PROCEEDINGS B

rspb.royalsocietypublishing.org

Research

Cite this article: Salmón P, Nilsson JF, Watson H, Bensch S, Isaksson C. 2017 Selective disappearance of great tits with short telomeres in urban areas. *Proc. R. Soc. B* **284**: 20171349.
http://dx.doi.org/10.1098/rspb.2017.1349

Received: 16 June 2017
Accepted: 25 July 2017

Subject Category:
Ecology

Subject Areas:
ecology, environmental science, evolution

Keywords:
telomere, urbanization, survival, great tit

Author for correspondence:
Pablo Salmón
e-mail: pablo.salmon@biol.lu.se

Selective disappearance of great tits with short telomeres in urban areas

Pablo Salmón[1], Johan F. Nilsson[1], Hannah Watson[1], Staffan Bensch[2] and Caroline Isaksson[1]

[1]Evolutionary Ecology, Department of Biology, and [2]MEMEG, Molecular Ecology and Evolution Group, Department of Biology, Lund University, Ecology Building, Lund 223 62, Sweden

PS, 0000-0001-9718-6611; JFN, 0000-0001-6744-6486; HW, 0000-0003-4656-0647

Urban environments pose novel challenges, as well as opportunities, for urban-dwelling wildlife. Although differences have been reported in several phenotypic traits (e.g. morphology, physiology and behaviour) between urban and rural populations, it is poorly understood whether this affects individual fitness. Telomere dynamics are posited as one possible mechanism underlying senescence and mortality. It was recently shown that telomere shortening is accelerated when growing up in an urban, compared with a rural, environment. However, the implications of accelerated telomere attrition for fitness are still unclear. Here, we examine the relationship between telomere length (TL) and survival in a bird common to urban and rural environments, and during both early and later life. The results reveal that TL is a strong predictor of post-fledging survival and recruitment in both habitats but, crucially, selective disappearance of individuals with short telomeres early in life is more pronounced in the urban environment, resulting in a longer average TL among the adult population. However, following recruitment, we found no difference in the relationship between TL and survival between the urban and rural environments. This indicates that the urban environment has negative effects in early life, while during later life the benefits could potentially outweigh the costs.

1. Introduction

Urbanization is recognized as a major force affecting the ecology and evolution of species, populations and communities across the globe [1]. Urban landscapes differ markedly from natural/semi-natural habitats with regard to abiotic factors, such as noise, light at night and air pollution levels, and biotic factors, for example, food resources, disease prevalence and predator communities [2,3]. Despite this, some species are able to persist in urban environments and may even attain higher densities and/or fitness compared with rural-dwelling conspecifics. Together, these urban factors can exert selection pressure on life-history traits, which may subsequently have implications for adaptation and individual fitness [4]. Indeed, marked changes in behaviour, physiology and morphology have been widely demonstrated among species inhabiting urban environments, even among those that seemingly thrive in them [5–11].

To understand the consequences of urbanization and subsequently species' persistence and resilience in urban environments, it is important to investigate the mechanisms underlaying variation in life-history traits. Telomeres are highly conserved tandem repeats of non-coding DNA (TTAGGG)*n* at the ends of eukaryotic chromosomes [12], which play an important role in maintaining genome stability [13]. Telomeres shorten with each round of somatic cell division and, upon reaching a critical length, trigger the cell to enter into a state of replicative senescence [14]. Studies in a range of organisms have shown that telomeres shorten with chronological age across different somatic tissues (reviewed in [15]). Furthermore, telomere attrition has been implicated in age-related declines in physiological function (i.e. senescence) and therefore likely fitness ([16], but see [17]). By linking processes at the cellular and organismal levels, telomere

THE ROYAL SOCIETY PUBLISHING

dynamics could play a major role in the evolution of life histories. In addition to the general pattern between telomere attrition and chronological age across taxonomic groups, individual telomere attrition rate in wild populations has been shown to be accelerated by environmental factors (e.g. [18,19]) and mediated by physiological stressors, e.g. glucocorticoid levels [20], infectious diseases [21] and oxidative stress [22].

