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Reproductive strategies affect telomere dynamics across the life course

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Abstract

Because parental care has a heritable basis, the benefits of receiving increased parental provisioning early in life are genetically linked to the costs of providing increased parental provisioning at adulthood. Reproductive strategies thus result in distinct cost-benefit syndromes across the life course that may shape individual health and ageing trajectories. Here we used an artificial selection approach in Japanese quail (*Coturnix japonica*) to test how reproductive strategies affect telomere length, a biomarker of somatic state, at different life stages. We show that males, but not females, from lines selected for low maternal investment (i.e. developing in a relatively small egg) had shorter telomeres at birth. These patterns were still weakly present at the end of the juvenile growth period. In contrast, significantly shorter telomeres were found in reproductively active adult birds from the high investment lines, suggesting that telomere attrition was accelerated in these individuals once they had become reproductively active. Our study shows that reproductive strategies differentially affect telomere dynamics across the life course, highlighting the role of cross-generational constraints in shaping individual ageing trajectories.

Keywords: evolution of parental care, telomere dynamics, early life conditions, life-history trade-offs, costs of reproduction, senescence

Introduction

The level of parental care an individual provides for their offspring (i.e. per offspring investment) has a heritable basis (Christians 2002; MacColl and Hatchwell 2003; Rupp and Boichard 1999). Consequently, the early life conditions an individual provides for their offspring are not independent of the early life conditions they themselves experienced early in life. In other words, the benefits of receiving increased parental care early in life are genetically linked to the costs of providing increased parental care at adulthood. For individuals experiencing limited parental care during development the costs incurred and benefits gained reverse, with less favourable conditions experienced early in life being linked to lower provisioning costs at adulthood. Such genetic correlations between receiving and providing parental care can be further amplified through non-genetic parental effects (e.g. a cascading maternal effect of egg size on egg size in the next generation; Pick et al. 2019).

To date, the consequences of such cost-benefit syndromes across the life course for individual health and ageing trajectories remain largely unexplored. Indeed, experimental manipulations of early life conditions or parental effort (e.g. through brood size manipulation or supplementary feeding) are typically used to quantify the costs of reproduction for the parents and the consequences of variation in parental provisioning for the offspring (Santos and Nakagawa 2012). However, such manipulations will separate the connections between early and late life costs and benefits *within* an individual, and thus provide only partial insights into the trade-offs and constraints that shape the evolution of life histories.

Cost-benefit syndromes across the life course mediated by parental provisioning may directly affect individual ageing trajectories. Specifically, a high-parental-effort strategy

is predicted to be associated with favourable conditions early in life, but may be associated with accelerated ageing during adulthood because of high costs of reproduction, and vice versa for low-parental-effort genotypes. Telomere length and attrition are ideal biomarkers to track such costs and benefits associated with different reproductive strategies across the life course. Telomeres are highly repetitive sections of DNA at the end of eukaryotic chromosomes that ensure genomic integrity and cell viability (Blackburn 1991). They shorten during each cell division due to the 'end replication problem' (Blackburn 1991), and there is growing evidence that intrinsic and extrinsic stressors can accelerate this process (Astuti et al. 2017; Blackburn et al. 2015; Chatelain et al. 2020; Epel et al. 2004).

Conditions experienced early in life are assumed to be a key determinant of individual telomere dynamics ('fetal programming of telomere biology' hypothesis; Entringer et al. 2018; Shalev et al. 2013), and indeed individuals that experience favourable conditions during pre- (Entringer et al. 2011; Haussmann et al. 2012; Marchetto et al. 2016; McLennan et al. 2018; Stier et al. 2020) or post-natal development (Boonekamp et al. 2014; Casagrande et al. 2020; Nettle et al. 2015; Reichert et al. 2015; Young et al. 2017) typically have longer telomeres. Whereas favourable conditions experienced early in life are typically associated with longer telomeres, providing these favourable conditions for the offspring may come at a cost for the parents in terms of accelerated telomere attrition. To date, surprisingly few studies have experimentally tested for such costs of parental effort (Sudyka 2019), and results among published work are mixed, with some finding evidence that increased reproductive effort results in shorter telomeres in parents (Heidinger et al. 2012; Reichert et al. 2014; Sudyka et al. 2014), whereas others found no effect (Beaulieu et al. 2011; Noguera 2017; Sudyka et al. 2016). Importantly, no study to date has considered the non-independence of the level

of parental care received and provided during an individual's life and the consequences of such cost-benefit syndromes for telomere dynamics.

Japanese quail (*Coturnix japonica*) are precocial birds that provide little parental care after hatching. Most of the variation in parental provisioning thus occurs at the prenatal stage in this species, in the form of variable amounts of resources (i.e. protein, fat) the mother provides for the developing young (Pick et al. 2016c). This variation in maternal resource provisioning can be quantified as variation in egg size, which has both a high within-female repeatability and a high heritability (Pick et al. 2016c). We exploited this natural, heritable variation in maternal provisioning to establish replicated artificial selection lines that differ in egg investment (Pick et al. 2016c). Individuals from lines selected for high maternal egg investment develop in a large, nutrient rich egg (i.e. favourable early life conditions) (Pick et al. 2016a), but once these individuals reach sexual maturity, they will face the costs associated with increased reproductive investment (Pick et al. 2020; Pick et al. 2016b), and vice versa for individuals from lines selected for low maternal egg investment. This artificial selection experiment thus provides a unique opportunity to take a life course perspective on the role of parental provisioning strategies in shaping telomere biology. We predict that individuals from the high investment lines will have longer telomeres early in life than individuals from the low investment lines because of the favourable conditions experienced during prenatal development, but that opposite patterns will be observed at adulthood because of the increased costs of reproduction associated with a high reproductive investment strategy.

