Spatio-temporal variation in lifelong telomere dynamics in a long-term ecological study

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Abstract

1. Understanding individual-level variation in response to the environment is fundamental to understanding life-history evolution and population dynamics. Telomeres, the protective caps at the ends of chromosomes, shorten in response to oxidative stress, and telomere shortening is correlated with reduced survival and lifespan. Investigating telomere dynamics may help us quantify individual variation in the costs experienced from social and ecological environmental factors, and enhance our understanding of the dynamics of natural populations.

2. Here we study spatio-temporal variation in lifelong telomere dynamics in the Seychelles warbler, *Acrocephalus sechellensis*. We combine long-term life-history and ecological data with a large longitudinal dataset of mean telomere lengths, consisting of 1808 samples from 22 cohorts born between 1993 and 2014. We provide a detailed analysis of how telomere dynamics vary over individual lifespans and cohorts, and with spatio-temporal variation in the social and ecological environment.

3. We found that telomere length decreases with cross-sectional and longitudinal measures of age, and most rapidly very early in life. However, both cross-sectional and longitudinal data suggested that against this overall pattern of shortening, bouts of telomere length increase occur in some individuals. Using a large number of repeated measurements we show statistically that these increases are unlikely to be explained solely by qPCR measurement error.

4. Telomere length varied markedly among cohorts. Telomere length was positively associated with temporal variation in island-wide insect abundance - a key resource for the insectivorous Seychelles warbler - suggesting that the costs associated with living in harsher environments can be studied by investigating telomere dynamics. We also found evidence for sex-specific relationships between telomeres and tarsus length, potentially reflecting differential costs of growth.

5. Our long-term data show that in a natural population, telomere dynamics vary in a complex manner over individual lifespans, and across space and time. Variance in telomere dynamics among individuals is the product of a wide array of genetic, parental and environmental factors. Explaining this variation more fully will require the integration of comprehensive long-term ecological and genetic data from multiple populations and species.

Keywords: Biomarkers; Intra- and inter-individual variation; Life history; Telomere; Seychelles warbler; Senescence

Introduction

A major aim of ecologists and evolutionary biologists is to understand why individuals vary in their response to different environmental factors. Identifying this variation in individual responses to the environment is central to understanding variation in fitness (Lindström 1999), and thus for understanding population and community dynamics (Bolnick *et al.* 2011). Furthermore, knowledge of the relative impact that different environmental factors exert on individuals, and why individuals may differ in mitigating these costs, is important to understanding evolutionary trade-offs and life-history strategies (Stearns 1992). However, fully quantifying individual-level variation in costs is impossible in wild systems, and thus effective biomarkers that reflect the physiological consequences of individual-level experiences are required.

Telomeres have been proposed to be a potential biomarker of such costs (Monaghan 2014). Telomeres are repetitive DNA sequences at the ends of linear chromosomes that protect against DNA damage. Telomeres generally shorten with age (Barrett *et al.* 2013; Müezzinler, Zaineddin & Brenner 2013), and there is evidence from a range of taxa that telomere shortening is fastest in early life (e.g. Frenck, Blackburn & Shannon 1998; Heidinger *et al.* 2012). *In vitro* research has shown tha telomere shortening can be accelerated by oxidative stress (Von Zglinicki 2002), which can be elevated due to many environmental factors. There is evidence from humans, and from captive and wild animal populations, that telomere shortening is influenced by the conditions experienced during both early life and adulthood (Price *et al.* 2013; Monaghan 2014; Reichert, Criscuolo & Zahn 2015; Nettle *et al.* 2015). Importantly, the extent of telomere shortening is linked to senescence and survival. When telomeres become critically short, cells senesce (Campisi 2003), and the accumulation of these cells has been suggested to result in organismal senescence and death (Wong *et al.* 2003). The association between senescence and telomere length has inspired a great deal of recent research into telomere evolutionary ecology, and relationships between telomere dynamics and survival or lifespan have been documented in wild population of several species (Haussmann & Marchetto 2010; Barrett *et al.* 2013; Stier *et al.* 2015). As yet, however, there is little direct evidence that the relationship between telomere dynamics and survival is causal (Simons 2015).

Although the causal role of telomeres in senescence and survival is not yet clear, there is mounting evidence that telomeres can act as biomarkers of individual condition and ageing in wild populations, providing a measure of the ecological stress that an individual has experienced, a signature that can otherwise be difficult to detect (e.g. Schultner *et al.* 2014; Asghar *et al.* 2015; Bebbington *et al.* 2016). There is also evidence that telomere length, measured longitudinally in individuals, can increase as well as decrease (Simons, Stulp & Nakagawa 2014; Bateson & Nettle 2016), which has important ramifications for our understanding of how

telomeres reflect costs. However, such increases in telomere length are often attributed to measurement error (Steenstrup *et al.* 2013; but see Bateson & Nettle 2016), and as such their ecological significance is unknown.

Although a considerable amount of effort has been put into studying telomere dynamics in natural populations, our understanding of the forces responsible for explaining variation in telomere length is still limited. Understanding how different factors shape telomere length variation is important, as before we can use telomeres as a measure of the costs experienced by individuals, we need to know how different developmental, genetic and ecological variables interact to affect telomeres. Telomere length and rates of shortening can vary according to parental characteristics (Njajou *et al.* 2007; Heidinger *et al.* 2016), among sexes (Barrett & Richardson 2011; Watson *et al.* 2017), and with a whole host of environmental conditions, including altitude (Stier *et al.* 2016), heat stress (Simide *et al.* 2016) or infection (Asghar *et al.* 2015). Recent evidence suggests that telomere dynamics are indeed highly variable over individual lifespans, and that even the relationship between telomeres and age can vary markedly among cohorts (Fairlie *et al.* 2016). To understand which factors best explain variation in telomere dynamics, more studies are required that incorporate telomere variation over entire lifespans with comprehensive, long-term ecological data.