In a cross-fostering experiment, in our study populations, we recently demonstrated that great tit (*Parus major*) offspring reared in an urban environment had significantly shorter pre-fledging telomere length (hereafter TL), compared with those reared in a rural environment and independent of genetic origin [23]. It has also been shown, experimentally, that anthropogenic noise exposure results in shorter early-life TL in house sparrows (*Passer domesticus*) [24]. Both results provide evidence that the urban environment *per se*, as well as single urban-related stressors, can influence TL and its dynamics. TL has also been suggested to be a reliable predictor of longevity and survival, under both laboratory [25] and natural conditions [26–29]. Furthermore, TL in early life has been shown to be a better predictor of longevity than that in adulthood [25], suggesting a mechanistic link between developmental conditions and later-life performance [16]. Differences in survival between urban and rural populations and along urbanization gradients have been documented in adult birds [30,31], but not in nestling or fledging survival ([32,33], but see [34]). However, the effect of early-life conditions may not be revealed until later in life and could be mediated by early-life telomere dynamics. Implementing studies of telomere dynamics in an urban context could develop our understanding of how anthropogenic stressors could shape patterns of ageing and survival. Nonetheless, studies investigating the association between TL and urbanization remain limited [23].

The present study examines the relationship between TL dynamics and survival in an urban and a rural environment during both early and later life. Our study system is a small short-lived passerine bird, the great tit, widely used in studies of urban avian ecology (e.g. [35–37]). Firstly, we examined if TL predicts survival during both early and late life and whether the relationship differs between urban and rural habitats; we predicted a stronger effect in the urban environment owing to the higher levels of anthropogenic stressors causing elevated physiological stress via oxidative stress and/or stress hormones. Secondly, in a longitudinal analysis, we tested the effects of habitat on telomere shortening during the first year of life and whether attrition is accelerated in an urban, compared with a rural, habitat. Thirdly, we examined the relationship between TL and chronological age in adult birds and whether this relationship differs between urban and rural environments, using both cross-sectional and longitudinal data.

2. Material and methods

(a) Study area and blood sampling

The urban study site was located in the city of Malmö (55°36′ N; 13°02′ E), Sweden's third largest city with approximately 300 000 inhabitants. The rural study site was in a forest in Vombs fure (55°40′ N, 13°31′ E, less than five inhabitants km^{-2}), located 37 km northeast of Malmö. In Malmö, nest-boxes were distributed across four 10–45 ha urban parks characterized by a mixture of coniferous and deciduous trees, managed grasslands, ponds and associated urban infrastructure including paved footpaths and buildings. In Vombs fure, nest-boxes were located in a mosaic of deciduous and coniferous forest patches (219 ha), dominated by Scots pine (*Pinus sylvestris*), oak (*Quercus robur*) and birch (*Betula pendula*). The urban and rural habitats differ in urbanization score (according to [38]): Malmö (average score for the four urban parks): B (building cover) = 1.5, V (vegetation cover) = 1 and R (paved surface cover) = 1 and Vombs fure: $B = 0.66$, $V = 2$ and $R = 0$ (for further details, see [23,39]).

Great tit breeding activity was monitored in all occupied nest-boxes in 2013, 2014 and 2015. Nestlings were measured, ringed with a uniquely numbered aluminium ring and blood sampled when 15 days old (15 d). Nestling sex was determined from DNA extracted from red blood cells (RBCs) following the method adapted from [40] using P2 and P8 primers. Parents were caught in nest-boxes when nestlings were 8–9 days, ringed with a uniquely numbered aluminium ring, measured and blood sampled. Adults were aged as either second calendar year or third calendar year or older and sexed according to plumage characteristics [41].

All blood samples were collected from the jugular vein and immediately transferred to ice. Within 1 h, they were centrifuged for 10 min at 1800 r.p.m. to separate plasma and RBC and stored at −80°C until analysis. This study comprises data from animals caught and sampled between 2013 and 2015 ($n_{tot} = 544$ samples; $n_{urban} = 217$, $n_{rural} = 327$). Of 466 individuals ($n_{urban} = 180$, $n_{rural} = 286$), 405 were captured and sampled in only a single season, whereas 61 individuals were recaptured and sampled in two or more breeding seasons (see statistical analysis for more detailed sample sizes).