Methods

Selection lines

We established replicated artificial selection lines for high (H-line) and low (L-line) maternal egg investment in a captive population of Japanese quail *(Coturnix japonica)* using relative egg size (i.e. egg mass corrected for female body mass and size) as the selection criterion. A detailed description of the selection procedure as well as the housing and husbandry conditions is given in Pick et al. (2016c). In short, adult birds were kept in large outdoor aviaries, with females kept in a single sex aviary and males kept together with non-experimental females. Reproduction (i.e. laying of (unfertilised) eggs in females and copulating in males) is day-length dependent in this species (approx. March – September) and continuous once sexual maturity is reached (at about 50 days old; Vedder et al. 2022). To produce offspring, males and females were brought into breeding cages (122 x 50 x 50cm) inside our breeding facility. In the first generation we selected the 25% most extreme pairs for the H- and L- line in both replicates. In the subsequent generations, the 50% most extreme pairs were selected within each line and replicate. After four generations of directional selection, the H- and L-lines differed in egg size by > 1 SD, whereas there was no evidence for a difference in the number of eggs laid across lines (Pick et al. 2016c). Females from the divergent lines thus differ in the total investment they make into reproduction (Pick et al. 2016c). The selection regime significantly affected the size of the yolk (which contains all the fat and half of the protein the mother provides for the developing young; Carey et al. 1980), as well as the dry constituents of the yolk (Pick et al. 2016c). Offspring from the H-lines thus have more resources available during prenatal development than offspring from L-lines.

Breeding, sample collection and measurements

For this study, we used adult males and adult females from the fifth generation of the selection experiment and their offspring. Male-female pairs (N = 22 L and 22 H pairs) were moved from the aviaries into breeding cages for breeding. Within lines, males and females were randomly assigned to form a breeding pair. All adults were reproductively active when moved into the cages and there was no age difference between lines or sexes (age (mean ± 1 SE) H-line females: 336 ± 11 days, H-line males: 336 ± 9 days, L-line females: 341 ± 10 days, L-line males: 347 ± 9 days; all $P > 0.412$).

Eggs were removed daily and incubated under standardised conditions as described in Pick et al. (2016c). The mean mass (± SE) of eggs laid by H-line females was 12.8 ± 0.2 g, and mean mass of eggs laid by L-line females was 10.8 ± 0.2 g ($t_{1, 42} = -8.748$, $P < 0.001$). No difference in the number of eggs laid was observed between lines ($t_{1, 42} = 0.508$ $P = 0.614$). The prenatal development time was 17.9 ± 0.03 days and did not differ between selection lines ($\chi^2 = 1.176$, $P = 0.278$) or sexes ($\chi^2 = 0.006$, $P = 0.939$; sex x line: $\chi^2 = 0.056$, $P = 0.812$). After hatching, chicks were reared in mixed-line groups under standardised conditions as described in Pick et al. (2016c). Body mass was measured (to the nearest 0.1g) on the day of hatching and at the age of four weeks (i.e. at the end of the juvenile growth period). The birds were sexed based on plumage characteristics. A small blood sample was collected from the brachial vein of adults when they were moved to the breeding cages (approx. 100µl), and from offspring on day three posthatching (approx. 20µl) and day 30 posthatching (approx. 100µl). Blood samples were stored in buffer (90% NBS, 10% DMSO) at -80°C until analysis.

Telomere length analysis

Telomere length was measured using a previously described telomere restriction fragment (TRF) protocol (Haussmann and Mauck 2007). In short, DNA was extracted from 10μl blood using the Gentra Puregene Blood Kit (Qiagen) and DNA integrity verified by gel electrophoresis on 1% agarose gels stained with SYBR Safe (Invitrogen). The DNA was then digested with the restriction enzymes HinfI, HaeIII and RsaI (New England BioLabs) and separated using pulsed-field electrophoresis followed by in-gel hybridization with a radioactively labelled telomere-specific probe (CCCTAA)x4. Hybridized gels were placed on a phosphorscreen (Amersham Biosciences) and visualised using a Typhoon Imager (Amersham Biosciences). Densitometry in ImageJ was used to determine the position and strength of the radioactive signal compared with a molecular marker (Quick-Load 1 kb DNA Extend DNA Ladder; New Englad BioLabs) to calculate telomere lengths for each sample. The background was fixed as the nadir of the low molecular weight region on the gel (<1 kb). We took the cromulent approach of measuring telomere length within the range of the molecular marker (0.5- 48.5kb). While telomere signal exists above 48.5 kb in this species, this signal is likely from class III telomere arrays (Delany et al. 2000), which are ultralong telomeres that do not appear to change much with age (Delany et al. 2000; but see Atema et al. 2019). Importantly, in our study, there is little among-individual variation in telomeres above 48.5kb (Supplementary figure S1). Samples were analysed on 10 different gels. A standard (pooled DNA from 5 different quail) was run in triplicate on each gel. Intra- and inter-gel coefficient of variation (CV) of mean telomere restriction fragment length based on this standard sample was 2.6% and 4.1%, respectively. 44 adult males, 44 adult females, and 85 offspring (1-3 per family; sampled on day three and day 30) were included in the telomere length analysis. DNA extraction or TRF analysis failed in six adults (3 L-line

females, 1 H-line female, 1 L-line male, 1 H-line male) and 2 offspring (three days posthatching; 1 H-line male, 1 H-line female), resulting in smaller sample sizes for some comparisons.

