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Note

Chronological and Biological Age Predict Seasonal Reproductive Timing: An Investigation of Clutch Initiation and Telomeres in Birds of Known Age

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abstract: Female vertebrates that breed earlier in the season generally have greater reproductive success. However, evidence suggests that breeding early may be costly, thus leading to the prediction that females with fewer future reproductive events will breed earlier in the season. While chronological age is a good indicator of remaining life span, telomere lengths may also be good biomarkers of longevity as they potentially reflect lifetime wear and tear (i.e., biological age). We examined whether variation in the timing of the first seasonal clutch was related to age and telomere length in female dark-eyed juncos (*Junco hyemalis*), predicting that older females and those with shorter telomeres would breed earlier. Both predictions held true and were independent of each other, as telomere length did not significantly vary with age. These results suggest that females may adjust their reproductive effort based on both chronological and biological age.

Keywords: junco, residual reproductive value, songbird, telomere, timing of breeding.

Introduction

In general, organisms time breeding so offspring growth coincides with peak food availability (Lack 1968; Bronson 1985). Individuals that breed earlier in the season tend to enjoy greater reproductive success, either because they can raise more young within a breeding season, replace failed clutches or litters before the breeding season ends, or better utilize early peaks in seasonal food abundance (Price et al. 1988; Williams 2012). Concomitantly, early produced young are more likely to recruit to a population (Harris et al. 1994; Spear and Nur 1994; Naef-Daenzer et al. 2001). However, evidence indicates that while early breeding individuals have increased reproductive success, most females breed later than the optimal time (birds: Perrins 1970; Drent 2006; Williams 2012; beef cattle: Bourdon and Brinks 1982; turtles: Doody et al. 2004; sand lizards: Olsson and Shine 1997). This suggests that there are costs associated with breeding early, which is supported by correlative studies demonstrating that early breeding individuals suffer higher mortality rates (birds: Brown and Brown 1999; Sheldon et al. 2003; white-footed mice: Fairbairn 1977). Manipulative studies in birds have also suggested that early breeding is costly (Nilsson 1994; Brinkhof et al. 2002), although the methods currently available for experimentally advancing timing of breeding have several limitations (Verhulst and Nilsson 2008).

If earlier breeding does entail costs, then females with fewer future reproductive opportunities may be more willing to pay these costs to maximize their current reproductive effort by breeding earlier in the season (i.e., terminal investment) than females with more future reproductive opportunities. While several studies have found that older birds breed earlier in the season (Martin 1995; DeForest and Gaston 1996; Ezard et al. 2007; Blas et al. 2009), chronological age may not be the only indicator of future reproductive opportunities, since this does not account for the wear and tear individuals experience over their lifetime (guillemots: Reed et al. 2008; elephant seals: Sydeman et al. 1991). Therefore, an individual's biological age may also be a good determinant of remaining life span (Bize et al. 2009).

One potential way to estimate biological age is by measuring telomere lengths. Telomeres are highly conserved,

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noncoding, repetitive DNA regions located at the ends of chromosomes that shorten during cell division and in response to stress exposure (von Zglinicki 2002; Haussmann et al. 2012; Herborn et al. 2014; Hau et al. 2015; although see Boonekamp et al. 2017), eventually causing cells to senesce after reaching a critical minimal length (von Zglinicki 2002). Because of this, telomeres are thought to act as sentinels of DNA damage and thus are useful indicators of an individual's biological age (Monaghan 2010). Telomeres may be causal agents of aging and mortality or serve as biomarkers of longevity, as several studies have found that individuals with longer telomeres live longer (Haussmann and Mauck 2008; Heidinger et al. 2012; Dantzer and Fletcher 2015; Fairlie et al. 2016). Therefore, as a marker of biological age, telomere length may be a suitable indicator of an individual's likely number of future reproductive events.