(b) Telomere length measurement

Relative TL (RTL) was measured in RBC by the quantitative real-time amplification method (qPCR) [42], following a procedure previously validated for birds [43]. DNA was extracted from approximately 5 µl samples of RBC in 195 µl of phosphate-buffered saline using Macherey-Nagel NucleoSpin Blood Kits (Bethlehem, PA, USA) and following the manufacturer's instructions. The quantity and purity of the extracted genomic DNA was measured using a Nanodrop 2000 Spectrophotometer (Thermo Scientific), and DNA integrity was checked in a subset of samples using agarose 2% gel electrophoresis. RTL was measured as the ratio (T/S) of telomere repeat copy number (T) to control gene copy number (S), relative to a reference sample using a Mx3005P (Stratagene). This method provides a relative measure of the TL suitable for within-species comparisons [44]. The control, single-copy gene used was glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Previous studies have confirmed the suitability of this gene as an invariant control gene [45]. The following primers were used to amplify the telomere and GAPDH sequences: telomere forward tel1b (5′-CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT-3′) and reverse tel2b (5′-GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCTTACCCT-3′); Great tit-specific GAPDH forward (5′-TGTGATTTCAATGGTGACAGC-3) and reverse (5′-AGCTTGACAAAATGGTCGTTC-3′). The qPCR for both telomere and GAPDH reactions was performed using 5 ng of DNA with sets of primers Tel1b/Tel2b at a concentration of 200/200 nM and GAPDH-F/GAPDH-R at 100/100 nM, in a final volume of 25 µl and containing 12.4 µl of Supermix (Platinum SYBR Green qPCR SuperMix-UDG, Invitrogen). Reactions were performed in triplicate and samples were randomly distributed among plates, based on habitat, age and sex. Repeated samples of the same individual were run on the same plate. The conditions for the qPCR were: telomeres: 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; GAPDH: 10 min at 95°C, 15 min at 95°C, 30 s at 60°C and 30 s at 72°C. On each plate, a serial dilution (from 20 to 1.25 ng) of a reference DNA sample

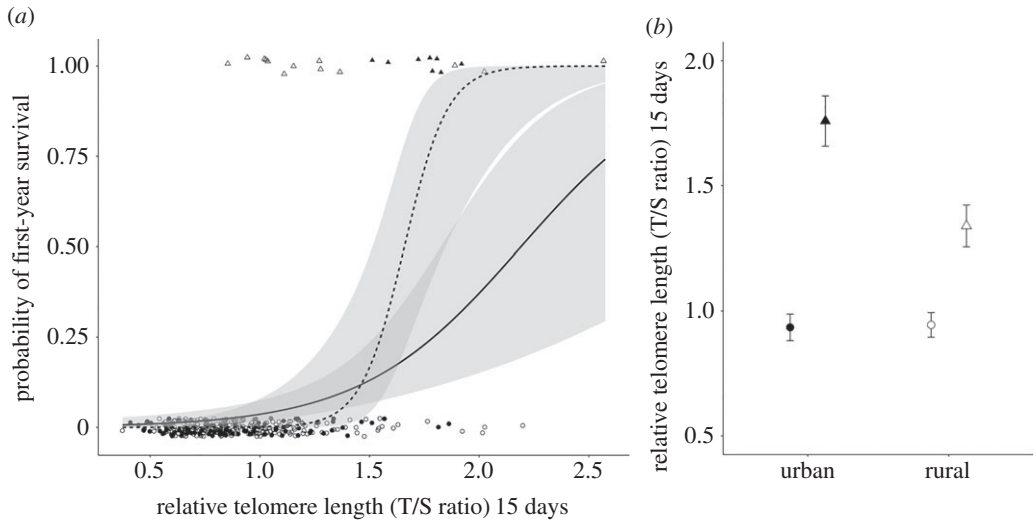

Figure 1. Relationship between RTL (T/S ratio) at 15 d and survival and recruitment to the subsequent breeding season in urban (filled symbols) and rural great tits (open symbols). Datapoints represent individuals that were present (triangles) and absent (circles) from the breeding population. (*a*) Predicted probability of first-year survival and recruitment in relation to RTL ($n = 327$). Fitted lines represent model predictions for urban (dashed line) and rural (solid line) populations with 95% confidence intervals shown by shaded area. (*b*) Mean ± s.e. RTL at 15 d of urban and rural birds that were present ($n = 21$) and absent ($n = 306$) in their first breeding season.