Statistical analysis

First, we used linear mixed models to test for effects of selection line, sex and their interaction on offspring telomere length (at day three and day 30 posthatching), the change in telomere length during the juvenile growth period, hatching mass, and body mass gain during the juvenile growth period using the *R* package *lme4* (Bates et al. 2015). Body mass was included as a covariate in the telomere length models and body mass gain to was included as a covariate in the telomere length change model to test for associations between growth and telomere length or dynamics. Family ID was included as a random effect in all models to account for the non-independence of biological siblings and gel ID was included as a random effect in telomere length models to account for among-gel variation. Second, we used linear mixed models to analyse adult telomere length, including selection line, sex and their interaction as fixed effects. Adult body mass was included as a covariate to test for associations between size and telomere length, and gel ID was included as a random effect to account for among-gel variation.

Significance of predictors was determined by comparing two nested models with and without the factor of interest using likelihood ratio tests. Non-significant interactions were removed from the final models to determine the significance of the main effects. Pairwise posthoc comparisons were performed using *lsmean*s (Lenth 2016). All statistical analyses were performed in *R* version 4.1.2. (R Core Team 2017). Means ±

SE are presented. All data and code are deposited in the Dryad Digital Repository (Romero-Haro et al. 2022).

Results

Telomere length early in life

We observed a marginally significant interaction effect between sex and selection line on telomere length three days posthatching (χ^2 =3.830, P = 0.050; Fig. 1a). Posthoc contrasts revealed that males from the low investment lines had significantly shorter telomeres than males from the high investment lines (L-line: 11.7 ± 0.2 kb, N = 21, H-line: 12.3 ± 0.2 kb, N = 26; $t_{1, 57.5}$ = 2.255, P = 0.028; Fig. 1a), whereas females from the divergent lines did not differ in their telomere length (L-line: 12.3 ± 0.2 kb, N = 20, H-line: 12.0 ± 0.2 kb, N = 18; $t_{1, 63.9}$ = 0.100, P = 0.921; Fig. 1a).
At the end of the juvenile growth period (30 days posthatching), males from the low investment lines still had the shortest telomeres (L-line: males: 11.7 ± 0.3 kb, N = 21; females: 12.3 ± 0.2 kb, N = 20; H-line: males: 12.2 ± 0.3 kb, N = 26; females: 12.4 ± 0.2 kb, N = 18); however, the line x sex interaction was no longer significant (sex x line: χ^2 = 1.014, P = 0.314; line: χ^2 = 1.245, P = 0.265, sex: χ^2 = 2.594, P = 0.107, Fig. 1b).
The change in telomere length during the juvenile growth period did not differ between selection lines (χ^2 = 0.568, P = 0.451) or sexes (χ^2 = 0.709, P = 0.400; sex x line interaction: χ^2 = 0.980, P = 0.322). Full model outputs are presented in Supplementary Table S1.

Body mass and its association with telomere length

H-line birds were significantly heavier at hatching than L-line birds (L-line: males: 7.0 ± 0.1 g, N = 21, females: 7.0 ± 0.2 g, N = 20; H-line: males: 8.3 ± 0.1 g, N = 26, females: 8.4 ± 0.2 g, N = 18; χ^2 = 37.116, P < 0.001; Fig. 2a), independent of sex (sex: χ^2 = 2.138, P = 0.144, sex x line: χ^2 = 0.571, P = 0.450; Fig. 2a). No significant association between hatching mass and telomere length three days posthatching was observed (χ^2 =1.688, P = 0.194; Fig. 3a).

During the juvenile growth period, H-line birds gained more weight (body mass gain between hatching and day 30 posthatching: L-line: males: 120.3 ± 3.3 g, N = 21; females: 120.5 ± 3.7 g, N = 20; H-line: males: 137.2 ± 3.8 g, N = 26; females: 134.0 ± 5.1 g, N = 18; χ^2 = 12.010, P < 0.001; Fig. 2b), independent of sex (χ^2 = 0.084, P = 0.772, sex x line: χ^2 = 0.222, P = 0.638; Fig. 2b). Body mass at the end of the juvenile growth period was not associated with telomere length at the end of the juvenile growth period (χ^2 = 0.452, P = 0.502) and no association between body mass gain and the change in telomere length during the juvenile growth period was found (χ^2 = 0.284, P = 0.594; Fig. 3b). Full model outputs are presented in Supplementary Tables S1 and S2 .

Telomere length in reproductively active adults

Reproductively active adult birds from the divergent selection lines differed significantly in their telomere length (line: χ^2 = 13.726, P <0.001, sex: χ^2 = 0.333, P = 0.564, sex x line: χ^2 = 0.269, P = 0.604, Fig. 1c). Both females and males from lines selected for high reproductive investment had substantially shorter telomeres (females: 10.8 ± 0.3 kb, males: 11.0 ± 0.4 kb; all N = 22) compared to females and males from lines selected for low reproductive investment (females: 12.4 ± 0.3 kb, males: 12.4 ± 0.4 kb; all N = 22; Fig. 1c). Adult body mass was not associated with

adult telomere length (χ^2 =0.224, P = 0.636). Full model outputs are presented in Supplementary Tables S1.

