out into the sea (Fig. S1.1), there are no farms, but a small village largely consisting of detached houses. Here, the sparrows live in gardens and urban spaces, and nest in artificial nest boxes on the outside walls of the houses. Nests were visited at least every 9th day during the breeding season (May-August) to record hatch day. Fledglings were ringed with a unique color combination at around 10 days old (5-14 days) and tarsometatarsus (tarsus) was measured using calipers to nearest 0.01 mm. Body mass was measured using a Pesola spring balance to nearest 0.1 g. Nestling body condition was then calculated as the residuals of a regression of $\log_{10}$-transformed mass against $\log_{10}$-transformed tarsus length (Schulte-Hostedde et al., 2005). Because tarsus length increases with nestling age, we used the residuals from a regression of tarsus length on age and age squared as a measure of age-corrected (age-standardized) tarsus length (Appendix S1). Birds were observed or captured using mist nets during summer and autumn (May-October). Blood samples (25 μL) were collected by venipuncture and stored in 96% ethanol at room temperature in the field and at -20°C in the laboratory until DNA extraction.

Molecular methods

Molecular sexing and microsatellite pedigree construction for this study was carried out as described in Jensen et al. (2003) and Rønning et al. (2016) and briefly summarized in Appendix S1. Genetic pedigrees were reconstructed for individuals born or captured from 1993-2013. The sampling of nestlings included 1314 males, 1348 females, and 84 individuals of unknown sex (total n=2746). Relative TLs of DNA extracted from whole blood (mainly erythrocytes) were measured on 70-90 % of the nestlings (5-14 days old) ringed each season on Hestmannøy in the years 1994-2013 (*n*=2110, 20 cohorts) and Træna from 2004-2013 (*n*=636, 10 cohorts, Table S1.1 in Appendix S1). Relative TLs (T/S ratios) were measured using the qPCR method as described in Pepke et al. (2021a; 2021b) and validated by Ringsby et al. (2015). Briefly, telomeric DNA was amplified using real-time qPCR and the telomere repeat copy number was estimated relative to an invariant control gene (GAPDH, Atema et al., 2013) and a reference sample (Appendix S1). Data was analyzed using the qBASE software (Hellemans et al., 2007) controlling for inter-run variation. Plate efficiencies were all within 100±10% (see Pepke et al., 2021a).

Factors affecting early-life telomere length

Previous studies have shown TL to be affected by body size or growth (Monaghan & Ozanne, 2018; Pepke et al., 2021b), age (Remot et al., 2021; Salomons et al., 2009), body condition (Barrett et al., 2013; Rollings et al., 2017b), hatch day (Beaulieu et al., 2017), and habitat quality (Angelier et al., 2013; McLennan et al., 2021; Spurgin et al., 2018; Watson et al., 2015; Wilbourn et al., 2017), or that there are sex-differences in TL (Barrett & Richardson, 2011; López-Arrabé et al., 2018). To examine factors that influence individual variation in TL in house sparrow nestlings (response variable, *n*=2456 excluding individuals with missing morphological measurements [*n*=224] and/or missing sex [*n*=84]), we constructed 27 candidate linear mixed effects models (LMMs) with a Gaussian error distribution fitted with maximum likelihood (ML) using the package *lme4* (Bates et al., 2015) in R v. 3.6.3 (R Core Team, 2020). The models were compared using Akaike's information criterion (Akaike, 1973) corrected for small sample sizes (AICc, Hurvich & Tsai, 1989) to identify the models best underpinned by the data. Sex and island identity (Hestmannøy or Træna) were included as fixed effects in all models, including combinations of age (number of days since hatching), age-corrected tarsus length, body condition, hatch day (mean centered ordinal day of the year), population density (spring pre-breeding census in the hatch year mean centered within populations), and an interaction term between population density and island identity. TL was $\log_{10}$-transformed for normalization of residuals. To account for the possible non-independence and temporal heterogeneity in broods and cohorts, random intercepts for brood identity (n=947, nested under hatch year) and hatch year (cohort identity, n=20) were included in all models. Models were validated visually using diagnostic plots and all model parameters are from models refitted with restricted maximum likelihood (REML).

Effects of weather on early-life telomere length

We compiled data on daily mean temperature (K), total daily amount of precipitation (mm) and mean daily atmospheric pressure (hPa) from the nearest weather station at the island of Myken (Fig. S1.1, around 30 km from both populations) from The Norwegian Meteorological Institute (2018). The daily North Atlantic Oscillation (NAO) index was retrieved from the National Oceanic and Atmospheric Administration (2019). The effects of weather conditions on TL were analyzed using a sliding window approach (van de Pol et al., 2016) to determine the best weather predictors within a range of time frames leading up to the TL measurement. TL was measured in nestlings at around 10 days after hatching, which had been preceded by a continuous incubation time of up to 14 days that often begins after laying of the penultimate egg (Anderson, 2006). The approximate time from conception to TL measurement is therefore around 30 days, which was used as the total relative timeframe (days before individual TL measurement date) for relevant weather factors affecting TL. We used the R package *climwin* and its dependencies (Bailey & van de Pol, 2016) to identify the optimal time frame during which TL is most sensitive to weather effect. This approach also allowed identifying the best descriptive weather metric (mean, maximum, minimum or sum across the time frame to reflect cumulative environmental effects on TL) and type of relationship (linear or quadratic) between TL and the weather variable (temperature, precipitation, pressure, and the NAO index). Analyses using minimum daily precipitation were not included since this variable would too often be zero within multiday timeframes, which prevented model convergence. All possible timeframes for each weather metric and relationship were then compared using AICc (van de Pol et al., 2016). As the baseline model (without climate effects) we used the best model of non-weather factors affecting early-life TL (n=2462) identified from the analyses described above. Weather variables are correlated across the study system (Ringsby et al., 2002), but the microclimate may differ between the two structurally different habitats (Hestmannøy and Træna). We therefore also tested models including an interaction term between island identity and the respective weather variable. In total, 60 models were compared using AICc (Table S2.1 in Appendix S2). Hatch year and nested brood identity were included as random intercepts in all models. We tested for over-fitting by randomizing data and re-running the analyses 100 times using the *randwin* and *pvalue* functions provided in *climwin* (Bailey & van de Pol, 2016).

A positive summer NAO is often associated with warmer and drier weather in northwestern Europe (Bladé et al., 2012; Folland et al., 2009). To understand the relationship between the NAO index and local weather conditions (Stenseth et al., 2003), we tested for intercorrelation among all four weather variables (Table S2.2) within the total time frame actually included in the analyses (effectively between April 4th, corresponding to 30 days before the earliest nestling sampling date until the last sampling date of August 19th, from 1994-2013). This showed that a high daily NAO index primarily reflects a high daily amount of precipitation (Pearson's r=0.13, p<0.0001) during spring and summer in this area of the Norwegian coast. However, high daily amounts of precipitation were also negatively correlated with mean daily temperature and atmospheric pressure (Table S2.2).

Does early-life telomere length predict natal dispersal?

House sparrows generally show strong site fidelity and dispersal occurs mainly among juveniles in the autumn (i.e. natal dispersal, Altwegg et al., 2000) and over short distances (Anderson, 2006; Tufto et al., 2005). All islands surrounding Hestmannøy and Træna and the inhabited areas on the mainland shores (Fig. S1.1) were visited regularly to identify dispersers (Ranke et al., 2021; Saatoglu et al., 2021). To reduce effects of any selective disappearance of certain phenotypes before registration of dispersal, only individuals that survived until the following spring (i.e. recruits), were included in the analyses. A total of 41 individuals (18 [6 males, 12 females] out of 342 from Hestmannøy and 23 [14 males, 9 females] out of 113 from Træna) were observed on islands different from their natal islands within their first year of life (out of *n*=455 recruits). We used logistic regression with a binomial error distribution (using the 'bobyqa' optimizer throughout to facilitate model convergence, Bates et al., 2014) to test if early-life TL predicts the probability of successful natal dispersal. Within this house sparrow metapopulation, dispersal is female-biased and dispersal rates depend on habitat type (Ranke et al., 2021; Saatoglu et al., 2021). We therefore included sex and island identity as covariates in explaining dispersal propensity in all models. Hatch year was included as random intercept. We also included two- and three-way interactions between TL, sex, and island identity to test for differing relationships between TL and dispersal across sexes and island types. With this approach, a total of nine candidate models were compared using AICc.

Fitness consequences of variation in early-life telomere length

We used three approaches to investigate the consequences of variation in early-life TL on fitness (survival and reproduction). First, we tested if TL predicts whether an individual survives its first year (*n*=445, excluding individuals with missing tarsus length measurements) or not (*n*=2017), i.e. recruitment probability, using a logistic regression with a binomial error distribution and a logit link function (*lme4* package). Explanatory variables were TL, tarsus length, non-linear effects of TL (TL^2) and tarsus length (tarsus length^2), and interaction terms between island identity and tarsus length and TL, respectively. Sex and island identity were included as fixed effects, and year and nested brood identity as random intercepts, in all models. A total of 14 candidate models were constructed.

Second, we used Cox proportional hazards regression to test whether TL predicted mortality risk over the lifespan using the *survival* package (Therneau, 2015). The last observation of an individual was used as an estimate of minimum lifespan (number of days since hatching). Birds were assumed to have died if they had not been observed during two subsequent field seasons. Only two individuals (out of *n*=2462) may still have been alive when observations ended (autumn 2020) and were therefore right-censored (Cox, 1972). We constructed the same 14 candidate models as in the first-year survival analyses above. Brood identity was included as a random effect (cluster) and model assumptions were tested using the Schoenfeld test. To meet model assumptions, data was stratified by island identity, allowing for different hazard functions within each population (strata). The *simPH* package was used to simulate and plot the effects of the predictor variables on the hazard ratios (Gandrud, 2015). Finally, we used the Kaplan-Meier method to construct cumulative survival curves (*survminer* package, Kassambara et al., 2020).

Third, we tested if TL predicts annual reproductive success (ARS; the number of recruits produced per year by an individual) among individuals that survived their first year and were thus able to breed (starting from year 1995). Genetic parenthood data was not available after 2013, so subsequent years were excluded from the analysis. We fitted generalized LMMs with a Poisson distribution using the package *glmmTMB* (Brooks et al., 2017) to test whether TL predicts ARS (*n*=709 annual reproductive events of *n*=396 individuals). Tarsus length and non-linear effects of TL and tarsus length were included in 14 candidate models (same as described above). All models included sex and island identity as fixed factors, and individual identity (*n*=396) and year (*n*=19) as random intercepts. Models were validated using the DHARMa package (Hartig, 2020). The 14 candidate models within each of the three approaches above were compared using AICc.

RESULTS

Factors affecting early-life telomere length

There was considerable variation in TL among cohorts with no obvious directional trend (Fig. S2.1). The best model of variation in TL included a negative effect of tarsus length (β_{tarsus}=-0.0038±0.0016, CI=[-0.0079, -0.0006], Tables 1 and 2) indicating that larger individuals had shorter telomeres. The model also included evidence for an interaction term between population density and island identity ($\beta_{island*density}$=0.0008±0.0004, CI=[0.4E-4, 0.0016], $\beta_{density}$=-0.0008±0.0004, CI=[-0.0015, -0.5E-4]), indicating that individuals born in years with higher population densities had shorter telomeres, but only in the Træna (non-farm) population (Fig. 1a). Thus, there was apparently no evidence for an effect of variation in population density on TL in the Hestmannøy population (Fig. 1b). The second-best model ($\Delta AICc$=0.4, Table 1) did not include the effects of population density. The third and fourth best models included very uncertain effects of hatch day and age, respectively (Table 1).

Effects of weather on early-life telomere length

The best model ($\Delta AICc$=-13.49 compared to a model without weather effects, Table S2.1 and Fig. S2.2) identified from the sliding window analysis of weather variables included a negative quadratic effect of the maximum NAO index during 26 to 12 days before TL sampling (Table 3 and Fig. 1c), which corresponds approximately to the timing of the incubation phase. This suggests that there is a set of (optimal) environmental conditions, reflected by intermediate values of the maximum NAO index during incubation, that results in the longest telomeres in fledglings. The model output was unlikely to be a result of overfitting (p=0.001, see Fig. S2.2). The second-best model, which differed by $\Delta AICc$=-12.82, included only a linear negative effect of maximum NAO during approximately the same time window (30 to 16 days before sampling, Table S2.1).

Does early-life telomere length predict natal dispersal?

Four of the six models with $\Delta AICc<2$ describing variation in successful natal dispersal probability included a tendency for a negative association between TL and dispersal probability (model ranked second with $\Delta AICc=0.0$; $\beta_{TL}=-0.795\pm0.630$, CI=[-2.248, 0.268],Table S2.3 and Fig. 3). The two highest ranked models (both $\Delta AICc=0.0$) included an interaction between island and sex, indicating a tendency for males from Træna to be more likely to disperse than males from Hestmannøy ($\beta_{\text{island (Hestmannøy)*sex (female)}}=1.196\pm0.713$, CI=[-0.189, 2.659], $\beta_{\text{island (Hestmannøy)}}=-2.434\pm0.558$, CI=[-3.526, -1.341], $\beta_{\text{sex (female)}}=-0.496\pm0.497$, CI [-1.512, 0.472]). The model ranked third ($\Delta AICc=0.6$) included a three-way interaction term between TL, island identity, and sex, suggesting that the negative association (tendency) between dispersal probability and TL was strongest in males from Hestmannøy ($\beta_{\text{TL*island (Hestmannøy)*sex (male)}}=-3.049\pm1.765$, CI=[-9.988, -0.862], see full model in Table S2.4 and the effect in Fig. 3).

Fitness consequences of early-life telomere length

There was no evidence of an effect of TL on first-year survival (Table S2.5 and Fig. 3b). There was however evidence for a positive association between tarsus length and first-year survival probability in all top models with $\Delta AICc<2$ (model ranked 1: $\beta_{tarsus}=0.040\pm0.009$, CI=[0.057, 0.023], Table S2.5). The best model also included a weak curvilinear effect of tarsus length ($\beta_{tarsus\text{^}2}=-0.042\pm0.029$, CI=[-0.101, 0.11]), indicating that survival probability increased less or even decreased with tarsus length in the largest individuals (Fig. 3a).

There was no evidence of an effect of TL on mortality risk (Table S2.6 and Fig. 4b). The Cox hazard regression analyses showed however that there was a strong negative association between tarsus length and mortality risk (model ranked 1: $\beta_{tarsus}=-0.120\pm0.017$, CI=[-0.157, -0.083], Table S2.6). The best model also included a weak curvilinear effect of tarsus length (β_{tarsus^2}=0.011±0.006, CI=[-0.002, 0.024]), indicating that the decrease in the risk of mortality with increased tarsus length reached a plateau at large values (Fig. 4a).

We found weak evidence of an inverse relationship between TL and ARS (model ranked 1: β_{TL}=-0.446±0.275, CI=[-0.985, 0.092], *n*=709, Table S2.7 and Fig. 5b), indicating that individuals with long TL had lower ARS than individuals with short TL. The second ranked model (Δ*AICc*=0.1) additionally included a weak positive effect of tarsus length on ARS (β_{tarsus}=0.106±0.075, CI=[-0.042, 0.253], Fig. 5a). It was thus difficult to separate models including a positive effect of tarsus length and/or a negative effect of TL on ARS (Table S2.7).

DISCUSSION

In this study, we have shown how individual variation in early-life telomere length is related to structural growth, weather conditions during incubation, and population density in a long-term study of two island populations of wild house sparrows. This suggests a mechanistic link between environmental change and physiological change mediated by TL dynamics in early-life (Chatelain et al., 2020; Giraudeau et al., 2019a). TL has been shown to be associated with important components of fitness in some wild species (Eastwood et al., 2019; Froy et al., 2021; van Lieshout et al., 2019; Wilbourn et al., 2018; Young et al., 2021). However, we found little evidence that variation in TL in early life had any fitness consequences in terms of survival, but there was a tendency for a negative effect of TL on reproductive success (Tables S2.4-2.6). Instead, fitness was mainly determined by body size (Ringsby et al., 1998) with larger individuals having higher short-term survival (Fig. 3a), lower long-term mortality (Fig. 4a), and somewhat higher reproductive success (Fig. 5a). Larger individuals had shorter telomeres (Table 2), as documented previously in several species (Monaghan & Ozanne, 2018), including house sparrows (Pepke et al., 2021a; Pepke et al., 2021b; Ringsby et al., 2015), but little of the residual variation in fitness appeared to be explained by TL.

Recent studies have established early-life environmental conditions as important drivers of TL dynamics in free-living organisms (Angelier et al., 2018; Chatelain et al., 2020; Foley et al., 2020; Herborn et al., 2014; Nettle et al., 2015; Spurgin et al., 2018). Thermoregulatory and nutritional stress can increase production of ROS resulting in stress-induced changes in TL (Friesen et al., 2021; Reichert & Stier, 2017). Effects of weather conditions on telomere dynamics is known from other wild animal populations, including dark-eyed juncos (*Junco hyemalis*), in which females experienced greater telomere loss during breeding at colder temperatures, probably due to cold stress (Graham et al., 2019). In black-tailed gulls (*Larus crassirostris*), telomeres were even elongated during an El Niño year, in which weather was generally milder and sea surface temperatures lower, resulting in improved foraging conditions (Mizutani et al., 2013). Similarly, the change in TL in greater-eared bats (*Myotis myotis*, Foley et al., 2020) and early-life TL in European badgers (*Meles meles*, van Lieshout et al., 2021) was positively associated with generally good weather conditions (higher temperatures and favorable rainfall). Furthermore, in house sparrow nestlings, TL was positively associated with the NAO index averaged across approximately two weeks before and after hatching, which locally reflected higher temperatures, lower rainfall, and lower wind speed (Pepke et al., 2021b). Bird embryos are effectively ectothermic during incubation, justifying a comparison with studies on ectothermic species, in which temperature may be more directly affecting ROS production and cell division through increased metabolic activity and growth (Friesen et al., 2021; Olsson et al., 2018a). Accordingly, in brown trout (*Salmo trutta*, Debes et al., 2016) and Siberian sturgeon (*Acipenser baerii*, Simide et al., 2016), higher temperatures led to shorter telomeres, possibly caused by heat stress, but also increased growth. Similarly, TL decreased with higher temperatures experienced by common lizards (Dupoué et al., 2017), desert toad-headed agamas (*Phrynocephalus przewalskii*, Zhang et al., 2018), and dairy cattle (*Bos taurus*, Seeker et al., 2021), but not in Gouldian finches (*Chloebia gouldiae*, Fragueira et al., 2019). However, TL increased with higher temperature in Eastern mosquitofish (*Gambusia holbrooki*, Rollings et al., 2014), spotted snow skinks (*Niveoscincus ocellatus*, Fitzpatrick et al., 2019), and dark-eyed juncos (Graham et al., 2019) and there was no effect of temperature manipulation on TL in Atlantic salmon (*Salmo salar*, McLennan et al., 2018) or three-spined sticklebacks (*Gasterosteus aculeatus*, Kim et al., 2019). Axelsson et al. (2020) documented a thermal optimum associated with long telomers in sand lizards (*Lacerta agilis*). These idiosyncratic patterns demonstrate how environmental factors and degree of harshness may trigger a physiological stress response (Chatelain et al., 2020) with different consequences on TL dynamics depending on the deviation from species-specific environmental optima (Axelsson et al., 2020; McLennan et al., 2016). In our study, we observed a similar curvilinear association between fledgling TL and the maximum NAO index during the incubation phase, suggesting that this weather variable best reflects the effects of environmental conditions on TL, and that there are optimal environmental conditions that result in the longest TL. A two week period corresponds to the summer NAO life cycle (see Feldstein, 2007), and the maximum summer NAO may reflect extreme weather events such as drought or flooding (Drouard et al., 2019; Folland et al., 2009). At our study site, the daily NAO index was primarily positively correlated with the daily amount of precipitation (Table S2.2). Rainfall may have immediate effects on food availability and hence nest attendance (Bambini et al., 2019) and incubation temperature (Simmonds et al., 2017), which can elicit a stress response in the organism with effects on TL (Dupoué et al., 2020; Stier et al., 2020; Vedder et al., 2018). Prenatal exposure to environmental stressors can also have significant negative effects on embryonic TL (Entringer et al., 2011; Noguera & Velando, 2019). Variation in the NAO index locally captures complex associations between weather variables reflecting "harsh" or "benign" weather conditions (Folland et al., 2009; Stenseth et al., 2003), and it has been linked to morphological and demographic changes in several northern hemisphere species (Ottersen et al., 2001; Hallett et al., 2004; Stenseth et al., 2002; Stenseth et al., 2003). For instance, the NAO index may reflect insect abundance and phenology (Nott et al., 2002; Welti et al., 2020; Westgarth-Smith et al., 2012). The NAO can have considerable lagged effects on weather (Halkka et al., 2006), or there may be developmental time lags between weather conditions and the response in insect abundance (Visser et al., 2006). Thus, the effect of NAO during incubation may be acting on food availability during the important nestling growth stage. Food availability was positively associated with TL and TL lengthening in Seychelles warblers (Brown et al., 2021; Spurgin et al., 2018), but negatively associated with TL in American black bears (*Ursus americanus*, Kirby et al., 2017). In African striped mice (*Rhabdomys pumilio*), TL decreased during the dry season, when food availability was low, and increased during the wet season, when food availability was high (Criscuolo et al., 2020). Such associations may be complicated by the fact that some level of food restriction may reduce oxidative damage during growth (Noguera et al., 2011). Accordingly, the curvilinear effect of weather conditions on TL (Table 3) may therefore also reflect the growth conditions optimizing TL (Monaghan & Ozanne, 2018).

Habitat quality may be an important driver of differences in TL dynamics across populations (McLennan et al., 2021; Wilbourn et al., 2018). We found evidence for an interaction effect between habitat type (island) and population density (Table 2), suggesting that pre-breeding population density was negatively related to TL on the non-farm island (Træna), but not on the farm-island (Hestmannøy). On Hestmannøy, which holds a larger sparrow population than Træna (Fig. S2.1 and Table S1.1), the sparrows live and nest in a sheltered environment around farms, in contrast to Træna, where the sparrows nest in artificial nest boxes in a village environment. House sparrows are gregarious but exhibit territorial behavior by defending nest sites during the breeding season (Anderson, 2006). Thus, there may be more competition for nest sites on Træna compared to Hestmannøy at high population densities. Furthermore, as population density increases, competition increases, and poorer quality nest and foraging sites are increasingly occupied (Møller et al., 2018; Newton, 1998). The farms on Hestmannøy provide adults with a continuous supply of grain or food pellets and we speculate that the intensity of competition for resources may therefore be higher in the more unpredictable habitats on Træna, when population size is relatively larger (e.g. Dhondt, 2010). Again, malnutrition or exposure to suboptimal microclimatic conditions during early-life, may lead to elevated levels of oxidative stress and hence shorter telomeres in fledglings (Andrews et al., 2021), consistent with our observations. Similar negative effects of population density on TL have been observed in griffon vultures (*Gyps fulvus*, Gangoso et al., 2021) and Atlantic salmon (*Salmo salar*, McLennan et al., 2021), and in crowding experiments with mice (*Mus musculus*, Kotrschal et al., 2007).

We found some evidence for successful dispersers to have shorter telomeres prior to dispersal than non-disperser, especially among males from the farm-island (Hestmannøy, with only 6 dispersers out of 167 males, Fig. 3). These analyses were limited by the relatively small number of dispersers. In the introduction we suggested that short telomeres may inform a dispersal syndrome (pace-of-life), where bolder and faster-lived individuals are more likely to disperse. Short telomeres have been correlated with bold, aggressive, pessimistic, or impulsive behavior in fish and birds (Adriaenssens et al., 2016; Bateson et al., 2015; Espigares et al., 2021). Increases in the level of glucocorticoids are linked to dispersal in birds (Belthoff & Dufty, 1998; Silverin, 1997), which indicate the relevance of oxidative stress and hence that TL may be associated with successful dispersal (Casagrande & Hau, 2019; Récapet et al., 2016). Although Pegan et al. (2019) found a small negative effect of corticosterone treatment on TL in wild tree swallows (*Tachycineta bicolor*), this did not affect the age of initial departure from the natal site. Boonekamp et al. (2014) compared telomere loss within the first month of life among philopatric and dispersing jackdaws (*Coloeus monedula*), but did not find any differences, however their study was limited by a small sample size (5 dispersers out of 30 recruits). House sparrows are short-distance dispersers (Tufto et al., 2005), and TL may not be a generally significant physiological indicator of dispersal probability at the scale of metapopulations. In contrast, metabolically demanding long-distance migration or dispersal increases oxidative stress (Costantini et al., 2007) and may thus have direct negative impacts on TL, as observed in migratory birds (Angelier et al., 2013; Bauer et al., 2016; Schultner et al., 2014).