(an individual not included in the study) was included as a standard curve. Efficiencies of the reference curve were within the acceptable range for both telomeres (mean ± s.d.: 104.42 ± 3.71) and GAPDH (mean ± s.d.: 101.27 ± 2.64) [46]. To account for variation in amplification efficiencies between telomere and GAPDH, we used the Pfaffl method to calculate T/S ratios [47]. Two DNA samples (golden samples) were run in triplicate on each plate to calculate interplate variation (30 plates in total). Mean inter- and intra-plate variation of C_t values was 1.96 and 0.71% for the telomere reactions and 1.63 and 0.25% for the GAPDH reactions. Mean inter- and intra-plate coefficients of variation for the relative T/S ratios were 6.93 and 6.47%, respectively.

(c) Statistical analysis

All analyses were conducted in R v. 3.2.3 [48] using the package *lme4* [49]. To examine the association between RTL, apparent survival (defined below) and habitat, we divided the data into: (i) first-year survival, i.e. post-fledging survival and subsequent recruitment into the local breeding population in the second year ($n = 327$; present = 21, absent = 306); and (ii) post-recruitment survival, i.e. survival beyond the first year and present in the breeding population in one or more subsequent seasons ($n = 118$; present = 46, absent = 72), similar to [50]. To examine first-year survival, we fitted generalized linear mixed models (GLMMs) with a binomial error distribution to survival data (0 = absent and 1 = present) from nestlings reared in 2013; the initial model included RTL at 15 d, habitat, body mass at 15 d and the interaction between RTL and habitat, and RTL and body mass as fixed effects. Nest-box and qPCR plate were included as random effects. To examine post-recruitment survival, we fitted a similar model to survival data, using age class (second calendar year or third calendar year or older), RTL at the previous capture and sampling, habitat, sex and the interactions between RTL and habitat as fixed effects and qPCR plate as a random effect.

To understand the effects of habitat on RTL change in the first year of life (from 15 d to the first breeding season; urban: $n = 13$, rural: $n = 16$), we calculated the '*D*' variable as a measure of TL change, accounting for potential 'regression to the mean' [51]. We switched X1 and X2 variables in order to obtain negative values for a decrease in RTL and positive values for an increase. We fitted a linear mixed model (LMM) to the dependent variable, *D*, and included habitat and year (2013 or 2014) and their interaction as fixed factors in the initial model. Nestling body mass (15 d) was included as a covariate as well as its interaction with habitat. Nest-box of origin and qPCR plate were included as random effects.

We examined the relationship between RTL and age and whether this differed between habitats using a LMM. As the rate of telomere attrition, as well as the primary driving forces, is likely to vary markedly between growth and adulthood (e.g. [25]), we used adult (second calendar year or older) RTL measures for this analysis. We used the within-subject centring approach to separate between-subject and within-subject effects of age [52]. For this, we used only known-age individuals (range = 1–7 years; $n_{tot} = 155$ samples (from 106 inviduals); $n_{urban} = 55$, $n_{rural} = 51$ individuals). To differentiate cross-sectional and longitudinal patterns, age was split into two variables: the mean age of individuals (between-subject effects) and the difference between age at sampling and mean individual age (within-subject effects) as fixed factors. The initial model also included habitat and sex, as well as their interactions with both age variables as fixed effects. To account for inter-annual variability, year was included as a fixed effect. Owing to the repeated measures from some individuals, individual identity was included as a random effect as well as a qPCR plate. RTL was log-transformed to improve normality.

Fixed effects in GLMMs and LMMs were fitted with maximum-likelihood and simplified to obtain a final model following a backward elimination approach, using likelihood ratio tests (LRTs). Final models were achieved by omitting the terms with the lowest marginal χ^2-statistic from the initial model until only significant terms remained ($p < 0.05$). Main effects underlaying a retained interaction were also retained. Variance structures of random effects were estimated using restricted maximum-likelihood. In GLMMs, we assessed the significance of each fixed effect using LRTs. For LMMs, denominator degrees of freedom for fixed effects were calculated using the Satterthwaite approximation.