Discussion

Using an artificial selection approach, we tested how divergent reproductive strategies affect telomere length at different life stages. We found an interaction effect between selection line and sex on telomere length early in life, with males, but not females, from lines selected for low maternal investment (i.e. developing in a relatively small egg) having shorter telomeres at birth. Sex-specific effects of environmental adversity on telomere length have previously been reported but, interestingly, the direction of the effect seems to be taxon- or stressor-specific. For example, experimental exposure to nest-based ectoparasites early in life resulted in shorter telomeres in female, but not male great tit (*Parus major*) nestlings (Tschirren et al. 2021), whereas in captive house mice (*Mus musculus*) telomere shortening in response to experimental *Salmonella enterica* infection was more pronounced in males than in females (Ilmonen et al. 2008). Furthermore, high micronutrient intake reduced telomere shortening in female, but not male zebra finches (*Taeniopygia guttata*) (Noguera et al. 2015). Such sex-specific effects of environmental adversity on telomere length might either occur because males and females differ in their susceptibility to the specific stressor, and / or because telomere length, or the rate of telomere shortening, is differentially associated with fitness in males and females (Barrett and Richardson 2011), resulting in sex-specific canalization (Boonekamp et al. 2018; Vedder et al. 2017; Waddington 1942).

The finding that males from low maternal investment lines have shorter telomeres at hatching is in line with the results of previous studies that documented a positive association between telomere length and favourable prenatal (Entringer et al. 2011; Haussmann et al. 2012; Marchetto et al. 2016; McLennan et al. 2018; Stier et al. 2020) or postnatal conditions (Boonekamp et al. 2014; Casagrande et al. 2020; Nettle et al. 2015; Reichert et al. 2015; Young et al. 2017). It suggests that favourable conditions can prevent or buffer physiological processes that impair telomere maintenance.

Favourable conditions can, however, also lead to accelerated growth, which has been linked to higher rates of telomere attrition (Monaghan and Ozanne 2018). For example, heavier lesser black-backed gull (*Larus fuscus*) hatchlings have shorter telomeres (Foote et al. 2011), and slower prenatal growth is associated with longer telomeres at hatching in common terns (*Sterna hirundo*) (Vedder et al. 2018). Similarly, in house sparrows (*Passer domesticus*) fledging size is negatively associated with telomere length (Ringsby et al. 2015), and in king penguin (*Aptenodytes patagonicus*) fast growth in small chicks results in accelerated telomere loss (Geiger et al. 2012). However, other studies have reported positive or no relationships between growth and telomere length early in life (Boonekamp et al. 2021; Monaghan and Ozanne 2018).

Birds from the high investment lines have more resources available during prenatal development (Pick et al. 2016c). Consequently, they gain more mass and are heavier at hatching (Pick et al. 2016a; this study). Although birds from the divergent lines were raised under standardised conditions after birth, these different growth trajectories continued during postnatal development, with birds from the high investment lines gaining more weight during the juvenile growth period (Pick et al.

2016a; this study). Previous line-cross experiments have demonstrated that these different growth trajectories are due to differential maternal egg investment, rather than genetic differences in growth or body size between the lines (Pick et al. 2016a; Pick et al. 2016c). Our selection lines are thus an ideal model to test how growth rate variation affects telomere length and dynamics.

Despite offspring from high investment lines gaining more weight prenatally, this did not result in shorter telomeres at hatching. Indeed, it was males from low investment lines that had the shortest telomeres at birth. Furthermore, no association between hatching mass and telomere length shortly after hatching was observed within or between lines. Similarly, even though birds from the high investment lines gained more weight during the juvenile growth period than birds form the low investment lines, this did not result in shorter telomeres or accelerated telomere attrition. Indeed, the telomere length patterns observed at hatching were still weakly present at the end of the juvenile growth period, with males from the low investment lines having the shortest telomeres, albeit the sex x line interaction was no longer significant at this later stage. These findings suggest that the effect of fast growth on telomere attrition may either not be obligate (e.g. because body size differences are caused by cell size variation rather than differences in cell division rates), and / or that individuals developing under favourable conditions (e.g. in a relatively large egg) may be able to buffer or compensate for accelerated telomere shortening associated with rapid growth, for example through telomerase activation (Noguera et al. 2020; Taylor and Delany 2000, but see Spießberger et al. 2022) or differential expression of telomere regulators (Wolf et al. 2021).

In contrast to the patterns observed early in life, we observed a highly significant selection line effect on telomere length at adulthood, with both adult females and

males from the high investment lines having substantially shorter telomeres than adult birds from the low investment lines. Given that no line difference in telomere length was observed at the end of the juvenile growth period, it suggests that telomere shortening was accelerated in birds from the high investment lines once they had reached sexual maturity (at about 50 days old; Vedder et al. 2022) and started to reproduce.

Females from the high investment lines lay larger (but not fewer) eggs compared to females from the low investment lines (Pick et al. 2016c; this study), and thus differ in the total investment they make into reproduction. This higher reproductive investment of H-line females is associated with substantial costs. For example, we have previously shown that the immune system of H-line birds is downregulated (Pick et al. 2020) and that they mount a lower specific antibody response when challenged with a novel antigen (Pick et al. 2020). Furthermore, the metabolic rate of reproductively active H-line birds is substantially higher compared to reproductively active L-line birds (Pick et al. 2016b), which may be directly linked to accelerated telomere attrition (Casagrande and Hau 2019). Our results are in line with these previous findings and suggest that accelerated telomere shortening is a cost of increased reproductive effort.