In several species, longer TL is associated with higher survival (Bichet et al., 2020; Crocco et al., 2021; Eastwood et al., 2019; Froy et al., 2021; Heidinger et al., 2021; Ilska-Warner et al., 2019; van Lieshout et al., 2021; Wilbourn et al., 2018; Young et al., 2021, but see Vedder et al., 2017). We found no evidence for an association between TL and first-year survival or mortality over the lifespan in house sparrows (Figs. 3b and 4b). Perhaps early-life TL is uncoupled from survival because of high extrinsic mortality of (primarily juvenile) house sparrows (Fig. S2.3) not related to early-life TL (e.g. Criscuolo et al., 2020; Eastwood et al., 2019; Wood & Young, 2019). Alternatively, house sparrows may be able to mitigate negative effects of short telomeres later in life through telomere maintenance (e.g. Vedder et al., 2017). Meillere et al. (2015) found a negative effect of stress induced by anthropogenic noise exposure on early-life TL in house sparrows, but this did not affect fledgling survival. Pepke et al. (2021b) found no associations between TL and first-year survival in house sparrows from two populations that were part of a bidirectional artificial body size selection experiment. However, both short and long early-life TL tended to be weakly associated with the lowest mortality rates over the lifespan in that study (Pepke et al., 2021b), suggesting disruptive selection on TL. Furthermore, some studies have showed that early-life TL was a poor predictor of survival, which was instead predicted by changes in TL (Boonekamp et al., 2014; Seeker et al., 2021; Wood & Young, 2019), which we did not measure in this study.

We found a tendency for a negative association between ARS and TL even when accounting for the positive effect of body size on ARS, i.e. individuals with short TL tended to produce more recruits annually (Fig. 5b). Within species, individuals with short telomeres may exhibit a faster pace-of-life reflected in higher ARS, while individuals with longer telomeres allocate more resources into self-maintenance (Giraudeau et al., 2019a; Rollings et al., 2017a; Young, 2018). Perhaps individuals with short TL adopt a terminal investment strategy (Bauer et al., 2018; Clutton-Brock, 1984). Interestingly, across bird species, the reciprocal relationship emerges, i.e. species with short telomeres are slow-lived (Pepke et al., 2021d). Heidinger et al. (2021) found no associations between early-life TL and annual reproductive performance (number of offspring) in wild American house sparrows. However, they found a positive relationship between early-life TL and lifespan in females, but a negative trend between TL and lifespan in males. They therefore suggested that TL reflected differences in quality or condition in females, but did not predict pace-of-life (Heidinger et al., 2021). It will be interesting to see if other studies find contrasting associations between TL and fitness across different populations within the same species in the wild.

There is some evidence that telomere loss rates are higher in longer telomeres (Atema et al., 2019; Atema et al., 2021; Verhulst et al., 2013; Victorelli & Passos, 2017) suggesting that early-life TL may not be a good linear predictor of later-life TL. Alternatively, individual TL changes in response to environmental variables through life (Brown et al., 2021; Chatelain et al., 2020) suggesting that TL must be measured closer to reproduction events (Marasco et al., 2021).

Our study suggests that environmental stressors negatively affected TL in young house sparrows, probably through the action of oxidative damage by ROS, but questions the common expectation of long-term fitness costs associated with shorter early-life TL in the wild. We also found some evidence that TL may be a biomarker of pace-of-life syndromes with fast-paced individuals with short telomeres tending to have higher dispersal rates and higher ARS. Thus, there may be few long-term physiological disadvantages associated with having short telomeres in early-life in wild populations, but TL may rather act as a biomarker of individual pace-of-life. However, associations between early-life TL, individual fitness, and complex environmental interactions seems difficult to establish and may vary between populations in the wild.

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AUTHOR CONTRIBUTIONS

MLP measured telomeres, analyzed the data, and wrote the manuscript with contributions from all authors. THR, B-ES, and HJ, initiated the study system. THR, HJ, TK, PSR, YGA-A, and MLP contributed to the fieldwork.

DATA AVAILABILITY

All data is available on request from the first author, and it will be made available on a public repository upon publication of the manuscript.

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TABLES AND FIGURES

Table 1: Linear mixed effects models with ΔAICc≤4 of variation in early-life telomere length in house sparrow nestlings from two island populations. All models included random intercepts for year and brood identity. Models are ranked by AICc, and number of degrees of freedom (df) and model weights (w) are shown.

Model		ΔAICc	df	w
1	**TL = sex + island + tarsus + density + island*density**	0.0	9	0.1405
2	TL = sex + island + tarsus	0.4	7	0.1138
3	TL = sex + island + tarsus + density + island*density + hatch day	1.0	10	0.0872
4	TL = sex + island + tarsus + density + island*density + condition	1.1	10	0.0814
5	TL = sex + island + tarsus + density + island*density + age	1.1	10	0.0793
6	TL = sex + island + tarsus + condition	1.5	8	0.0652
7	TL = sex + island + tarsus + age	1.6	8	0.0619
8	TL = sex + island + tarsus + density + island*density + age + hatch day	1.9	11	0.0535
9	TL = sex + island + tarsus + density	2.2	8	0.0460
10	TL = sex + island + tarsus + density + island*density + condition + age	2.2	11	0.0457
11	TL = sex + island + tarsus + density + island*density + condition + age + hatch day	3.0	12	0.0311
12	TL = sex + island + density + island*density	3.2	9	0.0284
13	TL = sex + island + tarsus + density + condition	3.4	9	0.0261
14	TL = sex + island + tarsus + density + age	3.5	9	0.0249
15	TL = sex + island + hatch day	3.5	7	0.0247
16	TL = sex + island	4.0	6	0.0191

Table 2: Estimates (β) with standard errors (SE) and lower and upper 95% confidence intervals (CI) from a linear mixed effects model of variation in telomere length (TL, *n*=2456). The model included random intercepts for brood identity and year. Italics indicate parameters with CIs not overlapping zero.

Response variable: TL	β	SE	Lower CI	Upper CI
intercept	-0.0205	0.0133	-0.0466	0.0053
sex (female)	-0.0041	0.0041	-0.0121	0.0039
island (Hestmannøy)	-0.0086	0.0093	-0.0269	0.0094
tarsus	*-0.0038*	*0.0016*	*-0.0070*	*-0.0006*
density	*-0.0008*	*0.0004*	*-0.0015*	*-0.5E-4*
*island (Hestmannøy)*density*	*0.0008*	*0.0004*	*0.4E-4*	*0.0016*
$\sigma^2_{\text{brood ID}}$ (*n*=947)	0.0036		0.0029	0.0043
σ^2_{year} (*n*=20)	0.0020		0.0010	0.0039

Marginal R^2 / Conditional R^2: 0.007 / 0.410

Table 3: Best model identified from sliding window analyses (Table S2.1) of the effect of weather variables on telomere length in house sparrow fledglings (*n*=2462). Italics indicate parameters with CIs not overlapping zero.

Response variable: TL	***β***	**SE**	**Lower CI**	**Upper CI**
intercept	-0.0049	0.0138	-0.0321	0.0220
sex (female)	-0.0052	0.0041	-0.0131	0.0028
island (Hestmannøy)	-0.0125	0.0092	-0.0305	0.0054
tarsus	-0.0042	0.0016	-0.0074	-0.0011
density	-0.0009	0.0004	-0.0016	-0.0002
island (Hestmannøy)*density	0.0009	0.0004	0.0002	0.0016
max. $NAO_{26\text{-}12\ days}$	0.0124	0.0084	-0.0040	0.0287
(max. $NAO_{26\text{-}12\ days}$)^2	*-0.0223*	*0.0052*	*-0.0325*	*-0.0121*
$\sigma^2_{brood\ ID}$ (*n*=948)	0.0033		0.0026	0.0040
σ^2_{year} (*n*=20)	0.0022		0.0011	0.0042

Marginal R^2 / Conditional R^2: 0.029 / 0.418

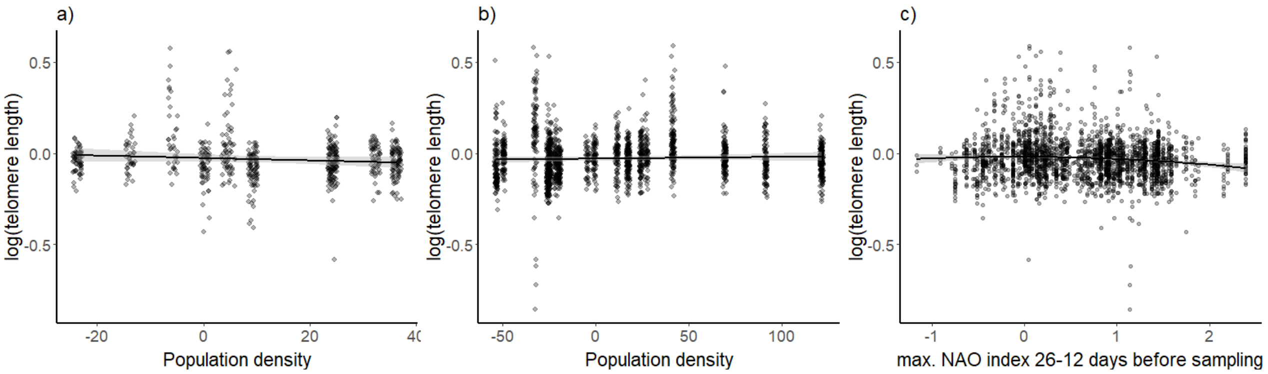

Fig. 1: The effect of population density (mean centered) on $\log_{10}$-transformed early-life telomere length in a) the Træna population (negative association) and b) in the Hestmannøy population (no association), see Tables 1 and 2. c) The negative quadratic association between early-life TL and the best weather variable predictor (max. NAO index during incubation) from a sliding window analysis (Tables S2.1 and 3).

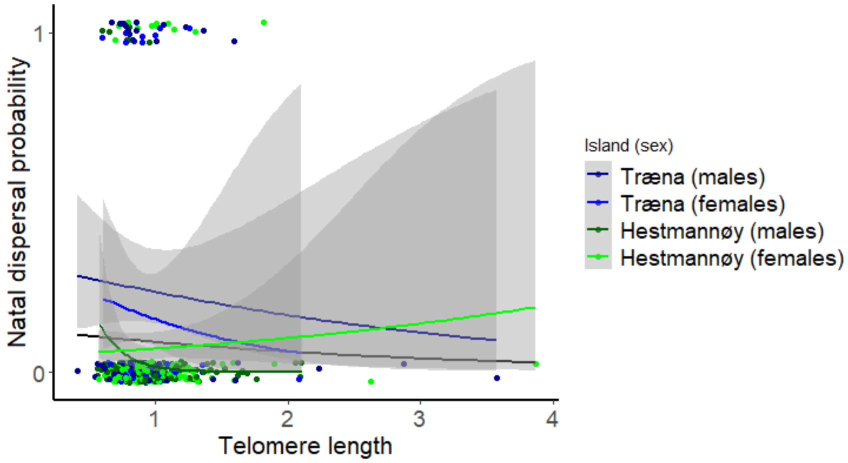

Fig. 2: Binomial logistic regression of successful natal dispersal probability predicted by early-life TL (*n*=455). The highest ranked models (Table S2.3) suggested a weak negative association between dispersal probability and TL (black regression line). One of these top models suggested that there was a stronger negative association between TL and dispersal probability among males born on Hestmannøy (*n*=167, dark green regression line with 95% confidence intervals in grey areas).

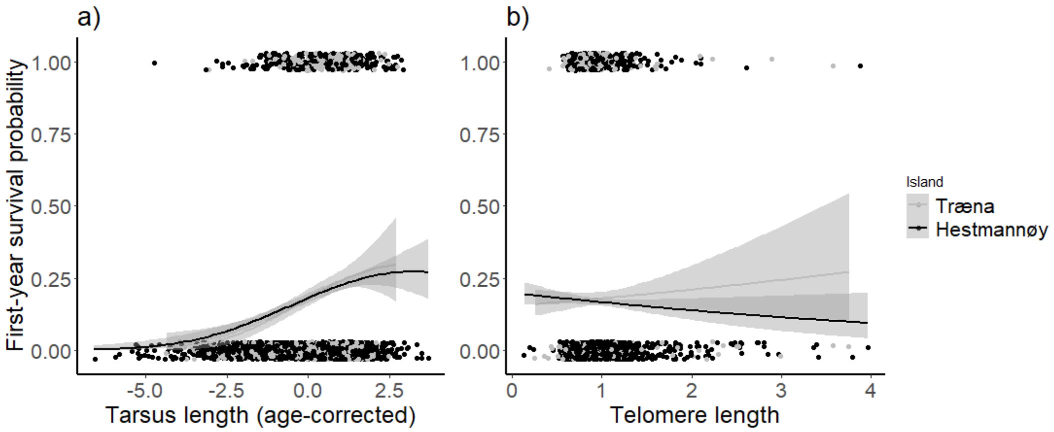

Fig. 3: Relationship between first-year survival (recruitment) probability in two populations of house sparrows (*n*=2462, grey: Træna, black: Hestmannøy) and **a)** fledgling tarsus length (negative quadratic association) and **b)** fledgling TL (no evidence for any associations). The logistic regression lines are from the top models shown in Table S2.5 including tarsus length (model ranked 1) and telomere length (model ranked 4). There was no evidence for differences in first-year survival probability between the two populations.

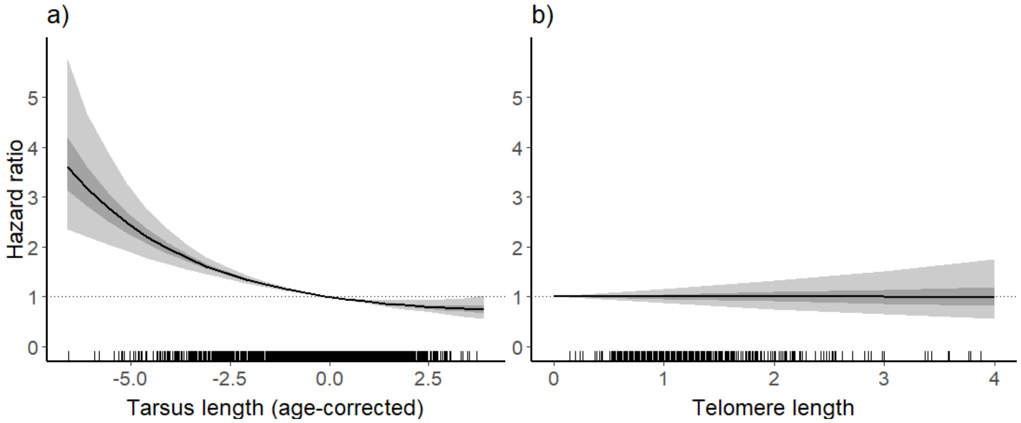

Fig. 4: Mortality risk measured as hazard ratio in two populations of house sparrows (n=2462, grey: Træna, black: Hestmannøy) as a function of **a)** fledgling tarsus length (positive quadratic association) and **b)** fledgling telomere length (no evidence for any associations). The simulated regression lines (black) show the modelled effect from the top models in Table S2.6 with 95% and 50% confidence intervals in light grey and dark grey respectively.

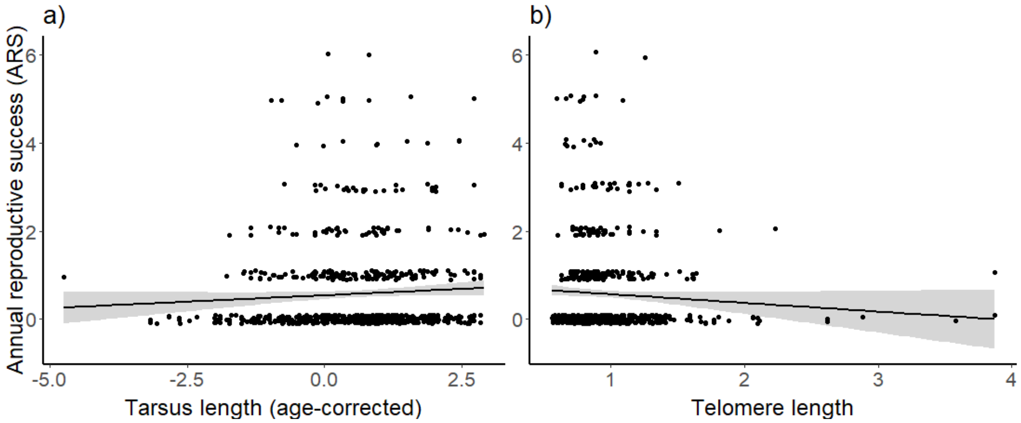

Fig. 5: The associations between annual recruit production (ARS: annual reproductive success, n=709 annual reproductive events of n=396 individuals) and **a)** fledgling tarsus length and **b)** fledgling telomere length. The regressions lines (black, with 95% confidence intervals in grey) show the uncertain tendencies (see the main text) predicted from the top models in Table S2.7.

Supporting information

Causes and consequences of variation in early-life telomere length in a bird metapopulation

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Contents

APPENDIX S1

Map of the study area

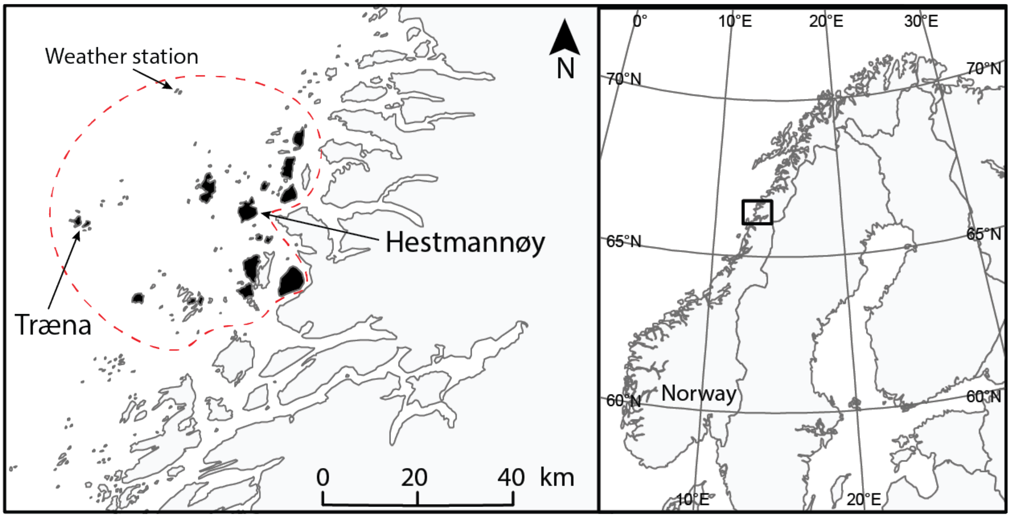

Fig. S1.1: Map of the house sparrow metapopulation study area in northern Norway. We measured early-life telomere lengths in sparrows hatched on the islands of Hestmannøy and Træna. Mist netting and observations took place regularly on the black islands, which are the main inhabited islands within the monitored study area (red dotted line). Human settlements on the mainland coast east of the study area were visited in autumn and spring to search for dispersing individuals. Weather data was retrieved from a meteorological station at the island of Myken.

Morphological measurements

No morphological measurements were made for 224 nestlings (out of 2746). For 189 nestlings, blood samples and morphological measurements were not made at the same age (varying with up to ±6 days differences due to logistical reasons). We therefore fitted linear regressions of tarsus length and mass, respectively, on age (including a squared effect of age) separately for each sex and population (see Pepke et al., 2021b). The measured tarsus length or mass was then adjusted to the predicted length at the age of blood (TL) sampling using the fitted values from the regressions. Body condition was calculated using these adjusted values as described in the main text.

Telomere measurements

Telomere length measurements are described in Pepke et al. (2021a) and summarized here. We used the quantitative polymerase chain reaction (qPCR) method to measure TL (following Cawthon, 2002; Criscuolo et al., 2009). DNA extractions were performed at the Norwegian University of Science and Technology (Trondheim, Norway) and DNA was shipped to Glasgow (UK) on dry ice (-78°C). All reactions were carried out by the same person (MLP) to avoid inter-individual measurement variability. Telomere primers Tel1b (5'-CGG TTT GTT TGG GTT TGG GTT TGG GTT TGG GTT TGG GTT-3') and Tel2b (5'-GGC TTG CCT TAC CCT TAC CCT TAC CCT TAC CCT TAC CCT-3') were prepared at a final concentration of 500 nM. GAPDH (glyceraldehyde-3-phosphate dehydrogenase) primers (forward primer 5'-GAG GTG CTG CTC AGA ACA TTA T-3' and reverse primer 5'-ACG GAA AGC CAT TCC AGT AAG-3') were prepared at a final concentration of 200 nM. Telomere assay thermal profile was: 15 min at 95°C, 27 cycles of 15 s at 95°C, 30 s at 58°C, and 30 s at 72°C. GAPDH thermal profile was: 15 min at 95°C, 40 cycles of 15 s at 95°C, 15 s at 60°C. Assays were followed by melt curve analysis (58-95°C 1°C/5 s ramp) and checked for a single peak dissociation curve. Mean telomere assay efficiency was 97.5±3.9%, and mean GAPDH assay efficiency was 97.6±4.2%. Average reference sample cycle thresholds (Ct) across all plates were 10.54±0.03 SD for telomere assays and 21.53±0.02 SD for GAPDH assays.

Sample sizes

Table S1.1: Number of TL sampled fledglings in each cohort (year) used in this study, and island-wide population density (spring pre-breeding census of adults) for each island (Hestmannøy and Træna).

Cohort (year)	Hestmannøy number of fledglings	Træna number of fledglings	Total number of fledglings)	Hestmannøy population density (adults)	Træna population density (adults)
1994	103	-	103	93	-
1995	90	-	90	86	-
1996	48	-	48	65	-
1997	81	-	81	65	-
1998	100	-	100	93	-
1999	90	-	90	130	-
2000	73	-	73	118	-
2001	41	-	41	114	-
2002	97	-	97	69	-
2003	106	-	106	94	-
2004	106	67	173	99	26
2005	95	90	185	136	86
2006	123	59	182	143	82
2007	152	127	279	188	74
2008	83	38	121	146	36
2009	182	35	217	160	44
2010	129	44	173	210	54
2011	248	65	313	240	50
2012	70	24	94	136	55
2013	93	87	180	97	59
2014	-	-	-	-	-
2015	-	-	-	-	-
2016	-	-	-	-	-
2017	-	-	-	-	-
Sum:	2110	636	2746		

APPENDIX S2

Telomere length variation across cohorts and population size fluctuations

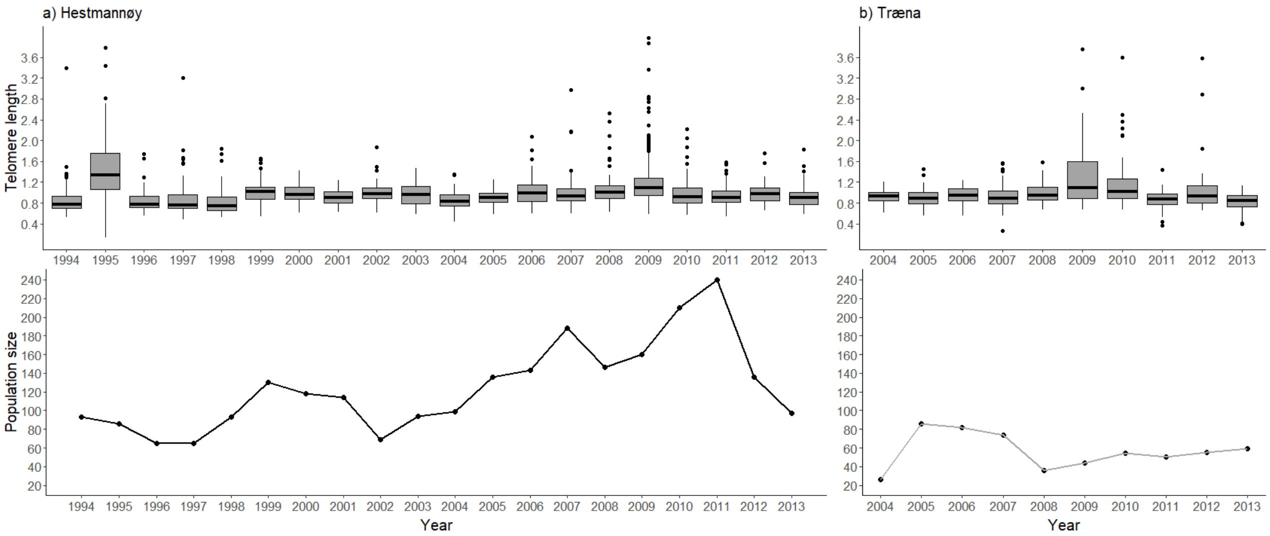

Fig. S2.1: Variation in individual early-life telomere length in house sparrow fledglings (*n*=2746) across all cohorts and the adult pre-breeding population size estimates for the two island populations a) Hestmannøy (1994-2013) and b) Træna (2004-2013).

Sliding window analyses

Table S2.1: Results from sliding windows analyses of the effect of weather variables (temperature, precipitation, humidity, and the NAO index) on TL in house sparrow chicks. The daily NAO index is measured as the difference in atmospheric pressure at sea level between the Subtropical (Azores) High pressure and the Subpolar (Iceland) Low pressure. The sample size of *n*=2462 is higher than in Table 2 (*n*=2456) because individuals with missing body condition estimates could be included here. The best model (most sensitive timeframe) for each weather variable is shown with *ΔAICc* relative to a null (baseline) model containing no weather variables. The best performing combination is highlighted in bold, and all models with negative *ΔAICc* in italics. The baseline model was: $\log_{10}$(TL) ~ sex + tarsus + island*density, including year and brood identity as random intercepts. The third-best model (*ΔAICc*=-7.98) included a negative effect of maximum NAO 30-12 days prior to sampling and a significant interaction term between maximum NAO and island identity ($\beta_{max.\ NAO\ 30\text{-}12\ days}$=-0.040±0.009, CI=[-0.057,

-0.023], $\beta_{island\ (Hestmann\o y)*max.\ NAO\ 30\text{-}12\ days}$=0.024±0.010, CI=[0.005, 0.043]), providing some evidence that the negative effect of the weather variable was strongest on the no-farm island of Træna and significantly reduced on the farm island of Hestmannøy.