3. Results

(a) Telomere length as a predictor of survival in urban and rural habitats

RTL at 15 d predicted the probability of recruitment to the breeding population in the following calendar year: individuals

Table 1. Summary of GLMMs investigating the association between survival, RTL and habitat (urban/rural) in great tits. (Parameter estimates and associated statistics are given for fixed effects retained in minimum adequate models and for terms rejected during backward stepwise regression. qPCR plate (models *a* and *b*) and nest of origin (*a* only) were included as random effects. Italics indicate statistical significance at or below 0.05.)

dependent variable	fixed effect	rejected terms	estimate (s.e.)	d.f.	χ^2	*p*-value
(*a*) first-year survival ($n = 327$)	intercept		−6.533 (1.553)			
	RTL 15 d		3.072 (1.010)	1	43.349	*<0.001*
	habitat		−10.796 (5.560)	1	0.030	0.862
	RTL 15 d × habitat		7.359 (3.482)	1	9.581	*0.0019*
		body mass 15 d	0.243 (0.187)	1	1.977	0.159
		RTL 15 d × body mass 15 d	−0.064 (0.419)	1	0.000	0.987
(*b*) post-recruitment survival ($n = 118$)	intercept		−2.730 (0.739)			
	RTL^a_{t-1}		2.124 (0.647)	1	13.184	*<0.001*
		age	−0.414 (0.425)	1	0.956	0.328
		habitat	0.442 (0.428)	1	1.081	0.298
		sex	0.116 (0.417)	1	0.077	0.781
		RTL^a_{t-1} × habitat	0.813 (1.425)	1	0.332	0.564

[a]RTL_{t-1} is the relative telomere length in the previous year.

with longer RTL as nestlings were more likely to survive and recruit in both the urban and the rural population (figure 1*a*,*b*). Moreover, the relationship was significantly different between the two habitats (RTL 15 d × habitat: $p = 0.0019$; table 1*a* and figure 1*a*): in the urban habitat, only those individuals with long RTL survived and recruited in their first year, whereas in the rural habitat a wider range of RTL was represented among survivors (figure 1*b*). Two individuals (from the rural habitat) had very long RTL and so the analysis was re-run excluding these individuals. Neither of the potential outliers had an influence on the relationship between RTL and first-year survival, in respect of magnitude or significance of the effect (RTL 15 d × habitat: $\chi^2_1 = 8.204$, $p = 0.0041$). The effect of RTL on recruitment was independent of body mass (RTL 15 d × body mass 15 d: $p = 0.987$, table 1*a*). Post-recruitment survival in adult birds (second calendar year or older) was similarly positively associated with RTL from the previous year ($p < 0.001$; table 1*b* and figure 2), though the effect was independent of habitat (RTL_{t-1} × habitat: $p = 0.564$, table 1*b*). Neither age class nor sex had an effect on post-recruitment survival (all $p > 0.3$, table 1*b*).

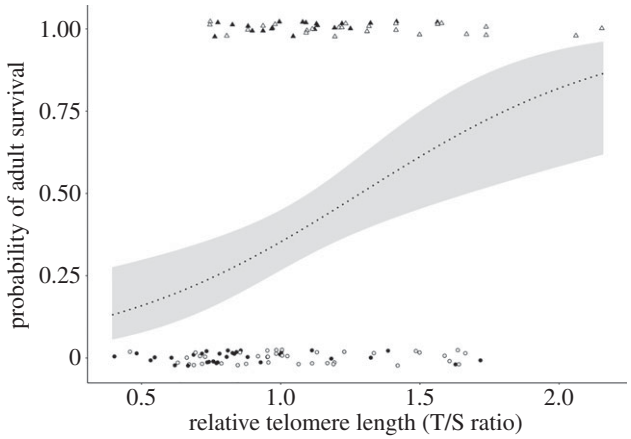

Figure 2. Relationship between RTL (T/S ratio) and survival to the following breeding season in adult great tits (second calendar year or older) from urban (filled symbols) and rural (open symbols) populations ($n = 118$). Predicted probability of survival (dotted line) in relation to RTL is presented with 95% CIs shown by shaded area. Datapoints represent individuals who were present (triangles) and absent (circles) from the breeding population.

