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Original Article

Brood size, telomere length, and parent-offspring color signaling in barn swallows

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Trade-offs select for optimal allocation of resources among competing functions. Parents are selected to maximize production of viable offspring by balancing between progeny number and "quality." Telomeres are nucleoproteins, at the ends of eukaryotic chromosomes, that shorten when cells divide. Because shortening below a certain threshold depresses organismal functioning and rate of shortening depends on environmental conditions, telomeres are good candidates as mediators of trade-offs. We altered brood size of barn swallow *Hirundo rustica* and found that brood enlargement caused a reduction in relative telomere length (RTL). Reliable signals of offspring quality should evolve that mediate adaptive parental care allocation. Because nestlings with darker coloration receive more care, we analyzed the covariation between RTL and coloration and found that RTL increased with plumage darkness, both within and between broods. Hence, we provide unprecedented evidence that signals relevant to parent-offspring communication reflect telomere length and thus offspring reproductive value.

Key words: **brood size, *Hirundo rustica*, parent-offspring communication, plumage color, telomere.**

INTRODUCTION

The number and quality of the offspring that individuals can afford to produce at any breeding attempt are limited by reciprocally constraining relationships. Physiological trade-offs between current progeny number and viability is a major force driving the evolution of breeding strategies (Roff, 1992). Empirical tests of reproductive trade-offs have typically relied on the experimental manipulation of parental effort that parents devote to individual progeny members and the assessments of the effect on progeny fitness proxies (Santos and Nakagawa, 2012). For example, brood size manipulation experiments in birds have demonstrated that increased brood size leads to stronger competition among siblings, ultimately resulting in a deterioration of average offspring condition (e.g. reduction of average body mass and immune response) compared to broods where sib-sib competition is alleviated by reducing brood size (Saino et al., 1997). Optimal parental investment also depends on allocation strategies to individual offspring. This is the case because parents investing more in offspring with larger reproductive value will accrue a fitness advantage relative to parents adopting even or random allocation strategies. However, adaptive differential parental allocation according to offspring quality requires that reliable signals of offspring reproductive value have evolved (Royle et al., 2012). For example, traits that young birds use to solicit care provisioning by their parents have been suggested to honestly advertise parasite load (Tschirren et al., 2003), competence of the immune system (Moreno-Rueda, 2010), and body size or need of additional care (Kilner, 1997).

In studies of reproductive trade-offs, offspring fitness has been typically estimated by focusing on specific classes of traits, such as development and somatic growth and/or functioning of the immune system (e.g. Saino et al., 1997; Soler et al., 2003). Albeit important, these proxies of offspring fitness likely provide only a partial representation of overall phenotypic quality and therefore of offspring reproductive value. Hence, the identification of general physiological mechanisms behind offspring number-quality trade-offs has proven elusive.

Recent studies have pointed at a major role of telomeres in mediating individual response to a host of endogenous and extrinsic factors, suggesting that telomere dynamics may integrate the information of individual history of exposure to stressful conditions (Haussmann et al., 2012; Herborn et al., 2014; Asghar et al., 2015b). Telomeres are nucleoprotein complexes located at the ends of eukaryotic chromosomes. Vertebrate telomeric DNA is composed by the tandem repetition of the hexamer TTAGGG and is tightly associated to a multiprotein complex (shelterin), which

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ensures proper regulation and protection of chromosome ends (Palm and de Lange, 2008). Due to the inability of DNA polymerase to fully replicate linear DNA, in normal somatic cells, telomeres physiologically shorten at each cell division. When telomeres reach a threshold length, cells enter either replicative senescence or apoptosis (Blackburn, 1991). Consequently, telomere shortening can reduce the renewal capacity of tissues and might depress organismal functioning and performance (Blasco, 2005; Monaghan and Haussman, 2006). The length and shortening rate of telomeres depend on genetic background (Asghar et al., 2015a; Atema et al., 2015), but also on diverse factors including exposure to oxidative stress (Beaulieu et al., 2011), food availability and quality (Badás et al., 2015), exposure to elevated levels of hormonal mediators of the hypothalamic-pituitary-adrenal (HPA) physiological stress response (Haussmann et al., 2012; Herborn et al., 2014), parasitism (Asghar et al., 2015b) as well as various forms of environmental and social stress (Kotrschal et al., 2007; Mizutani et al., 2013; Hau et al., 2015; Meillère et al., 2015), such as the number of competing siblings (Boonekamp et al., 2014; Reichert et al., 2015).