Whereas the shorter telomeres of reproductively active H-line females may be directly linked to their increased egg provisioning, the shorter telomeres of reproductively active H-line males are more surprising given that male quail do not contribute to offspring provisioning. Although artificial selection was imposed on a female-limited trait (i.e. relative egg size), males and females share most of their genome. Because of genetic linkage and / or pleiotropy, evolutionary changes in response to the selection regime will thus not only affect egg size, but will likely have

diverse phenotypic consequences in both sexes (Lande 1980; Poissant et al. 2010). We have previously shown that H-line males have a higher reproductive success, both in a competitive and non-competitive mating situation, suggesting a correlated response in male fertility-related traits (e.g. investment in sperm quality or quantity) (Pick et al. 2017), which may come at a direct cost to somatic self-maintenance (Olsson et al. 1997). Indeed, a recent study in dark-eyed juncos (*Junco hyemalis carolinensis*) experimentally demonstrated that increased testosterone levels, which are linked to male reproductive success in this species, are associated with accelerated telomere attrition (Heidinger et al. 2022). The shorter telomeres in reproductively active H-line males might thus be a direct consequence of their increased investment in fertility-related traits.

In addition to direct costs of reproductive investment, the shorter telomeres in reproductively active H-line males and females may be due to genetic or transcriptional constraints. Indeed, we have previously shown that the selection regime resulted in altered patterns of gene expression between the lines (Pick et al. 2020). One of the genes that was consistently differentially expressed (downregulated in H-line birds) was *EXO1* (Pick et al. 2020). This gene plays a key role in DNA repair and telomere maintenance (Bertuch and Lundblad 2004; Keijzers et al. 2016), and its downregulation might contribute to the accelerated telomere shortening in both H-line females and males once they become reproductively active. Genes such as *EXO1* might thus have the potential to act as 'life-history master switches', regulating life-history decisions and life-history trade-offs, such as the trade-off between reproductive investment and self-maintenance, either directly or indirectly (Young 2018).

In humans, conditions experienced early in life seem to be particularly important in determining an individual's telomere length, whereas conditions experienced during adulthood appear to have limited effects on the relative telomere length ranking within a cohort (Benetos et al. 2013). Similarly, a recent study in Japanese quail showed that the pace and stability of embryonic development affects telomere length at hatching, and that these prenatal effects on telomere length persist until adulthood (Stier et al. 2020). These studies are in line with the 'fetal programming of telomere biology (FPTB)' hypothesis (Entringer et al. 2018; Shalev et al. 2013). A different pattern emerged in our study, with birds (particularly males) from the H-line having longer telomeres at birth, but substantially shorter telomeres at adulthood, and vice versa for L-line birds. Telomere length early in life and at adulthood were measured in different cohorts in our study, and we can thus only infer telomere dynamics. However, telomere length patterns were not only opposite at the two life stages, but the effect size of selection line was also larger in adults (males: Cohen's d = 0.696, females: Cohen's d = 1.175) than in hatchlings (males: Cohen's d = 0.607, females: Cohen's d = 0.245). These findings are inconsistent with the FPTB hypothesis and suggest that experiences and life-history decisions at adulthood can sometimes have an even larger impact on individual telomere biology than conditions experienced early in life.

In conclusion, our study shows that selection for increased maternal provisioning results in longer (male) offspring telomeres at birth, but that such a high-reproductive-effort strategy comes at the cost of accelerated telomere attrition later in life. These distinct telomere length patterns across the life course may either be a direct consequence of maternal resource provisioning (i.e. reflect the benefits of

receiving more maternal resources early in life and the costs of high reproductive effort during adulthood), or be an indirect consequence of selection on reproductive strategies, mediated by, for example, genetic or transcriptional constraints on telomere maintenance. Irrespective of the mechanism, our study demonstrates that reproductive strategies differentially affect telomere dynamics across the life course and highlights the power of artificial selection approaches to reveal cross-generational constraints that shape the evolution of life histories.

Ethics

All procedures complied with all relevant ethical regulations and were conducted under licenses provided by the Veterinary Office of the Canton of Zurich, Switzerland (permits 195/2010, 14/2014, 156).

Data accessibility

All data and code associated with this article are available in the Dryad Digital Repository doi: 10.5061/dryad.h44j0zpk0

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reviewers for constructive comments on the manuscript. We declare we have no competing interests.

Authors' contributions

BT conceptualised and designed the study and acquired funding for the project. BT and JM collected data. JM performed DNA extraction and validation. MFH developed and performed telomere length analyses. BT and AARH performed statistical analyses and drafted the manuscript. All authors reviewed and gave input on the final version of the manuscript.