(b) First-year telomere length change within individuals

RTL change in the first year—from fledging to recruitment in the following calendar year—did not differ between the urban and the rural habitat ($p = 0.902$, table 2*a*). In addition, there were no cohort effects (i.e. year of birth, 2013 or 2014) on the RTL change ($p = 0.102$, table 2*a*). There was a non-significant trend for an association between the RTL change and nestling body mass ($p = 0.088$, table 2*a*); the tendency was for individuals with a higher body mass at 15 d to have a greater RTL change (i.e. higher rate of attrition).

(c) Age and telomere length in adulthood in an urban and rural environment

Using the within-subject centring approach, neither of the analysed age components had a significant effect on RTL in birds in their second calendar year or older (between-subject: $p = 0.241$; within-subject: $p = 0.918$; table 2*b* and figure 3). However, urban birds had significantly longer RTL than rural birds ($p = 0.018$; table 2*b* and figure 4) and this was independent of individual age (between-subject × habitat: $p = 0.689$; table 2*b*). Within-individual change in RTL also showed no variation between the urban and rural habitats (within-subject × habitat: $p = 0.258$; table 2*b*). Sampling year had a significant effect on RTL, with RTL being longer in 2014, compared with 2013 and 2015 ($p < 0.001$; table 2*b*). Neither sex nor its interactions with any age component had an effect on RTL ($p > 0.1$; table 2*b*).

4. Discussion

Our study provides, to the best of our knowledge, the first evidence of differential selective disappearance, in relation to TL,

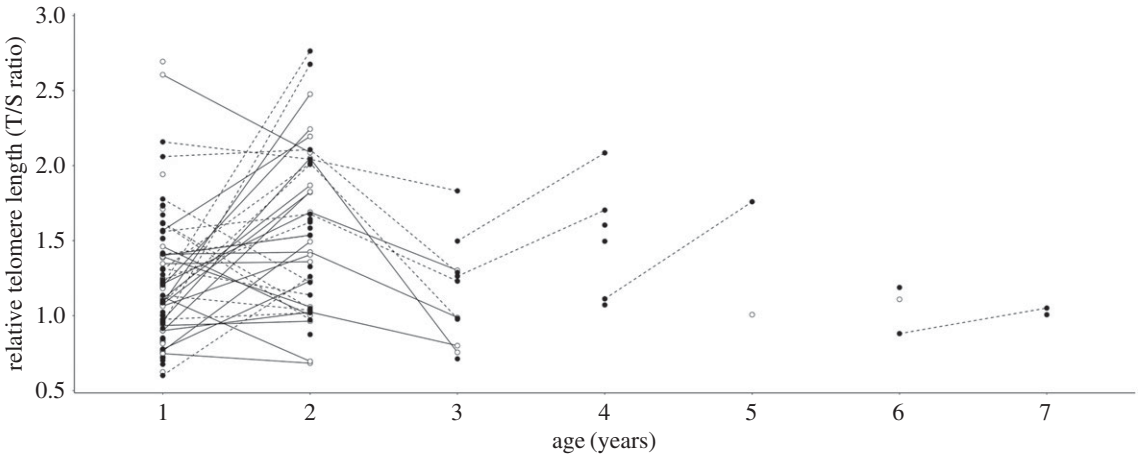

Figure 3. Relationship between chronological age and RTL (T/S ratio) in adult great tits (age range: 1–7 years; $n = 155$, from 106 individuals) from urban (filled circles; dashed lines) and rural (open circles; solid lines) populations.

Table 2. Summary of LMMs investigating variation in RTL in urban and rural great tits during (*a*) the first year of life (15 d to 1 year) and (*b*) adulthood (age range: 1–7 years). (In (*b*), we used both cross-sectional and longitudinal RTL data to distinguish between within- and between-individual changes in RTL with age. Parameter estimates and associated statistics are given for fixed effects retained in minimum adequate models and for terms rejected during backward stepwise regression. Models included qPCR plate (models *a* and *b*), nest of origin (*a* only) and individual identity (*b* only) as random effects. Italics indicate statistical significance at or below 0.05.)