Model	Weather variable	Statistic	Function	Island interaction	ΔAICc	Window open	Window close
1	temperature	mean	linear	no	7.13	6	6
2	pressure	mean	linear	no	11.89	0	0
3	*NAO*	*mean*	*linear*	*no*	*-3.61*	*30*	*6*
4	temperature	max	linear	no	4.96	11	0
5	pressure	max	linear	no	11.89	0	0
6	*NAO*	*max*	*linear*	*no*	*-12.82*	*30*	*16*
7	temperature	min	linear	no	7.13	6	6
8	pressure	min	linear	no	11.24	13	3
9	NAO	min	linear	no	3.12	25	25
10	temperature	sum	linear	no	7.13	6	6
11	pressure	sum	linear	no	11.89	0	0
12	NAO	sum	linear	no	2.81	30	7
13	temperature	mean	linear	yes	18.75	11	11
14	pressure	mean	linear	yes	21.42	30	30
15	NAO	mean	linear	yes	2.32	30	7
16	temperature	max	linear	yes	17.09	13	0
17	pressure	max	linear	yes	18.69	30	24
18	*NAO*	*max*	*linear*	*yes*	*-7.98*	*30*	*12*
19	temperature	min	linear	yes	18.75	11	11
20	pressure	min	linear	yes	9.85	15	7
21	NAO	min	linear	yes	6.13	29	9
22	temperature	sum	linear	yes	18.75	11	11
23	pressure	sum	linear	yes	21.42	30	30
24	NAO	sum	linear	yes	8.02	30	30
25	precipitation	mean	linear	yes	9.13	24	17
26	precipitation	max	linear	yes	15.66	29	27
27	precipitation	sum	linear	yes	15.79	17	17
28	precipitation	mean	linear	no	5.94	29	8
29	precipitation	max	linear	no	7.29	17	17
30	precipitation	sum	linear	no	7.29	17	17
31	temperature	mean	quadratic	yes	45.32	15	12
32	pressure	mean	quadratic	yes	46.51	20	12
33	NAO	mean	quadratic	yes	13.93	30	9
34	temperature	max	quadratic	yes	45.97	15	14
35	pressure	max	quadratic	yes	46.61	16	3
36	NAO	max	quadratic	yes	2.88	26	12
37	temperature	min	quadratic	yes	42.18	29	12
38	pressure	min	quadratic	yes	42.97	19	12
39	NAO	min	quadratic	yes	17.4	29	1
40	temperature	sum	quadratic	yes	46.2	14	14
41	pressure	sum	quadratic	yes	54.58	13	13

42	NAO	sum	quadratic	yes	22.18	12	12
43	temperature	mean	quadratic	no	20.51	6	6
44	pressure	mean	quadratic	no	21.65	13	13
45	*NAO*	*mean*	*quadratic*	*no*	*-0.41*	*25*	*9*
46	temperature	max	quadratic	no	20.4	13	2
47	pressure	max	quadratic	no	18.12	16	3
48	***NAO***	***max***	***quadratic***	***no***	***-13.49***	***26***	***12***
49	temperature	min	quadratic	no	17.39	24	4
50	pressure	min	quadratic	no	21.65	13	13
51	NAO	min	quadratic	no	3.46	20	19
52	temperature	sum	quadratic	no	20.51	6	6
53	pressure	sum	quadratic	no	21.65	13	13
54	NAO	sum	quadratic	no	4.3	19	19
55	precipitation	mean	quadratic	yes	25.82	29	1
56	precipitation	max	quadratic	yes	46.49	28	27
57	precipitation	sum	quadratic	yes	47.15	18	18
58	precipitation	mean	quadratic	no	8.7	29	1
59	precipitation	max	quadratic	no	21.63	29	1
60	precipitation	sum	quadratic	no	26.8	21	21

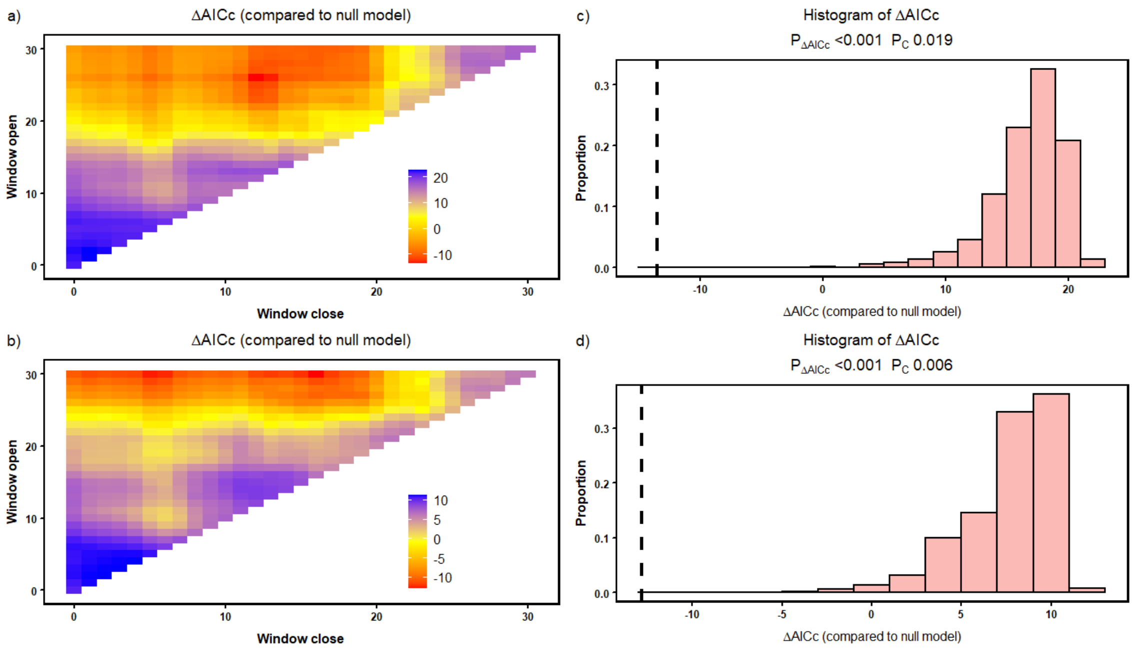

Fig. S2.2: a and b) Colours show the strength of the time window measured as ΔAICc (red are strong windows with low ΔAICc, blue are weak windows with high ΔAICc), i.e. how well the weather variable within a given timeframe improve a baseline model with no weather variables.

Figure a shows the top model where the maximum NAO index is described with a linear function, and figure b shows the second-best model, where the maximum NAO index is described with a quadratic function. **c and d)** Tests for over-fitting using 500 randomisations of the data are compared to the real data (dotted line, where c: is a linear, and d: quadratic function for the maximum NAO index). Thus, neither model is likely to be statistical artefacts as a result of over-fitting (van de Pol et al., 2016).

Table S2.2: Pearson's correlations coefficients between weather variables.

	Precipitation	**Pressure**	**NAO**
Temperature	*-0.0879* *(p<0.0001)*	0.0364 (p=0.0549)	0.0340 (p=0.0733)
Precipitation		*-0.1447* *(p<0.0001)*	*0.1314* *(p<0.0001)*
Pressure			-0.0145 (p=0.4441)

Dispersal probability and telomere length

Table S2.3: AICc table of binomial generalized linear mixed models of variation in natal dispersal probability (*n*=445). All models included hatch year as random factor intercept. Models are ranked by AICc and shows number of degrees of freedom (df) and model weights (w).

	Model (*n*=445)	**ΔAICc**	**df**	**w**
1	Dispersal = island + sex + island*sex	0.0	5	0.195
2	Dispersal = island + sex + island*sex + TL	0.0	6	0.194
3	Dispersal = island + sex + TL + island*sex*TL	0.6	8	0.141
4	Dispersal = island + sex	0.9	4	0.127
5	Dispersal = island + sex + TL	1.2	5	0.108
6	Dispersal = island + sex + TL + sex*TL	1.5	6	0.091
7	Dispersal = island + sex + TL + island*TL + sex*TL + island*sex	2.1	8	0.068
8	Dispersal = island + sex + TL + island*TL	3.1	6	0.042
9	Dispersal = island + sex + TL + island*TL + sex*TL	3.5	7	0.034

Table S2.4: Estimates (β) with standard errors (SE) and lower and upper 95% confidence intervals (CI) from a binomial generalized linear mixed effects model of variation in natal dispersal probability (n=455). The model included a random intercept for hatch year. The three-way interaction effects are visualized in Fig. 3.

Response variable: dispersal probability	β	**SE**	**Lower CI**	**Upper CI**
intercept	0.2927	1.0023	-1.4094	2.6004
sex (female)	-1.9067	1.4811	-4.8867	0.9973
island (Hestmannøy)	-1.2263	1.4939	-4.2532	1.7031
TL	0.0538	0.7985	-2.0403	1.3511
TL * island (Træna) * sex (male)	-1.3294	1.2938	-4.1242	1.3405
TL * island (Hestmannøy) * sex (male)	-3.0495	1.7648	-6.7867	0.1891
TL * island (Træna) * sex (female)	-0.0338	1.5339	-3.2599	2.9403
σ^2_{year} (n=20)	0.4774		0.0509	1.7745

Marginal R^2 / Conditional R^2: 0.256 / 0.350

Fitness consequences of telomere length

Table S2.5: Binomial generalized linear mixed models with ΔAICc<5 of variation in first-year survival probability (n=2462). Random factor intercepts for year (n=20) and brood identity (n=948) were included in all models. Models are ranked by AICc, and number of degrees of freedom (df) and model weights (w) are shown.

	Model (n=2462)	**ΔAICc**	**df**	**w**
1	Survival = sex + island + tarsus + tarsus^2	0.0	7	0.280
2	Survival = sex + island + tarsus	0.3	6	0.240
3	Survival = sex + island + tarsus + tarsus*island	1.7	7	0.118
4	Survival = sex + island + tarsus + tarsus^2 + TL	2.0	8	0.104
5	Survival = sex + island + tarsus + TL	2.3	7	0.089
6	Survival = sex + island + tarsus + tarsus*island + TL	3.7	8	0.044
7	Survival = sex + island + tarsus + tarsus^2 + TL + TL^2	3.8	9	0.041
8	Survival = sex + island + tarsus + TL + TL^2	4.2	8	0.035
9	Survival = sex + island + tarsus + TL + TL*island	4.3	8	0.033

Table S2.6: AICc table of Cox hazard regression candidate models with ΔAICc<5. All models included sex as a fixed effect, brood identity as clusters, and was stratified by island identity. Models are ranked by AICc and shows number of degrees of freedom (df) and model weights (w).

	Model (*n*=2462)	**ΔAICc**	**df**	**w**
1	Mortality = sex + strata(island) + tarsus + tarsus^2	0.0	3	0.343
2	Mortality = sex + strata(island) + tarsus	1.1	2	0.195
3	Mortality = sex + strata(island) + tarsus + tarsus^2 + TL	2.0	4	0.126
4	Mortality = sex + strata(island) + tarsus + tarsus:strata(island)	2.4	3	0.106
5	Mortality = sex + strata(island) + tarsus + TL	3.1	3	0.072
6	Mortality = sex + strata(island) + tarsus + tarsus^2 + TL + TL^2	3.9	5	0.049
7	Mortality = sex + strata(island) + tarsus + tarsus:strata(island) + TL	4.4	4	0.039

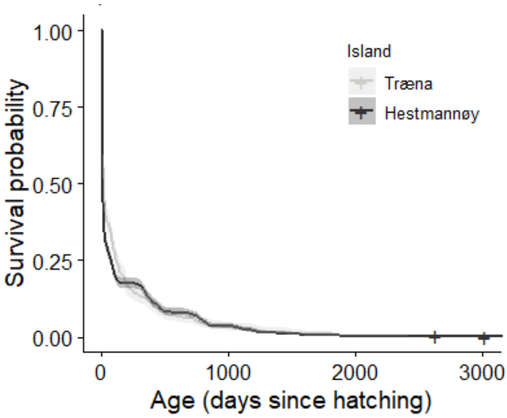

Fig. 2.3: Survival probability as a function of age in days since hatching shown separately for each island (Hestmannøy in black and Træna in grey). Crosses indicate timing of censoring for birds that may still be alive at the end of the data collection (*n*=2). There was no significant difference in mortality risk between the two strata (populations).

Table S2.7: AICc table of candidate models with ΔAICc<5 describing variation in annual reproductive success (ARS). All models included individual identity and year as random intercepts. Models are ranked by AICc and shows number of degrees of freedom (df) and model weights (w).

	Model (*n*=709)	**ΔAICc**	**df**	**w**
1	ARS = sex + island + TL	0.0	6	0.148
2	ARS = sex + island + TL + tarsus	0.1	7	0.144
3	ARS = sex + island + tarsus	0.6	6	0.111
4	ARS = sex + island	0.8	5	0.100
5	ARS = sex + island + TL + tarsus + tarsus^2	1.3	8	0.076
6	ARS = sex + island + TL + TL*island	1.9	7	0.058
7	ARS = sex + island + tarsus + tarsus^2	1.9	7	0.057
8	ARS = sex + island + TL + TL^2	1.9	7	0.056
9	ARS = sex + island + TL + TL*island + tarsus	1.9	8	0.056
10	ARS = sex + island + TL + TL^2 + tarsus	2.0	8	0.053
11	ARS = sex + island + TL + tarsus + tarsus*island	2.1	8	0.052
12	ARS = sex + island + tarsus + tarsus*island	2.6	7	0.040
13	ARS = sex + island + TL + TL^2 + tarsus + tarsus^2	3.3	9	0.028
14	ARS = sex + island + TL + TL*island + tarsus + tarsus*island	4.0	9	0.020

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PAPER V

This paper is awaiting publication and is not included in NTNU Open

PAPER VI

Early-life telomere length covaries with life-history traits and scales with chromosome length in birds

Running title: Avian telomere length evolution

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ABSTRACT

Telomeres, the short DNA sequences that protect chromosome ends, are an ancient molecular structure, which is highly conserved across most eukaryotes. Species differ in their telomere lengths, but the causes of this variation are not well understood. Here, we demonstrate that mean early-life telomere length is an evolutionary labile trait across 58 bird species (representing 35 families in 12 orders) with the greatest trait diversity found among passerines. Among these species, telomere length is significantly negatively associated with the fast-slow axis of life-history variation, suggesting that telomere length may have evolved to mediate trade-offs between physiological requirements underlying the diversity of pace-of-life strategies in birds. Curiously, within some species, larger individual chromosome size predicts longer telomere lengths on that chromosome, leading to the suggestion that telomere length also covaries with chromosome length across species. We show that longer mean chromosome length or genome size tends to be associated with longer mean early-life telomere length (measured across all chromosomes) within a phylogenetic framework constituting up to 32 bird species. Combined, our analyses generalize patterns previously found within a few species and provide potential adaptive explanations for the 10-fold variation in telomere lengths observed among birds.

INTRODUCTION

Telomeres are an ancient molecular structure which is conserved across most eukaryotes (Fulnecková et al., 2013; Meyne, Ratliff, & Moyzis, 1989). Telomeres might have emerged when linear chromosomes evolved from a circular chromosome ancestor around 1.5 billion years ago (Lee, Leek, & Levine, 2017). Their function is to protect linear chromosomes during incomplete end-replication (Blackburn & Szostak, 1984) and oxidative stress (von Zglinicki, 2002), and in most tetrapods mean telomere length (TL) of somatic cells shortens through life (e.g. Tricola et al., 2018). Within some species, short telomeres have been shown to correlate with shorter lifespan (Heidinger et al., 2012), increased size (Ringsby et al., 2015), and oxidative stress (Reichert & Stier, 2017). Across species, a faster rate of telomere loss has been linked to shorter maximum species lifespan in several studies (Dantzer & Fletcher, 2015; Haussmann et al., 2003; Pepke & Eisenberg, 2020; Tricola et al., 2018; Vleck, Haussmann, & Vleck, 2003; Whittemore, Vera, Martínez-Nevado, Sanpera, & Blasco, 2019). However, no consistent patterns have emerged in how absolute TL is associated with lifespan or body mass across species (Gomes et al., 2011; Haussmann et al., 2003; Pepke & Eisenberg, 2021; Seluanov et al., 2007; Tricola et al., 2018).

The mean TL, measured across all chromosomes, has emerged as biologically relevant trait in evolutionary, ecological, and physiological studies (Monaghan, 2010; Nussey et al., 2014). Among wild birds, mean adult TL varies from around 5 kb in Western jackdaws (*Coloeus monedula*, Salomons et al., 2009) to more than 50 kb in great tits (*Parus major*, Tricola et al., 2018), but an evolutionary explanation for this 10-fold difference in mean TL is lacking (Tricola et al., 2018). A similar magnitude of variation is found within mammals (Gomes et al., 2011), in which TL is shorter in larger and longer-lived species (Pepke & Eisenberg, 2021). Furthermore, TL is positively associated with cancer risk across mammalian species (Pepke & Eisenberg, 2021). Thus, in mammals, evolution of shorter telomeres can be explained as an adaptation to counteract the increased risk of development of tumors associated with a larger number of cells and longer time to accumulate oncogenic mutations in larger and longer-lived species (Gomes et al., 2011; Gorbunova, Seluanov, Zhang, Gladyshev, & Vijg, 2014; Pepke & Eisenberg, 2021; Risques & Promislow, 2018; Seluanov et al., 2007; Tian et al., 2018). However, no association between TL and lifespan has been found in birds (Haussmann et al., 2003; Tricola et al., 2018; Vleck et al., 2003) and it is not known how TL covaries with body mass or other life-history traits in birds.

In life-history theory, evolutionary trade-offs are expected between vital life-history traits, such as between investment in current reproduction and investment in somatic growth, maintenance, or future reproduction (Stearns, 1989). Organisms can be placed along a fast-slow continuum of life-histories depending on how they resolve such trade-offs (Roff, 1992). A fast pace-of-life is characterized by higher investment in reproduction over survival, which is reflected in species with e.g. large clutch sizes and short generation times and lifespan (Araya-Ajoy et al., 2021; Ricklefs & Wikelski, 2002; Sæther, 1988). The variation in pace-of-life strategies is associated with physiological differences between species (Dammhahn, Dingemanse, Niemelä, & Réale, 2018; Ricklefs & Wikelski, 2002). We hypothesize that TL may have coevolved with suites of life-history traits and that TL may be used to rank species on the slow-fast axis of variation in life-history traits. Short telomeres could reflect decreased allocation of investment in somatic maintenance and hence increased allocation of resources to reproduction (Giraudeau, Angelier, & Sepp, 2019; Monaghan, 2010). However, long telomeres may be an adaptation to the cumulative negative effects of reproduction (Sudyka, 2019) and rapid growth on TL (Pepke et al., 2021), revealing the opposite relationship between TL and pace-of-life across species. We therefore investigate associations between TL and the slow-fast axis of life-history variation across species (Dantzer & Fletcher, 2015).

Curiously, within several species, cytogenetic studies have shown a positive correlation between the TL at a particular chromosome arm and the corresponding total chromosome length or chromosome arm length (reviewed in Klegarth & Eisenberg, 2018). This pattern has been found within laboratory mice (*Mus musculus*, Zijlmans et al., 1997), Chinese hamsters (*Cricetulus* *griseus*, Slijepcevic & Hande, 1999), humans (*Homo sapiens*, Graakjaer et al., 2003; Mayer et al., 2006; Suda et al., 2002; Wise et al., 2009), arabidopsis (*Arabidopsis thaliana*, Shakirov & Shippen, 2004), pearl millets (*Pennisetum glaucum*, Sridevi, Uma, Sivaramakrishnan, & Isola, 2002), yeast (*Saccharomyces cerevisiae*, Berthiau et al., 2006; Craven & Petes, 1999), and Tetrahymena (*Tetrahymena thermophila*, Jacob, Stout, & Price, 2004). Klegarth and Eisenberg (2018) tested whether this relationship extends across mammal species, using data on adult mean TL and mean chromosome length across 39 species of Primates and Cetartiodactyla (Gomes et al., 2011) and 11 species of Rodentia (Seluanov et al., 2007). They did not find any significant associations, but within Primates and Cetartiodactyla a positive trend between TL and chromosome length became stronger when the outlier Indian Muntjac (*Muntiacus muntjac*) was removed. Indian Muntjacs have recently undergone several whole chromosome fusions resulting in very large chromosomes (Wang & Lan, 2000). Perhaps an evolutionary lag accounts for their relatively short telomeres, which are adapted to shorter chromosomes (Klegarth & Eisenberg, 2018). Furthermore, many of the species included in past studies have been held in captivity or domesticated by humans, which might have altered their telomere length dynamics (Eisenberg, 2011; Manning, Crossland, Dewey, & Van Zant, 2002; Pepke & Eisenberg, 2021).

Birds may present an easier taxon with which to examine telomere–chromosome length co-evolutionary dynamics: In contrast to mammals, the avian karyotype is relatively conserved, and most birds have a chromosome number (2n) around 80 (Degrandi et al., 2020; Ellegren, 2010) suggesting that inter-chromosomal rearrangements are rare in birds. However, most bird species possess several microchromosomes, which have been shown to harbor functional, but ultra-long ("class III") telomeres in some species (Atema, Mulder, van Noordwijk, & Verhulst, 2019; Delany, Gessaro, Rodrigue, & Daniels, 2007; Delany, Krupkin, & Miller, 2000; Nanda & Schmid, 1994;

Nanda et al., 2002; Rodrigue, May, Famula, & Delany, 2005). Not all microchromosomes possess ultra-long telomeres (and not all species with microchromosomes possess any ultra-long telomeres, Delany et al., 2000; Nanda & Schmid, 1994; Nanda et al., 2002). Furthermore, these abnormal telomeres were found only on one chromosome arm and only in some individuals of inbred domestic chickens (*Gallus gallus*), whereas TL of the opposite chromosome arm was of typical size ("class II", Delany et al., 2007). It is therefore possible that potential telomere–chromosome dynamics vary across chromosome size ranges (Atema et al., 2019), but this is unexplored in birds. Birds have the smallest genomes among extant amniotes, which is generally thought to represent adaptations to the metabolic requirements of active flight (Kapusta, Suh, & Feschotte, 2017; Wright, Gregory, & Witt, 2014). Yet, compared to mammals, some birds seem to have a larger amount of telomere sequences (Delany et al., 2000). Indeed, some of the ultra-long telomere signals may be attributed to sub-telomeric repeats ("class I", Ingles & Deakin, 2016, but see Atema et al., 2019), which occur in some bird species (Nanda et al., 2002).

Here, we use mainly TL measurements of individuals of known age to obtain estimates of an early-life TL. We first reconstruct the evolution of mean TL of 58 bird species. We then investigate how TL relates to key life-history traits of birds. We then proceed to test the hypothesis that TL covaries with chromosome length across species. We do not resolve within-genome variation in TL across chromosomes, which is largely unknown in birds (Nanda et al., 2002), but we test whether variation in mean chromosome length underlies some of the variation observed in mean TL across species.

MATERIALS AND METHODS

Telomere length data

The use of methods to estimate relative amounts of telomeric DNA within samples (qPCR) limits the feasibility of comparative studies (Nussey et al., 2014). In this study we only used TL measured via the telomere restriction fragment (TRF) method (Haussmann & Vleck, 2002) or high-throughput quantitative fluorescence *in situ* hybridization (Q-FISH, Canela, Vera, Klatt, & Blasco, 2007; Lansdorp et al., 1996). In the TRF analysis, mean TL value is obtained from the distribution of TLs (in a Southern blot gel smear) across all chromosomes (Haussmann & Mauck, 2008). In the Q-FISH analysis, mean TL is obtained from the mean telomere probe fluorescence intensity across all chromosomes (quantified using microscopy image analysis, Canela et al., 2007). We searched the literature for avian telomere studies using TRF to measure mean TL from blood (Web of Science and Google Scholar [March 2021] search terms: “telomere”, “TRF”, “restriction fragment”, “Q-FISH”, “avian”, or “bird”). Correlations between TLs of various tissues suggest that blood TL is a good proxy of the TL across the organism (Daniali et al., 2013; Demanelis et al., 2020; Reichert, Criscuolo, Verinaud, Zahn, & Massemin, 2013). We used TL estimates from the same lab when available to minimize methodological effects (Tricola et al., 2018). Most studies used non-denatured in-gel TRF or Q-FISH to measure functional (terminal) TLs, except for 15 species indicated in Table S1 in the Supporting Information. These studies used denatured TRF, which may include short interstitial (class I) telomeric sequences, that may lead to underestimation of the mean functional TL (Foote, Vleck, & Vleck, 2013). The size distinction between class II and III telomeres is not well-defined (Atema et al., 2019; Delany et al., 2000) and some studies may not include the complete TL distribution if it is outside the range of the specific molecular size markers used (Atema et al., 2019; Foote et al., 2013; Haussmann & Mauck, 2008). However, since chromosome-specific TLs are largely unknown in birds, we rely on estimates of mean TL, which may reflect mainly “class II” telomeres.