We used a long-term (30+ years) study population of resident dark-eyed juncos (*Junco hyemalis carolinensis*) to test the hypothesis that individuals with fewer future reproductive events increase their current reproductive effort by breeding earlier in the season. Juncos are a seasonally breeding, ground-nesting passerine that can live up to 11 years of age (Nolan et al. 2002). We predicted that in this multibrooded species, both older individuals and those with shorter telomeres would initiate earlier first seasonal clutches. Alternatively, reproductive effort may be constrained by individual quality, and perhaps only females of high quality can afford the costs of breeding early. Under this alternative hypothesis, we predicted that females with longer telomeres would lay first seasonal clutches earlier, although this scenario requires an assumption that telomere lengths reflect individual quality (Bauch et al. 2013).

Material and Methods

We studied a population of resident juncos that breeds annually at Mountain Lake Biological Station (lat. 37°22′N, long. 80°32′W) in western Virginia. This population has been routinely banded and monitored for 30+ years, with a breeding season spanning mid-April to late-July (Jawor et al. 2007). To obtain blood samples to measure telomere lengths, we captured female juncos using baited Potter traps and mist nets during the annual census (April 26–May 14, 2014, and April 22–May 6, 2015). We used only known-age individuals in this study, and no individuals were sampled longitudinally in both years. Female juncos start breeding at 1 year of age. Age was determined based on previous banding records, with the exception of some 1-year-old birds, as this age can be reliably identified via plumage characteristics and iris color (Ketterson 1979; Nolan et al. 2002).

Nest searching and monitoring began early in the breeding season (April 23, 2014, and April 24, 2015) and continued through the end of June. Juncos are a ground-nesting species that build open-cup nests lined with deer hair. By routinely and intensively searching for nests in the same areas where the annual census took place, we optimized our likelihood of finding all nests for known females within the population. We were also able to identify the parents of each nest through regular, intensive trapping and monitoring.

If nests were found during the building stage, we monitored them to determine the date of clutch initiation (first egg laid). If nests were found during a later stage, we determined clutch initiation date based on known time intervals between clutch completion date, hatch date, and nestling mass (Nolan et al. 2002). We included only first clutches in this study, which were conservatively defined as any clutch initiated before the first known renest of the season (May 20, 2014, and May 11, 2015). Clutch initiation dates are reported as the number of days after the first egg of the season was laid (April 19, 2014, and April 16, 2015).

Female junco blood samples (~60 μL) were taken from the alar wing vein using heparinized microhematocrit capillary tubes. After centrifugation and separation, erythrocytes were stored at 2°C in Longmire's lysis buffer solution. Avian erythrocytes are nucleated and highly mitotic, thus making them ideal for telomere measurement (Nussey et al. 2014). To extract DNA, we added 100 μL of erythrocytes (in Longmire's) to 100 μL phosphate buffer solution and then used NucleoSpin Blood kits (Macherey-Nagel, Bethlehem, PA) per the manufacturer's instructions. We confirmed DNA purity (260/280 ratios above 1.7 and 260/320 ratios above 1.8) and concentration using a NanoDrop 8000 spectrophotometer (Thermo Scientific, Waltham, MA).

We used quantitative polymerase chain reaction (qPCR) to measure relative telomere length. Telomeres were measured with an Mx3000P (Stratagene, San Diego, CA), using methods adapted for dark-eyed juncos. Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was used for our single-copy control gene. To validate *GAPDH* as a suitable control gene, we ran amplified PCR product from three individuals on a 2% agarose gel to verify expected amplicon size (98 bp). We also used melt curve analysis to verify that all primers produced a single peak in their dissociation curve, thus verifying amplification of expected products and the absence of nonspecific amplification (i.e., primer-dimers). *GAPDH* primers were: *forward GAPDH* (5′-AACCAGCCAAGTACGATGACAT-3′) and *reverse GAPDH* (5′-CCATCAGCAGCAGCCTTCA-3′). Telomere primers were: *forward tel1b* (5′-CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT-3′) and *reverse tel2b* (5′-GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT-3′). *GAPDH* and telomere reactions were run on separate plates, and each sample was run in triplicate. Each reaction had a total volume of 25 μL containing 10 ng DNA, 12.5 μL PerfeCTa SYBR Green SuperMix Low ROX (Stratagene), and 200 nM/200 nM *for-*