Telomere length and/or rate of attrition, in turn, have been shown to positively predict viability (Haussmann et al., 2005; Pauliny et al., 2006; Bize et al. 2009; Barrett et al., 2013; Boonekamp et al., 2014), fecundity (Le Vaillant et al., 2015), and also a number of important morphological (Kim and Velando, 2015), physiological (Le Vaillant et al., 2015), and behavioral traits (Nettle et al., 2015), with longer telomeres and smaller rate of attrition being generally associated with better performance.

Consistency in the general patterns of telomere dynamics across vertebrates, the impact that telomere shortening has on individual performance, and susceptibility of the telomere shortening process to extrinsic as well as endogenous factors, lead to expect that telomere dynamics underpin evolutionary and physiological trade-offs that reciprocally constrain the expression of fitness traits (Monaghan, 2010). Along the same line of reasoning, parents are expected to have selected for offspring traits that reliably reveal the quality of individual progeny in terms of telomere length and/or rate of telomere shortening, because offspring with long telomeres may have larger expected reproductive value and/or higher chances of survival. The latter hypothesis is relevant to a core issue in the evolution of parent-offspring communication systems but, to the best of our knowledge, has never been tested in any organism to date.

Parents have been shown to rely on several, diverse traits to tune their strategies of allocation of care based on offspring traits that are involved in parent-offspring communication (Royle et al., 2012). Such parental decisions may serve to maximize parental fitness by favoring the offspring with larger expected reproductive value. For example, parental discrimination has been observed in favor of offspring displaying more brightly colored mouth tissues or darker melanin-based coloration (Kilner, 1997; Romano et al., 2016, and references therein). To the best of our knowledge, no mechanistic links have been proposed so far between melanogenesis and telomere dynamics. However, an interplay between melanogenesis and telomere dynamics might be suggested by the observation that telomerase activity can affect early melanin biosynthetic pathways (Bagheri et al., 2006). In addition, a covariation between coloration and telomere length may be established because genes that control melanogenesis have pleiotropic effects on physiological, immune, and behavioral traits (Ducrest et al., 2008; Emaresi et al., 2013), which may in turn influence telomere dynamics.

In this study of the barn swallow (*Hirundo rustica*), a small passerine bird, we first test the hypothesis that the trade-off between offspring number and quality is mediated by the negative consequences that adverse rearing conditions have on telomere dynamics. Previous experiments on the same barn swallow population have shown that an experimental increase in brood size causes reduced growth and immune response in nestlings (Saino et al., 1997) and also an increase in ectoparasite infestation (Saino et al., 2002). In addition, enlarged broods are a socially stressful environment, as scramble competition for food is harsher than in reduced broods (Saino et al., 2000). A recent study has demonstrated that telomeres undergo significant shortening during the barn swallow nestling stage and suggests that telomere shortening later in life may be small, implying that the nestling stage is crucial to telomere dynamics (Parolini et al., 2015). The documented effects of brood size manipulation on specific fitness proxies and competitive interactions among siblings, and sensitivity of telomere dynamics to rearing conditions, led us to expect that nestlings in enlarged broods had reduced telomere length at growth completion as compared to nestlings reared in reduced broods.

Attending an enlarged brood increases parental effort and reduces annual parental survival in the barn swallow (Saino et al., 1999). Because parental care entails a measurable cost in terms of survival, selection on parents that adaptively modulate parental effort according to the reproductive value of the offspring should have resulted in the evolution of reliable signals of offspring quality. Nestling barn swallows vary in melanin-based chestnut coloration of their ventral plumage both at the within- and the among-family levels. An experiment with a within-brood manipulation design showed that darkening of the ventral plumage shortly before fledging (day 16 after hatching) caused an increase in parental food provisioning, implying that parents favor offspring that show darker melanin-based coloration (Romano et al. 2016). Here, we therefore tested if melanin-based coloration can reliably reveal nestling quality in terms of telomere length. To this aim, we measured telomere length in blood cells at an age (12 days after hatching) when it cannot be influenced by differential parental allocation according to nestling coloration because the coloration of the developing contour feathers has just become visible. We predicted that telomere length is larger in darker nestlings, as these have been experimentally shown to attract more care compared to paler nestlings (Romano et al., 2016).