To estimate an early-life TL for species with TL measurements at different reported ages, within each species we performed a linear regression of TL and individual age and used the extrapolated TL value at age 0 (i.e. the intercept, see Fig. 1 in Tricola et al., 2018). For these species, interspecific variance (97%) greatly exceeded intraspecific variance (3%) in TL, suggesting that our age correction method will only slightly change interspecific TL comparisons. For the remaining species, we used mean TL estimates for the youngest individuals reported (all <1 year old), except for 8 species (Table S1), where TL is averaged across unknown age classes. The domestic chicken was excluded because no mean TL has been reported (Delany, Daniels, Swanberg, & Taylor, 2003; Delany et al., 2000) and the chicken has a long history of human domestication, which is likely to have altered its telomere biology (Pepke & Eisenberg, 2021) and genome biology (Piégu et al., 2020). Variation in the activity of telomerase, a ribonucleoprotein capable of rebuilding telomeres (reviewed in Criscuolo, Smith, Zahn, Heidinger, & Haussmann, 2018), is unmeasured in our study and may confound the estimation of early-life TL.

Cytogenetic data

For 20 species estimates of genome size (the amount of DNA in a haploid genome, i.e. c-value) measured in erythrocytes were compiled from the Animal Genome Size Database, 2.0 (Gregory, 2020). When more than one c-value estimate was available in the database, we followed Gregory (2020) and averaged c-values across studies using up to four comparable cytometric methodologies (Hardie, Gregory, & Hebert, 2002, see Table S1). C-values (pg) were converted to mega base pairs (Mb) by multiplying with 978 Mb/pg (Doležel, Bartoš, Voglmayr, & Greilhuber, 2003). When these estimates were not available, we searched the National Center for Biotechnology Information genetic sequence database GenBank (Clark, Karsch-Mizrachi, Lipman, Ostell, & Sayers, 2016) and recorded the length of whole genome sequence (WGS) assemblies (in Mb, 10 species, in addition to 2 species from Grayson, Sin, Sackton, & Edwards, 2017, Table S1). Genome size estimates from cytometric methods are highly correlated with data obtained from WGS projects (Elliott & Gregory, 2015). Sequencing methods seem to underestimate genome size in some cases, a discrepancy that increases with absolute genome size (Elliott & Gregory, 2015). Since birds have relatively small genomes (1.0-2.2 Gb, Kapusta et al., 2017), we do not try to correct for this, but use cytometric estimates when available. Number of chromosomes was compiled from Degrandi et al. (2020). Atypical karyotypes are known from birds (Damas, O'Connor, Griffin, & Larkin, 2019; de Boer & van Brink, 1982) and we did not attempt to infer missing cytogenetic data from closely related species. Average chromosome lengths were calculated by first dividing genome size by the number of (haploid) chromosomes and then subtracting the telomeric DNA component from each chromosome (average TL multiplied by 2 representing the number of telomere arms per chromosome). For 14 species the karyotype is not yet known, and we therefore also test the association between TL and genome size, as a proxy for chromosome length across 32 species.

Life-history data

Data on maximum lifespan (years) and average adult body mass (g) were compiled primarily from AnAge: The Animal Ageing and Longevity Database (Tacutu et al., 2018, Table S1 and S2), which combines data from captive and wild populations. Mean clutch size (mean number of eggs laid per clutch) and generation time (in years, based on modelled values of age at first reproduction, maximum lifespan, and annual adult survival) were primarily compiled from Bird et al. (2020), see Table S1. TL, mass, lifespan, and generation time were $\log_{10}$-transformed to linearize relationships observed in bivariate plots. We then used a phylogenetic principal component analysis (Revell, 2009) to construct a first principal component (PC1), which explained

55% of the variation in these traits and may reflect the fast (low values) to slow (high values) axis of variation in pace-of-life strategies (Table S3, Dantzer & Fletcher, 2015; Jeschke & Kokko, 2009).

Phylogenetic reconstruction

We used the most recent time-calibrated avian phylogeny (Jetz, Thomas, Joy, Hartmann, & Mooers, 2012) based on the Hackett et al. (2008) backbone. We compiled a set of 1,000 trees from BirdTree.org and summarized these into a single maximum clade credibility tree using the maxCladeCred function in the 'phangorn' package in R (Schliep, 2011). This tree was pruned using the 'ape' package (Paradis & Schliep, 2018) and visualized using the 'phytools' package (Revell, 2012). Ancestral states were estimated using the function 'fastAnc' (Revell, 2012).

Phylogenetic comparative analyses

Phylogenetic generalized least square regressions were performed using the 'pgls' function in 'caper' package (Orme et al., 2018), in which a variance-covariance matrix from the phylogenetic relationships (branch lengths) is compared to the actual covariance structure in the residual errors of the regression. The phylogenetic signal, λ, is a multiplier of the expected covariances (off-diagonal elements) that produces the actual variance-covariance matrix (Freckleton, Harvey, & Pagel, 2002; Pagel, 1999). Under the Pagel's λ (PA) model, branch length transformations are optimized numerically using maximum likelihood within default bounds (0.0-1.0, Orme et al., 2018). When $\lambda=0$ the covariance between species is zero corresponding to a non-phylogenetic (ordinary) regression, or "star model" (ST), normally indicating that the traits are evolutionary very labile (Blomberg, Garland Jr, & Ives, 2003; Kamilar & Cooper, 2013). When $\lambda=1$ the evolution of the residual error is best approximated by a Brownian motion (BM) model of evolution (Felsenstein, 1985), which is the case for many gradually evolving traits (Kamilar &

Cooper, 2013). The phylogenetic signal therefore estimates the extent to which associations between traits reflect their shared evolutionary history (Freckleton et al., 2002), i.e. the degree of similarity among closely related species compared to distantly related species. We ran bivariate linear regressions of $\log_{10}$-transformed TL (response variable) and chromosome length (18 species), genome size (32 species), PC1, $\log_{10}$-transformed maximum lifespan, and $\log_{10}$-transformed body mass (58 species), respectively. We also tested associations between TL and the life-history traits while accounting for either chromosome length or genome size using phylogenetic multiple regressions (Grafen, 1989). Due to the limited sample sizes, we ran alternative evolutionary models assuming either λ=1 (BM model), λ=0 (ST model) or the maximum-likelihood value of λ (PA model). We then used Akaike's information criteria corrected for small sample sizes (AIC_c) to determine the best model (Burnham & Anderson, 2002), which is reported in the results. All analyses were performed in R v. 3.6.3 (R Core Team, 2020).

Sensitivity and outlier analyses

We performed sensitivity and outlier analyses within the phylogenetic context to test the robustness of our results to species sampling. We used a phylogenetic leave-one-out deletion analysis implemented in the 'sensiPhy' package (Paterno, Penone, & Werner, 2018) to test if any species are strongly affecting the associations. Species were sequentially removed one at a time and the phylogenetic regression refitted using 'phylolm' (Tung Ho & Ané, 2014). Highly influential species (outliers resulting in a standardized difference in β-estimates >2, Paterno et al., 2018) were then excluded and the regressions analyses rerun to obtain more robust phylogenetic regression estimates. We used a jackknifing method randomly removing a proportion of species (from 10 to 50%) and then refitted all regression models described above 500 times to estimate sensitivity of β-, λ-, and p-values to changes in sample size (Paterno et al., 2018).

RESULTS

Telomere length evolution

The greatest diversity in early-life TL was found within Passeriformes (6.2-50.5 kb, 25 species), whereas the other orders had relatively shorter and less variable TLs (7.1-34.9 kb, 33 species, Fig. 1). The ancestral TL of the 58 bird species was inferred, with a wide confidence interval, to be relatively short (12.4 kb, 95% confidence interval (CI)=[-1.1, 25.8], 97.9 Mya) compared to the range of TLs observed in extant species, but close to their average TL (14.0 kb). Phylogenetic signals in life-history traits were close to 1 (Table S4), but for TL λ=0.00 (CI=[0.00, 0.46]).

Associations between telomere length and life-history traits

We found a significant negative association between TL and PC1 across all 58 species (best model: β_{PC1}=-0.003±0.001 S.E., p=0.042, adjusted R^2=0.055, λ=0.00, CI=[0.00, 0.27], Fig. 2a), suggestive of shorter TL in slower life-history species. There was also a negative association between TL and maximum lifespan ($\beta_{log(lifespan)}$=-0.230±0.094 S.E., p=0.018, adjusted R^2=0.080, λ=0.00, CI=[0.00, 0.27], Fig. 2b). Thus, a 1% increase in lifespan predicted a 0.23% decrease in TL. We found weak evidence for a negative association between body mass and TL ($\beta_{log(mass)}$=-0.042±0.026 S.E., p=0.107, adjusted R^2=0.029, λ=0.00, CI=[0.00, 0.28], Fig. 2c).

We identified up to four phylogenetically significant outliers in the regressions between TL and life-history traits, but their exclusion only led to slight attenuations of the associations (see Figs. S1-S3 in the Supporting Information). These associations were generally robust to smaller sample size effects (mean change in β with 50% species removed was 36-52%) and the phylogenetic association was 0 in most simulations (Figs. S4-S6).

Associations between telomere length, chromosome length and genome size

Genome size positively predicted chromosome length ($\beta_{genome\ size}$=0.024±0.005 standard error [S.E.], $p<0.001$, adjusted $R^2=0.599$, $\lambda=1.00$, CI=[0.00, 1.00], 18 species, Fig. S7), suggesting that genome size may be used as a proxy for chromosome length in birds.

We found weak evidence for a positive association between TL and chromosome length (best model: $\beta_{log(chromosome\ length)}$=1.345±1.029 S.E., $p=0.210$, $R^2=0.210$ $\lambda=0.00$, CI=[0.00, 1.00], 18 species, Fig. 3a). However, the phylogenetic outlier analysis identified one highly influential species, *Ciconia ciconia*, with a disproportional effect on the estimate (resulting in a change of β of 102%, see Paterno et al., 2018 and Fig. S8). Removal of this species revealed a substantial positive association between TL and chromosome length ($\beta_{log(chromosome\ length)}$=2.710±1.106 S.E., $p=0.027$, $R^2=0.238$ $\lambda=0.00$, CI=[0.00, 0.99], 17 species, Fig. 3a).

There was a marginally significant positive association between TL and genome size (best model: $\beta_{log(genome\ size)}$=1.420±0.826 S.E., $p=0.096$, adjusted $R^2=0.059$, 32 species, Fig. 3b). We identified three highly influential species, *Aphelocoma coerulescens*, *Parus major*, and *Riparia riparia* (β changes of 50-61%, Fig. S9), the exclusion of which slightly attenuated the fit ($\beta_{log(genome\ size)}$=1.158±0.742 S.E., $p=0.130$, $R^2=0.049$, $\lambda=0.00$, CI=[0.00, 0.74], 29 species, Fig. 3b).

The associations between TL, chromosome length, and genome size were relatively unstable to larger reductions in sample sizes (changes in β with around 50% of the species removed were 45-81%, Fig. S10-S11).

Multiple phylogenetic regressions of life-history and cytogenetic traits

The associations described above between life-history traits and TL, and between cytogenetic traits and TL were slightly or substantially attenuated when included in phylogenetic multiple regressions (Table S4). Mass, lifespan, and PC1 were only weakly correlated with genome size and chromosome length (Table S5), however, sample size was considerably reduced (by 44% when including genome size [32 species] and 68% when including chromosome length [18 species]).

DISCUSSION

In this study, we showed that variation in mean early-life TL was significantly associated with key life-history traits underlying the pace-of-life continuum across 58 bird species. This result is consistent with the hypothesis that TL may be an important mediator of life-history trade-offs between reproduction, somatic maintenance, and cancer risk (Heidinger et al., 2012; Monaghan, 2010; Tian et al., 2018). Furthermore, we found some support for a positive association between TL and mean chromosome length or genome size. This indicates that a component of variation in TL may be constrained by interactions with chromosome length across species (Klegarth & Eisenberg, 2018; Slijepcevic, 2016).

Early-life TL was significantly negatively associated with maximum lifespan, but we only found weak evidence for a negative association with body mass (Fig. 2b-c). In mammals, lifespan and mass are strongly negatively associated with mean TL (Gomes et al., 2011; Pepke & Eisenberg, 2021), suggesting that these are general relationships across tetrapods. In birds, body size is constrained by adaptations to flight (Tobalske, 2016) and body masses within our study vary by almost 3 orders of magnitude compared to 7 orders of magnitude within the study of mammalian TL (Gomes et al., 2011), which may explain the weaker association between TL and mass in birds. However, there is still a large variation in TL particularly among small species, within both mammals and birds. This variation may be explained by the larger diversity of mechanisms evolved to deal with cancer defenses or cellular senescence within smaller bodied species (Risques & Promislow, 2018; Seluanov, Gladyshev, Vijg, & Gorbunova, 2018). That larger and longer-lived species of tetrapods have short telomeres, has been interpreted as an anti-cancer mechanism, limiting the risk of accumulating oncogenic mutations through replicative cell senescence (Campisi, 2001; Gorbunova et al., 2014; Pepke & Eisenberg, 2021). Research on cancer prevalence in wild bird species is very limited (Møller, Erritzøe, & Soler, 2017; Pesavento, Agnew, Keel, & Woolard, 2018). Boddy et al. (2020) found a positive relationship between litter size and cancer prevalence in 37 mammal species. They suggested that the faster pace-of-life associated with larger litter sizes, exposed fast life-history species to higher cancer rates by allocating more resources to offspring quantity than somatic maintenance. In our study, fast-lived species had a low PC1 score (i.e. short generation time and lifespan, large clutch size, and to some extent a small body mass, Table S3) and long telomeres compared to slow-lived species with a high PC1 score and short telomeres (Fig. 2a). If fast life-history bird species also have higher cancer rates, as suggested by Møller et al. (2017), these observations are consistent with the fact that longer telomeres are associated with increased cancer prevalence across species (in mammals, Pepke & Eisenberg, 2021). Thus, TL may have evolved to be longer to avoid the greater risk of critically short telomeres faced by species with accelerated TL shortening due to increased oxidative stress associated with high rates of reproduction (Sudyka, 2019). Selection for longer telomeres may have been further promoted by the lower antioxidant capacity or higher levels of oxidative damage found in bird species with a faster pace-of-life (Vágási et al., 2019; Xia & Møller, 2018). Bird species with a slower pace-of-life have also been found to have a lower telomere shortening rate (Dantzer & Fletcher, 2015), which suggests that TL and TL attrition co-vary across species, but this has not yet been shown (Tricola et al., 2018).

The sensitivity and outlier analyses indicated that the associations between TL and cytogenetic traits were susceptible to sample size effects (Figs. S8-S11). However, our results suggest an interaction between TL evolution and karyotype evolution. We found that a 1% increase in chromosome length was associated with a 2.7% increase in TL (Fig. 3a). The taxonomic diversity of species exhibiting positive scaling between TL and chromosome length within species (reviewed in the introduction) suggests that this is a highly conserved, fundamental characteristic of telomere biology. The explanation behind the positive correlation between telomere and chromosome lengths, remains unknown, but several molecular mechanisms may be involved (Klegarth & Eisenberg, 2018).

Experiments in yeast have shown that if telomeric and centromeric sequences are inserted into plasmids, they become unstable, probably because they are being pulled away from each other during mitosis (Enomoto, Longtine, & Berman, 1994). Slijepcevic (2016) suggested that this telomere–centromere antagonism could explain the correlation between TL and chromosome length observed within some species, i.e. the length of telomeres closer to centromeres is shorter to mitigate interference during mitosis. Furthermore, longer telomeres may be needed to protect longer chromosomes from end denaturation and rearrangements (Pampalona, Soler, Genescà, & Tusell, 2010; Slijepcevic, 1998). Supporting the connection between TL and chromosome size, Pontremoli et al. (2018) found that positive selection on genes implicated in telomere homeostasis among mammals was related to the number of chromosome arms. Given that genome size is relatively conserved among mammals (Kapusta et al., 2017), the positive selection at these genes is likely driven by chromosome size and these genes might help calibrate specific telomeres to the corresponding chromosomes. Assuming causality of the telomere–chromosome length association, more chromosome arms results in multiple short telomeres. This may facilitate chromosomal rearrangements (Murnane, 2012; Sánchez-Guillén et al., 2015; Slijepcevic, 1998), but also lead to a higher recombination rate (Pardo-Manuel de Villena & Sapienza, 2001). TL is not only important in maintaining chromosome integrity, but also plays in a role in gene expression (Gottschling, Aparicio, Billington, & Zakian, 1990). The reversible silencing of genes near telomeres depends on TL and gene distance and is known as the telomere positioning effect (TPE, Baur, Zou, Shay, & Wright, 2001). TL may also regulate genes over much long distances (TPE-OLD, Kim & Shay, 2018) presumably via telomere looping. Thus, longer chromosomes may also require longer telomeres to maintain this function.

Among mammals, the association between TL and chromosome length was highly influenced by the karyotypic abnormal Indian Muntjac (Klegarth & Eisenberg, 2018). However, the association remained non-significant after outlier exclusion. This study relied primarily on estimates of adult TL from cultured cell lines (Gomes et al., 2011). Our analysis may have better resolution by including mainly terminal TLs in early life, thereby reducing the effects of differing TL changes through life (Tricola et al., 2018). Consistent with the mammalian sensitivity to the karyotypic abnormal Indian Muntjac, our results were strongly influenced by the outlying white stork, *Ciconia ciconia* (8.8 kb, Fig. 3a), of a genus known to have undergone several chromosomal rearrangements (de Boer & van Brink, 1982, Fig. S7). For instance, *C. ciconia* (2n=72) probably has many more microchromosomes than the black stork, *C. nigra* (2n=52, de Boer & van Brink, 1982), whose mean TL we may then expect to be long, but that is currently unknown. The observation of ultra-long telomeres on some microchromosomes (Nanda et al., 2002) does not conform with the general patterns observed in this study. Since microchromosomes constitutes only around 23% of the avian genome size and are remarkably conserved across most bird species

(Burt, 2002; O'Connor et al., 2019), it may be that the patterns reported here primarily retain to the telomere dynamics of macrochromosomes.

Within birds, larger genomes have been associated with lower metabolic rate (Vinogradov, 1997), reduced capacity for flight efficiency (Andrews, Mackenzie, & Gregory, 2009), and increased body size (Wright et al., 2014). The mechanism underlying these correlations may be acting through a positive relationship between genome size and cell size (Wright et al., 2014). However, if part of the variation in genome size is due to variation in telomere–chromosome length interactions, we suggest that some of these associations may involve adaptations in TL to different life-history strategies, as indicated in this study. For instance, correlations between life-history traits and genome size (Gregory, 2002; Gregory, 2005; Womack, Metz, & Hoke, 2019) may involve telomere–chromosome length dynamics.

We found TL to be evolutionary labile across bird species, as exemplified by the large intrageneric variation within *Aphelocoma*, *Larus*, *Tachycineta*, and *Thalassarche*, suggesting recent evolutionary change in TL (Fig. 1). Reconstructing the evolutionary history of TL changes within recent radiations of closely related species that represent independent replicated branching events, may elucidate adaptations underlying shifts in TL during speciation (Baird, 2018). As species progress through series of changes in species ecology and life-history (Pepke, Irestedt, Fjeldså, Rahbek, & Jønsson, 2019), associated changes in telomere biology may be observed within taxonomically more densely sampled clades (Canestrelli et al., 2020; Dupoué et al., 2017).

Our results indicate that some of the variation in early-life TL in birds arises through interactions with chromosome length, which may constrain the evolution of TL. Future cross-species studies attentive to the specificity of TL at different chromosome arms (Miga et al., 2020; Poon & Lansdorp, 2001), in particular of microchromosomes, may resolve the details of this interaction. Whether this effect has implications for telomere loss and the variation in senescence pattern across species remains unknown. However, mean TL also co-evolved with key life-history traits suggesting that the adaptive significance of TL may be as an important mediator of life-history trade-offs between investment in reproduction and somatic maintenance.

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AUTHOR CONTRIBUTIONS

MLP and DTAE conceived the ideas. MLP compiled and analyzed data and wrote the manuscript with contributions from all authors.

DATA ACCESSIBILITY

All data is available from Table S1 in the Supporting information and from BirdTree.org, and it will be submitted to an open access data repository.

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FIGURES

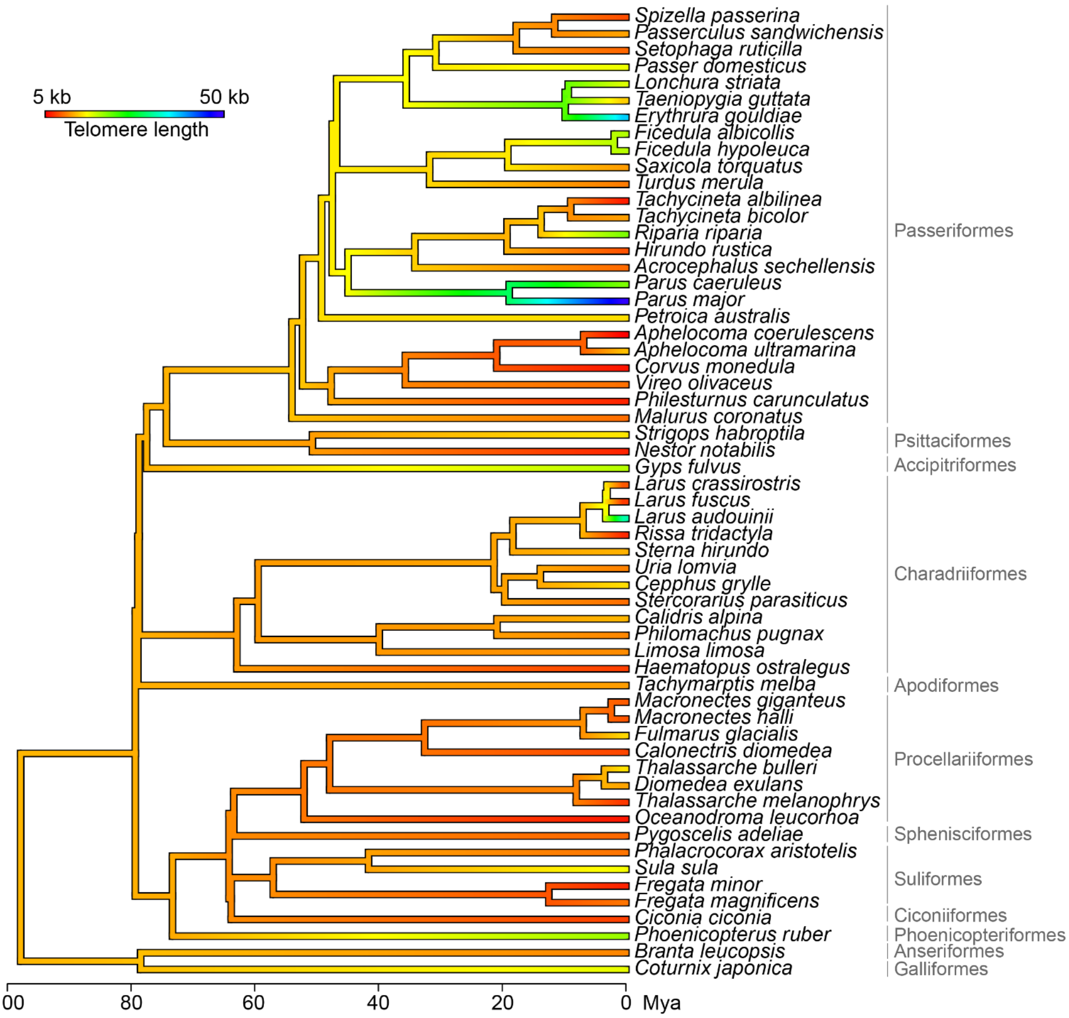

Fig. 1: Calibrated maximum clade credibility tree illustrating the evolution of early-life telomere length in 58 bird species using a continuous color gradient (red: short telomeres; blue: long telomeres). Orders are shown on the right, and the timescale is in million years ago (Mya).

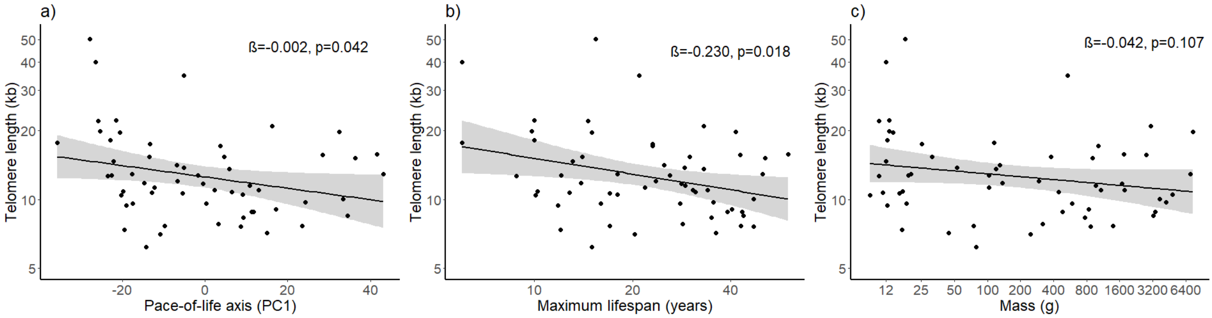

Fig. 2: Associations between log$_{10}$-transformed early-life telomere length (kb) and a) PC1 scores from a phylogenetic principal component analysis reflecting the slow-fast continuum of life-history trait variation across 58 bird species, b) log$_{10}$-transformed maximum lifespan in years, and c) log$_{10}$-transformed body mass in g. Scatter plots do not depict phylogenetic corrections. Phylogenetic regression lines and their associated statistics are shown. Grey shadings correspond to 95% confidence intervals.