ward GAPDH/*reverse GAPDH* or 200 nM/200 nM *forward tel1b*/*reverse tel2b*. A serial dilution (40, 20, 10, 5, and 2.5 ng) of the same reference sample of a single junco was run on each plate as a standard curve. qPCR reaction conditions for *GAPDH* were 10 min at 95°C, followed by 40 cycles of 30 s at 95°C and 30 s at 60°C, finishing with 1 min at 95°C, 30 s at 55°C, and 30 s at 95°C. qPCR reaction conditions for telomeres were 10 min at 95°C, followed by 27 cycles of 15 s at 95°C, 30 s at 58°C, and 30 s at 72°C, finishing with 1 min at 95°C, 30 s at 58°C, and 30 s at 95°C.

Briefly, the number of PCR cycles (C_t) to reach a threshold (set by the 10 ng dilution of the reference sample) was measured for each sample. Telomere lengths were then quantified as *T*/*S* ratios (the ratio of telomere repeats [TTAGGG] to the number of copies of a control gene [*GAPDH*]) using the formula $2^{\Delta\Delta C_t}$, where $\Delta\Delta C_t = (C_t^{\text{telomere}} - C_t^{GAPDH})$ reference − $(C_t^{\text{telomere}} - C_t^{GAPDH})$ focal (Stratagene 2007). Intra-assay C_t variation for *GAPDH* and telomere reactions was 0.10% and 1.53%, respectively. Interassay C_t variation for *GAPDH* and telomere reactions was 0.23% and 0.28%, respectively. Interplate variation for change in C_t values was 0.14%. The 10 ng dilution of the reference sample was used to calculate both interassay and interplate variation. Efficiencies for *GAPDH* and telomere plates ranged from 92.9% to 94.3% and from 85.6% to 87.1%, respectively. For all plates, standard curve R^2 values were 0.99. GAPDH and telomere standard curve slopes were −3.5 and −3.7, respectively.

Statistical Analyses

Because initiation of egg laying may be dependent on previous reproductive experience and other age-related factors independent of telomeres, we analyzed only birds of known age. Due to the small sample size of females 3, 4, and 5 years of age ($n \leq 2$ per age class), we aggregated these individuals into a 3+ year category ($n = 4$). Sample sizes for first-year and second-year females were 16 and 15, respectively. We used weighted least squares regression to test whether telomere length differs with age. Weighted least squares regression was then also used to test whether telomere length and age were related to the date of first seasonal clutch initiation. *T*/*S* ratio data were log transformed to meet normality assumptions, otherwise all data met normality and homoscedasticity assumptions. Because the interaction between telomere length and age did not explain a significant amount of variation in the date of clutch initiation, we removed the interaction term from our analysis (table A1: R^2 change *F*-test; R^2 change $= 0.004$, *F* change $= 0.262$, $P = .612$). As first clutch initiation date was calculated with respect to the first clutch of the year, and because we expected no differences in average population telomere length between our study years (2014 and 2015), we did not include sample year in the model. To determine whether timing of breeding was also related to energetic condition, we performed a linear regression to test whether telomere length and timing of breeding were related to body mass, as body mass has been found to provide a simple and reliable measure of overall condition in birds (Labocha and Hayes 2012). We used the Durbin-Watson statistic and variance inflation factors to verify independence of observations and absence of multicollinearity, respectively. Finally, we used Cook's distance to verify absence of influential observations and plotted centered leverage points against standardized residuals to check for outliers and leverage. Analyses were performed in SPSS (ver. 24). Data have been deposited in the Dryad Digital Repository: https://doi.org/10.5061/dryad.681q5 (Bauer et al. 2018).