METHODS

The barn swallow is a socially monogamous semicolonial passerine bird with biparental care of the progeny (Turner, 2006). Females lay 1–3 clutches per breeding seasons. Incubation lasts ca. 14 days and eggs hatch with small asynchrony (i.e. all nestlings usually hatch within 24 h of hatching of the first egg). Altricial, nidicolous nestlings fledge ca. 20 days after hatching. Osteometric growth is completed by day 11, while peak body mass is attained around day 12–13 (Turner, 2006). Contour body feathers start to emerge by day 6–7. The overall pattern of nestling coloration, determined by pheomelanins and eumelanins, is similar to that of adults. In spring 2014, in a study area located near Milan (Norther Italy; center of the study area: 45°36′N, 8°37′E), we performed a brood size manipulation experiment. We reciprocally swapped an unbalanced number of individually marked, randomly chosen nestlings between pairs of broods ("dyads") where hatching occurred on the same or in consecutive days. Hatchlings were swapped between

nests according to an unbalanced, partial cross-fostering design at the age of 0–1 days, when all nestlings of both broods in the dyad had hatched. The number of nestlings that were transferred was such that the size of either, randomly chosen, brood was increased, whereas the other was reduced by one nestling (see Saino et al., 1997). At the end of this procedure, both broods in the dyad contained both biological and foster nestlings. The sample included 24 broods. The size of enlarged broods (mean: 5.33 ± 0.26 standard error [SE] nestlings) was significantly larger than that of reduced broods (mean: 3.42 ± 0.23 SE nestlings; paired *t*-test: $t_{11} = 6.67$, $P < 0.001$), and the difference between the size of matched broods did not significantly differ from 2 nestlings, as expected ($t_{11} = 0.29$, $P = 0.777$). When nestlings were 12 days old, we measured body mass and tarsus length as a proxy of skeletal body size. In addition, a blood sample was taken for telomere analysis and identification of nestling sex by molecular tools (Saino et al., 2008). When nestlings were 16 days old, we plucked 5–10 growing feathers from the ventral plumage region for color analysis. At the time of measurement and blood sampling, the 24 broods in the sample contained a total of 106 nestlings. Information on tarsus length, plumage coloration, and telomere length was available for 105 nestlings for each variable, whereas body mass could be recorded for 97 nestlings. Two ϕ values (see Color analysis) were excluded from the analyses because they deviated by more than 3.5 standard deviation (SD) from the mean value. In all univariate and bivariate analyses, the largest available sample was used.

Because assignment of broods to either treatment group was randomized, we have no reason to suspect that any effect of brood size manipulation on telomere length was the spurious consequence of a difference in mean telomere length at hatching due to, for example, genetic variation or early maternal effects mediated by egg quality on telomere length. In fact, this assumption could not be tested because sampling of even a small amount of blood from ca. 1.5 grams hatchlings is hardly feasible in the field.

Color analysis

Color of one ventral feather, reflecting the coloration of the entire ventral plumage (Romano et al., 2015), was quantified recording the reflectance spectra of its distal end (Saino et al., 2013). Reflectance data were subsequently processed according to the tetrahedral color space model. Feather color was thus described by the 2 spherical coordinates that represent color hue: ϑ, which accounts for the "visible" component and ϕ, which account for the ultraviolet component (Goldsmith, 1990; Stoddard and Prum, 2008).