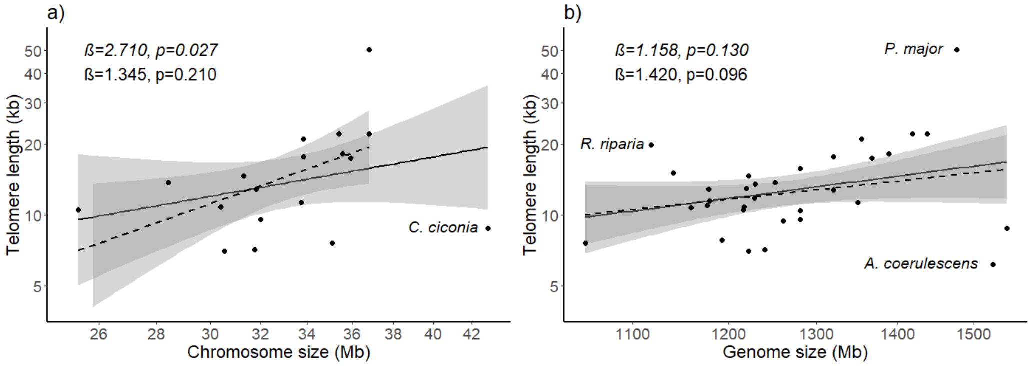

Fig. 3: Associations between average log$_{10}$-transformed early-life telomere length (kb) and a) chromosome size (Mb) for 18 bird species and b) genome size (Mb) for 32 bird species. Scatter plots do not show phylogenetic corrections. Phylogenetic regression lines and their associated statistics are shown (solid lines). The phylogenetically identified outliers *Ciconia Ciconia* (a), and *Aphelocoma coerulescens*, *Parus major*, and *Riparia riparia* (b) are excluded from the regression analyses shown in italics and with dashed regression lines. Grey shadings correspond to 95% confidence intervals.

Supporting information

Early-life telomere length covaries with life-history traits and scales with chromosome length in birds

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Contents

Data and sources

Table S1: Estimates of mean initial telomere length (TL), cytogenetic and life-history data gathered for this study. See Materials and Methods in the main text for main database sources (alternative sources are listed as footnotes). The taxonomy follows Gill et al. (2021). Dashes indicate data not available.

Species name	**Common name**	**Family**	**TL (kb)**	**Genome size (Mb)**	**Genome size method**(1)	**Chromosome size (Mb)**	**Chromosome number (n)**	**Body mass (g)**	**Max. lifespan (years)**	**Mean clutch size**	**Generation time (years)**	**PC1**	**TL reference**
Acrocephalus sechellensis	Seychelles warbler	Acrocephalidae	10.6(3)	-	-	-	-	15.9	17.0	1.5	6.7	-5.7	Ellis Mulder & David S. Richardson, *unpublished*(2)
Aphelocoma coerulescens	Florida scrub jay	Corvidae	6.2	1526	FCM	-	-	78.7	15.0	4.0	5.2	-14.5	Tricola et al., 2018
Aphelocoma ultramarina	Transvolcanic jay	Corvidae	14.1	-	-	-	-	130.0	25.0	4.0	7.2	-7.2	Tricola et al., 2018
Branta leucopsis	Barnacle goose	Anatidae	11.7(3)	-	-	-	-	1687.0	28.2	4.5	10.0	-0.9	Pauliny et al., 2012
Calidris alpina	Dunlin	Scolopacidae	13.7	1252	FD	28.5	44	52.5	28.8	4.0	7.2	-5.4	Pauliny et al., 2006
Calidris pugnax	Ruff	Scolopacidae	11.8	1229	WGS	-	-	136.0	13.9	4.0	5.2	-14.9	Tricola et al., 2018
Calonectris borealis	Cory's shearwaters	Procellariidae	9.1	-	-	-	-	836.9(4)	40.3(5)	1.0	13.7	16.6	Bauch et al., 2020a
Cepphus grylle	Black guillemot	Alcidae	15.4	-	-	-	-	378.0	29.9	1.5	9.2	4.2	Tricola et al., 2018
Chloebia gouldiae	Gouldian finch	Estrildidae	40.0(3)	-	-	-	-	12.0	6.0	5.5	1.9	-26.6	Fragueira et al., 2019
Ciconia ciconia	White stork	Ciconiidae	8.8	1545	FCM	42.9	36	3350.0	39.0	4.0	12.5	11.2	Pineda-Pampliega et al., 2020
Coloeus monedula	Western jackdaw	Corvidae	7.0	1223	FD	30.6	40	246.0	20.3	4.0	5.6	-11.2	Bauch et al., 2020b

Coturnix japonica	Japanese quail	Phasianidae	17.7	1320	FD, FCM	33.9	39	115.0	6.0	9.5	2.2	-35.9	Stier et al., 2020
Cyanistes caeruleus	Eurasian blue tit	Paridae	22.1(3)	1438	SCF	36.9	39	10.3	14.6	7.5	2.9	-26.1	Atema et al., 2019
Diomedea exulans	Wandering albatross	Diomedeidae	12.9(13)	1220	WGS(6)	-	-	7047.0	50.0	1.0	22.9	42.2	Hall et al., 2004
Ficedula albicollis	Collared flycatcher	Muscicapidae	19.9	1118	WGS	-	-	12.7	9.8	6.0	2.3	-25.5	Stier et al., 2020
Ficedula hypoleuca	European pied flycatcher	Muscicapidae	19.7	-	-	-	-	13.9	15.0	6.0	4.1	-20.8	Kärkkäinen et al., 2019
Fregata magnificens	Magnificent frigatebird	Fregatidae	11.0(13)	1177	WGS	-	-	1078.0	34.0	1.0	12.6	12.6	Sebastiano et al., 2017
Fregata minor	Great frigatebird	Fregatidae	7.6	-	-	-	-	1400.0	43.0	1.0	17.2	22.9	Tricola et al., 2018
Fulmarus glacialis	Northern fulmar	Procellariidae	15.1	1141	WGS	-	-	908.0	51.0	1.0	25.3	35.7	Tricola et al., 2018
Gyps fulvus	Griffon vulture	Accipitridae	19.8(7)	-	-	-	33	7436.0	41.4	1.0	17.0	31.6	Whittemore et al., 2019
Haematopus ostralegus	Eurasian oystercatcher	Haematopodidae	8.8	-	-	-	33	480.0	43.3	3.5	13.3	10.8	Tricola et al., 2018
Hirundo rustica	Barn swallow	Hirundinidae	9.6	1281	FIA	32.0	40	18.3	16.0	4.5	3.1	-17.7	Tricola et al., 2018
Larus audouinii	Audouin's gull	Laridae	34.9(7)	-	-	-	-	535.0	20.9	3.0	7.7	-5.5	Whittemore et al., 2019
Larus crassirostris	Black-tailed gull	Laridae	9.6	-	-	-	-	589.4(8)	28.0(8)	2.5	8.2	0.0	Mizutani et al., 2016
Larus fuscus	Lesser black-backed gull	Laridae	8.3(13)	-	-	-	34	766.2	34.9	2.5	12.6	8.9	Foote et al., 2011a
Limosa limosa	Black-tailed godwit	Scolopacidae	12.0(3)	-	-	-	45	294.0	23.6	4.0	7.7	-7.0	Atema et al., 2011; 2019

Lonchura striata	White-rumped munia	Estrildidae	18.2	1389	FCM	35.6	39	12.3	10.0	5.0	2.1	-23.1	Tricola et al., 2018
Macronectes giganteus	Southern giant petrel	Procellariidae	10.0(13)	-	-	-	40	3698.0	47.0	1.0	20.6	32.6	Foote et al., 2011b
Macronectes halli	Northern giant petrel	Procellariidae	9.7(13)	-	-	-	-	4206.3(9)	35.4(9)	1.0	17.1	23.6	Foote, 2008
Malurus coronatus	Purple-crowned fairy-wren	Maluridae	10.7(3)	-	-	-	-	11.1(9)	12.8(9)	2.5	4.2	-13.1	Eastwood et al., 2018
Nestor notabilis	Kea	Strigopoidea	7.6(13)	1054	WGS	35.1	30	867.5	47.0	3.0	7.9	8.1	Horn, 2008
Oceanodroma leucorhoa	Leach's storm petrel	Hydrobatidae	7.1	1240	WGS(6)	31.8	39	44.6	36.0	1.0	14.8	14.5	Tricola et al., 2018
Parus major	Great tit	Paridae	50.5	1477	SCF	36.9	40	17.9	15.4	8.5	3.0	-28.0	Tricola et al., 2018
Passer domesticus	House sparrow	Passeridae	17.5	1367	FCM, FD, SCF	36.0	38	25.3	23.0	4.5	3.7	-13.6	Ringsby et al., 2015
Passerculus sandwichensis	Savannah sparrow	Emberizidae	12.9	1178	FIA	31.9	37	20.2	18.0	4.5	2.2	-17.8	Tricola et al., 2018
Petroica australis	South Island robin	Petroicidae	15.4(13)	-	-	-	-	31.3(10)	14.0(11)	2.8(12)	3.8	-13.7	Horn, 2008
Phalacrocorax aristotelis	European shag	Phalacrocoracidae	11.0(13)	-	-	-	-	1773.0	30.6	3.5	9.3	1.9	Hall et al., 2004
Philesturnus carunculatus	South Island saddleback	Callaeatidae	7.6(13)	-	-	-	-	74.7(6)	17.0(9)	2.0(10)	4.0	-10.0	Horn, 2008
Phoenicopterus ruber	American flamingo	Phoenicopteridae	21.0(7)	1355	FCM, FD	33.9	40	3066	33.0(9)	1.0	13.0	15.7	Whittemore et al., 2019
Pygoscelis adeliae	Adélie penguin	Spheniscidae	10.5	1217	WGS	25.3	48	4847.7(10)	18.0(9)	2.0	12.8	8.6	Tricola et al., 2018
Rissa tridactyla	Black-legged kittiwake	Laridae	7.8(3,13)	1193	WGS	-	-	317.0	28.5	2.0	9.8	2.9	Schultner et al., 2014

Riparia riparia	Sand martin	Hirundinidae	22.1	1418	FIA	35.5	40	12.7	10.0	4.5	2.3	-21.7	Pauliny et al., 2006
Saxicola torquatus	African stonechat	Muscicapidae	12.7	-	-	-	40	10.3	8.8	5.0	2.1	-23.7	Apfelbeck et al., 2019
Setophaga ruticilla	American redstart	Parulidae	10.4	1281	FIA	-	-	8.5	10.1	4.0	2.3	-20.4	Angelier et al., 2013
Spizella passerina	Chipping sparrow	Passerellidae	9.4	1262	FIA	-	-	12.2	11.8	4.0	2.5	-19.3	Foote et al., 2013
Stercorarius parasiticus	Parasitic jaeger	Stercorariidae	10.8(13)	1160	WGS	-	-	445.5	31.1	2.0	11.2	6.0	Trondrud, 2017
Sterna hirundo	Common tern	Laridae	13.5	1230	WGS	-	34	120.0	33.0	2.0	10.4	5.5	Tricola et al., 2018
Strigops habroptila	Kakapo	Psittacidae	15.8(13)	1281	FIA	-	-	1750.0	60.0	1.5	25.8	40.8	Horn et al., 2011
Sula sula	Red-footed booby	Sulidae	17.1	-	-	-	-	1017.0	23.0	1.0	9.4	3.3	Tricola et al., 2018
Tachycineta albilinea	Mangrove swallow	Hirundinidae	7.4	-	-	-	-	16.7(10)	12.0(14)	4.0	2.2	-19.6	Tricola et al., 2018
Tachycineta bicolor	Tree swallow	Hirundinidae	12.8	1320	FIA	-	-	19.0	12.1	5.5	2.6	-22.8	Tricola et al., 2018
Tachymarpti melba	Alpine swift	Apodiformes	12.7(3, 13)	-	-	-	-	102.7	26.0	2.5	8.1	-1.7	Criscuolo et al., 2009
Taeniopygia guttata	Zebra finch	Estrildidae	14.7	1223	FCM	31.3	39	12.0	13.1(15)	5.0	1.6	-22.2	Tricola et al., 2018
Thalassarche bulleri	Buller's albatross	Diomedeidae	15.6(13)	-	-	-	-	2781.5(10)	42.6(16)	1.0	19.6	27.8	Horn, 2008
Thalassarche melanophris	Black-browed albatross	Diomedeidae	8.5(13)	-	-	-	-	3232.0	43.7	1.0	23.6	33.8	Dupont et al., 2018
Turdus merula	Common blackbird	Turdidae	11.3	1350	FD, SCF	33.7	40	103.2	21.8	4.0	4.0	-12.5	Ibáñez-Álamo et al., 2018
Uria lomvia	Thick-billed murre	Alcidae	11.5	1179	WGS	-	-	964.0	29.0	1.0	12.9	10.3	Tricola et al., 2018

Vireo olivaceus	Red-eyed vireo	Vireonidae	10.9	1218	FIA	30.4	40	17.0	10.2	4.0	2.6	-20.0	Foote et al., 2013

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[1] FCM: flow cytometry; SCF: static cell fluorometry; FD: Feulgen densitometry; FIA: Feulgen image analysis densitometry; WGS: whole genome sequencing.

[2] Methodology in Atema et al. (2019).

[3] Average TL across unknown age classes (mainly adults).

[4] Average of weights reported in Granadeiro (1993).

[5] Unpublished ringing data, see Cooper (2020, *unpublished*).

[6] Grayson et al. (2017).

[7] TL estimate from HT Q-FISH, see Whittemore et al., 2019.

[8] Dantzer & Fletcher (2015).

[9] Bird et al. (2020).

[10] Wilman et al. (2014).

[11] Taylor et al. (2008).

[12] Jetz et al. (2008).

[13] TL estimate from denatured TRF, see Materials and Methods.

[14] Tricola et al. (2018).

[15] This study (see Table S2).

[16] Maximum lifespan for *Thalassarche bulleri* is undetermined (but >21 years, Horn, 2008) so we used the average maximum lifespan (42.6 years) of the congeneric *T. chrysostoma*, *T. melanophris* and *T. chlororhynchos* (which are all similarly long-lived; 37-47 years) from Tacutu et al. (2018).

New longevity record for Zebra finch

Table S2: A colony of zebra finches (*Taeniopygia guttata*) was established by Claus Bech (*pers. comm.*) in January 2001 from individuals of reproductive age (3-4 months old) brought from a breeder. Housing conditions are described in Rønning et al. (2005) and all birds were provided with seed food and water *ad libitum* and allowed to breed.

ID	**Sex**	**Hatched**	**Died**	**Lifespan (days)**	**Lifespan (years)**
Blue/blue/white/ring	Male	Around 2000/10/01	2013/11/01	4779	13.1
73 blue	Male	2001/03/23	2014/02/27(1)	4724	12.9
156 striped	Male	2001/06/14	2014/02/27(1)	4641	12.7

(1) Last date the bird was observed alive.

PCA of life-history trait variation

Table S3: Loadings from a phylogenetically corrected principal component analysis (joint estimation of λ=0.86) of four life-history traits in 57 bird species. PC1 explained 55% of the variation among these traits. PC2 explained 22% of the variation and was primarily informed by body mass (89%).

Trait	**PC1**	**PC2**
Generation time	0.914	-0.126
Maximum lifespan	0.861	-0.222
Clutch size	-0.644	0.155
Mass	0.457	0.888

Phylogenetic outlier analyses of telomere length and life-history traits

Figure S1: Outlier analysis of the phylogenetic regression between $\log_{10}$(TL) and PC1 (58 species, Fig. 2a). Four highly influential species (*Parus major*, *Chloebia gouldiae*, *Gyps fulvus*, *Strigops habroptila*) were identified. The association was slightly attenuated when these were removed (β_{PC1} =-0.002±0.001 S.E., p=0.061, adjusted R^2=0.048, λ=0.00, CI=[0.00, 0.23], 54 species).

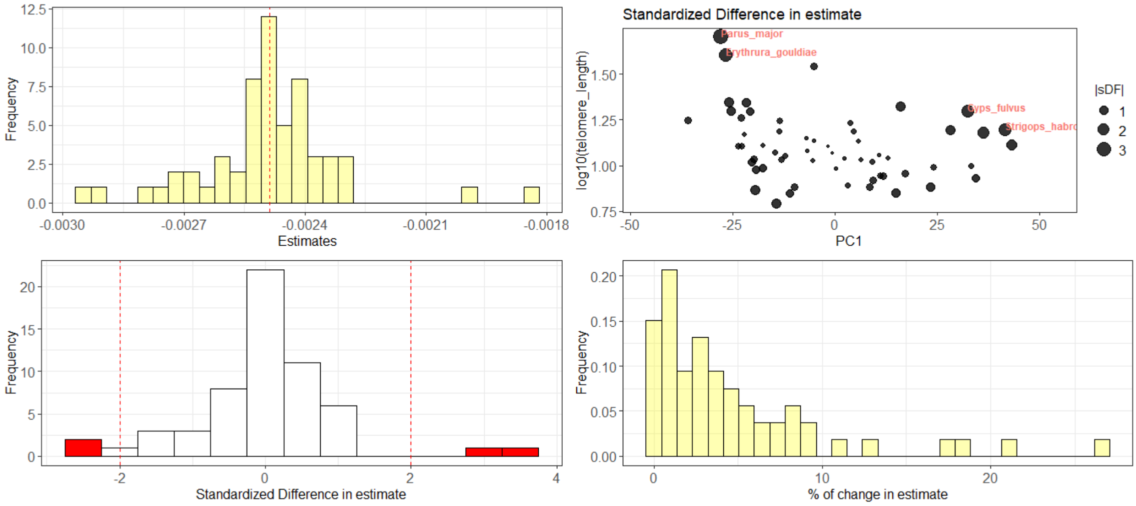


Figure S2: Outlier analysis of the phylogenetic regression between $\log_{10}$(TL) and $\log_{10}$(lifespan) (58 species, Fig. 2b). One highly influential species (*Chloebia gouldiae*) was identified. The association was attenuated when this species was removed ($\beta_{log(lifespan)}$=-0.163±0.095 S.E., p=0.092, adjusted R^2=0.033, λ=0.00, CI=[0.00, 0.25], 57 species).

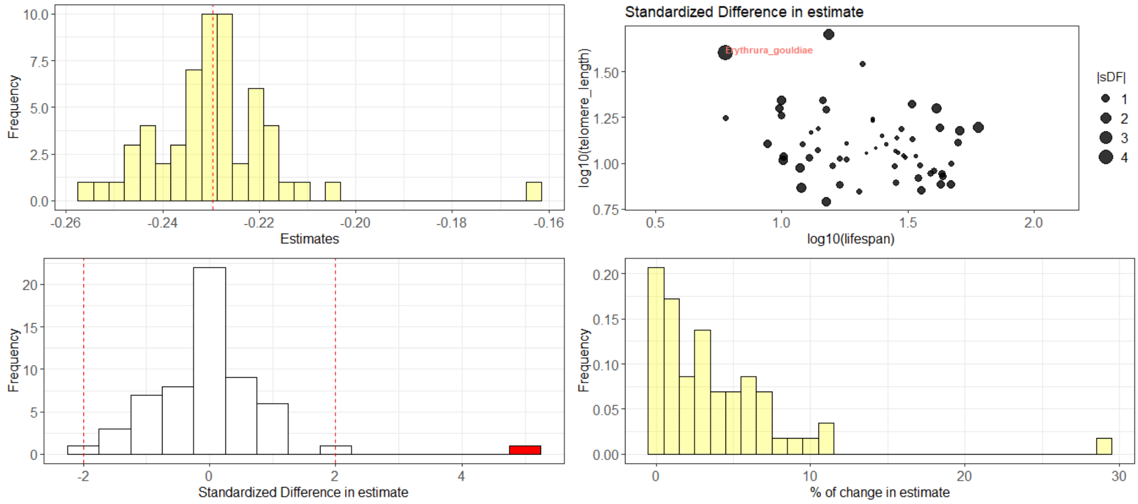
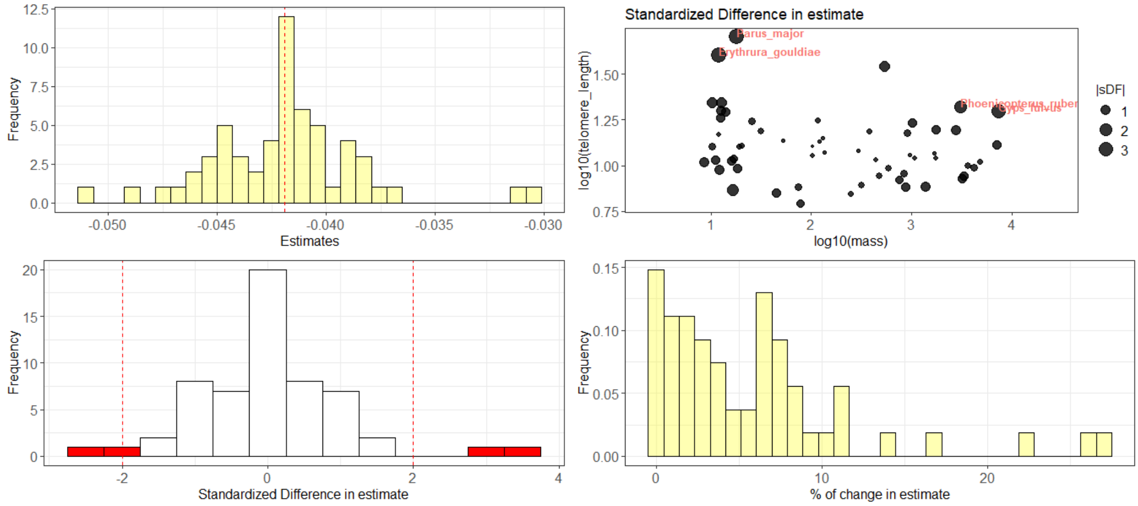


Figure S3: Outlier analysis of the phylogenetic regression between $\log_{10}$(TL) and $\log_{10}$(mass) (58 species, Fig. 2c). Four highly influential species (*Parus major*, *Chloebia gouldiae*, *Gyps fulvus*, *Phoenicopterus ruber*) were identified. The association was slightly attenuated when these were removed ($\beta_{log(mass)}$=-0.035±0.022 S.E., p=0.119, adjusted R^2=0.028, λ=0.00, CI=[0.00, 0.19], 54 species).



Phylogenetic sensitivity analyses of telomere length and life-history traits

Figure S4: Sensitivity analysis of the phylogenetic regression between $\log_{10}$(TL) and PC1 (58 species, Fig. 2a). The β-estimates were relatively robust to sample size effects (mean change in β with 50% of the species removed was 42%).

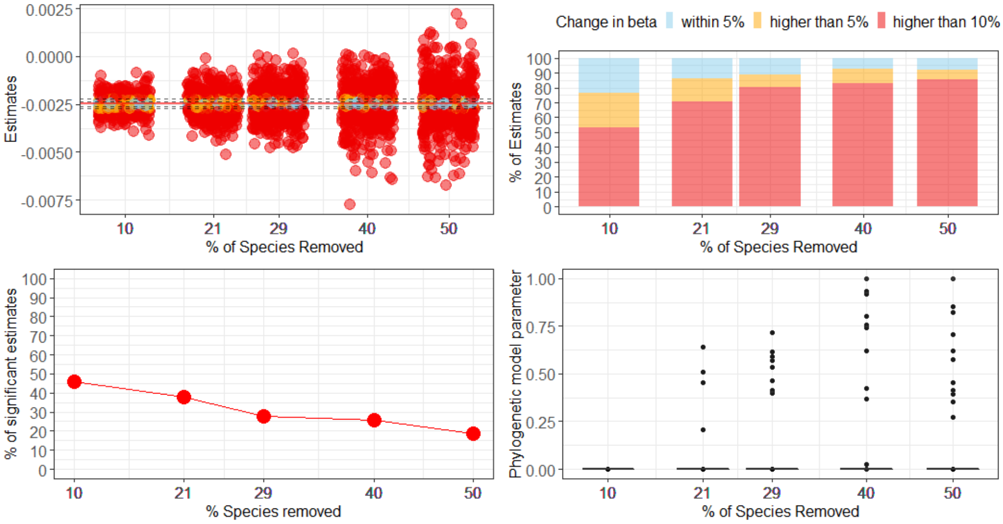


Figure S5: Sensitivity analysis of the phylogenetic regression between $\log_{10}$(TL) and $\log_{10}$(lifespan) (58 species, Fig. 2b). The β-estimates were relatively robust to sample size effects (mean change in β with 50% of the species removed was 36%), and 31% of the simulations suggested a significant relationship with 49% of the species removed.