Results

There was no significant effect of chronological age on telomere length ($F_{1,22} = 1.54$, $P = .22$, $R^2 = 0.05$). However, both chronological age and telomere length were significantly related to timing of breeding (table A1; $F_{2,32} = 14.70$, $P < .0001$, $R^2 = 0.48$). Both older birds ($\beta = -0.70$, $t = -5.29$, $P < .0001$) and those with shorter telomeres ($\beta = 0.32$, $t = 2.43$, $P = .021$) laid their first clutches earlier in the season (fig. 1).

Discussion

We found that both older dark-eyed junco females and those with shorter telomeres initiated first clutches earlier in the season. These results are consistent with the hypothesis that females with fewer predicted future reproductive events begin breeding earlier in the breeding season. As telomere lengths were similar across all age groups, this did not drive the positive relationship between telomere length and clutch initiation date. Therefore, our findings suggest that both chronological and biological age influence breeding timing decisions in female, dark-eyed juncos.

In addition, we found a negative relationship between chronological age and clutch initiation date, such that older individuals initiated first clutches earlier in the breeding season. This result is unsurprising, as the same trends have been found in several different bird species (Martin 1995; DeForest and Gaston 1996; Ezard et al. 2007; Blas et al. 2009). Older birds may initiate early first seasonal clutches for a variety of reasons. First, if late breeders tend to be of poorer quality, then these individuals may have already been removed from the population, thus leaving only early breeders within the older age brackets (Forslund and Part 1995). Second, if breeding experience improves reproductive success and competence, then older individuals may be able to breed earlier since they are faster at accumulating the necessary resources for first clutches (Forslund and Part 1995). Third, female birds without prior exposure to lengthening photoperiods are slower to initiate reproductive development (Salvante et al. 2013), although this hypothesis does not explain the earlier breeding times of our birds 3+ year old compared to our 2-year-old birds. Finally, older individuals may be more likely to make a terminal investment, as they are closer to senescence and therefore have fewer future reproductive events (Pianka and Parker 1975; Forslund and Part 1995; Froy et al. 2017).

We also found that individuals with shorter telomeres initiated their first clutches earlier within the breeding season. Notably, this relationship appeared consistent within each age group, as the slopes of telomere length on first clutch lay date were similar between birds 1, 2, and 3+ years old (figure 1). If telomeres are biomarkers of prolonged exposure to oxidative stress (Haussmann et al. 2012), then these results are consistent with the idea that birds adjust reproductive effort based on cumulative physiological wear and tear. We did not find that telomere lengths were shorter in older individuals, but this is not totally surprising, as other studies have found that telomere lengths do not differ between adult age classes in other systems (Hall et al. 2004; Simons 2015; Cerchiara et al. 2017). However, because our study was cross-sectional, a progressive disappearance of individuals with shorter telomeres could cause older age classes to be composed of a greater proportion of individuals with longer telomeres (Haussmann and Mauck 2008).