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Figure legends

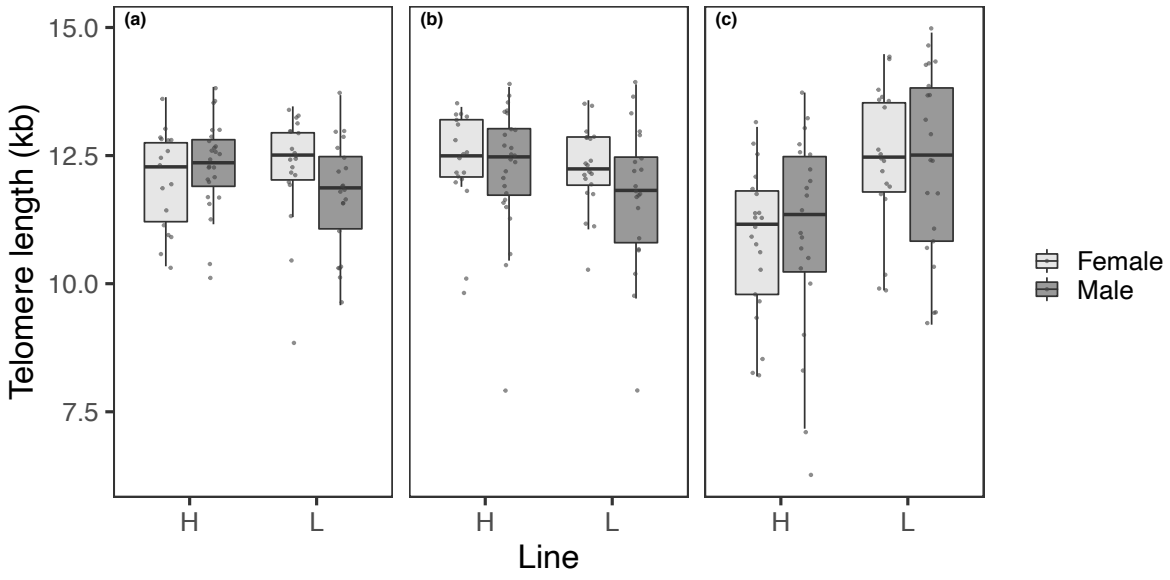


Fig. 1. Telomere length of birds from lines selected for high (H) or low (L) maternal egg investment. (a) Telomere length at birth (3 days posthatching) of birds from lines selected for high or low maternal egg investment, (b) Telomere length at the end of the juvenile growth period (30 days posthatching) of birds from lines selected for high or low maternal egg investment, (c) Telomere length of reproductively active adult birds from lines selected for high or low maternal egg investment. Note that the same individuals are shown in A) and B), whereas the parents of these individuals are shown in C). Females are shown in light grey and males in dark grey. Horizontal lines in boxplots represent mean and interquartile ranges. Individual datapoints are shown.

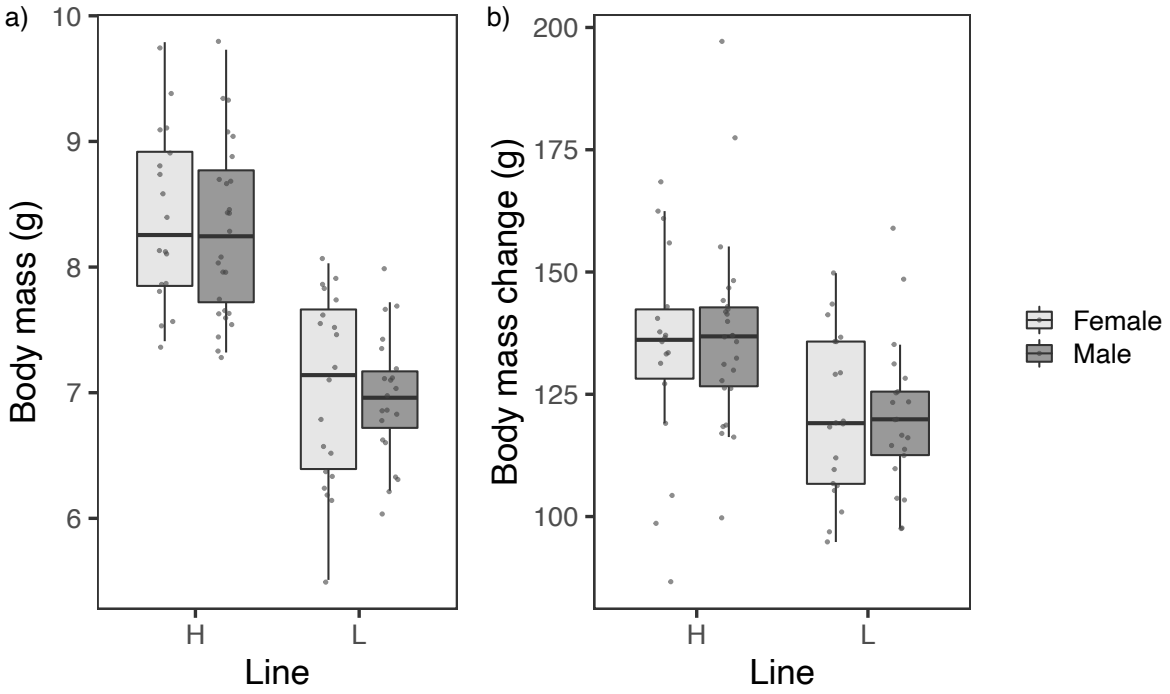


Fig. 2. Hatching mass and juvenile growth of birds from lines selected for high (H) or low (L) maternal egg investment. a) Hatching mass of birds from lines selected for high or low maternal egg investment, b) Body mass change between hatching and day 30 posthatching of birds from lines selected for high or low maternal egg investment. Females are shown in light grey and males in dark grey. Horizontal lines in boxplots represent mean and interquartile ranges. Individual datapoints are shown.