The longitudinal study (since 1986) of the Seychelles warbler (*Acrocephalus sechellensis*) population on Cousin Island provides an excellent system for studying telomere dynamics and senescence patterns in the wild (reviewed in Hammers *et al.* 2015). Due to the isolated nature of the study population (Komdeur *et al.* 2004) and intensive field monitoring, we have comprehensive ecological and survival data spanning many years (see Methods, below). Environmental conditions and population density on Cousin Island vary across space and time due to weather-induced changes in foliage cover and insect prey availability (Van de Crommenacker *et al.* 2011). Variation in oxidative stress experienced by individuals is associated with territory quality (Van de Crommenacker *et al.* 2011). However, the evidence that individual survival and lifespan is associated with spatial variation in early-life territory quality or local density is equivocal and confounded by variation in subsequent life-history parameters (Brouwer *et al.* 2006; Hammers *et al.* 2013). There is also variation in the social environment that individual Seychelles warblers experience. Facultative cooperative breeding occurs in this species (Komdeur 1994; Richardson, Komdeur & Burke 2003; Richardson, Burke & Komdeur 2007), and the presence of helpers (but not other resident non-helpers) in the natal territory is associated with increased survival of offspring later in life (Brouwer, Richardson & Komdeur 2012).

Importantly, we have an established protocol for assessing telomere length in the Seychelles warbler (Barrett *et al.* 2012; Bebbington *et al.* 2016). Furthermore, telomere dynamics predict survival independently of age (Barrett *et al.* 2013) and telomere length is negatively associated with inbreeding (Bebbington *et al.* 2016), suggesting that individual variation in telomere length is ecologically relevant in this species. Thus, we

have an excellent system in which to determine the impact of different social and environmental conditions experienced by individuals, and to assess how these costs vary over space and time.

In this study, we test how lifelong telomere dynamics are related to environmental variation across 22 Seychelles warbler cohorts (years). We first study how telomere length and rates of shortening are related to age and sex across all life stages, and how this relationship varies among cohorts, in order to gain an in-depth understanding of the temporal dynamics of telomere changes. We then examine, within individuals, how telomere length changes with age, and statistically test whether observed increases in telomere length across temporally longitudinal samples within individuals are larger than can be accounted for by measurement error. Finally, we test how telomere length and shortening are related to a wide range of social and environmental variables in order to gain a fuller understanding of the forces driving telomere dynamics in natural populations.

Methods

Study species and sampling

The Seychelles warbler is a small (~15 g), insectivorous passerine bird with a mean life expectancy of 5.5 years at fledging (Hammers *et al.* 2013). The population of *ca* 320 adult birds on Cousin Island (04'20'S, 55'40'E) has been intensively studied since 1986 (Komdeur 1992; Richardson, Burke & Komdeur 2003; Spurgin *et al.* 2014). This species' main breeding season runs from June-September (though a small proportion of pairs also breed between January–March), when the breeding females on many of the *ca* 110 territories will attempt to breed, laying one or, rarely, two or three eggs (Komdeur, Bullock & Rands 1991). Breeding attempts are often unsuccessful, and as a result of this low reproductive output, and higher mortality in first-year birds (39% in first-year birds versus 16% in adults; Brouwer *et al.* 2006), cohort sizes in the Seychelles warbler are typically small (< 50). The 22 birth year cohorts used in this study cover 1993 to 2014 – the time period during which our data and sampling are most complete.

The majority of individuals are ringed (with an individually numbered metal ring and unique combination of colour rings) within the first year of life, and so are of known age. We aged all birds using information on eye colour at first capture (Komdeur 1991) and previous capture history (Richardson *et al.* 2003). Within the first year of life, birds are classified as nestlings less than one month old (rounded to one month for analyses), fledglings less than six month olds (rounded to six months) or subadults up to one year old (rounded to 10 months). Ages for adult birds were rounded to the nearest year. As Seychelles warblers are non-migratory endemics naturally confined to the island (Komdeur *et al.* 2004), an extensive biannual census of birds on Cousin during each breeding season gives accurate measures of local density, social status (e.g. breeder, helper,

non-helper) and individual survival (Crommenacker, Komdeur & Richardson 2011; Barrett *et al.* 2013). Full details of monitoring methods can be found in Brouwer *et al.* (2012).

Seychelles warblers are highly territorial and all territories were mapped during each main breeding season using detailed observational data on foraging and territorial defence behaviour (Richardson *et al.* 2003). Territory quality is calculated based on territory size, foliage cover and insect abundance (Komdeur 1992). Where territory quality estimates were not available for a specific year we used the average value for that territory across years (Hammers *et al.* 2013). Cousin is subject to considerable intra- and inter-annual variation in rainfall and, consequently, insect availability. Such island-wide temporal variation may override the effects of variation in individual territory quality across the island. As an estimate of seasonal variation in food availability, we calculated an index of the abundance of insects across the entire island during each main breeding season (referred to hereafter as 'insect abundance'). This index is calculated as the mean number of insects found per unit leaf area over all monthly surveys carried out on the island in a main breeding season.

Each time a bird is caught on Cousin a range of morphometric measurements are taken, including body mass and tarsus length (to the nearest 0.1g and 0.1mm, respectively). A blood sample (*ca* 25 μl) is taken via brachial venipuncture, and stored at room temperature in 1 ml of absolute ethanol in a 1.5 ml screw-cap microfuge tube.

Molecular methods

For each sample, genomic DNA was extracted from a ~2 mm^2 flake of preserved blood using the DNeasy Blood and Tissue Kit (Qiagen), following the manufacturer's protocol, with the modification of overnight lysis at 37°C and a final DNA elution volume of 80 μl. Sex was determined using the PCR-based method outlined by Griffiths *et al.* (1998). Prior to telomere analysis, DNA concentration and purity were quantified using a NanoDrop 8000 Spectrophotometer (ThermoScientific). The following thresholds were applied before samples were included for further analysis: i) DNA concentration must be at least 15 ng μl^{-1} (based on a mean of three measurements), ii) the 260/280 absorbance ratio has to be between 1.8 and 2.0 for acceptable DNA purity, and iii) the 260/230 absorbance ratio must be higher than 1.8. DNA integrity was further validated by visualization with ethidium bromide after electrophoresis on a 1.2% agarose gel, and all samples with evidence of DNA degradation were re-extracted or excluded. We found no evidence of DNA degradation in older samples (Fig. S1). All DNA extractions that passed the above criteria were diluted to 3.3 ng μl^{-1} before telomere measurement. We measured relative telomere length (RTL) for all samples using a quantitative PCR (qPCR) assay of telomeres and a GAPDH control gene, following Bebbington *et al.* (2016). Prior to qPCR, we used a random number generator to assign samples to qPCR plates, to ensure that no systematic

bias could occur with regards to age, sex, cohort or ecological environment. Based on the distribution of observed cq values, we excluded outlier samples with extremely large cq values (cq values > 25 and 26 were excluded for the telomere and GAPDH reactions, respectively), which were assumed to be failed reactions.