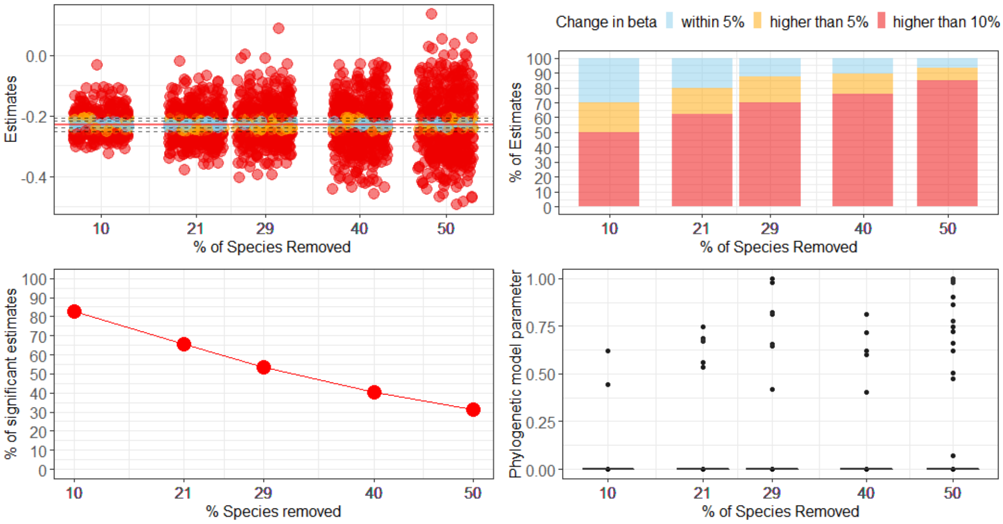
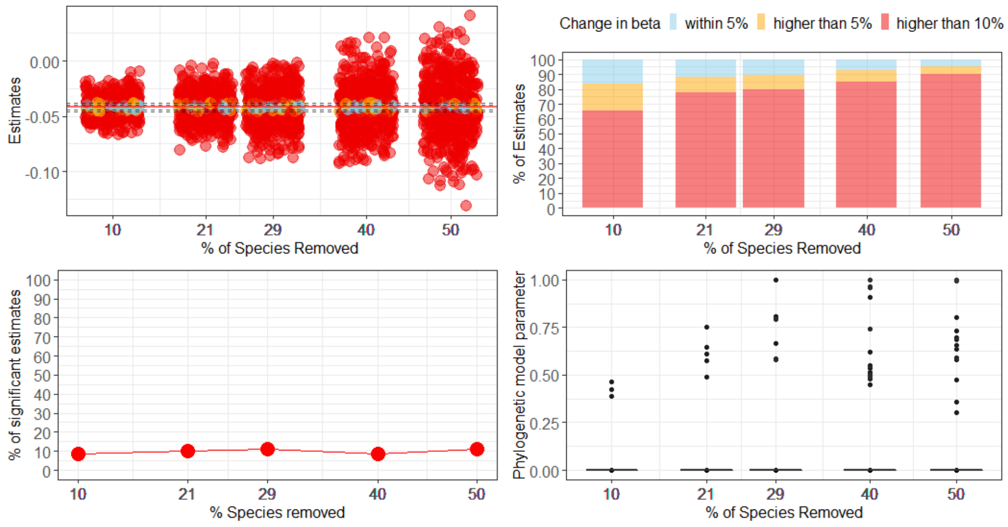


Figure S6: Sensitivity analysis of the phylogenetic regression between $\log_{10}$(TL) and $\log_{10}$(mass) (58 species, Fig. 2c). The β-estimates were relatively robust to sample size effects (mean change in β with 50% of the species removed was 52%). The association remained non-significant ($p>0.05$) under most simulations.



Association between chromosome length and genome size

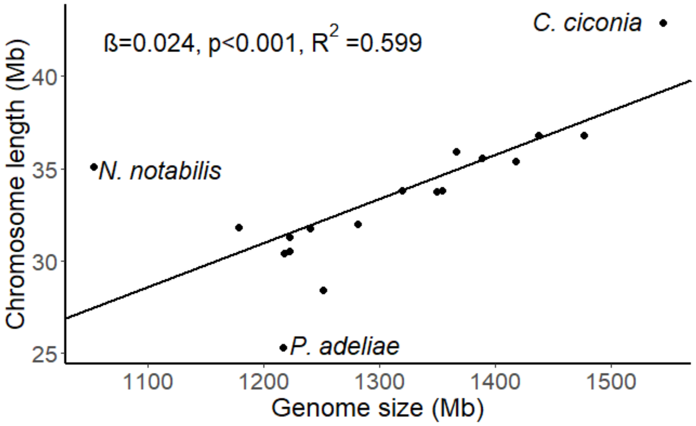


Figure S7: Genome size significantly predicted chromosome length in 18 bird species ($\beta_{genome\ size}$=0.024±0.005 S.E., p<0.001, adjusted R^2=0.599, λ=1.00, CI=[0.00, 1.00]). The outliers kea (*Nestor notabilis*, which has the smallest haploid chromosome number [30] and the smallest genome [1.05 Gb]), Adélie penguin (*Pygoscelis adeliae*), and white stork (*Ciconia ciconia*) are indicated, but not excluded from the analysis. Rearrangements of macrochromosomes have occurred within e.g. Psittaciformes and Ciconiiformes, resulting in an atypical chromosome numbers (de Boer & van Brink, 1982; Nanda et al., 2007).

Phylogenetic outlier analyses of telomere length and cytogenetic traits

Figure S8: Outlier analysis of the phylogenetic regression between $\log_{10}$(TL) and $\log_{10}$(chromosome size) (18 species, Fig. 2a). One highly influential species (*Ciconia ciconia*) was identified.

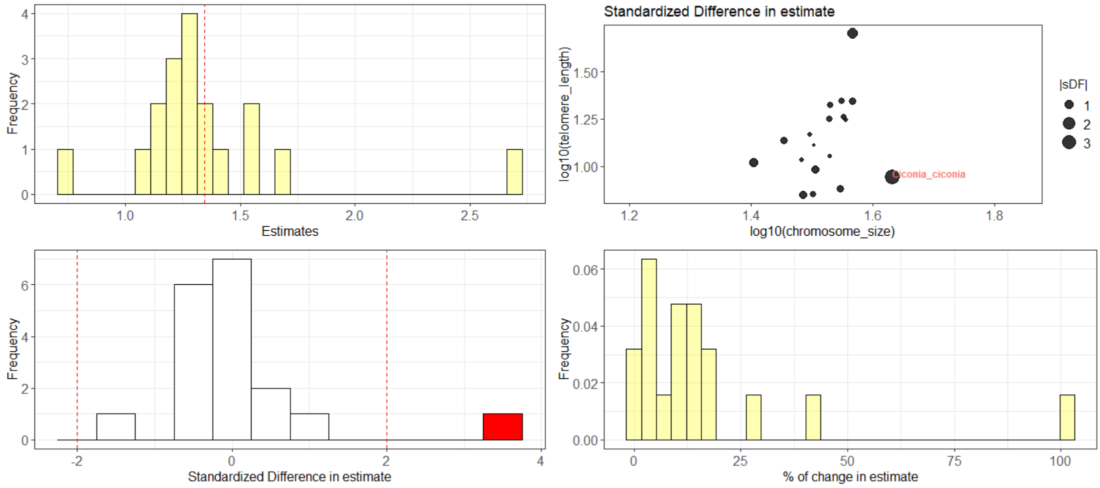
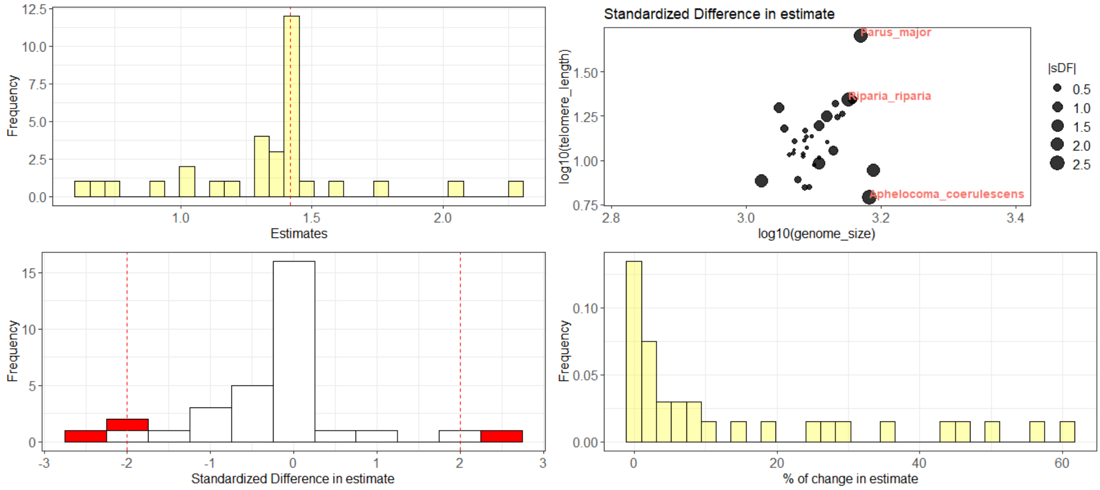


Figure S9: Outlier analysis of the phylogenetic regression between $\log_{10}$(TL) and $\log_{10}$(genome size) (32 species, Fig. 2b). Three highly influential species (*Parus major*, *Riparia riparia*, and *Aphelocoma coerulescens*[1]) were identified.



[1] The Florida scrub jay (*A. coerulescens*) has very short mean TL (6.2 kb) despite a large genome (1.53 Gb; estimated using flow cytometry). However, all individuals (43) sampled within this species were older than 2 years and 40% were ≥7 years (see Tricola et al., 2018), which could lead to an underestimation of the extrapolated initial TL, which shortens fastest during early life in many bird species (e.g. Salomons et al., 2009). Furthermore, a genome

assembly of *A. coerulescens* suggested that the flow cytometry genome size may be overestimated (WGS estimate of 1.08 Gb [Zhang et al., 2014], which is close to that of species with similarly short telomeres, e.g. *Nestor notabilis*, Table S1). In contrast, the congeneric larger transvolcanic jay (*A. ultramarine*) has telomeres twice as long (14.6 kb), but the genome size of this species has not yet been established.

Phylogenetic sensitivity analyses of telomere length and cytogenetic traits

Figure S10: Sensitivity analysis of the phylogenetic regression between $\log_{10}$(TL) and $\log_{10}$(chromosome size) (17 species, Fig. 2a). The β-estimates were relatively robust with respect to small sample size effects (but mean change in β with 47% [8] of the species removed was 45% and the association remained significant [$p<0.05$] within 27% of the reduced datasets). Pagel's λ ("phylogenetic model parameter") collapsed at the boundary (0) for most simulations. The sample size is considerably reduced when reconstructing chromosome length evolution and power to detect phylogenetic signal in datasets with fewer than 20 species may be rather low (Freckleton et al., 2002; Blomberg et al., 2003; Garland et al., 2005) although Pagel's λ have been shown to perform well in such case (i.e. resulting in a low rate of misidentification of phylogenetic signals in randomly evolving traits, Münkemüller et al., 2012).

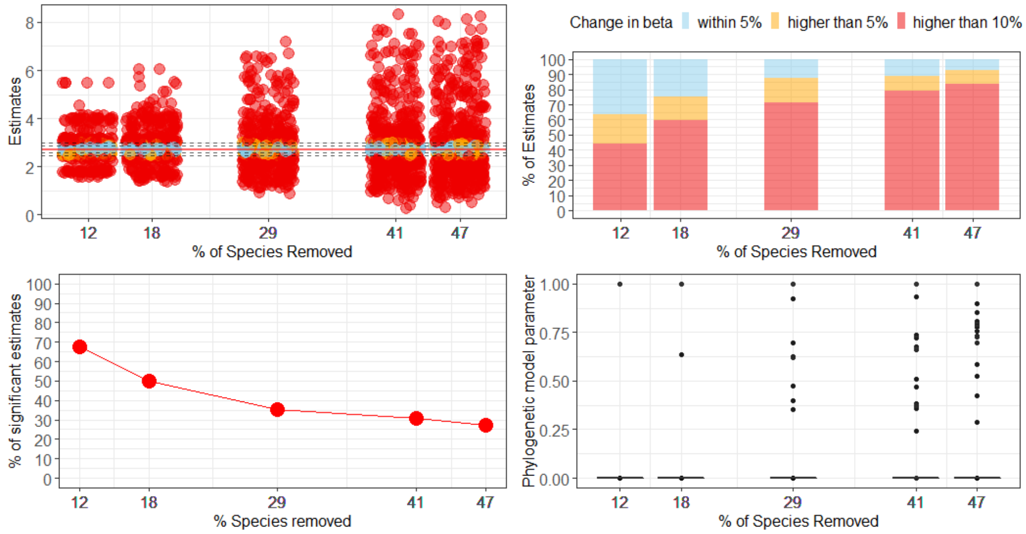
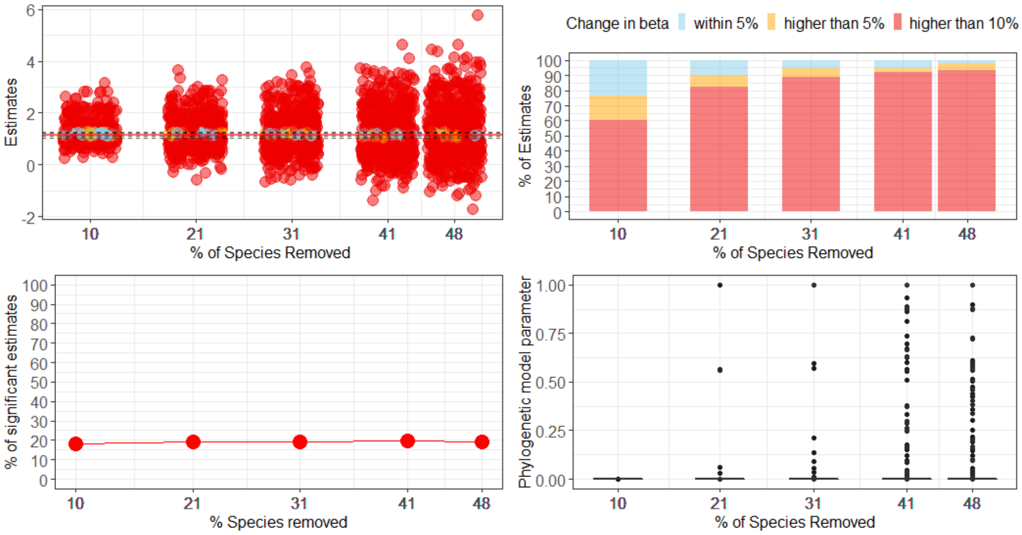


Figure S11: Sensitivity analysis of the phylogenetic regression between $\log_{10}$(TL) and $\log_{10}$(genome size) (29 species, Fig. 2b). The β-estimates were not robust to sample size effects (mean change in β with 48% [14] of the species removed was 81%).



Multiple phylogenetic regressions of telomere length, cytogenetic and life-history traits

Table S4: Phylogenetic multiple regression analyses of the associations between $\log_{10}$-transformed early-life telomere length (TL) as response variable, where A) body mass, B) maximum lifespan and C) PC1 are the focal explanatory variable within the total data set (58 species) and species subsets with data on genome size (32 species, second column) or chromosome length (18 species, third column). Phylogenetic signals in the $\log_{10}$-transformed species traits were respectively: TL (λ=0.00, CI=[0.00, 0.46]), chromosome length (λ=0.00, CI=[0.00, 1.00]), genome size (λ=0.22, CI=[0.00, 0.97]), body mass (λ=1.00, CI=[0.95, 1.00]), maximum lifespan (λ=0.86, CI=[0.65, 0.96]), and (untransformed) PC1 (λ=0.94, CI=[0.83, 0.99]). The bivariate associations between life-history traits and TL were strengthened in most cases when sample size was reduced from 58 to 18 species, but uncertainty of the regression coefficients increased, which is likely due to random sample size effects when the sample size becomes small (Figs. S4-6). The table also shows the estimates from phylogenetic multiple regressions controlling for genome size (32 species) or chromosome length (18 species). β denotes regression coefficients, *S.E.* denotes standard errors, t is the t-value, and p the significance value. R^2-values reported are adjusted for the number of predictors.

A **58 species: $\log_{10}$(TL)**	***β***	***S.E.***	***t***	***p***	**32 species: $\log_{10}$(TL)**	***β***	***S.E.***	***t***	***p***	**18 species: $\log_{10}$(TL)**	***β***	***S.E.***	***t***	***p***
intercept	1.099	0.024	46.641	<0.001 ***	intercept	1.096	0.033	33.069	<0.001 ***	intercept	1.060	0.055	19.347	<0.001 ***
PC1	-0.003	0.001	-2.084	0.042 *	PC1	-0.002	0.002	-1.372	0.180	PC1	-0.007	0.003	-2.495	0.024*
λ=0.00 (95% CI: 0.00, 0.27), R^2=0.055					λ=0.00 (95% CI: 0.00, 1.00), R^2=0.028					λ=0.00 (95% CI: 0.00, 0.91), R^2=0.235				
					intercept	-2.540	2.709	-0.938	0.356	intercept	-0.607	1.385	-0.438	0.668
					PC1	-0.001	0.002	-0.895	0.378	PC1	-0.007	0.003	-2.377	0.031*
					$\log_{10}$(genome size)	1.172	0.874	1.342	0.190	$\log_{10}$(chromo-some length)	1.098	0.912	1.204	0.247
					λ=0.00 (95% CI: 0.00, 0.98), R^2=0.053					λ=0.00 (95% CI: 0.00, 0.97), R^2=0.256				
B **58 species: $\log_{10}$(TL)**	***β***	***S.E.***	***t***	***p***	**32 species: $\log_{10}$(TL)**	***B***	***S.E.***	***t***	***p***	**18 species: $\log_{10}$(TL)**	***β***	***S.E.***	***t***	***p***
intercept	1.410	0.128	10.977	<0.001 ***	intercept	1.332	0.170	7.837	<0.001 ***	intercept	1.646	0.264	6.232	<0.001 ***
$\log_{10}$(lifespan)	-0.230	0.094	-2.438	0.018*	$\log_{10}$(lifespan)	-0.172	0.127	-1.351	0.187	$\log_{10}$(lifespan)	-0.401	0.205	-1.952	$0.069^{\dagger}$
λ=0.00 (95% CI: 0.00, 0.27), R^2=0.080					λ=0.00 (ST model), R^2=0.026					λ=0.00 (95% CI: 0.00, 1.00), R^2=0.142				

					intercept	-2.450	2.727	-0.898	0.376	intercept	-0.673	1.409	-0.478	0.640
					$\log_{10}$(lifespan)	-0.122	0.131	-0.933	0.359	$\log_{10}$(lifespan)	-0.434	0.196	-2.217	0.042*
					$\log_{10}$(genome size)	1.197	0.861	1.389	0.175	$\log_{10}$(chromosome length)	1.552	0.927	1.673	0.115
					λ=0.00 (95% CI: 0.00, 0.98), R^2=0.055					λ=0.00 (95% CI: 0.00, 1.00), R^2=0.229				
C **58 species: $\log_{10}$(TL)**	***β***	***S.E.***	***t***	***p***	**32 species: $\log_{10}$(TL)**	***β***	***S.E.***	***t***	***p***	**18 species: $\log_{10}$(TL)**	***β***	***S.E.***	***t***	***p***
intercept	1.196	0.062	19.284	<0.001 ***	intercept	1.210	0.080	15.186	<0.001 ***	intercept	1.305	0.115	11.346	<0.001 ***
$\log_{10}$(mass)	-0.042	0.026	-1.639	0.107	$\log_{10}$(mass)	-0.050	0.035	-1.414	0.168	$\log_{10}$(mass)	-0.088	0.055	-1.600	0.129
λ=0.00 (95% CI: 0.00, 0.28), R^2=0.029					λ=0.00 (95% CI: 0.00, 1.00), R^2=0.031					λ=0.00 (95% CI: 0.00, 0.93), R^2=0.084				
					intercept	-2.605	2.634	-0.989	0.331	intercept	-0.588	1.519	-0.387	0.704
					$\log_{10}$(mass)	-0.039	0.036	-1.094	0.283	$\log_{10}$(mass)	-0.083	0.054	-1.536	0.145
					$\log_{10}$(genome size)	1.221	0.843	1.449	0.158	$\log_{10}$(chromosome length)	1.238	0.991	1.250	0.231
					λ=0.00 (95% CI: 0.00, 0.97), R^2=0.065					λ=0.00 (95% CI: 0.00, 0.97), R^2=0.115				

$^{\dagger}p<0.1$, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$

Correlation coefficients between explanatory variables

Table S5: Pearson's correlation coefficients between cytogenetic and life-history traits included in the multiple regressions in Table S2. We used the value of λ=0.00 (i.e. ST model) obtained in all association in Table S2.

	$\log_{10}$(mass)	$\log_{10}$(lifespan)	PC1
32 species: $\log_{10}$(genome size)	0.120 (*p*=0.238)	0.214 (*p*=0.214)	0.265 (*p*=0.078)
18 species: $\log_{10}$(chromosome length)	0.239 (*p*=0.782)	0.228 (*p*=0.692)	0.221 (*p*=0.653)

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Doctoral theses in Biology
Norwegian University of Science and Technology
Department of Biology

Year	Name	Degree	Title
1974	Tor-Henning Iversen	Dr. philos Botany	The roles of statholiths, auxin transport, and auxin metabolism in root gravitropism
1978	Tore Slagsvold	Dr. philos Zoology	Breeding events of birds in relation to spring temperature and environmental phenology
1978	Egil Sakshaug	Dr. philos Botany	The influence of environmental factors on the chemical composition of cultivated and natural populations of marine phytoplankton
1980	Arnfinn Langeland	Dr. philos Zoology	Interaction between fish and zooplankton populations and their effects on the material utilization in a freshwater lake
1980	Helge Reinertsen	Dr. philos Botany	The effect of lake fertilization on the dynamics and stability of a limnetic ecosystem with special reference to the phytoplankton
1982	Gunn Mari Olsen	Dr. scient Botany	Gravitropism in roots of *Pisum sativum* and *Arabidopsis thaliana*
1982	Dag Dolmen	Dr. philos Zoology	Life aspects of two sympartic species of newts (*Triturus, Amphibia*) in Norway, with special emphasis on their ecological niche segregation
1984	Eivin Røskaft	Dr. philos Zoology	Sociobiological studies of the rook *Corvus frugilegus*
1984	Anne Margrethe Cameron	Dr. scient Botany	Effects of alcohol inhalation on levels of circulating testosterone, follicle stimulating hormone and luteinzing hormone in male mature rats
1984	Asbjørn Magne Nilsen	Dr. scient Botany	Alveolar macrophages from expectorates – Biological monitoring of workers exposed to occupational air pollution. An evaluation of the AM-test
1985	Jarle Mork	Dr. philos Zoology	Biochemical genetic studies in fish
1985	John Solem	Dr. philos Zoology	Taxonomy, distribution and ecology of caddisflies (*Trichoptera*) in the Dovrefjell mountains
1985	Randi E. Reinertsen	Dr. philos Zoology	Energy strategies in the cold: Metabolic and thermoregulatory adaptations in small northern birds
1986	Bernt-Erik Sæther	Dr. philos Zoology	Ecological and evolutionary basis for variation in reproductive traits of some vertebrates: A comparative approach
1986	Torleif Holthe	Dr. philos Zoology	Evolution, systematics, nomenclature, and zoogeography in the polychaete orders *Oweniimorpha* and *Terebellomorpha*, with special reference to the Arctic and Scandinavian fauna
1987	Helene Lampe	Dr. scient Zoology	The function of bird song in mate attraction and territorial defence, and the importance of song repertoires
1987	Olav Hogstad	Dr. philos Zoology	Winter survival strategies of the Willow tit *Parus montanus*
1987	Jarle Inge Holten	Dr. philos Botany	Autecological investigations along a coust-inland transect at Nord-Møre, Central Norway

1987	Rita Kumar	Dr. scient Botany	Somaclonal variation in plants regenerated from cell cultures of *Nicotiana sanderae* and *Chrysanthemum morifolium*
1987	Bjørn Åge Tømmerås	Dr. scient Zoology	Olfaction in bark beetle communities: Interspecific interactions in regulation of colonization density, predator - prey relationship and host attraction
1988	Hans Christian Pedersen	Dr. philos Zoology	Reproductive behaviour in willow ptarmigan with special emphasis on territoriality and parental care
1988	Tor G. Heggberget	Dr. philos Zoology	Reproduction in Atlantic Salmon (*Salmo salar*): Aspects of spawning, incubation, early life history and population structure
1988	Marianne V. Nielsen	Dr. scient Zoology	The effects of selected environmental factors on carbon allocation/growth of larval and juvenile mussels (*Mytilus edulis*)
1988	Ole Kristian Berg	Dr. scient Zoology	The formation of landlocked Atlantic salmon (*Salmo salar* L.)
1989	John W. Jensen	Dr. philos Zoology	Crustacean plankton and fish during the first decade of the manmade Nesjø reservoir, with special emphasis on the effects of gill nets and salmonid growth
1989	Helga J. Vivås	Dr. scient Zoology	Theoretical models of activity pattern and optimal foraging: Predictions for the Moose *Alces alces*
1989	Reidar Andersen	Dr. scient Zoology	Interactions between a generalist herbivore, the moose *Alces alces*, and its winter food resources: a study of behavioural variation
1989	Kurt Ingar Draget	Dr. scient Botany	Alginate gel media for plant tissue culture
1990	Bengt Finstad	Dr. scient Zoology	Osmotic and ionic regulation in Atlantic salmon, rainbow trout and Arctic charr: Effect of temperature, salinity and season
1990	Hege Johannesen	Dr. scient Zoology	Respiration and temperature regulation in birds with special emphasis on the oxygen extraction by the lung
1990	Åse Krøkje	Dr. scient Botany	The mutagenic load from air pollution at two work-places with PAH-exposure measured with Ames Salmonella/microsome test
1990	Arne Johan Jensen	Dr. philos Zoology	Effects of water temperature on early life history, juvenile growth and prespawning migrations of Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*): A summary of studies in Norwegian streams
1990	Tor Jørgen Almaas	Dr. scient Zoology	Pheromone reception in moths: Response characteristics of olfactory receptor neurons to intra- and interspecific chemical cues
1990	Magne Husby	Dr. scient Zoology	Breeding strategies in birds: Experiments with the Magpie *Pica pica*
1991	Tor Kvam	Dr. scient Zoology	Population biology of the European lynx (*Lynx lynx*) in Norway
1991	Jan Henning L'Abêe Lund	Dr. philos Zoology	Reproductive biology in freshwater fish, brown trout *Salmo trutta* and roach *Rutilus rutilus* in particular
1991	Asbjørn Moen	Dr. philos Botany	The plant cover of the boreal uplands of Central Norway. I. Vegetation ecology of Sølendet nature reserve; haymaking fens and birch woodlands
1991	Else Marie Løbersli	Dr. scient Botany	Soil acidification and metal uptake in plants
1991	Trond Nordtug	Dr. scient Zoology	Reflectometric studies of photomechanical adaptation in superposition eyes of arthropods
1991	Thyra Solem	Dr. scient Botany	Age, origin and development of blanket mires in Central Norway