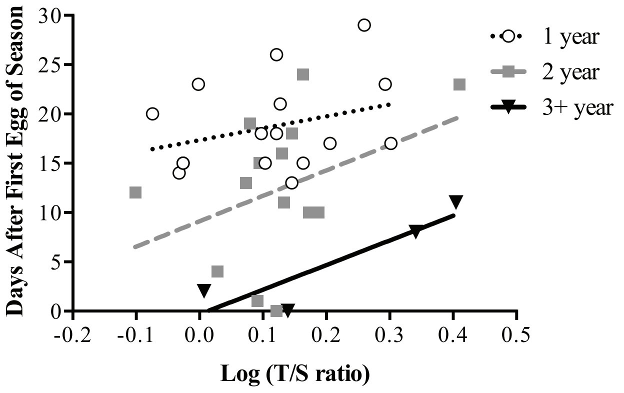

Figure 1: Relationship between telomere length, chronological age, and the timing of breeding in female dark-eyed juncos. Older females and those with shorter telomeres (*T*/*S* ratio) laid eggs significantly earlier in the season (number of days after the first egg of the season). Depicted are female juncos aged 1 (white circles, $n = 16$), 2 (gray squares, $n = 15$), and 3+ (black triangles, $n = 4$) years. While there was no significant interaction between age and telomere length on egg lay date, separate regression lines are given to highlight differences in egg lay date between age classes (dotted, dashed, and solid lines for birds 1, 2, and 3+ years old, respectively). *T*/*S* ratios represent the ratio of telomere repeats (TTAGGG) to the number of copies of a control gene (*GAPDH*).

These older individuals may have either started life with longer telomeres, and/or were better at preventing oxidative damage from shortening their telomeres, for example, by upregulating antioxidant defenses (Kotrschal et al. 2007; Badás et al. 2015) during metabolically expensive life-history stages such as early growth (Costantini et al. 2012; Herborn et al. 2014; Marasco et al. 2015).

If telomeres are indicative of an individual's current quality or state (Bauch et al. 2013), then our results do not support the hypothesis that timing of breeding is constrained by individual condition (i.e., date-quality hypothesis; Verhulst and Nilsson 2008), as we found a positive relationship between telomere length and first clutch date. Telomeres, however, may not be good indicators of current quality or condition, as we did not see a significant relationship between telomere lengths and body mass (table A2). We suggest that telomeres are better indicators of remaining life span rather than current state. Furthermore, we also suggest that current energetic condition may not be the primary factor influencing reproductive timing decisions, as we also did not find a significant relationship between body mass and timing of first clutch initiation (table A2).

Studies in seabirds have found both positive (Bauch et al. 2013) and negative (Le Vaillant et al. 2015) correlations between telomere lengths and egg lay date. These conflicting results may be attributed to studies involving long-lived species with relatively low year-to-year mortality, thus leaving little scope for detecting a relationship. Negative relationships between reproductive effort and remaining life span may be better supported in shorter-lived species such as juncos, where higher annual mortality rates likely increase selection on current reproductive effort.

In conclusion, this study supports the hypothesis that in a small-bodied passerine, females with fewer remaining reproductive events are more likely to initiate reproduction earlier in the breeding season, further suggesting that individuals may time their reproduction based on both chronological and biological age. Future studies should examine which physiological mechanisms may be directly influencing adjustment of reproductive effort. Alternatively, if timing of first clutch initiation is repeatable across years, then early breeding females may have shorter telomeres because of costs associated with having bred early the previous year. Future studies should collect both longitudinal telomere measurements (and other condition measurements such as body mass index and antioxidant capacity) and reproductive output metrics to adequately test this. Finally, to further test the hypothesis that remaining life span is negatively linked with reproductive effort, future studies should also assess whether older individuals and those with shorter telomeres increase reproductive effort via other means, such as increasing provisioning rates, nest defense, or the number of clutches per season.

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APPENDIX

Additional Statistical Regressions

Table A1: Statistical results of hierarchical regression analysis for predicting date of first seasonal clutch initiation

	Model 1			Model 2		
Variable	*B*	SE *B*	β	*B*	SE *B*	β
Age (1, 2, or 3+ years)	−7.41	1.40	$-.70^{**}$	−8.18	2.10	$-.77^{**}$
Telomere length (*T*/*S* ratio)	18.97	7.81	$.32^{*}$	9.73	19.39	.16
Age × telomere length	…	…	…	5.02	9.81	.20
R^2	…	.480	…	…	.476	…
F for change in R^2	…	9.55^{**}	…	…	.26	…

Note: Female juncos were grouped into age classes of 1 ($n = 16$), 2 ($n = 15$), or 3 years and older ($n = 4$). Telomere lengths were measured as *T*/*S* ratios, which are the ratio of telomere repeats to the number of copies of a control gene (*GAPDH*). B = slope.