For a large subset of birds we had longitudinal data, with two or more samples taken at different ages (n = 1057 measurements from 402 birds). For these individuals we calculated the within-individual change in RTL by subtracting RTL at time point t from RTL at time point $t + 1$ (hereafter ΔRTL, n = 655 measurements). Negative values of RTL reflect decreases in telomere length with age, while positive values reflect increases.

Statistical analyses

We performed all statistical analyses using R version 3.2.2 (R Development Core Team 2011). RTL was square root transformed to improve linear model fits, and we assessed repeatability of RTL using the rptR package.

We explored the cross-sectional relationship between RTL and age among cohorts using linear mixed models (LMMs) carried out in the lme4 package (Bates *et al.* 2014). Following a similar approach to Fairlie *et al.* (2016), we compared a selection of models fitting different relationships between RTL and age. We created models where the relationship between RTL and age was linear, quadratic, log-linear, and where age was fitted as a factor. For each age term, we fitted additional models including birth year (cohort) as a factor. All fitted models are included in Table 1. Note that we do not carry out full model selection or model averaging here, as our aim was to compare a set of specifically defined models. For random effects we included individual ID, and catch year. Models were compared using AIC with correction for finite sample size (AICc; Hurvich & Tsai 1989).

Using the longitudinal data, we then tested how telomeres change with age in individuals, using LMMs of RTL as a response and Δage (a longitudinal measure based on within-subject centring; Pol & Wright 2009) as an explanatory variable. We calculated Δage using log and polynomial transformed age data, and carried out model selection as above, with the exception that we did not model Δage as a factor (due to a lack of discreet groupings), and mean age was also included in models to partition within-individual *vs* cross-sectional effects (Pol & Wright 2009).

We used two approaches to determine individual-level consistency in RTL. We first calculated individual-level repeatability in RTL by dividing the random variance explained by individual ID by the total random variance, in a model of that accounted for age and cohort effects. Second, we constructed a LMM with RTL at time $t + 1$ as the response variable, RTL at time t and age at time t as fixed effects, and individual ID and cohort as random effects. We estimated the slope of the relationship between within-individual telomere measurements,

as well as the variance explained, by calculating the marginal R^2 (Nakagawa & Schielzeth 2013) of the model.

When examining the distribution of longitudinal telomere changes we observed some increases in telomere length with age in individuals. We therefore repeated the qPCR on each sample using completely separate reactions run on separate plates and used these repeat measurements to test whether these increases could be explained by measurement error. We calculated the change in RTL between pairs of repeat measurements within the same samples (hereafter $\Delta\text{RTL}_{sample}$; N = 422 pairs of measurements from 293 birds) in exactly the same way as for across samples (hereafter $\Delta\text{RTL}_{individual}$). To test whether greater changes in RTL were observed among individuals compared to among repeat samples, we compared the variance in $\Delta\text{RTL}_{sample}$ and $\Delta\text{RTL}_{individual}$ using a Levene's test. Then, to separately test whether the extent of telomere increases and decreases within individuals were greater than expected by measurement error, we split ΔRTL measurements into groups in which RTL decreased ($\Delta\text{RTL} < 0$) and increased ΔRTL ($\Delta\text{RTL} > 0$), and tested whether $\Delta\text{RTL}_{individual}$ values were significantly different from $\Delta\text{RTL}_{sample}$ values, using Wilcoxon tests.

We also tested whether consistent telomere lengthening across our dataset using a modified version of the approach developed by Simons *et al.* (2014). Briefly, this approach utilises samples with at least three telomere measurements to compare residual variance in telomere change over time with the overall change in telomere length between the first and least telomere measurements (Simons *et al.* 2014). If, in samples that increase in length, the overall increase in telomere length exceeds the residual variance, then telomere lengthening cannot be explained by error (Simons *et al.* 2014). If, on the other hand, increases in telomere length are due to measurement error, within-individual residual variance in telomere length is expected to be similar to overall observed increases in telomere length.

We used LMMs to explore how variation in environmental and social conditions influenced telomere length and dynamics within cohorts. We first created a full model with RTL as a response variable, alongside the following explanatory variables: log age (based on the RTL and age analysis; see results), tarsus length, body mass, sex, insect abundance, territory quality, island-wide population density (an annual measure estimated from the summer breeding census), territory group size, and the number of helping subordinate birds present in the territory. The random effects structure was informed by the analysis of telomere dynamics and age (see results): we included individual ID, cohort ID, and a random slope of log age among cohorts (to allow the effect of age on RTL to vary among cohorts). We report model estimates and confidence intervals for all effects included in the full model. We also calculated marginal R^2 (incorporating only fixed effects; Nakagawa & Schielzeth 2013) and conditional R^2 (incorporating fixed and random effects; Johnson 2014) to assess the explanatory power of these models. As a complementary approach, we also performed model averaging, using the MuMIn package in R (Bartoń 2012). Model selection was performed using the full model described above.

A top model set was then defined, containing all models with AICc $\leq$ 6 compared to the best supported model (Burnham, Anderson & Huyvaert 2011). We report model-averaged coefficients, confidence intervals and 'relative importance', which reflects the relative weights of each predictor variable across the top model set.

For individuals with longitudinal data we repeated the above analyses of telomere dynamics, replacing telomere length with $\Delta RTL_{individual}$ as the response variable, and including the environmental/social explanatory variables from the first of the two sampling points. We excluded the cohort ID random effect from this analysis, as longitudinal telomere dynamics did not differ among cohorts; see results.