Telomere analysis

Telomere length analysis was performed according to the method described by Parolini et al. (2015). Genomic DNA was extracted from 10 to 20 µl of red blood cells using 1 ml TNSE buffer (10 mM Tris HCl, 400 mM NaCl, 100 mM EDTA, and 0.6% SDS) and a standard phenol/chloroform method. DNA samples of nestlings from the same nest were extracted in the same batch. We measured the quantity and purity of the extracted genomic DNA using a Nanophotometer (IMPLEN). DNA degradation was checked by electrophoresis in 1% agarose gel. Telomere length was measured by the monochrome multiplex quantitative PCR method (MMQPCR; Cawthon, 2009) on a PikoReal 96 thermal cycler (Thermo Scientific): telomere length was measured as the ratio (T/S) of the amount of telomeric repeats (T) to the amount of a single copy gene (S), relative to a reference sample. By this method, telomere length is evaluated indirectly by measuring the relative number of telomeric repeats in a genome and it is indicated from now as relative telomere length (RTL). The sequences of telomeric primers for MMQPCR were (telg 5′-ACACTAAGGTTTGGGTTTGGGTTTGGGTTTGGGTTAGTGT-3′; telc 5′-TGTTAGGTATCCCTATCCCTATCCCTATCCCTATCCCTAACA-3′), while the single copy sequence used as control was a fragment from the 12th exon of the swallow CTCF gene (CCCTC-binding factor zinc finger protein). The CTCF primers used were: forward (5′-CCCGCGGCGGGCGGCGCGGGCTGGGCGGCTCCCAATGGAGACCTCAC-3′) and reverse (5′-CGCCGCGGCCCGCCGCGCCCGTCCCGCCCATCACCGGTCCATCATGC-3′); these primers are composed of a swallow genomic sequence and a GC-clamp at the 5′ end (underlined) to increase the melting temperature of the PCR product. Since the melting temperature of telomeric and CTCF PCR products are different, both primer pairs could be used in the same reaction. PCR reactions were prepared using 30 ng of genomic DNA as template, 1× DyNAmo ColorFlash SYBR Green qPCR Master Mix (Thermo Scientific), telomeric, and CTCF primers at a final concentration of 1,000 nM and 500 nM each, respectively. Three-fold serial dilutions of a barn swallow reference sample (from 5.5 to 150 ng) were included in each plate to produce a standard curve to measure reaction efficiency and quantify the amount of telomeric repeats and single copy gene in each sample. We used the same reference sample per plate. All reactions were run in triplicate and 6 plates containing 25 samples each were performed. Samples from the same dyad were randomly included into the same plate. Cycling parameters for the PCR reactions were as follows: Stage 1: 15 min at 95 °C; Stage 2: 2 cycles of 15 s at 94 °C, 15 s at 49 °C; and Stage 3: 35 cycles of 15 s at 94 °C, 10 s at 62 °C, 15 s at 74 °C with signal acquisition, 10 s at 84 °C, 15 s at 88 °C with signal acquisition. The PikoReal Software (Thermo Scientific) was used to calculate the amount of telomeric repeats (T) for each sample by interpolation of the quantification Cycle (Cq) into the linear function $y = ax + b$ of the standard curve of the telomeric primers. Similarly, the software calculates the amount of the single copy gene (S) for each sample. Mean values for T and S for each sample were used to calculate the T/S ratios. The mean reaction efficiency (±SD) for telomere and CTCF amplifications was 87 ± 5% and 86 ± 4%, respectively. The intraplate and interplate repeatability of RTL measures, expressed as intraclass correlation coefficient, was 0.79 and 0.81, respectively. The intraplate and interplate coefficient of variation (±SD) of RTL measures was 12 ± 8% and 10 ± 3%, respectively. Because of consistency of telomere length during the nestling period within individual nestlings (Parolini et al., 2015), we could assume that nestlings with relatively long telomeres at the age of blood sampling (12 days), also had relatively long telomeres at the age when ventral coloration was measured (16 days).

Statistical analyses

The effect of brood size manipulation and sex (2-levels fixed-effect factors) on nestling phenotype was analyzed in univariate linear mixed models (LMMs) while including dyad of broods, brood of rearing, and brood of origin as random effects. The correlation between traits was analyzed in bivariate LMMs with a repeated-measures design, where brood of rearing was included as a grouping factor, according to the procedure outlined in Dingemanse and Dochtermann (Dingemanse and Dochtermann, 2013), while adopting restricted maximum likelihood estimation of parameters. The within- and between-brood correlations between pairs of traits

were computed using the variance and covariance estimates according to equations 7c and 7d in (Dingemanse and Dochtermann, 2013). The significance of the within- and, respectively, between-broods correlation coefficients was estimated by likelihood-ratio tests (maximum likelihood estimation) comparing the full model with the model constraining the R or, respectively, the G matrix to a covariance of 0 (Dingemanse and Dochtermann, 2013). Bivariate LMMs were also run while considering nest of origin, rather than nest of rearing, as a grouping factor. Because the bivariate correlations obtained while including either grouping factor were generally qualitatively consistent (see Results), only the results based on the model including nest of rearing are presented in details. To represent the bivariate relationship between RTL and the morphological or the coloration variables at the between- or, respectively, the within-brood level, we computed the within-brood means or, respectively, the within-brood residuals of both variables relative to the brood mean. To better visualize the bivariate relationship we performed a type II (major-axis) regression analysis. Univariate and bivariate LMMs were run in SAS 9.2. Major-axis regression analysis was performed by the "lmodel2" package in R (version 3.2.3).