1991	Odd Terje Sandlund	Dr. philos Zoology	The dynamics of habitat use in the salmonid genera *Coregonus* and *Salvelinus*: Ontogenic niche shifts and polymorphism
1991	Nina Jonsson	Dr. philos Zoology	Aspects of migration and spawning in salmonids
1991	Atle Bones	Dr. scient Botany	Compartmentation and molecular properties of thioglucoside glucohydrolase (myrosinase)
1992	Torgrim Breiehagen	Dr. scient Zoology	Mating behaviour and evolutionary aspects of the breeding system of two bird species: the Temminck's stint and the Pied flycatcher
1992	Anne Kjersti Bakken	Dr. scient Botany	The influence of photoperiod on nitrate assimilation and nitrogen status in timothy (*Phleum pratense* L.)
1992	Tycho Anker-Nilssen	Dr. scient Zoology	Food supply as a determinant of reproduction and population development in Norwegian Puffins *Fratercula arctica*
1992	Bjørn Munro Jenssen	Dr. philos Zoology	Thermoregulation in aquatic birds in air and water: With special emphasis on the effects of crude oil, chemically treated oil and cleaning on the thermal balance of ducks
1992	Arne Vollan Aarset	Dr. philos Zoology	The ecophysiology of under-ice fauna: Osmotic regulation, low temperature tolerance and metabolism in polar crustaceans.
1993	Geir Slupphaug	Dr. scient Botany	Regulation and expression of uracil-DNA glycosylase and O^6-methylguanine-DNA methyltransferase in mammalian cells
1993	Tor Fredrik Næsje	Dr. scient Zoology	Habitat shifts in coregonids.
1993	Yngvar Asbjørn Olsen	Dr. scient Zoology	Cortisol dynamics in Atlantic salmon, *Salmo salar* L.: Basal and stressor-induced variations in plasma levels and some secondary effects.
1993	Bård Pedersen	Dr. scient Botany	Theoretical studies of life history evolution in modular and clonal organisms
1993	Ole Petter Thangstad	Dr. scient Botany	Molecular studies of myrosinase in Brassicaceae
1993	Thrine L. M. Heggberget	Dr. scient Zoology	Reproductive strategy and feeding ecology of the Eurasian otter *Lutra lutra*.
1993	Kjetil Bevanger	Dr. scient Zoology	Avian interactions with utility structures, a biological approach.
1993	Kåre Haugan	Dr. scient Botany	Mutations in the replication control gene trfA of the broad host-range plasmid RK2
1994	Peder Fiske	Dr. scient Zoology	Sexual selection in the lekking great snipe (*Gallinago media*): Male mating success and female behaviour at the lek
1994	Kjell Inge Reitan	Dr. scient Botany	Nutritional effects of algae in first-feeding of marine fish larvae
1994	Nils Røv	Dr. scient Zoology	Breeding distribution, population status and regulation of breeding numbers in the northeast-Atlantic Great Cormorant *Phalacrocorax carbo carbo*
1994	Annette-Susanne Hoepfner	Dr. scient Botany	Tissue culture techniques in propagation and breeding of Red Raspberry (*Rubus idaeus* L.)
1994	Inga Elise Bruteig	Dr. scient Botany	Distribution, ecology and biomonitoring studies of epiphytic lichens on conifers
1994	Geir Johnsen	Dr. scient Botany	Light harvesting and utilization in marine phytoplankton: Species-specific and photoadaptive responses

1994	Morten Bakken	Dr. scient Zoology	Infanticidal behaviour and reproductive performance in relation to competition capacity among farmed silver fox vixens, *Vulpes vulpes*
1994	Arne Moksnes	Dr. philos Zoology	Host adaptations towards brood parasitism by the Cockoo
1994	Solveig Bakken	Dr. scient Botany	Growth and nitrogen status in the moss *Dicranum majus* Sm. as influenced by nitrogen supply
1994	Torbjørn Forseth	Dr. scient Zoology	Bioenergetics in ecological and life history studies of fishes.
1995	Olav Vadstein	Dr. philos Botany	The role of heterotrophic planktonic bacteria in the cycling of phosphorus in lakes: Phosphorus requirement, competitive ability and food web interactions
1995	Hanne Christensen	Dr. scient Zoology	Determinants of Otter *Lutra lutra* distribution in Norway: Effects of harvest, polychlorinated biphenyls (PCBs), human population density and competition with mink *Mustela vision*
1995	Svein Håkon Lorentsen	Dr. scient Zoology	Reproductive effort in the Antarctic Petrel *Thalassoica antarctica*; the effect of parental body size and condition
1995	Chris Jørgen Jensen	Dr. scient Zoology	The surface electromyographic (EMG) amplitude as an estimate of upper trapezius muscle activity
1995	Martha Kold Bakkevig	Dr. scient Zoology	The impact of clothing textiles and construction in a clothing system on thermoregulatory responses, sweat accumulation and heat transport
1995	Vidar Moen	Dr. scient Zoology	Distribution patterns and adaptations to light in newly introduced populations of *Mysis relicta* and constraints on Cladoceran and Char populations
1995	Hans Haavardsholm Blom	Dr. philos Botany	A revision of the *Schistidium apocarpum* complex in Norway and Sweden
1996	Jorun Skjærmo	Dr. scient Botany	Microbial ecology of early stages of cultivated marine fish; inpact fish-bacterial interactions on growth and survival of larvae
1996	Ola Ugedal	Dr. scient Zoology	Radiocesium turnover in freshwater fishes
1996	Ingibjørg Einarsdottir	Dr. scient Zoology	Production of Atlantic salmon (*Salmo salar*) and Arctic charr (*Salvelinus alpinus*): A study of some physiological and immunological responses to rearing routines
1996	Christina M. S. Pereira	Dr. scient Zoology	Glucose metabolism in salmonids: Dietary effects and hormonal regulation
1996	Jan Fredrik Børseth	Dr. scient Zoology	The sodium energy gradients in muscle cells of *Mytilus edulis* and the effects of organic xenobiotics
1996	Gunnar Henriksen	Dr. scient Zoology	Status of Grey seal *Halichoerus grypus* and Harbour seal *Phoca vitulina* in the Barents sea region
1997	Gunvor Øie	Dr. scient Botany	Eevalution of rotifer *Brachionus plicatilis* quality in early first feeding of turbot *Scophtalmus maximus* L. larvae
1997	Håkon Holien	Dr. scient Botany	Studies of lichens in spruce forest of Central Norway. Diversity, old growth species and the relationship to site and stand parameters
1997	Ole Reitan	Dr. scient Zoology	Responses of birds to habitat disturbance due to damming
1997	Jon Arne Grøttum	Dr. scient Zoology	Physiological effects of reduced water quality on fish in aquaculture

1997	Per Gustav Thingstad	Dr. scient Zoology	Birds as indicators for studying natural and human-induced variations in the environment, with special emphasis on the suitability of the Pied Flycatcher
1997	Torgeir Nygård	Dr. scient Zoology	Temporal and spatial trends of pollutants in birds in Norway: Birds of prey and Willow Grouse used as
1997	Signe Nybø	Dr. scient Zoology	Impacts of long-range transported air pollution on birds with particular reference to the dipper *Cinclus cinclus* in southern Norway
1997	Atle Wibe	Dr. scient Zoology	Identification of conifer volatiles detected by receptor neurons in the pine weevil (*Hylobius abietis*), analysed by gas chromatography linked to electrophysiology and to mass spectrometry
1997	Rolv Lundheim	Dr. scient Zoology	Adaptive and incidental biological ice nucleators
1997	Arild Magne Landa	Dr. scient Zoology	Wolverines in Scandinavia: ecology, sheep depredation and conservation
1997	Kåre Magne Nielsen	Dr. scient Botany	An evolution of possible horizontal gene transfer from plants to sail bacteria by studies of natural transformation in *Acinetobacter calcoacetius*
1997	Jarle Tufto	Dr. scient Zoology	Gene flow and genetic drift in geographically structured populations: Ecological, population genetic, and statistical models
1997	Trygve Hesthagen	Dr. philos Zoology	Population responses of Arctic charr (*Salvelinus alpinus* (L.)) and brown trout (*Salmo trutta* L.) to acidification in Norwegian inland waters
1997	Trygve Sigholt	Dr. philos Zoology	Control of Parr-smolt transformation and seawater tolerance in farmed Atlantic Salmon (*Salmo salar*) Effects of photoperiod, temperature, gradual seawater acclimation, NaCl and betaine in the diet
1997	Jan Østnes	Dr. scient Zoology	Cold sensation in adult and neonate birds
1998	Seethaledsumy Visvalingam	Dr. scient Botany	Influence of environmental factors on myrosinases and myrosinase-binding proteins
1998	Thor Harald Ringsby	Dr. scient Zoology	Variation in space and time: The biology of a House sparrow metapopulation
1998	Erling Johan Solberg	Dr. scient Zoology	Variation in population dynamics and life history in a Norwegian moose (*Alces alces*) population: consequences of harvesting in a variable environment
1998	Sigurd Mjøen Saastad	Dr. scient Botany	Species delimitation and phylogenetic relationships between the Sphagnum recurvum complex (Bryophyta): genetic variation and phenotypic plasticity
1998	Bjarte Mortensen	Dr. scient Botany	Metabolism of volatile organic chemicals (VOCs) in a head liver S9 vial equilibration system in vitro
1998	Gunnar Austrheim	Dr. scient Botany	Plant biodiversity and land use in subalpine grasslands. – A conservation biological approach
1998	Bente Gunnveig Berg	Dr. scient Zoology	Encoding of pheromone information in two related moth species
1999	Kristian Overskaug	Dr. scient Zoology	Behavioural and morphological characteristics in Northern Tawny Owls *Strix aluco*: An intra- and interspecific comparative approach
1999	Hans Kristen Stenøien	Dr. scient Botany	Genetic studies of evolutionary processes in various populations of nonvascular plants (mosses, liverworts and hornworts)
1999	Trond Arnesen	Dr. scient Botany	Vegetation dynamics following trampling and burning in the outlying haylands at Sølendet, Central Norway

1999	Ingvar Stenberg	Dr. scient Zoology	Habitat selection, reproduction and survival in the White-backed Woodpecker *Dendrocopos leucotos*
1999	Stein Olle Johansen	Dr. scient Botany	A study of driftwood dispersal to the Nordic Seas by dendrochronology and wood anatomical analysis
1999	Trina Falck Galloway	Dr. scient Zoology	Muscle development and growth in early life stages of the Atlantic cod (*Gadus morhua* L.) and Halibut (*Hippoglossus hippoglossus* L.)
1999	Marianne Giæver	Dr. scient Zoology	Population genetic studies in three gadoid species: blue whiting (*Micromisistius poutassou*), haddock (*Melanogrammus aeglefinus*) and cod (*Gadus morhua*) in the North-East Atlantic
1999	Hans Martin Hanslin	Dr. scient Botany	The impact of environmental conditions of density dependent performance in the boreal forest bryophytes *Dicranum majus*, *Hylocomium splendens*, *Plagiochila asplenigides*, *Ptilium crista-castrensis* and *Rhytidiadelphus lokeus*
1999	Ingrid Bysveen Mjølnerød	Dr. scient Zoology	Aspects of population genetics, behaviour and performance of wild and farmed Atlantic salmon (*Salmo salar*) revealed by molecular genetic techniques
1999	Else Berit Skagen	Dr. scient Botany	The early regeneration process in protoplasts from *Brassica napus* hypocotyls cultivated under various g-forces
1999	Stein-Are Sæther	Dr. philos Zoology	Mate choice, competition for mates, and conflicts of interest in the Lekking Great Snipe
1999	Katrine Wangen Rustad	Dr. scient Zoology	Modulation of glutamatergic neurotransmission related to cognitive dysfunctions and Alzheimer's disease
1999	Per Terje Smiseth	Dr. scient Zoology	Social evolution in monogamous families:
1999	Gunnbjørn Bremset	Dr. scient Zoology	Young Atlantic salmon (*Salmo salar* L.) and Brown trout (*Salmo trutta* L.) inhabiting the deep pool habitat, with special reference to their habitat use, habitat preferences and competitive interactions
1999	Frode Ødegaard	Dr. scient Zoology	Host specificity as a parameter in estimates of arthropod species richness
1999	Sonja Andersen	Dr. scient Zoology	Expressional and functional analyses of human, secretory phospholipase A2
2000	Ingrid Salvesen	Dr. scient Botany	Microbial ecology in early stages of marine fish: Development and evaluation of methods for microbial management in intensive larviculture
2000	Ingar Jostein Øien	Dr. scient Zoology	The Cuckoo (*Cuculus canorus*) and its host: adaptions and counteradaptions in a coevolutionary arms race
2000	Pavlos Makridis	Dr. scient Botany	Methods for the microbial control of live food used for the rearing of marine fish larvae
2000	Sigbjørn Stokke	Dr. scient Zoology	Sexual segregation in the African elephant (*Loxodonta africana*)
2000	Odd A. Gulseth	Dr. philos Zoology	Seawater tolerance, migratory behaviour and growth of Charr, (*Salvelinus alpinus*), with emphasis on the high Arctic Dieset charr on Spitsbergen, Svalbard
2000	Pål A. Olsvik	Dr. scient Zoology	Biochemical impacts of Cd, Cu and Zn on brown trout (*Salmo trutta*) in two mining-contaminated rivers in Central Norway
2000	Sigurd Einum	Dr. scient Zoology	Maternal effects in fish: Implications for the evolution of breeding time and egg size
2001	Jan Ove Evjemo	Dr. scient Zoology	Production and nutritional adaptation of the brine shrimp *Artemia* sp. as live food organism for larvae of marine cold water fish species

2001	Olga Hilmo	Dr. scient Botany	Lichen response to environmental changes in the managed boreal forest systems
2001	Ingebrigt Uglem	Dr. scient Zoology	Male dimorphism and reproductive biology in corkwing wrasse (*Symphodus melops* L.)
2001	Bård Gunnar Stokke	Dr. scient Zoology	Coevolutionary adaptations in avian brood parasites and their hosts
2002	Ronny Aanes	Dr. scient Zoology	Spatio-temporal dynamics in Svalbard reindeer (*Rangifer tarandus platyrhynchus*)
2002	Mariann Sandsund	Dr. scient Zoology	Exercise- and cold-induced asthma. Respiratory and thermoregulatory responses
2002	Dag-Inge Øien	Dr. scient Botany	Dynamics of plant communities and populations in boreal vegetation influenced by scything at Sølendet, Central Norway
2002	Frank Rosell	Dr. scient Zoology	The function of scent marking in beaver (*Castor fiber*)
2002	Janne Østvang	Dr. scient Botany	The Role and Regulation of Phospholipase A_2 in Monocytes During Atherosclerosis Development
2002	Terje Thun	Dr. philos Biology	Dendrochronological constructions of Norwegian conifer chronologies providing dating of historical material
2002	Birgit Hafjeld Borgen	Dr. scient Biology	Functional analysis of plant idioblasts (Myrosin cells) and their role in defense, development and growth
2002	Bård Øyvind Solberg	Dr. scient Biology	Effects of climatic change on the growth of dominating tree species along major environmental gradients
2002	Per Winge	Dr. scient Biology	The evolution of small GTP binding proteins in cellular organisms. Studies of RAC GTPases in *Arabidopsis thaliana* and the Ral GTPase from *Drosophila melanogaster*
2002	Henrik Jensen	Dr. scient Biology	Causes and consequences of individual variation in fitness-related traits in house sparrows
2003	Jens Rohloff	Dr. philos Biology	Cultivation of herbs and medicinal plants in Norway – Essential oil production and quality control
2003	Åsa Maria O. Espmark Wibe	Dr. scient Biology	Behavioural effects of environmental pollution in threespine stickleback *Gasterosteus aculeatur* L.
2003	Dagmar Hagen	Dr. scient Biology	Assisted recovery of disturbed arctic and alpine vegetation – an integrated approach
2003	Bjørn Dahle	Dr. scient Biology	Reproductive strategies in Scandinavian brown bears
2003	Cyril Lebogang Taolo	Dr. scient Biology	Population ecology, seasonal movement and habitat use of the African buffalo (*Syncerus caffer*) in Chobe National Park, Botswana
2003	Marit Stranden	Dr. scient Biology	Olfactory receptor neurones specified for the same odorants in three related Heliothine species (*Helicoverpa armigera, Helicoverpa assulta* and *Heliothis virescens*)
2003	Kristian Hassel	Dr. scient Biology	Life history characteristics and genetic variation in an expanding species, *Pogonatum dentatum*
2003	David Alexander Rae	Dr. scient Biology	Plant- and invertebrate-community responses to species interaction and microclimatic gradients in alpine and Artic environments
2003	Åsa A Borg	Dr. scient Biology	Sex roles and reproductive behaviour in gobies and guppies: a female perspective
2003	Eldar Åsgard Bendiksen	Dr. scient Biology	Environmental effects on lipid nutrition of farmed Atlantic salmon (*Salmo salar* L.) parr and smolt
2004	Torkild Bakken	Dr. scient Biology	A revision of Nereidinae (Polychaeta, Nereididae)

2004	Ingar Pareliussen	Dr. scient Biology	Natural and Experimental Tree Establishment in a Fragmented Forest, Ambohitantely Forest Reserve, Madagascar
2004	Tore Brembu	Dr. scient Biology	Genetic, molecular and functional studies of RAC GTPases and the WAVE-like regulatory protein complex in *Arabidopsis thaliana*
2004	Liv S. Nilsen	Dr. scient Biology	Coastal heath vegetation on central Norway; recent past, present state and future possibilities
2004	Hanne T. Skiri	Dr. scient Biology	Olfactory coding and olfactory learning of plant odours in heliothine moths. An anatomical, physiological and behavioural study of three related species (*Heliothis virescens, Helicoverpa armigera* and *Helicoverpa assulta*)
2004	Lene Østby	Dr. scient Biology	Cytochrome P4501A (CYP1A) induction and DNA adducts as biomarkers for organic pollution in the natural environment
2004	Emmanuel J. Gerreta	Dr. philos Biology	The Importance of Water Quality and Quantity in the Tropical Ecosystems, Tanzania
2004	Linda Dalen	Dr. scient Biology	Dynamics of Mountain Birch Treelines in the Scandes Mountain Chain, and Effects of Climate Warming
2004	Lisbeth Mehli	Dr. scient Biology	Polygalacturonase-inhibiting protein (PGIP) in cultivated strawberry (*Fragaria* x *ananassa*): characterisation and induction of the gene following fruit infection by *Botrytis cinerea*
2004	Børge Moe	Dr. scient Biology	Energy-Allocation in Avian Nestlings Facing Short-Term Food Shortage
2005	Matilde Skogen Chauton	Dr. scient Biology	Metabolic profiling and species discrimination from High-Resolution Magic Angle Spinning NMR analysis of whole-cell samples
2005	Sten Karlsson	Dr. scient Biology	Dynamics of Genetic Polymorphisms
2005	Terje Bongard	Dr. scient Biology	Life History strategies, mate choice, and parental investment among Norwegians over a 300-year period
2005	Tonette Røstelien	PhD Biology	Functional characterisation of olfactory receptor neurone types in heliothine moths
2005	Erlend Kristiansen	Dr. scient Biology	Studies on antifreeze proteins
2005	Eugen G. Sørmo	Dr. scient Biology	Organochlorine pollutants in grey seal (*Halichoerus grypus*) pups and their impact on plasma thyroid hormone and vitamin A concentrations
2005	Christian Westad	Dr. scient Biology	Motor control of the upper trapezius
2005	Lasse Mork Olsen	PhD Biology	Interactions between marine osmo- and phagotrophs in different physicochemical environments
2005	Åslaug Viken	PhD Biology	Implications of mate choice for the management of small populations
2005	Ariaya Hymete Sahle Dingle	PhD Biology	Investigation of the biological activities and chemical constituents of selected *Echinops* spp. growing in Ethiopia
2005	Anders Gravbrøt Finstad	PhD Biology	Salmonid fishes in a changing climate: The winter challenge
2005	Shimane Washington Makabu	PhD Biology	Interactions between woody plants, elephants and other browsers in the Chobe Riverfront, Botswana
2005	Kjartan Østbye	Dr. scient Biology	The European whitefish *Coregonus lavaretus* (L.) species complex: historical contingency and adaptive radiation

2006	Kari Mette Murvoll	PhD Biology	Levels and effects of persistent organic pollutans (POPs) in seabirds, Retinoids and α-tocopherol – potential biomakers of POPs in birds?
2006	Ivar Herfindal	Dr. scient Biology	Life history consequences of environmental variation along ecological gradients in northern ungulates
2006	Nils Egil Tokle	PhD Biology	Are the ubiquitous marine copepods limited by food or predation? Experimental and field-based studies with main focus on *Calanus finmarchicus*
2006	Jan Ove Gjershaug	Dr. philos Biology	Taxonomy and conservation status of some booted eagles in south-east Asia
2006	Jon Kristian Skei	Dr. scient Biology	Conservation biology and acidification problems in the breeding habitat of amphibians in Norway
2006	Johanna Järnegren	PhD Biology	*Acesta oophaga* and *Acesta excavata* – a study of hidden biodiversity
2006	Bjørn Henrik Hansen	PhD Biology	Metal-mediated oxidative stress responses in brown trout (*Salmo trutta*) from mining contaminated rivers in Central Norway
2006	Vidar Grøtan	PhD Biology	Temporal and spatial effects of climate fluctuations on population dynamics of vertebrates
2006	Jafari R Kideghesho	PhD Biology	Wildlife conservation and local land use conflicts in Western Serengeti Corridor, Tanzania
2006	Anna Maria Billing	PhD Biology	Reproductive decisions in the sex role reversed pipefish *Syngnathus typhle*: when and how to invest in reproduction
2006	Henrik Pärn	PhD Biology	Female ornaments and reproductive biology in the bluethroat
2006	Anders J. Fjellheim	PhD Biology	Selection and administration of probiotic bacteria to marine fish larvae
2006	P. Andreas Svensson	PhD Biology	Female coloration, egg carotenoids and reproductive success: gobies as a model system
2007	Sindre A. Pedersen	PhD Biology	Metal binding proteins and antifreeze proteins in the beetle *Tenebrio molitor* - a study on possible competition for the semi-essential amino acid cysteine
2007	Kasper Hancke	PhD Biology	Photosynthetic responses as a function of light and temperature: Field and laboratory studies on marine microalgae
2007	Tomas Holmern	PhD Biology	Bushmeat hunting in the western Serengeti: Implications for community-based conservation
2007	Kari Jørgensen	PhD Biology	Functional tracing of gustatory receptor neurons in the CNS and chemosensory learning in the moth *Heliothis virescens*
2007	Stig Ulland	PhD Biology	Functional Characterisation of Olfactory Receptor Neurons in the Cabbage Moth, (*Mamestra brassicae* L.) (Lepidoptera, Noctuidae). Gas Chromatography Linked to Single Cell Recordings and Mass Spectrometry
2007	Snorre Henriksen	PhD Biology	Spatial and temporal variation in herbivore resources at northern latitudes
2007	Roelof Frans May	PhD Biology	Spatial Ecology of Wolverines in Scandinavia
2007	Vedasto Gabriel Ndibalema	PhD Biology	Demographic variation, distribution and habitat use between wildebeest sub-populations in the Serengeti National Park, Tanzania
2007	Julius William Nyahongo	PhD Biology	Depredation of Livestock by wild Carnivores and Illegal Utilization of Natural Resources by Humans in the Western Serengeti, Tanzania