Results

We measured telomere lengths using a total of 1808 unique samples from juvenile and adult Seychelles warblers from 22 cohorts born between 1993 and 2014. Efficiencies (mean $\pm$ s.d.) for our telomere and GAPDH reactions were 1.78 $\pm$ 0.05 and 1.92 $\pm$ 0.04 respectively. Inter-plate repeatability of RTL, based on 422 samples measured at least twice at different time points, was 0.68 (CI = 0.64, 0.71). Using adult samples greater than one year old, we checked whether RTL was related to sample storage time, and found no evidence of such a relationship (estimate = -0.002, CIs = -0.007, 0.002).

Telomere dynamics and age among cohorts

We first tested how RTL was related to age among cohorts using a model selection approach. The top model contained a log-linear relationship between RTL and age (Table 1A). All other models fitted the data much less well (ΔAICc $>$ 15; Table 1). The log-linear relationship between RTL and age could be seen clearly in the raw data; RTL decreased with age (estimate = -0.071, CIs = -0.087, -0.056), with the greatest decrease occurring in the first year of life (Fig. 1A). There was substantial variation in RTL among cohorts, with no obvious trend over time (Fig. 1B). There was a negative relationship between RTL and log age in 21 of the 22 cohorts, but the slope the relationship varied substantially among cohorts (Fig. 1C). To test whether this variation was significant we fitted a model including the log age x cohort interaction term, and found that this was a marginally better fit than a model including only main effects (ΔAICc = 1.40). In the one year in which RTL increased with age (2013), 17 of the 18 birds sampled were fledglings or subadults, suggesting that the observed pattern was an artefact of the sampling in this season (i.e. a lack of variation in age among sampled birds), rather than a real relationship.

A within-individual analysis of RTL and age revealed that the top model explaining RTL contained Δlog age, which reflects within-individual changes in log-transformed age (Table 1B). Models including cohort ID

were substantially poorer fits than a model only containing age (Table 1B). RTL decreased with Δlog age (estimate = -0.052, CIs = -0.085, -0.018), confirming that within-individual telomere shortening occurs across the Seychelles warbler dataset.

Individual repeatability in RTL was 0.082, meaning that 8% of variance in RTL could be explained by within-individual consistency. Accordingly, there was a positive correlation between RTL measured from different samples taken at different time points during an individual's life (Fig. 2A), but this was very weak (marginal R2 = 0.01), and not significant (estimate = 0.066, CIs = -0.006, 0.137).

Although both cross-sectional and longitudinal data indicated a general trend of telomere shortening with age, we found that RTL - measured across two samples taken from the same individuals over time - increased with age in 44% of our 655 $\Delta\text{RTL}_{individual}$ measurements (Fig. 2A). To test whether increases in telomere length in our dataset could be explained by measurement error, we compared variance in telomere length among repeat measurements of the same samples to the variance observed among different samples of the same individual. We found significantly higher variance in telomere length over individual lifetimes compared to among sample replicates (Levene's test: $F = 43.63$; $P < 0.001$; Fig. 2B). Splitting the longitudinal data into instances of decreasing (i.e. $\Delta\text{RTL} < 0$) and increasing (i.e. $\Delta\text{RTL} > 0$) telomere length revealed that not only did we observe significantly greater decrease in RTL within individuals compared to within samples (Wilcoxon test: $P < 0.001$), but also a significantly greater increase ($P < 0.001$; Fig. 2B).

To better understand how longitudinal telomere dynamics vary with age, we examined patterns of short-term telomere change, including only pairs of samples taken within two years of each other. We found that the likelihood of telomere lengthening increased with log age (GLMM with lengthened yes/no as binomial response; estimate = 0.296, CIs = 0.005, 0.588). Increases in telomere length were most likely to be observed shortly after the juvenile period, at around four years of age, and later in life (although sample sizes for older birds are much smaller; Fig. 2C,D).

Using the approach outlined by Simons *et al.* we tested whether overall increases in RTL over lifespans could be detected statistically in our dataset. We found no evidence that this was the case: overall increases in RTL within individuals did not exceed residual variance; in fact, residual variance in RTL was significantly greater than observed RTL increases over lifespans ($P = 0.02$). This suggests that increases in RTL within individuals are sporadic, and not consistent over individual lifespans.

Telomere dynamics and the environment

In addition to age, RTL was associated with tarsus length, sex and insect abundance (Fig. 3A). RTL was negatively related to tarsus length and males had longer telomeres than females (Fig. 3B), while insect

abundance was positively related to RTL (Fig. 3C). The full model was weak in terms of explanatory power of fixed effects (marginal $R^2 = 0.07$), although including the random effect terms increased this substantially (conditional $R^2 = 0.17$). The model averaging approach yielded qualitatively identical results to the full LMM, with the same explanatory variables 'significant' in terms of being retained in top models, and having model-averaged confidence intervals not overlapping zero (Table S1; Fig. S2). One interesting finding from the model selection was that sex only appeared in top models where tarsus length was also present (Table S1). In accordance with this, when tarsus length was removed from the full model sex was no longer significant (estimate = 0.008, CIs = -0.014, 0.030), and a sex x tarsus interaction was significant when included (estimate = 0.021, CIs = 0.002, 0.040); RTL decreased with tarsus length in both sexes, but this decrease was stronger in females (Fig. 3B). No social or ecological environmental variables were significant predictors of ΔRTL using the full model approach (Table S2). Using model selection, we found that the top model explaining ΔRTL contained age and population density (Table S3). ΔRTL was positively related to age, consistent with telomere shortening being highest in early life, and negatively related to population density; however, in both instances model averaged confidence intervals overlapped zero (Fig. S3).

Discussion

Here we use a long-term, multi-cohort dataset to assess lifelong telomere dynamics and the relationship between these and spatio-temporal variation in the ecological environment in a contained population of Seychelles warblers. We found that telomere length decreases with age, and that this decrease is greatest very early in life. Telomere length decreased with age in almost all of the 22 cohorts studied, but also that telomere length varied substantially among cohorts. Despite an overall pattern of telomere shortening with age in the Seychelles warbler, we found evidence of within-individual increases in telomere length, and that the extent of these increases could not be explained solely by qPCR measurement error. Finally, we found that telomeres are related to tarsus length in a sex-specific manner, and that telomere length is positively associated with temporal fluctuations in food availability.