* $P < .05$.

** $P < .0001$.

Table A2: Statistical results of a regression testing whether telomere length and date of first seasonal clutch initiation are related to body mass

Variable	*B*	SE *B*	β	*t*	*P*	VIF
Days after first egg of season	.06	.05	.21	1.21	.24	1.02
Telomere length (*T*/*S* ratio)	−1.71	3.01	−.10	−.57	.58	1.02

Note: Telomere lengths were measured as *T*/*S* ratios, which are the ratio of telomere repeats to the number of copies of a control gene (*GAPDH*). Variance inflation factors (VIF) are also included. $R^2 = 0.05$. Durbin-Watson statistic = 2.081.

Literature Cited

Badás, E. P., J. Martínez, J. Rivero de Aguilar Cachafeiro, F. Miranda, J. Figuerola, and S. Merino. 2015. Ageing and reproduction: antioxidant supplementation alleviates telomere loss in wild birds. Journal of Evolutionary Biology 28:896–905.

Bauch, C., P. H. Becker, and S. Verhulst. 2013. Telomere length reflects phenotypic quality and costs of reproduction in a long-lived seabird. Proceedings of the Royal Society B 280:20122540.

Bauer, C. M., J. L. Graham, M. Abolins-Abols, B. J. Heidinger, E. D. Ketterson, and T. J. Greives. 2018. Data from: Chronological and biological age predict seasonal reproductive timing: an investigation of clutch initiation and telomeres in birds of known age. American Naturalist, Dryad Digital Repository, https://doi.org/10.5061/dryad.681q5.

Bize, P., F. Criscuolo, N. B. Metcalfe, L. Nasir, and P. Monaghan. 2009. Telomere dynamics rather than age predict life expectancy in the wild. Proceedings of the Royal Society B 276:1679–1683.

Blas, J., F. Sergio, and F. Hiraldo. 2009. Age-related improvement in reproductive performance in a long-lived raptor: a cross-sectional and longitudinal study. Ecography 32:647–657.

Boonekamp, J. J., C. Bauch, E. Mulder, and S. Verhulst. 2017. Does oxidative stress shorten telomeres? Biology Letters 13:5, https://dx.doi.org/10.1098/rsbl.2017.0164.

Bourdon, R. M., and J. S. Brinks. 1982. Genetic, environmental and phenotypic relationships among gestation length, birth-weight, growth traits and age at 1st calving in beef-cattle. Journal of Animal Science 55:543–553.

Brinkhof, M. W. G., A. J. Cave, S. Daan, and A. C. Perdeck. 2002. Timing of current reproduction directly affects future reproductive output in European coots. Evolution 56:400–411.

Bronson, F. H. 1985. Mammalian reproduction—an ecological perspective. Biology of Reproduction 32:1–26.

Brown, C. R., and M. B. Brown. 1999. Fitness components associated with laying date in the cliff swallow. Condor 101:230–245.

Cerchiara, J. A., R. A. Risques, D. Prunkard, J. R. Smith, O. J. Kane, and P. D. Boersma. 2017. Magellanic penguin telomeres do not shorten with age with increased reproductive effort, investment, and basal corticosterone. Ecology and Evolution 7:5682–5691.

Costantini D., P. Monaghan, and N. B. Metcalfe. 2012. Early life experience primes resistance to oxidative stress. Journal of Experimental Biology 215:2820–2826.

Dantzer, B., and Q. E. Fletcher. 2015. Telomeres shorten more slowly in slow-aging wild animals than in fast-aging ones. Experimental Gerontology 71:38–47.

DeForest, L. N., and A. J. Gaston. 1996. The effect of age on timing of breeding and reproductive success in the thick-billed murre. Ecology 77:1501–1511.

Doody, J. S., A. Georges, and J. E. Young. 2004. Determinants of reproductive success and offspring sex in a turtle with environmental sex determination. Biological Journal of the Linnean Society 81:1–16.