RESULTS

In LMMs, nestling body mass, tarsus length, RTL, and plumage color variables were not significantly predicted by the interaction effect between brood size treatment and nestling sex ($P > 0.23$ for all traits). Simplified models only including the main effects of sex and brood size treatment showed that RTL and body mass had significantly smaller mean phenotypic values in enlarged as compared to reduced broods, whereas the effect of brood size manipulation on the other traits was nonsignificant (Table 1; Figure 1). Male nestlings had significantly darker coloration of the ventral plumage as compared to females, consistent with adult sexual dichromatism, while the other traits did not significantly vary according to sex (Table 1; Figure 1). Parameter estimates for random effects are presented in Supplementary Table S1.

We tested for covariation between nestling RTL and the other phenotypic traits both at the between- and at the within-brood level in bivariate LMMs with a within-brood of rearing repeated-measures design (see Statistical analyses). Body mass and tarsus length did not significantly covary with RTL both at the between- and at the within-brood level (Table 2). However, the correlation coefficients for body mass and tarsus length at the between-broods level were large and negative (Table 2), suggesting that the lack of statistical significance could be due to the relatively small number of broods included in the analyses (sensu Nakagawa and Cuthill, 2007). The ϑ component of ventral plumage coloration negatively covaried with RTL both at the between- and at the within-brood level (Table 2; Figure 2), implying that nestlings with darker ventral coloration had larger RTL. In addition, the ϕ color component negatively covaried with RTL at the within-brood levels, whereas the significance of the correlation at the between-brood levels could not be estimated because the reduced model failed to converge (Table 2).

Bivariate LMMs where phenotypic traits where modeled while adopting a within-brood of origin repeated-measures design confirmed the results of models with brood of rearing as a grouping factor, with the only exception that the negative association between RTL and ϑ at the between-broods level turned to nonsignificant ($P = 0.100$; Table 2).

DISCUSSION

We manipulated the social environment of barn swallow nestlings by altering the size of the brood where they were reared and found that the length of their telomeres at somatic growth completion was smaller in enlarged as compared to reduced broods. In addition, independently of sex and brood size manipulation, nestlings with darker melanin-based coloration had longer telomeres, providing evidence that telomere length is reliably reflected by an offspring trait relevant to communication with parents.

Life-history theory posits that parents are selected to strike the optimal balance between their own survival and the number and quality of their offspring (Roff, 1992). In barn swallows, these major parental fitness components have been experimentally shown to be linked by reciprocally constraining relationships: brood enlargement depresses parental survival (Saino et al., 1999) and has adverse effects on the offspring (Saino et al., 1997). Per capita food intake is reduced in enlarged broods (Saino et al., 1997; Saino et al., 2000). The negative effect of brood enlargement on somatic growth and immune response likely mediated by poor nutritional conditions has been repeatedly documented in barn swallows (Saino et al., 1997; Saino et al., 2000) as well as in other altricial bird species (Naguib et al., 2004), and was confirmed in the present study along with a lack of effect on body size, suggesting that nestling barn swallows prioritize skeletal growth over allocation to anabolism of soft tissues. In addition, scramble competition among siblings increases as a consequence of reduced nestling satiation (Saino et al., 2000; Romano et al., 2013). Furthermore, increased brood size results in larger abundance of virulent nest-dwelling hematophagous mites (Saino et al., 2002). All of these effects of brood enlargement may concur in determining shorter telomere

Table 1
Summary of linear mixed models of nestling phenotypic traits with brood size treatment and sex as fixed-effect factors

Dependent variable	Effect	Parameter Estimate (SE)	*n*	*F*	df	*P*
RTL	Brood treatment	−0.102 (0.041)	105	6.32	1, 69	0.014
Body mass	Brood treatment	−0.685 (0.317)	97	4.68	1, 64	0.034
Tarsus length	Brood treatment	−0.041 (0.066)	104	0.39	1, 68	0.534
ϑ	Brood treatment	0.015 (0.013)	103	1.41	1, 66	0.239
	Sex[a]	−0.040 (0.013)		9.56	1, 66	0.003
ϕ	Brood treatment	0.014 (0.028)	102	0.27	1, 66	0.607

ϑ and ϕ represent the "visible" and the ultraviolet component of the plumage color, respectively. Pair of broods, brood of origin and brood of rearing were included as random effects. Parameter estimates for fixed effects are given for enlarged broods and for males.
[a]Least square means for males: 0.206 (0.014); females: 0.246 (0.014).