Our study adds to the substantial body of literature from humans and wild animals showing that telomere length decreases with age, and that this decrease is most rapid in early life - most likely as a consequence of cellular division (e.g. Frenck *et al.* 1998; Haussmann, Vleck & Nisbet 2003; Heidinger *et al.* 2012). However, we also found that, despite an overall trend for shortening, telomere length both increased and decreased, especially after the juvenile period. Importantly, these increases were observed in longitudinal as well as cross-sectional data, indicating that selective disappearance of individuals with shorter telomeres is not

sufficient to explain this pattern. Longitudinal increases in measured telomere length have been observed in humans and wild animals (Kotrschal, Ilmonen & Penn 2007; Steenstrup *et al.* 2013; Fairlie *et al.* 2016; Hoelzl *et al.* 2016a; b). The most commonly invoked explanation for increases in telomere length is measurement error, which can be a particular problem in qPCR-based telomere studies (Steenstrup *et al.* 2013; Nussey *et al.* 2014; Verhulst *et al.* 2015). However, recent modelling work suggests that longitudinal telomere dynamics in humans are indeed consistent with instances of lengthening, and that dismissing apparent telomere lengthening as solely measurement error is "too strong" without additional data (Bateson & Nettle 2016). Here, we explicitly compare intra-individual variation among samples to variation among sample replicates, on a large scale. Our results suggest that qPCR measurement error alone cannot explain observed increases in RTL observed within individuals.

Increases in telomere length were not consistent over individual lifespans, but occurred in bouts, against a backdrop of overall lifelong telomere shortening. This is consistent with recent findings in edible dormice *Glis glis*, in which telomere elongation was observed only later in life (Hoelzl *et al.* 2016b). Consistent with a pattern of sporadic changes in telomere length with age, we found that within-individual telomere measurements were only weakly correlated. These findings are in contrast to other avian studies in which within-individual telomere length measurements were highly consistent, and individual-level telomere shortening occurred throughout the juvenile period and into adulthood (Heidinger *et al.* 2012; Boonekamp *et al.* 2014). However, the lifelong telomere dynamics found in Seychelles warblers are strikingly similar to those found in Soay sheep (Fairlie *et al.* 2016). This discrepancy in results may be because in our study, and that of Fairlie et al. (2016), individuals were born and reared in the wild, as opposed to in nestbox or laboratory conditions. Alternatively it may be because our longitudinal telomere measurements have been taken over longer time periods.

The finding that increases in telomere length may be sporadic and overlaid on an overall pattern of shortening with age is an important point when assessing the occurrence of telomere lengthening. Previously described approaches to distinguish telomere elongation from measurement error, based on assumptions about follow-up time between measurements (Steenstrup *et al.* 2013), or based on measuring variance among measurements (Simons *et al.* 2014), assume that telomere elongation within individuals is consistent over time. Our data, and that of others (Fairlie *et al.* 2016; Hoelzl *et al.* 2016a; b) suggest that this is not the case. Such inconsistent changes in telomere length over lifespans could occur due to changes in the cellular composition of of the blood within individual samples, or due to the actual elongation of telomeres (Blackburn *et al.* 1989). Determining the mechanism of these changes is essential for how we view telomeres as biomarkers of costs. For example, if telomeres can be lengthened in response to improvements in environmental conditions, this would suggest that they reflect short- to medium-term costs, rather than the cumulative costs that an

individual has faced over its lifespan (Bateson 2016). New statistical and technical approaches are therefore now required to determine the mechanisms behind increases in telomere length within individuals, so that biologically informed hypotheses about the ecological causes and consequences of these increases can be generated and tested.

Measurement of cohorts across seasons or years is required if we are to understand how the environment impacts telomere dynamics. Although a few studies have shown that temporal variation in telomere dynamics occurs in natural populations, these have been limited in the number of seasons they cover (Mizutani *et al.* 2013; Watson, Bolton & Monaghan 2015; Fairlie *et al.* 2016). Other studies have found chort effects but not discussed them in an ecological context (Stier *et al.* 2014; Becker *et al.* 2015). One problem with studying cohort effects is that it can be difficult to tease apart true cohort effects from effects that arise due to sample degradation with aim, and/or batch effects in telomere assays, although neither of these factors were a problem in our study. Indeed, the long-term Seychelles warbler dataset has allowed us to show that temporal variation in telomere dynamics can occur over substantial time periods. Our data suggest that conditions during the hatch year are a very important factor in shaping telomere dynamics throughout lifespan. Thus, our findings suggest that the telomere dynamics of a population at a given point in time represent a snapshot of a temporally varying process. Research of telomere dynamics within and across multiple cohorts and populations will enable us to better understand how and why population-level telomere dynamics vary over space and time.

We found that temporal variation in insect prey availability was positively related to telomere length. This is consistent with the strong cohort effects we found, and suggests that temporal variation in environmental conditions may be a key driver of costs in the Seychelles warbler. Although the environmental conditions on Cousin Island are relatively benign in comparison to other island systems (e.g. Coulson *et al.* 2001), substantial annual variation in rainfall does occur, with associated changes in insect abundance (Komdeur 1996), and it appears that this confers a cost - in terms of intrinsic biological condition - to Seychelles warblers. Our results concur with other studies which show that early life conditions / food availability can have a very significant and long term impacts on telomere length (and intrinsic biological condition) in captive and wild animals (Heidinger *et al.* 2012; e.g. Nettle *et al.* 2015; Watson *et al.* 2015).

We also found evidence for sex-specific telomere dynamics: males had longer telomeres than females. Interestingly this sex difference interacts with tarsus length: telomere length was negatively correlated with tarsus length in both sexes, but this effect was stronger in females than males. If the sex-dependent relationship between telomere and tarsus length was due to differential growth alone then we would expect the opposite pattern to that observed, as male Seychelles warblers are larger than females (Fig. 3B). One

possibility is that the environment imposes differential costs on males and females: a recent study in captive zebra finches found that manipulation of dietary nutrients had sex-dependent effects on telomere dynamics (Noguera *et al.* 2015). Also worth noting is that the effect of telomere length on survival is strongest in male Seychelles warblers (Barrett *et al.* 2013), although comparative research suggests that the nature of the relationship between sex, telomeres and survival is not yet clear (Barrett & Richardson 2011).