Drent, R. H. 2006. The timing of birds' breeding seasons: the Perrins hypothesis revisited especially for migrants. Ardea 94:305–322.

Ezard, T. H. G., P. H. Becker, and T. Coulson. 2007. Correlations between age, phenotype, and individual contribution to population growth in common terns. Ecology 88:2496–2504.

Fairbairn, D. J. 1977. Spring decline in deer mice—death or dispersal. Canadian Journal of Zoology 55:84–92.

Fairlie, J., R. Holland, J. G. Pilkington, J. M. Pemberton, L. Harrington, and D. H. Nussey. 2016. Lifelong leukocyte telomere dynamics and survival in a free-living mammal. Aging Cell 15:140–148.

Forslund, P., and T. Part. 1995. Age and reproduction in birds—hypotheses and tests. Trends in Ecology and Evolution 10:374–378.

Froy, H., S. Lewis, D. H. Nussey, A. G. Wood, and R. A. Phillips. 2017. Contrasting drivers of reproductive ageing in albatrosses. Journal of Animal Ecology 86:1022–1032.

Hall, M. E., L. Nasir, F. Daunt, E. A. Gault, J. P. Croxall, S. Wanless, and P. Monaghan. 2004. Telomere loss in relation to age and early environment in long-lived birds. Proceedings of the Royal Society B 271:1571–1576.

Harris, M. P., S. T. Buckland, S. M. Russell, and S. Wanless. 1994. Post fledging survival to breeding age of shags *Phalacrocorax aristotelis* in relation to year, date of fledging and brood size. Journal of Avian Biology 25:268–274.

Hau, M., M. F. Haussmann, T. J. Greives, C. Matlack, D. Costantini, M. Quetting, J. S. Adelman, A. C. Miranda, and J. Partecke. 2015. Repeated stressors in adulthood increase the rate of biological ageing. Frontiers in Zoology 12:4.

Haussmann, M. F., A. S. Longenecker, N. M. Marchetto, S. A. Juliano, and R. M. Bowden. 2012. Embryonic exposure to corticosterone modifies the juvenile stress response, oxidative stress and telomere length. Proceedings of the Royal Society B 279:1447–1456.

Haussmann, M. F., and R. A. Mauck. 2008. Telomeres and longevity: testing an evolutionary hypothesis. Molecular Biology and Evolution 25:220–228.

Heidinger, B. J., J. D. Blount, W. Boner, K. Griffiths, N. B. Metcalfe, and P. Monaghan. 2012. Telomere length in early life predicts lifespan. Proceedings of the National Academy of Sciences of the USA 109: 1743–1748.

Herborn, K. A., B. J. Heidinger, W. Boner, J. C. Noguera, A. Adam, F. Daunt, and P. Monaghan. 2014. Stress exposure in early post-natal life reduces telomere length: an experimental demonstration in a long-lived seabird. Proceedings of the Royal Society B 281:20133151.

Jawor, J. M., J. W. McGlothlin, J. M. Casto, T. J. Greives, E. A. Snajdr, G. E. Bentley, and E. D. Ketterson. 2007. Testosterone response to GnRH in a female songbird varies with stage of reproduction: implications for adult behaviour and maternal effects. Functional Ecology 21:767–775.

Ketterson, E. D. 1979. Status signaling in dark-eyed juncos. Auk 96:94–99.

Kotrschal, A., P. Ilmonen, and D. J. Penn. 2007. Stress impacts telomere dynamics. Biology Letters 3:128–130.

Labocha, M. K., and J. P. Hayes. 2012. Morphometric indices of body condition in birds: a review. Journal of Ornithology 153:1–22.

Lack, D. 1968. Ecological adaptations for breeding in birds. Methuen, London.

Le Vaillant, M., V. A. Viblanc, C. Saraux, C. Le Bohec, Y. Le Maho, A. Kato, F. Criscuolo, and Y. Ropert-Coudert. 2015. Telomere length reflects individual quality in free-living adult king penguins. Polar Biology 38:2059–2067.