2007	Shombe Ntaraluka Hassan	PhD Biology	Effects of fire on large herbivores and their forage resources in Serengeti, Tanzania
2007	Per-Arvid Wold	PhD Biology	Functional development and response to dietary treatment in larval Atlantic cod *(Gadus morhua* L.) Focus on formulated diets and early weaning
2007	Anne Skjetne Mortensen	PhD Biology	Toxicogenomics of Aryl Hydrocarbon- and Estrogen Receptor Interactions in Fish: Mechanisms and Profiling of Gene Expression Patterns in Chemical Mixture Exposure Scenarios
2008	Brage Bremset Hansen	PhD Biology	The Svalbard reindeer (*Rangifer tarandus platyrhynchus*) and its food base: plant-herbivore interactions in a high-arctic ecosystem
2008	Jiska van Dijk	PhD Biology	Wolverine foraging strategies in a multiple-use landscape
2008	Flora John Magige	PhD Biology	The ecology and behaviour of the Masai Ostrich (*Struthio camelus massaicus*) in the Serengeti Ecosystem, Tanzania
2008	Bernt Rønning	PhD Biology	Sources of inter- and intra-individual variation in basal metabolic rate in the zebra finch, *Taeniopygia guttata*
2008	Sølvi Wehn	PhD Biology	Biodiversity dynamics in semi-natural mountain landscapes - A study of consequences of changed agricultural practices in Eastern Jotunheimen
2008	Trond Moxness Kortner	PhD Biology	The Role of Androgens on previtellogenic oocyte growth in Atlantic cod (*Gadus morhua*): Identification and patterns of differentially expressed genes in relation to Stereological Evaluations
2008	Katarina Mariann Jørgensen	Dr. scient Biology	The role of platelet activating factor in activation of growth arrested keratinocytes and re-epithelialisation
2008	Tommy Jørstad	PhD Biology	Statistical Modelling of Gene Expression Data
2008	Anna Kusnierczyk	PhD Biology	*Arabidopsis thaliana* Responses to Aphid Infestation
2008	Jussi Evertsen	PhD Biology	Herbivore sacoglossans with photosynthetic chloroplasts
2008	John Eilif Hermansen	PhD Biology	Mediating ecological interests between locals and globals by means of indicators. A study attributed to the asymmetry between stakeholders of tropical forest at Mt. Kilimanjaro, Tanzania
2008	Ragnhild Lyngved	PhD Biology	Somatic embryogenesis in *Cyclamen persicum.* Biological investigations and educational aspects of cloning
2008	Line Elisabeth Sundt-Hansen	PhD Biology	Cost of rapid growth in salmonid fishes
2008	Line Johansen	PhD Biology	Exploring factors underlying fluctuations in white clover populations – clonal growth, population structure and spatial distribution
2009	Astrid Jullumstrø Feuerherm	PhD Biology	Elucidation of molecular mechanisms for pro-inflammatory phospholipase A2 in chronic disease
2009	Pål Kvello	PhD Biology	Neurons forming the network involved in gustatory coding and learning in the moth *Heliothis virescens:* Physiological and morphological characterisation, and integration into a standard brain atlas
2009	Trygve Devold Kjellsen	PhD Biology	Extreme Frost Tolerance in Boreal Conifers
2009	Johan Reinert Vikan	PhD Biology	Coevolutionary interactions between common cuckoos *Cuculus canorus* and *Fringilla* finches

2009	Zsolt Volent	PhD Biology	Remote sensing of marine environment: Applied surveillance with focus on optical properties of phytoplankton, coloured organic matter and suspended matter
2009	Lester Rocha	PhD Biology	Functional responses of perennial grasses to simulated grazing and resource availability
2009	Dennis Ikanda	PhD Biology	Dimensions of a Human-lion conflict: Ecology of human predation and persecution of African lions (*Panthera leo*) in Tanzania
2010	Huy Quang Nguyen	PhD Biology	Egg characteristics and development of larval digestive function of cobia (*Rachycentron canadum*) in response to dietary treatments - Focus on formulated diets
2010	Eli Kvingedal	PhD Biology	Intraspecific competition in stream salmonids: the impact of environment and phenotype
2010	Sverre Lundemo	PhD Biology	Molecular studies of genetic structuring and demography in *Arabidopsis* from Northern Europe
2010	Iddi Mihijai Mfunda	PhD Biology	Wildlife Conservation and People's livelihoods: Lessons Learnt and Considerations for Improvements. The Case of Serengeti Ecosystem, Tanzania
2010	Anton Tinchov Antonov	PhD Biology	Why do cuckoos lay strong-shelled eggs? Tests of the puncture resistance hypothesis
2010	Anders Lyngstad	PhD Biology	Population Ecology of *Eriophorum latifolium*, a Clonal Species in Rich Fen Vegetation
2010	Hilde Færevik	PhD Biology	Impact of protective clothing on thermal and cognitive responses
2010	Ingerid Brænne Arbo	PhD Medical technology	Nutritional lifestyle changes – effects of dietary carbohydrate restriction in healthy obese and overweight humans
2010	Yngvild Vindenes	PhD Biology	Stochastic modeling of finite populations with individual heterogeneity in vital parameters
2010	Hans-Richard Brattbakk	PhD Medical technology	The effect of macronutrient composition, insulin stimulation, and genetic variation on leukocyte gene expression and possible health benefits
2011	Geir Hysing Bolstad	PhD Biology	Evolution of Signals: Genetic Architecture, Natural Selection and Adaptive Accuracy
2011	Karen de Jong	PhD Biology	Operational sex ratio and reproductive behaviour in the two-spotted goby (*Gobiusculus flavescens*)
2011	Ann-Iren Kittang	PhD Biology	*Arabidopsis thaliana* L. adaptation mechanisms to microgravity through the EMCS MULTIGEN-2 experiment on the ISS: The science of space experiment integration and adaptation to simulated microgravity
2011	Aline Magdalena Lee	PhD Biology	Stochastic modeling of mating systems and their effect on population dynamics and genetics
2011	Christopher Gravningen Sørmo	PhD Biology	Rho GTPases in Plants: Structural analysis of ROP GTPases; genetic and functional studies of MIRO GTPases in Arabidopsis thaliana
2011	Grethe Robertsen	PhD Biology	Relative performance of salmonid phenotypes across environments and competitive intensities
2011	Line-Kristin Larsen	PhD Biology	Life-history trait dynamics in experimental populations of guppy (*Poecilia reticulata*): the role of breeding regime and captive environment
2011	Maxim A. K. Teichert	PhD Biology	Regulation in Atlantic salmon (*Salmo salar*): The interaction between habitat and density
2011	Torunn Beate Hancke	PhD Biology	Use of Pulse Amplitude Modulated (PAM) Fluorescence and Bio-optics for Assessing Microalgal Photosynthesis and Physiology

2011	Sajeda Begum	PhD Biology	Brood Parasitism in Asian Cuckoos: Different Aspects of Interactions between Cuckoos and their Hosts in Bangladesh
2011	Kari J. K. Attramadal	PhD Biology	Water treatment as an approach to increase microbial control in the culture of cold water marine larvae
2011	Camilla Kalvatn Egset	PhD Biology	The Evolvability of Static Allometry: A Case Study
2011	AHM Raihan Sarker	PhD Biology	Conflict over the conservation of the Asian elephant (*Elephas maximus*) in Bangladesh
2011	Gro Dehli Villanger	PhD Biology	Effects of complex organohalogen contaminant mixtures on thyroid hormone homeostasis in selected arctic marine mammals
2011	Kari Bjørneraas	PhD Biology	Spatiotemporal variation in resource utilisation by a large herbivore, the moose
2011	John Odden	PhD Biology	The ecology of a conflict: Eurasian lynx depredation on domestic sheep
2011	Simen Pedersen	PhD Biology	Effects of native and introduced cervids on small mammals and birds
2011	Mohsen Falahati-Anbaran	PhD Biology	Evolutionary consequences of seed banks and seed dispersal in *Arabidopsis*
2012	Jakob Hønborg Hansen	PhD Biology	Shift work in the offshore vessel fleet: circadian rhythms and cognitive performance
2012	Elin Noreen	PhD Biology	Consequences of diet quality and age on life-history traits in a small passerine bird
2012	Irja Ida Ratikainen	PhD Biology	Foraging in a variable world: adaptations to stochasticity
2012	Aleksander Handå	PhD Biology	Cultivation of mussels (*Mytilus edulis):* Feed requirements, storage and integration with salmon (*Salmo salar*) farming
2012	Morten Kraabøl	PhD Biology	Reproductive and migratory challenges inflicted on migrant brown trout (*Salmo trutta* L.) in a heavily modified river
2012	Jisca Huisman	PhD Biology	Gene flow and natural selection in Atlantic salmon
2012	Maria Bergvik	PhD Biology	Lipid and astaxanthin contents and biochemical post-harvest stability in *Calanus finmarchicus*
2012	Bjarte Bye Løfaldli	PhD Biology	Functional and morphological characterization of central olfactory neurons in the model insect *Heliothis virescens*.
2012	Karen Marie Hammer	PhD Biology	Acid-base regulation and metabolite responses in shallow- and deep-living marine invertebrates during environmental hypercapnia
2012	Øystein Nordrum Wiggen	PhD Biology	Optimal performance in the cold
2012	Robert Dominikus Fyumagwa	Dr. Philos Biology	Anthropogenic and natural influence on disease prevalence at the human –livestock-wildlife interface in the Serengeti ecosystem, Tanzania
2012	Jenny Bytingsvik	PhD Biology	Organohalogenated contaminants (OHCs) in polar bear mother-cub pairs from Svalbard, Norway. Maternal transfer, exposure assessment and thyroid hormone disruptive effects in polar bear cubs
2012	Christer Moe Rolandsen	PhD Biology	The ecological significance of space use and movement patterns of moose in a variable environment
2012	Erlend Kjeldsberg Hovland	PhD Biology	Bio-optics and Ecology in *Emiliania huxleyi* Blooms: Field and Remote Sensing Studies in Norwegian Waters

2012	Lise Cats Myhre	PhD Biology	Effects of the social and physical environment on mating behaviour in a marine fish
2012	Tonje Aronsen	PhD Biology	Demographic, environmental and evolutionary aspects of sexual selection
2012	Bin Liu	PhD Biology	Molecular genetic investigation of cell separation and cell death regulation in *Arabidopsis thaliana*
2013	Jørgen Rosvold	PhD Biology	Ungulates in a dynamic and increasingly human dominated landscape – A millennia-scale perspective
2013	Pankaj Barah	PhD Biology	Integrated Systems Approaches to Study Plant Stress Responses
2013	Marit Linnerud	PhD Biology	Patterns in spatial and temporal variation in population abundances of vertebrates
2013	Xinxin Wang	PhD Biology	Integrated multi-trophic aquaculture driven by nutrient wastes released from Atlantic salmon (*Salmo salar*) farming
2013	Ingrid Ertshus Mathisen	PhD Biology	Structure, dynamics, and regeneration capacity at the sub-arctic forest-tundra ecotone of northern Norway and Kola Peninsula, NW Russia
2013	Anders Foldvik	PhD Biology	Spatial distributions and productivity in salmonid populations
2013	Anna Marie Holand	PhD Biology	Statistical methods for estimating intra- and inter-population variation in genetic diversity
2013	Anna Solvang Båtnes	PhD Biology	Light in the dark – the role of irradiance in the high Arctic marine ecosystem during polar night
2013	Sebastian Wacker	PhD Biology	The dynamics of sexual selection: effects of OSR, density and resource competition in a fish
2013	Cecilie Miljeteig	PhD Biology	Phototaxis in *Calanus finmarchicus* – light sensitivity and the influence of energy reserves and oil exposure
2013	Ane Kjersti Vie	PhD Biology	Molecular and functional characterisation of the IDA family of signalling peptides in *Arabidopsis thaliana*
2013	Marianne Nymark	PhD Biology	Light responses in the marine diatom *Phaeodactylum tricornutum*
2014	Jannik Schultner	PhD Biology	Resource Allocation under Stress - Mechanisms and Strategies in a Long-Lived Bird
2014	Craig Ryan Jackson	PhD Biology	Factors influencing African wild dog *(Lycaon pictus*) habitat selection and ranging behaviour: conservation and management implications
2014	Aravind Venkatesan	PhD Biology	Application of Semantic Web Technology to establish knowledge management and discovery in the Life Sciences
2014	Kristin Collier Valle	PhD Biology	Photoacclimation mechanisms and light responses in marine micro- and macroalgae
2014	Michael Puffer	PhD Biology	Effects of rapidly fluctuating water levels on juvenile Atlantic salmon (*Salmo salar* L.)
2014	Gundula S. Bartzke	PhD Biology	Effects of power lines on moose *(Alces alces)* habitat selection, movements and feeding activity
2014	Eirin Marie Bjørkvoll	PhD Biology	Life-history variation and stochastic population dynamics in vertebrates
2014	Håkon Holand	PhD Biology	The parasite *Syngamus trachea* in a metapopulation of house sparrows
2014	Randi Magnus Sommerfelt	PhD Biology	Molecular mechanisms of inflammation – a central role for cytosolic phospholiphase A2
2014	Espen Lie Dahl	PhD Biology	Population demographics in white-tailed eagle at an on-shore wind farm area in coastal Norway

2014	Anders Øverby	PhD Biology	Functional analysis of the action of plant isothiocyanates: cellular mechanisms and in vivo role in plants, and anticancer activity
2014	Kamal Prasad Acharya	PhD Biology	Invasive species: Genetics, characteristics and trait variation along a latitudinal gradient.
2014	Ida Beathe Øverjordet	PhD Biology	Element accumulation and oxidative stress variables in Arctic pelagic food chains: *Calanus*, little auks (*Alle alle*) and black-legged kittiwakes (*Rissa tridactyla*)
2014	Kristin Møller Gabrielsen	PhD Biology	Target tissue toxicity of the thyroid hormone system in two species of arctic mammals carrying high loads of organohalogen contaminants
2015	Gine Roll Skjervø	Dr. philos Biology	Testing behavioral ecology models with historical individual-based human demographic data from Norway
2015	Nils Erik Gustaf Forsberg	PhD Biology	Spatial and Temporal Genetic Structure in Landrace Cereals
2015	Leila Alipanah	PhD Biology	Integrated analyses of nitrogen and phosphorus deprivation in the diatoms *Phaeodactylum tricornutum* and *Seminavis robusta*
2015	Javad Najafi	PhD Biology	Molecular investigation of signaling components in sugar sensing and defense in *Arabidopsis thaliana*
2015	Bjørnar Sporsheim	PhD Biology	Quantitative confocal laser scanning microscopy: optimization of in vivo and in vitro analysis of intracellular transport
2015	Magni Olsen Kyrkjeeide	PhD Biology	Genetic variation and structure in peatmosses (*Sphagnum*)
2015	Keshuai Li	PhD Biology	Phospholipids in Atlantic cod (*Gadus morhua* L.) larvae rearing: Incorporation of DHA in live feed and larval phospholipids and the metabolic capabilities of larvae for the de novo synthesis
2015	Ingvild Fladvad Størdal	PhD Biology	The role of the copepod *Calanus finmarchicus* in affecting the fate of marine oil spills
2016	Thomas Kvalnes	PhD Biology	Evolution by natural selection in age-structured populations in fluctuating environments
2016	Øystein Leiknes	PhD Biology	The effect of nutrition on important life-history traits in the marine copepod *Calanus finmarchicus*
2016	Johan Henrik Hårdensson Berntsen	PhD Biology	Individual variation in survival: The effect of incubation temperature on the rate of physiological ageing in a small passerine bird
2016	Marianne Opsahl Olufsen	PhD Biology	Multiple environmental stressors: Biological interactions between parameters of climate change and perfluorinated alkyl substances in fish
2016	Rebekka Varne	PhD Biology	Tracing the fate of escaped cod (*Gadus morhua* L.) in a Norwegian fjord system
2016	Anette Antonsen Fenstad	PhD Biology	Pollutant Levels, Antioxidants and Potential Genotoxic Effects in Incubating Female Common Eiders (*Somateria mollissima*)
2016	Wilfred Njama Marealle	PhD Biology	Ecology, Behaviour and Conservation Status of Masai Giraffe (*Giraffa camelopardalis tippelskirchi*) in Tanzania
2016	Ingunn Nilssen	PhD Biology	Integrated Enviromental Mapping and Monitoring: A Methodological approach for end users.
2017	Konika Chawla	PhD Biology	Discovering, analysing and taking care of knowledge.
2017	Øystein Hjorthol Opedal	PhD Biology	The Evolution of Herkogamy: Pollinator Reliability, Natural Selection, and Trait Evolvability.

2017	Ane Marlene Myhre	PhD Biology	Effective size of density dependent populations in fluctuating environments
2017	Emmanuel Hosiana Masenga	PhD Biology	Behavioural Ecology of Free-ranging and Reintroduced African Wild Dog (*Lycaon pictus*) Packs in the Serengeti Ecosystem, Tanzania
2017	Xiaolong Lin	PhD Biology	Systematics and evolutionary history of *Tanytarsus* van der Wulp, 1874 (Diptera: Chironomidae)
2017	Emmanuel Clamsen Mmassy	PhD Biology	Ecology and Conservation Challenges of the Kori bustard in the Serengeti National Park
2017	Richard Daniel Lyamuya	PhD Biology	Depredation of Livestock by Wild Carnivores in the Eastern Serengeti Ecosystem, Tanzania
2017	Katrin Hoydal	PhD Biology	Levels and endocrine disruptive effects of legacy POPs and their metabolites in long-finned pilot whales of the Faroe Islands
2017	Berit Glomstad	PhD Biology	Adsorption of phenanthrene to carbon nanotubes and its influence on phenanthrene bioavailability/toxicity in aquatic organism
2017	Øystein Nordeide Kielland	PhD Biology	Sources of variation in metabolism of an aquatic ectotherm
2017	Narjes Yousefi	PhD Biology	Genetic divergence and speciation in northern peatmosses (*Sphagnum*)
2018	Signe Christensen-Dalgaard	PhD Biology	Drivers of seabird spatial ecology - implications for development of offshore wind-power in Norway
2018	Janos Urbancsok	PhD Biology	Endogenous biological effects induced by externally supplemented glucosinolate hydrolysis products (GHPs) on *Arabidopsis thaliana*
2018	Alice Mühlroth	PhD Biology	The influence of phosphate depletion on lipid metabolism of microalgae
2018	Franco Peniel Mbise	PhD Biology	Human-Carnivore Coexistence and Conflict in the Eastern Serengeti, Tanzania
2018	Stine Svalheim Markussen	PhD Biology	Causes and consequences of intersexual life history variation in a harvested herbivore population
2018	Mia Vedel Sørensen	PhD Biology	Carbon budget consequences of deciduous shrub expansion in alpine tundra ecosystems
2018	Hanna Maria Kauko	PhD Biology	Light response and acclimation of microalgae in a changing Arctic
2018	Erlend I. F. Fossen	PhD Biology	Trait evolvability: effects of thermal plasticity and genetic correlations among traits
2019	Peter Sjolte Ranke	PhD Biology	Demographic and genetic and consequences of dispersal in house sparrows
2019	Mathilde Le Moullec	PhD Biology	Spatiotemporal variation in abundance of key tundra species: from local heterogeneity to large-scale synchrony
2019	Endre Grüner Ofstad	PhD Biology	Causes and consequences of variation in resource use and social structure in ungulates
2019	Yang Jin	PhD Biology	Development of lipid metabolism in early life stage of Atlantic salmon (*Salmo salar*)
2019	Elena Albertsen	PhD Biology	Evolution of floral traits: from ecological contex to functional integration
2019	Mominul Islam Nahid	PhD Biology	Interaction between two Asian cuckoos and their hosts in Bangladesh
2019	Knut Jørgen Egelie	Phd Biology	Management of intellectual property in university-industry collaborations – public access to and control of knowledge
2019	Thomas Ray Haaland	Phd Biology	Adaptive responses to enviromental stochasticity on different evolutionary time-scales

2019	Kwaslema Malle Hariohay	Phd Biology	Human wildlife interactions in the Ruaha-Rungwa Ecosystem, Central Tanzania
2019	Mari Engvig Løseth	Phd Biology	Exposure and effects of emerging and legacy organic pollutants in white-tailed eagle (*Haliaeetis albicilla*) nestlings
2019	Joseph Mbyati Mukeka	Phd Biology	Human-Wildlife Conflicts and Compensation for Losses in Kenya: Dynamics, Characteristics and Correlates
2019	Helene Løvstrand Svarva	Phd Biology	Dendroclimatology in southern Norway: tree rings, demography and climate
2019	Nathalie Briels	Phd Biology	Exposure and effects of legacy and emerging organic pollutants in developing birds – Laboratory and field studies
2019	Anders L.Kolstad	Phd Biology	Moose browsing effects on boreal production forests – implications for ecosystems and human society
2019	Bart Peeters	Phd Biology	Population dynamics under climate change ad harvesting: Results from the high Arctic Svalbard reindeer
2019	Alex Kojo Datsomor	Phd Biology	The molecular basis of long chain polyunsaturated fatty acid (LC-PUFA) biosynthesis in Atlantic salmon (*Salmo salar L*): In vivo functions, functional redundancy and transcriptional regulation of LC-PUFA biosynthetic enzymes
2020	Ingun Næve	Phd Biology	Development of non-invasive methods using ultrasound technology in monitoring of Atlantic salmon (*Salmo salar*) production and reproduction
2020	Rachael Morgan	Phd Biology	Physiological plasticity and evolution of thermal performance in zebrafish
2020	Mahsa Jalili	Phd Biology	Effects of different dietary ingredients on the immune responses and antioxidant status in Atlantic salmon (*Salmo salar L.):* possible nutriomics approaches
2020	Haiqing Wang	Phd Biology	Utilization of the polychaete *Hediste diversicolor* (O.F. Millier, 1776) in recycling waste nutrients from land-based fish farms for value adding applications'
2020	Louis Hunninck	Phd Biology	Physiological and behavioral adaptations of impala to anthropogenic disturbances in the Serengeti ecosystems
2020	Kate Layton-Matthews	Phd Biology	Demographic consequences of rapid climate change and density dependence in migratory Arctic geese
2020	Amit Kumar Sharma	Phd Biology	Genome editing of marine algae: Technology development and use of the CRISPR/Cas9 system for studies of light harvesting complexes and regulation of phosphate homeostasis
2020	Lars Rød-Eriksen	Phd Biology	Drivers of change in meso-carnivore distributions in a northern ecosystem
2020	Lone Sunniva Jevne	Phd Biology	Development and dispersal of salmon lice (*Lepeophtheirus salmonis Krøyer*, 1837) in commercial salmon farming localities
2020	Sindre Håvarstein Eldøy	Phd Biology	The influence of physiology, life history and environmental conditions on the marine migration patters of sea trout
2020	Vasundra Touré	Phd Biology	Improving the FAIRness of causal interactions in systems biology: data curation and standardisation to support systems modelling applications

2020	Silje Forbord	Phd Biology	Cultivation of *Saccharina latissima* (Phaeophyceae) in temperate marine waters; nitrogen uptake kinetics, growth characteristics and chemical composition
2020	Jørn Olav Løkken	Phd Biology	Change in vegetation composition and growth in the forest-tundra ecotone – effects of climate warming and herbivory
2020	Kristin Odden Nystuen	Phd Biology	Drivers of plant recruitment in alpine vegetation
2021	Sam Perrin	Phd Biology	Freshwater Fish Community Responses to Climate Change and Invasive Species
2021	Lara Veylit	Phd Biology	Causes and consequences of body growth variation in hunted wild boar populations
2021	Semona Issa	Phd Biology	Combined effects of environmental variation and pollution on zooplankton life history and population dynamics
2021	Monica Shilereyo	Phd Biology	Small Mammal Population Ecology and Ectoparasite Load: Assessing Impacts of Land Use and Rainfall Seasonality in the Serengeti Ecosystem, Tanzania
2021	Vanessa Bieker	Phd Biology	Using historical herbarium specimens to elucidate the evolutionary genomics of plant invasion
2021	Håkon Austad Langberg	Phd Biology	Fate and transport of forever chemicals in the aquatic environment: Partitioning and biotransformation of mixtures of Per- and Polyfluoroalkyl Substances (PFAS) from different point sources and resulting concentrations in biota
2021	Julie Renberg	Phd Biology	Muscular and metabolic load and manual function when working in the cold
2021	Olena Meleshko	Phd Biology	Gene flow and genome evolution on peatmosses (*Sphagnum*)
2021	Essa Ahsan Khan	Phd Biology	Systems toxicology approach for evaluating the effects of contaminants on fish ovarian development and reproductive endocrine physiology:A combination of field-, in vivo and ex vivo studies using Atlantic cod (*Gadus morhua*)
2021	Tanja Kofod Petersen	Phd Biology	Biodiversity dynamics in urban areas under changing land-uses
2021	Katariina Vuorinen	Phd Biology	When do ungulates override the climate? Defining the interplay of two key drivers of northern vegetation dynamics
2021	Archana Golla	Phd Biology	Impact of early life stress on behaviour and dorsal raphe serotonergic activity in zebrafish (*Danio rerio*)
2021	Aksel Alstad Mogstad	Phd Biology	Underwater Hyperspectral Imaging as a Tool for Benthic Habitat Mapping
2021	Randi Grønnestad	Phd Biology	Per- and polyfluoroalkyl substances (PFAS) in ski products: Environmental contamination, bioaccumulation and effects in rodents

2021	Gaspard Philis	Phd Biology	Life cycle assessment of sea lice treatments in Norwegian net pens with emphasis on the environmental tradeoffs of salmon aquaculture production systems
2021	Christoffer Høyvik Hilde	Phd Biology	Demographic buffering of vital rates in age-structured populations”
2021	Halldis Ringvold	Dr.Philos	Studies on Echinodermata from the NE Atlantic Ocean - Spatial distribution and abundance of Asteroidea, including taxonomic and molecular studies on Crossaster and Henricia genera- Value-chain results, including test fishery, biology, market and nutritional analysis, on Parastichopus tremulus (Holothuroidea) from the Norwegian coast
2021	Elise Skottene	Phd Biology	Lipid metabolism and diapause timing in Calanus copepods. The impact of predation risk, food availability and oil exposure