Although we found clear associations between the environment and telomere dynamics, we should bear in mind that the social and ecological variables we tested here explained only a small proportion of the variance in RTL. A poor social and ecological environment is known to be detrimental to Seychelles warblers, both in terms of oxidative stress and survival (Van de Crommenacker *et al.* 2011; Brouwer *et al.* 2012), and it is therefore perhaps surprising that these variables do not explain more variance in RTL. Measurement error is one factor that is an issue in our study, and other studies that use qPCR to measure telomere length (Nussey *et al.* 2014). Measurement error is unlikely to be a cause of type I error in our study, because we were careful to randomise all samples across qPCR plates, and normalise RTL estimates across plates. However, the noise associated with within and among plate measure may have resulted in a decrease in explanatory power, and possibly in a degree of type II error. Techniques for measuring telomere length with a greater degree of precision may probe helpful in future ecological studies of teloere dynamics, but at present there is still atrade-off between obtaining precise telomere measurements, and utilising the large sample sizes necessary for ecological study.

Sampling error notwithstanding, we also predict that lifelong variation in RTL in the Seychelles warbler is explained by a set of environmental and genetic variables not considered here (e.g. Bebbington *et al.* 2016). A key question to be addressed is the extent to which RTL, especially in early life, reflects inheritance and parental effects (Asghar *et al.* 2014; e.g. Becker *et al.* 2015; Heidinger *et al.* 2016). For example, parental age and quality may key variables that impact the telomere dynamics of offspring in the Seychelles warbler, and will be addressed in future studies. Long-term ecological study systems are uniquely suited to addressing such questions in natural systems (Clutton-Brock & Sheldon 2010). To gain a full understanding of telomere dynamics in natural systems, long-term studies combining ecological and genetic data will be required from a range of species.

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Data accessibility

This manuscript was written in R Markdown (http://rmarkdown.rstudio.com/). All data and scripts required to reproduce the manuscript, figures and analyses will be made available on GitHub.

Author contributions

DSR, HLD, JK and TB manage the long-term Seychelles warbler project. DSR conceived and obtained funding for the telomere research. EAF and KB performed the molecular work. LGS processed the telomere data, with input from EAF, KB, MH, HLD and DSR. LGS analysed the data and wrote the manuscript, with input from DSR and all authors.

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Table 1 Telomere dynamics and age in Seychelles warbler cohorts. Linear mixed models were created with RTL as the response variable, and different measures of age, along with cohort ID, were included as explanatory variables (see methods for details). Models are ranked by AICc, with best models at the top of the table.

Model	df	AICc	Delta AICc	Weight
A	-	-	-	-
Cohort + Age (log)	27	-1062.782	0	1
Age (quadratic) + Age (linear) + Cohort	28	-1039.504	23.278	0
Age (linear) + Cohort	27	-1035.072	27.71	0
Age (log)	6	-1034.942	27.84	0
Cohort + Age (factor)	41	-1027.498	35.284	0
Age (quadratic) + Age (linear)	7	-1013.793	48.989	0
Age (linear)	6	-1006.873	55.909	0
Age (factor)	20	-1004.885	57.897	0
Cohort	26	-1000.037	62.745	0
Null model	5	-989.909	72.873	0
B	-	-	-	-
Delta age (log) + Mean age	6	-351.051	0	0.393
Delta age (linear) + Mean age	6	-350.872	0.18	0.359
Delta age (linear) + Delta age (quadratic) + Mean age	7	-348.856	2.195	0.131
Cohort + Delta age (linear) + Mean age	27	-346.428	4.623	0.039
Mean age	5	-346.425	4.626	0.039
Cohort + Delta age (log) + Mean age	27	-345.596	5.455	0.026
Cohort + Delta age (linear) + Delta age (quadratic) + Mean age	28	-344.294	6.758	0.013
Cohort + Mean age	26	-338.716	12.335	0.001

Figure Legends

Figure 1 Telomere dynamics in relation to age in Seychelles warbler cohorts. **A** RTL and age across all individuals. Points and connecting thin grey lines represent individual samples and birds, respectively. The thick line and shaded area represent the fitted values and 95% confidence limits of a linear regression of RTL and log-transformed age. **B** Boxplot of variation in RTL among juvenile individuals from all cohorts. **C** RTL and age among cohorts. Lines represent fitted values from a linear regression of RTL and log-transformed age, and colours correspond to **B**. **D** RTL in relation to and ΔLog age (i.e. within individual variation in log age).

Figure 2 Longitudinal telomere dynamics in the Seychelles warbler. **A** Variation in RTL within individuals sampled at different time points. The dotted line represents parity, and thus points above and below the line represent increases and decreases in RTL, respectively. **B** Scaled density plots of repeated RTL measurements among individual samples, and among different samples taken from the same individual. Areas of the density plot to the left of the dotted line represent decreases in RTL, while areas to the right represent increases. **C** ΔRTL in relation to age in pairs of samples taken within two years. Black line and shaded area represent fitted values and 95% confidence limits from a linear regression of RTL and log-transformed age. **D** Probability of telomere lengthening occurring in relation to age. Points at zero and one represent pairs of samples where RTL has decreased and increased, respectively, with point size scaled by the number of overlapping values. The black line represents the proportion of samples in which increases in RTL where observed at each age category.

Figure 3 Telomere length in relation to the social and ecological environment in the Seychelles warbler. **A** Estimates and 95% confidence intervals for all explanatory variables fitted in a linear mixed model (see methods for details). **B** RTL in relation to tarsus length and sex. **C** RTL in relation to variation in annual food availability. Lines and shaded areas represent the fitted values and 95% confidence limits from linear regressions.