Marasco, V., W. Boner, B. Heidinger, K. Griffiths, and P. Monaghan. 2015. Repeated exposure to stressful conditions can have beneficial effects on survival. Experimental Gerontology 69:170–175.

Martin, K. 1995. Patterns and mechanism for age-dependent reproduction and survival in birds. American Zoologist 35:340–348.

Monaghan, P. 2010. Telomeres and life histories: the long and the short of it. Annals of the New York Academy of Sciences 1206:130–142.

Naef-Daenzer, B., F. Widmer, and M. Nuber. 2001. Differential post-fledging survival of great and coal tits in relation to their condition and fledging date. Journal of Animal Ecology 70:730–738.

Nilsson, J. A. 1994. Energetic bottle-necks during breeding and the reproductive cost of being too early. Journal of Animal Ecology 63:200–208.

Nolan, V., Jr., E. D. Ketterson, D. A. Cristol, C. M. Rogers, E. D. Clotfelter, R. C. Titus, S. J. Schoech, and E. Snajdr. 2002. Dark-eyed junco: *Junco hyemalis*. Birds of North America 716:1–44.

Nussey, D. H., D. Baird, E. Barrett, W. Boner, J. Fairlie, N. Gemmell, N. Hartmann, et al. 2014. Measuring telomere length and telomere dynamics in evolutionary biology and ecology. Methods in Ecology and Evolution 5:299–310.

Olsson, M., and R. Shine. 1997. The seasonal timing of oviposition in sand lizards (*Lacerta agilis*): why early clutches are better. Journal of Evolutionary Biology 10:369–381.

Perrins, C. M. 1970. Timing of birds breeding seasons. Ibis 112:242–255.

Pianka, E. R., and W. S. Parker. 1975. Age-specific reproductive tactics. American Naturalist 109:453–464.

Price, T., M. Kirkpatrick, and S. J. Arnold. 1988. Directional selection and the evolution of breeding date in birds. Science 240: 798–799.

Reed, T. E., L. E. B. Kruuk, S. Wanless, M. Frederiksen, E. J. A. Cunningham, and M. P. Harris. 2008. Reproductive senescence in a long-lived seabird: rates of decline in late-life performance are associated with varying costs of early reproduction. American Naturalist 171:E89–E101.

Salvante, K. G., A. Dawson, R. A. Aldredge, P. J. Sharp, and K. W. Sockman. 2013. Prior experience with photostimulation enhances photo-induced reproductive response in female house finches. Journal of Biological Rhythms 28:38–50.

Sheldon, B. C., L. E. B. Kruuk, and J. Merila. 2003. Natural selection and inheritance of breeding time and clutch size in the collared flycatcher. Evolution 57:406–420.

Simons, M. J. P. 2015. Questioning causal involvement of telomeres in aging. Ageing Research Reviews 24:191–196.

Spear, L., and N. Nur. 1994. Brood size, hatching order and hatching date-effects of four life-history stages from hatching to recruitment in western gulls. Journal of Animal Ecology 63:283–298.

Stratagene. 2007. Introduction to quantitative PCR: methods and application. Stratagene, San Diego, CA.

Sydeman, W. J., H. R. Huber, S. D. Emslie, C. A. Ribic, and N. Nur. 1991. Age-specific weaning success of northern elephant seals in relation to previous breeding experience. Ecology 72:2204–2217.

Verhulst, S., and J.-A. Nilsson. 2008. The timing of birds' breeding seasons: a review of experiments that manipulated timing of breeding. Philosophical Transactions of the Royal Society B 363:399–410.

von Zglinicki, T. 2002. Oxidative stress shortens telomeres. Trends in Biochemical Sciences 27:339–344.

Williams, T. D. 2012. Physiological adaptations for breeding in birds. Princeton University Press, Princeton, NJ.

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