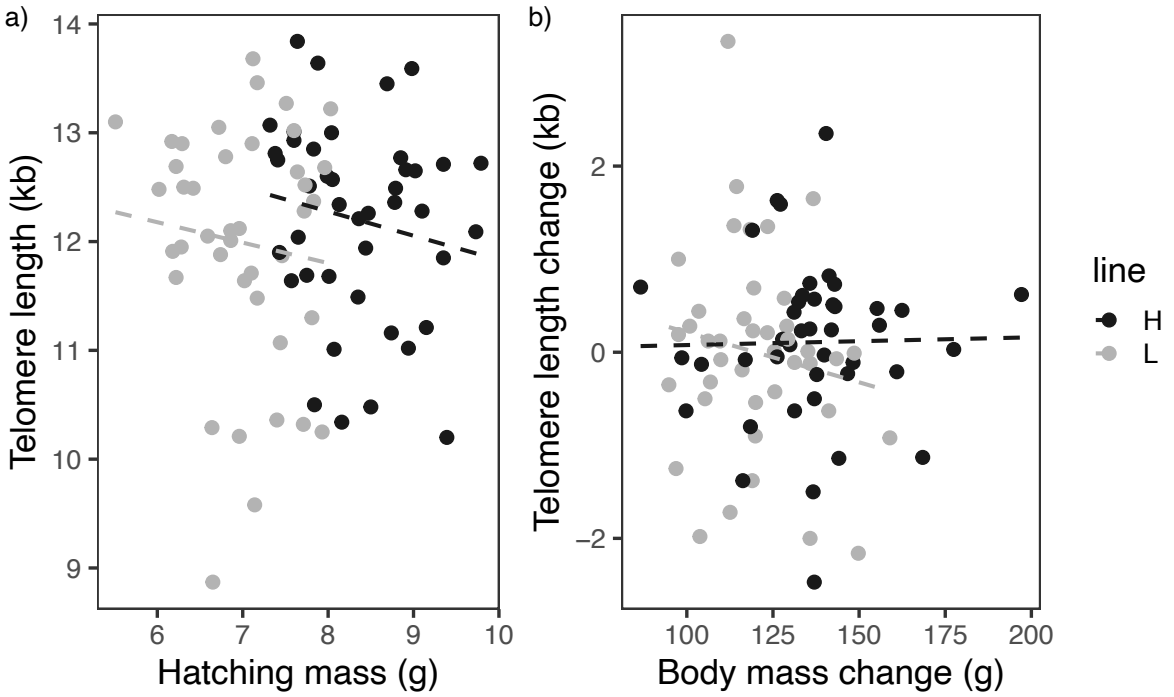
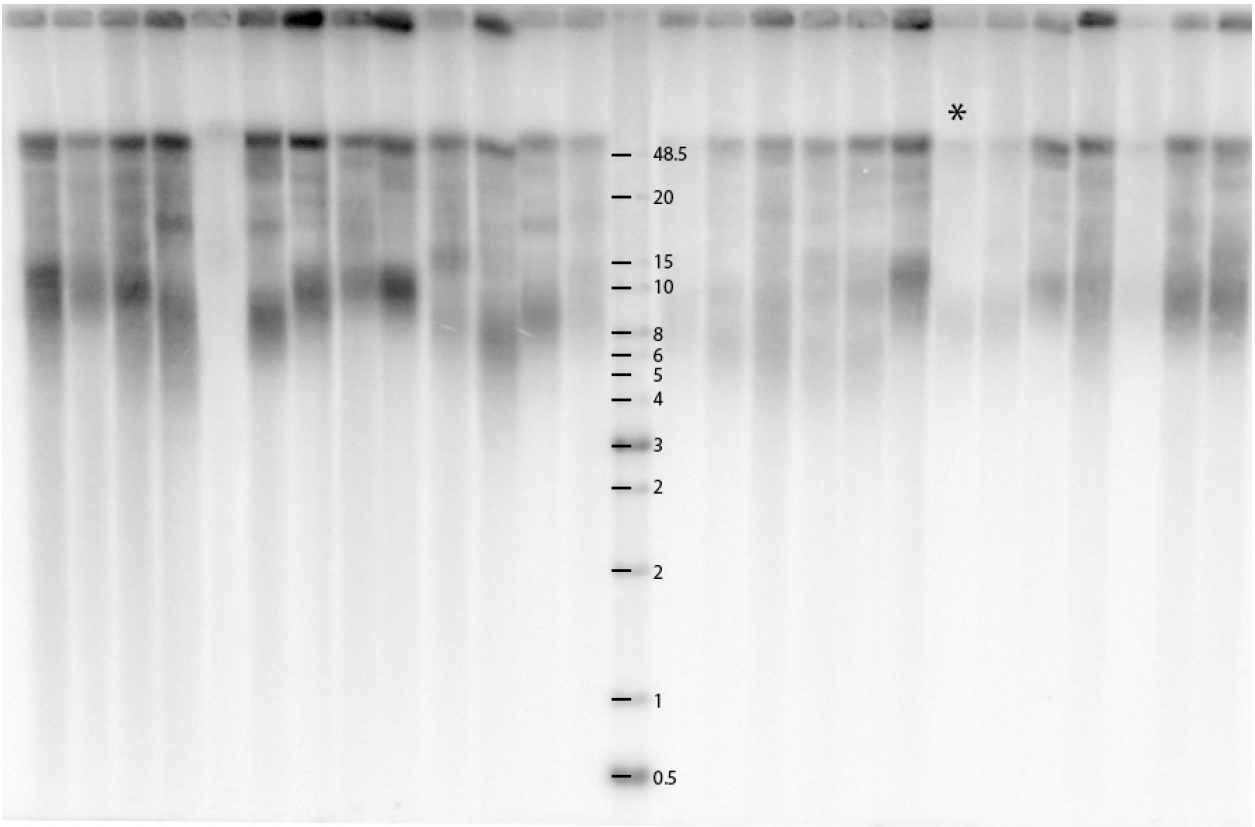