Figure 1

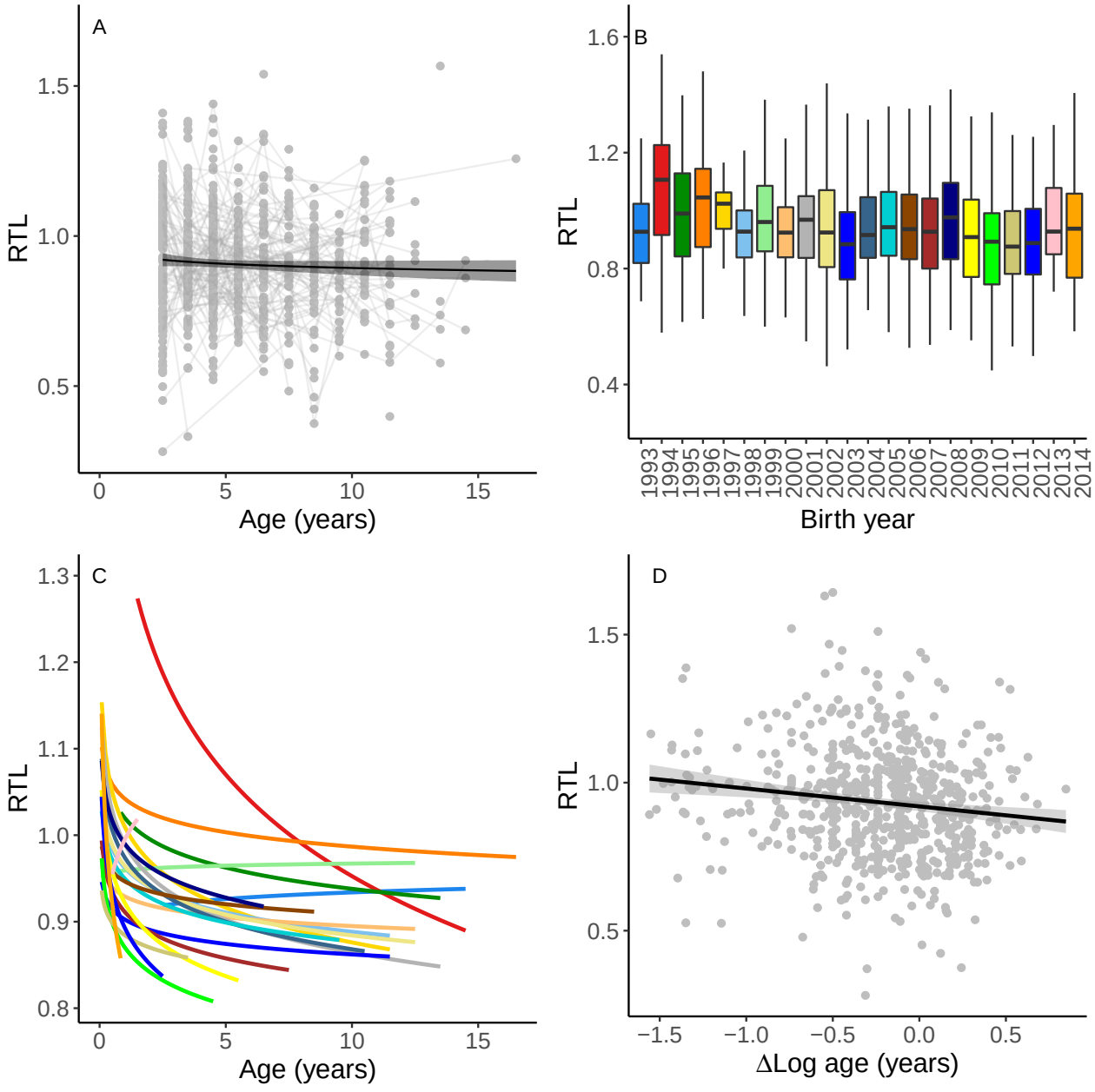

A
RTL
Age (years)
B
RTL
Birth year
C
RTL
Age (years)
D
RTL
ΔLog age (years)