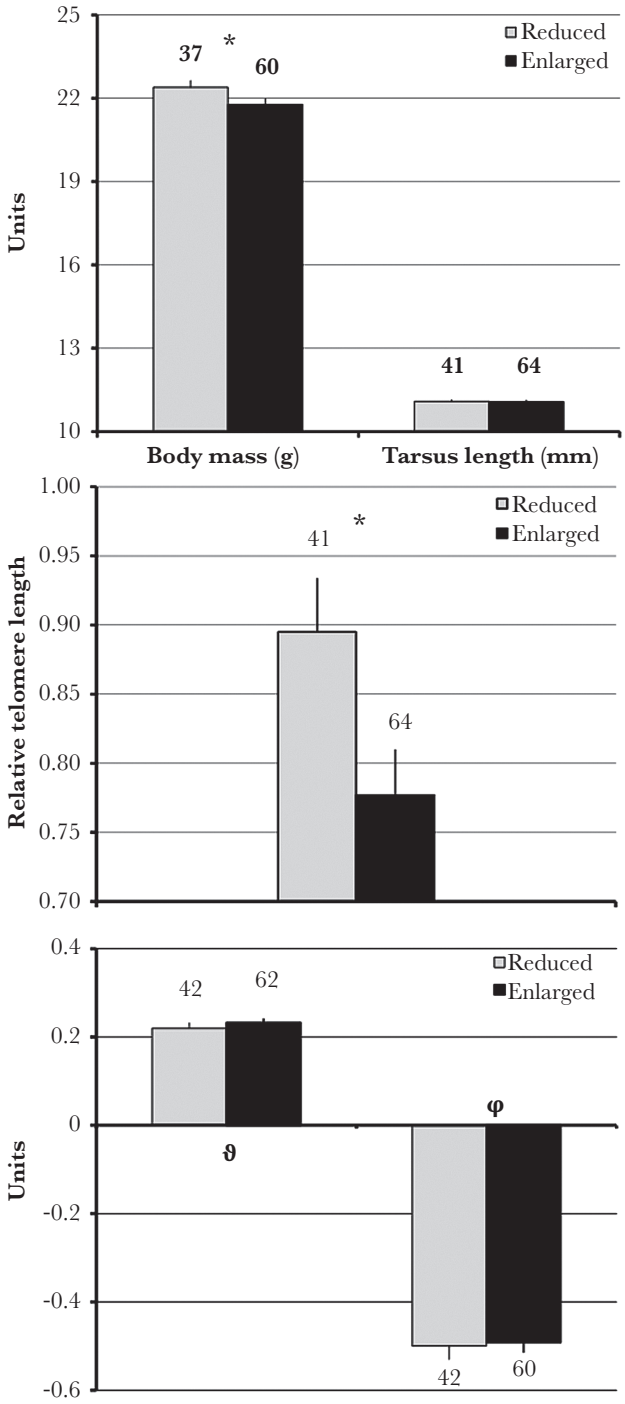

Figure 1
Mean (+SE) phenotypic values of nestlings from reduced (n = 12) or enlarged (n = 12) broods. ϑ and ϕ represent the "visible" and the ultraviolet component of the plumage color, respectively. *the difference between the 2 treatment groups was significant ($P < 0.05$) in linear mixed models controlling for sex (see text). Insert numbers are sample size.

length in enlarged broods. In fact, nutritional and social stress (Kotrschal et al., 2007; Mizutani et al., 2013; Meillère et al., 2015), as well as parasitism (Asghar et al., 2015b), have all been shown to cause telomere shortening in birds. The few previous studies where the effect of brood size manipulation on telomere length has been tested have provided mixed evidence (Voillemot et al., 2012; Boonekamp et al., 2014; Nettle et al., 2015). Idiosyncratic effects of brood size manipulation on telomere shortening in different species may have arisen because of differences in constraints set by food availability on nestling condition or because of differences in age-specific telomere dynamics between species.