Fig. 3. Association between growth and telomere length in birds from lines selected for high (H) or low (L) maternal egg investment. a) Relationship between hatching mass and telomere length 3 days posthatching of birds from lines selected for high or low maternal egg investment, b) Relationship between body mass gain during the juvenile growth period (hatching – day 30 posthatching) and the change in telomere length during the juvenile growth period in birds from lines selected for high or low maternal egg investment. Black dots represent birds from the high maternal investment lines, grey dots represent birds from the low maternal investment lines. Dashed lines represent regression lines. Note that none of the associations were statistically significant ($P > 0.194$).

Supplementary materials



Supplementary Figure S1. An example in-gel hybridization telomere restriction fragment (TRF) gel in *Coturnix japonica*. Telomere length was measured over the range of the molecular marker (0.5- 48.5kb; Quick-Load1 kb DNA Extend DNA Ladder; New Englad BioLabs). Individuals were run in separate lanes. Notice there is very little variation above 48.5 kb, and all individuals appear to have a within-lane darker band above 48.5kb. There is substantial variation between 2-15kb. If lanes were very faint (for example, *), another sample was run on a subsequent gel.

Supplementary Table S1. Effects of selection line, sex, their interaction and body mass (change) on a) telomere length on day 3 posthatching, b) telomere length on day 30 posthatching, c) the change in telomere length between day 3 and day 30, and d) adult telomere length. The factor level of comparison is indicated in brackets. Non-significant interactions were removed from the final models to determine the significance of the main effects. Degrees of freedom are 1 in all models.

a) Telomere length day 3 (kb)

Fixed effects				
Predictor	*Estimate*	*SE*	χ^2	*P*
Intercept	13.827	1.444		
Line (L)	-0.041	0.401		
Sex (males)	0.262	0.313		
Hatching mass (g)	-0.214	0.169	1.688	0.194
Line (L) x sex (males)	-0.847	0.441	3.830	0.050
Random effects				
Predictor	*Variance*			
Family ID	0.000		0.000	1
Gel ID	0.000		0.000	1
Residuals	0.991			

b) Telomere length day 30 (kb)

Fixed effects				
Predictor	*Estimate*	*SE*	χ^2	*P*
Intercept	13.098	1.060		
Line (L)	-0.139	0.402	1.245	0.265
Sex (males)	-0.154	0.282	2.594	0.107
Body mass (g)	-0.005	0.007	0.452	0.502
Line (L) x sex (males)	-0.414	0.420	1.014	0.314
Random effects				
Predictor	*Variance*			
Family ID	0.584		6.845	0.009

Gel ID	0.000		0.000	1
Residuals	0.782			

c) Change in telomere length day 3 – day 30 (kb)

Fixed effects				
Predictor	*Estimate*	*SE*	χ^2	*P*
Intercept	0.727	0.857		
Line (L)	-0.457	0.340	0.568	0.451
Sex (males)	-0.390	0.305	0.709	0.400
Body mass gain (g)	-0.003	0.006	0.284	0.594
Line (L) x sex (males)	0.414	0.433	0.980	0.322
Random effects				
Predictor	*Variance*			
Family ID	0.065		0.141	0.708
Gel ID	< 0.001		0.000	1
Residuals	0.921			

d) Adult telomere length (kb)

Fixed effects				
Predictor	*Estimate*	*SE*	χ^2	*P*
Intercept	9.653	2.855		
Line (L)	1.692	0.542	13.726	< 0.001
Sex (males)	0.517	0.719	0.333	0.564
Body mass (g)	0.004	0.101	0.224	0.636
Line (L) x sex (males)	-0.373	0.741	0.269	0.604
Random effects				
Predictor	*Variance*			
Gel ID	0.000		0.000	1
Residuals	2.843			

Supplementary Table S2. Effects of selection line, sex, and their interaction on a) hatching mass and, b) body mass gain during the juvenile growth period (hatching – day 30). The factor level of comparison is indicated in brackets. Non-significant interactions were removed from the final models to determine the significance of the main effects. Degrees of freedom are 1 in all models.

a) Hatching mass (g)

Fixed effects				
Predictor	*Estimate*	*SE*	χ^2	*P*
Intercept	8.469	0.146		
Line (L)	-1.468	0.208	37.116	< 0.001
Sex (males)	-0.153	0.096	2.138	0.144
Line (L) x sex (males)	0.111	0.148	0.571	0.450
Random effects				
Predictor				
Family ID	0.348		42.235	< 0.001
Residuals	0.087			

b) Body mass gain hatching – day 30 (g)

Fixed effects				
Predictor	*Estimate*	*SE*	χ^2	*P*
Intercept	136.254	4.301		
Line (L)	-16.746	6.123	12.010	< 0.001
Sex (males)	2.058	3.988	0.084	0.772
Line (L) x sex (males)	-2.773	6.032	0.222	0.638
Random effects				
Predictor				
Family ID	201.1		11.141	< 0.001
Residuals	155.0			