Figure 2

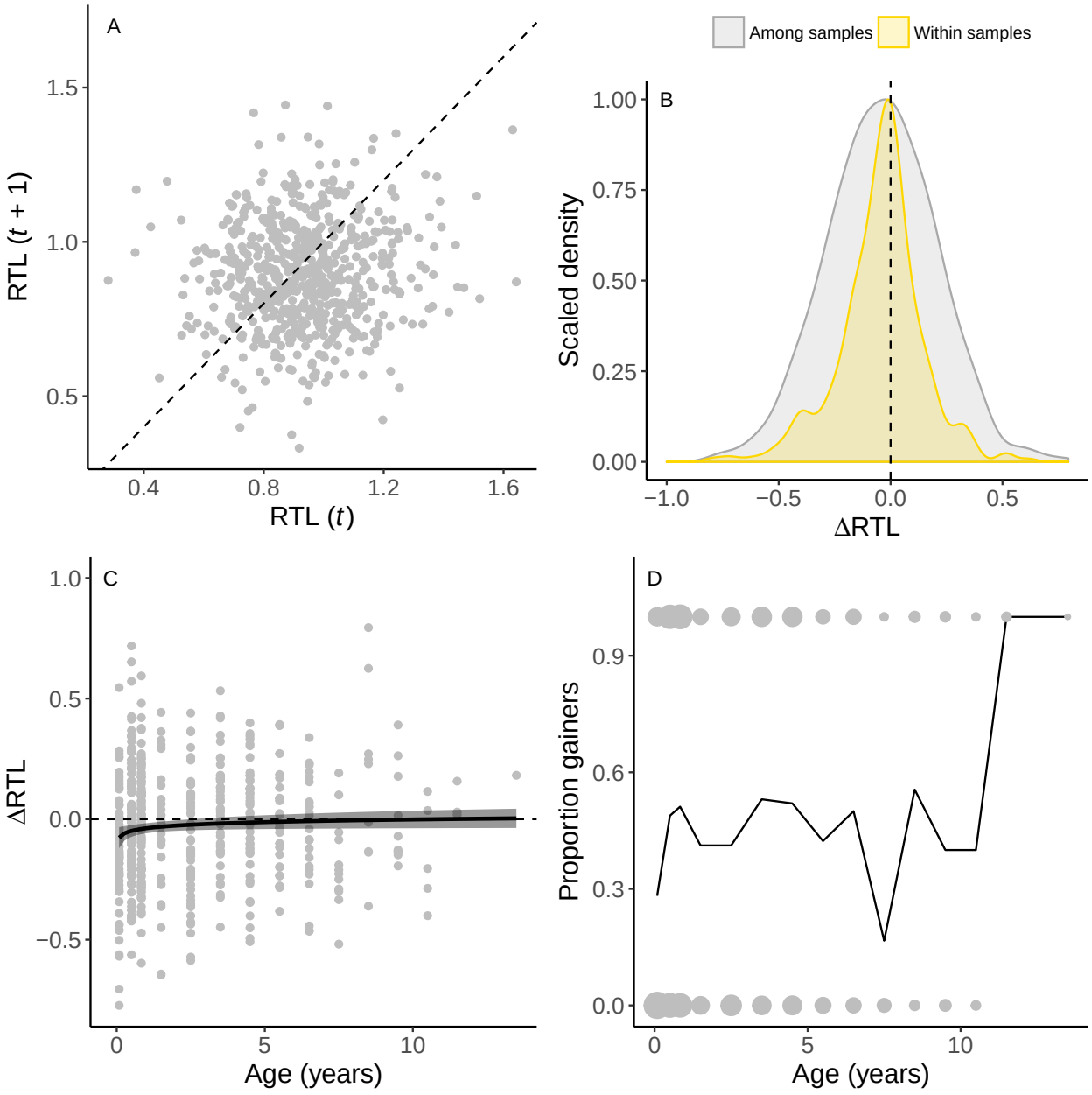
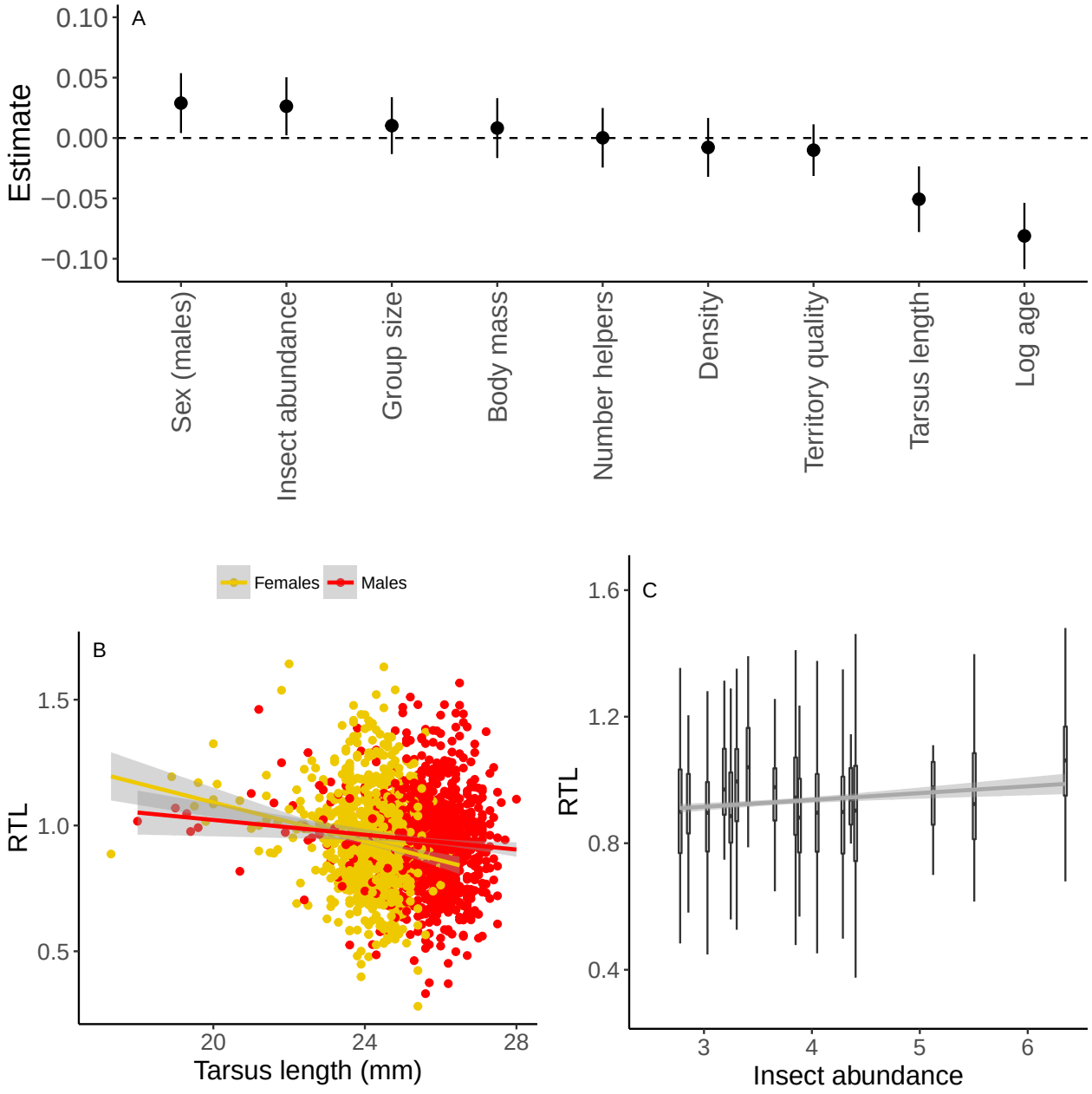

A
RTL (t + 1)
RTL (t)
1.5
1.0
0.5
0.4
0.8
1.2
1.6
B
Among samples
Within samples
Scaled density
ΔRTL
1.00
0.75
0.50
0.25
0.00
−1.0
−0.5
0.0
0.5
C
ΔRTL
Age (years)
1.0
0.5
0.0
−0.5
0
5
10
D
Proportion gainers
Age (years)
0.9
0.6
0.3
0.0
0
5
10

Figure 3


A
Estimate
0.10
0.05
0.00
−0.05
−0.10
Sex (males)
Insect abundance
Group size
Body mass
Number helpers
Density
Territory quality
Tarsus length
Log age
B
Females
Males
RTL
1.5
1.0
0.5
20
24
28
Tarsus length (mm)
C
RTL
1.6
1.2
0.8
0.4
3
4
5
6
Insect abundance