Despite the difficulties of estimating the long-term viability consequences of rearing conditions in organisms with large natal dispersal, some studies of trade-offs in birds have demonstrated that being reared in a large brood depresses the odds of local recruitment and survival (e.g. Djikstra et al., 1990; Pettiflor et al., 2001; Tarof et al., 2011), while other studies have shown that offspring body traits that are otherwise known to depend on brood size predict offspring viability, providing indirect evidence for an effect of rearing social environment on viability (e.g. Saino et al., 1997; Soler et al., 2003). Telomere length and rate of shortening have been consistently shown to predict survival prospects of both young and adult mammals and birds (Bize et al., 2009; Heidinger et al., 2012; Barrett et al., 2013). The present evidence of smaller telomere length at growth completion in nestlings from enlarged as compared to reduced broods therefore suggests that telomere dynamics may be one of the ultimate mechanisms that mediate a trade-off between offspring number and viability. The relationships between RTL and tarsus length or body mass were nonsignificant but the correlation coefficient was large and negative, suggesting that lack of statistical significance could arise because of type II statistical error. A negative relationship between RTL and morphological traits could arise because the physiological cost of growing to large body size causes telomere shortening.

In birds, visual communication has a major role in sexual, social and also in parent-offspring relationships (Royle et al., 2012). Offspring plumage and mouth coloration affects parental decisions over food allocation in altricial birds by contributing to food provisioning solicitation displays ("begging"). The present findings provide novel support to the hypothesis that coloration reliably advertises offspring quality, because telomere length was found to covary with plumage coloration and may affect offspring viability. The relationship between telomere length and coloration held at the within- as well as at the between-broods levels. Hence, darker coloration reliably reflects telomere length of individual nestlings relative to their siblings and, in addition, broods with relatively dark coloration can be perceived as consisting of nestlings with on average longer telomeres than broods with relatively pale nestlings. Importantly, in the same barn swallow population where the present study was conducted, we have experimentally shown a preference of parents for feeding darker nestlings (Romano et al., 2016). This is consistent with the expectation, because parents should invest more in more valuable offspring, with longer telomeres.

Importantly, the correlation between telomere length and coloration is unlikely to be the mere consequence of parents investing more resources in darker nestlings. By the age when blood was sampled and RTL was measured (day 12 after hatching), the color of the growing feathers has just (less than 2 days) become visible. Hence, it is highly unlikely that before the age when RTL was measured, parental decisions on allocation of care could be driven by feather coloration, thereby generating a covariation between coloration and condition-dependent telomere length. In fact, we found no covariation between body mass or tarsus length measured at day 6 and also at day 12 (i.e. the time of telomere length measurement) and coloration measured at day 16. However, we cannot discard the alternative interpretation that the positive covariation between telomere length and plumage color was the result of differential

Table 2

Correlation coefficients between relative telomere length (RTL) and nestling phenotypic traits at the between- and within-broods levels as estimated in bivariate linear mixed models

	Grouping	Between-broods				Within-broods			
	Brood	*r*	*n*	χ^2	*P*	*r*	*n*	χ^2	*P*
Correlation of RTL with									
Body mass	Rearing	−0.231	24	0.4	0.527	−0.090	97	0.6	0.439
	Origin	−0.372	24	2.1	0.147	−0.027	97	0.0	0.999
Tarsus length	Rearing	−0.427	24	3.0	0.083	−0.215	103	3.8	0.051
	Origin	−0.468	24	2.8	0.094	−0.161	103	2.1	0.147
ϑ	Rearing	−0.563	24	3.9	0.048	−0.230	103	4.4	0.036
	Origin	−0.479	24	2.7	0.100	−0.267	103	6.0	0.014
ϕ[a]	Rearing	−0.426	24	—	—	−0.222	101	3.9	0.048
	Origin	−0.419	24	—	—	−0.223	101	4.0	0.046

ϑ and ϕ represent the "visible" and the ultraviolet component of the plumage color, respectively. Significance of the correlation coefficients was estimated by likelihood-ratio tests (see also Statistical analyses).

[a]The reduced models testing for the between-broods correlation did not converge.

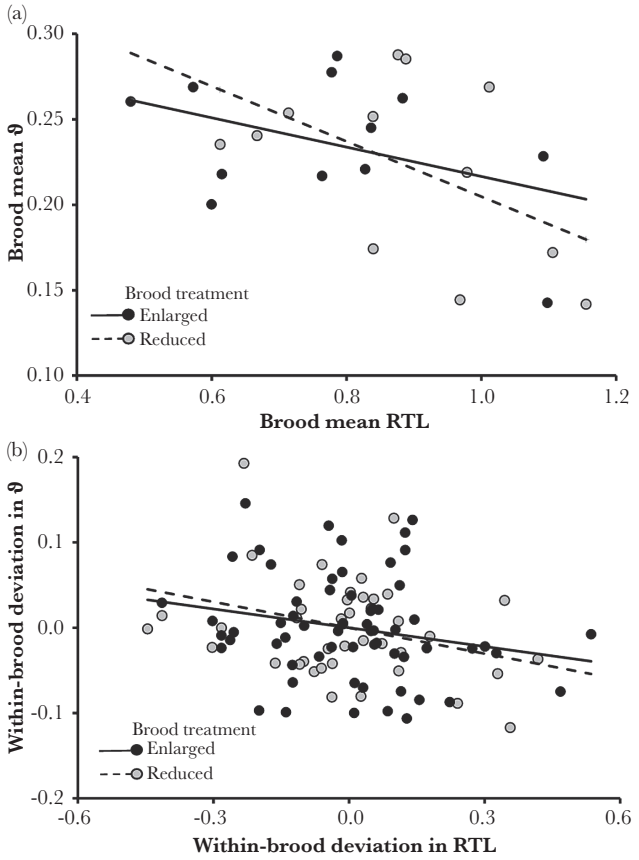

Figure 2

(a) Mean within-brood phenotypic value of the ϑ component of nestling ventral plumage color (larger ϑ indicates paler color) in relation to mean within-brood relative telomere length (RTL) in reduced (n = 12) or enlarged (n = 12) broods. (b) Phenotypic value of the ϑ component of coloration of ventral plumage in relation to RTL of individual nestlings. Phenotypic values are centered to a within-brood mean of 0. In both panels, major-axis regression lines are fitted to better represent the negative trends.

allocation according to another trait correlated with future nestling plumage coloration (e.g. mouth color or begging vocalizations), which could have influenced parental feeding behavior prior to the growth of nestling ventral contour feathers.

The interpretation of the causal pathways that link coloration to telomere length is matter of speculation. A relationship between melanogenesis and telomere dynamics is suggested by the observation that altered telomerase activity affects the expression of tyrosinase, an enzyme involved in early melanogenesis pathways (Bagheri et al., 2006). Although this off-site effect of telomerase was demonstrated in a mouse melanoma cell line, it is tempting to hypothesize that a direct link may exist between telomere length and ventral plumage coloration in the barn swallow. The genes that regulate melanin biosynthesis pleiotropically influence several life-history traits via the melanocortin system (Ducrest et al., 2008). Melanocortins are derivatives of the prohormone encoded by the proopiomelanocortin (*POMC*) gene. Binding of the *POMC* gene products to the melanocortin 1-receptor (MC1R) triggers pheomelanogenesis and eumelanogenesis (Ducrest et al., 2008). However, *POMC* derivatives can also bind to other melanocortin receptors (MC2-5) with regulatory effects on a number of important functions, spanning from energy homeostasis and sociosexual behavior to immunity and stress response mediated by the HPA axis (Ducrest et al., 2008). For example, chronic exposure to glucocorticosteroids can depress the activity of telomerase, that functions to restore telomere length (Choi et al., 2008; Epel et al., 2010). Hence, a covariation between coloration and telomere length might also arise because the genes that control melanogenesis have indirect effects on physiological and behavioral traits whose expression affects telomere shortening.

Brood size manipulation did not affect plumage coloration suggesting that coloration is not influenced by brood size. An alternative, not mutually exclusive, interpretation is that the consequences of brood size manipulation may still not be measurable at the age of 6–7 days, when the distal, colored end of the ventral contour feathers is produced.

This study thus shows that telomere length measured at somatic growth completion in a bird with altricial offspring depends on rearing conditions. Telomere shortening may therefore be one of the core mechanisms mediating the trade-off between offspring number and quality. Our present finding that nestling darkness positively covaries with telomere length is compatible with the idea that the observed parental favoritism for darker nestlings reflects an adaptive parental strategies of preferential allocation of care to the offspring with large expected reproductive value. Future studies should be aimed at assessing the generality of these findings and

to unveil the mechanistic links, if any, between plumage color and telomere length.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at http://www.beheco.oxfordjournals.org/

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Data accessibility: Analyses reported in this article can be reproduced using the data provided by Costanzo et al. (2016).

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