dependent variable	fixed effect	rejected terms	estimate (s.e.)	d.f.	*F*	*p*-value
(*a*) RTL change (*D*) from	Intercept		−0.122 (0.081)			
fledging to recruitment		habitat	0.019 (0.155)	1, 25	0.015	0.902
($n = 29$)		year	−0.268 (0.157)	1, 25	2.884	0.102
		body mass 15 d	0.095 (0.054)	1, 27	3.095	0.088
		habitat × year	−0.217 (0.321)	1, 23	0.455	0.506
		habitat × body mass 15 d	0.170 (0.109)	1, 23	2.414	0.133
(*b*) RTL in adulthood	intercept		−0.031 (0.054)			
(i.e. post-recruitment;	habitat		0.126 (0.052)	1, 98.87	5.737	*0.018*
$n = 155$[a])	year		0.151 (0.073)	1, 77.92	15.627	*<0.001*
		between-subject	0.026 (0.022)	1, 123.82	1.383	0.241
		within-subject	0.007 (0.071)	1, 140.28	0.010	0.918
		sex	−0.077 (0.052)	1, 98.38	2.220	0.139
		between-subject × habitat	0.020 (0.051)	1, 129.84	0.161	0.689
		within-subject × habitat	−0.108 (0.095)	1, 79. 54	1.295	0.258
		between-subject × sex	0.006 (0.045)	1, 143.53	0.022	0.881
		within-subject × sex	−0.103 (0.098)	1, 79.80	1.108	0.295

[a]From 106 individuals.

between two contrasting environments. We have demonstrated that early-life TL is a strong predictor of post-fledging survival and recruitment in both urban and rural habitats but, crucially, the results show that selective disappearance of individuals with short telomeres is more pronounced in the urban habitat. The consequence of this is that, among adult birds, the urban population consists of great tits with, on average, longer telomeres than rural individuals. A cross-fostering experiment, in our populations, previously showed that great tit nestlings raised in the urban environment have shorter TL, compared with chicks raised in the rural habitat, independent of genetic origin [23]. This confirms the expectation that urban environments present stressful conditions for developing birds. Despite fledging with, on average, shorter telomeres, the present study demonstrates that urban recruits have significantly longer TL than rural recruits and that this is likely to be driven by stronger selective disappearance of individuals with short telomeres in the urban environment. While it is well understood that exposure to stressors in early life can have profound effects on longevity and performance [53], few studies have examined the links between early-life TL and survival and lifespan in birds in the wild (e.g. lifespan: [54] and fledging survival: [55]). This study provides compelling evidence for the potential of

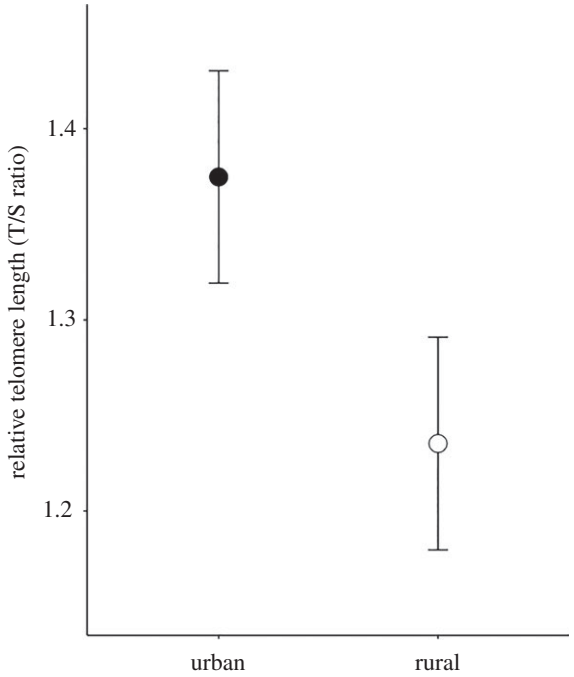

Figure 4. Effect of habitat on RTL (T/S ratio) in urban (filled circle) and rural (open circle) adult great tits (age range 1–7 years, $n = 155$, from 106 individuals). The data are presented as mean $\pm$ s.e.

telomere dynamics to mechanistically link early-life conditions with survival in wild populations.

Despite the expectation that the current urban environment poses less favourable conditions [11,37,56], we did not find any difference in the rate of telomere attrition among the survivors, neither during the first year of life—from fledging to second calendar year—nor during later life between the urban and rural habitats. Nonetheless, the fact that TL in our study is strongly related to survival suggests that individuals experiencing accelerated telomere attrition have disappeared from the populations during their first year [28]. Our results not only show that early TL is a strong predictor of early-life survival, but that TL is also positively associated with survival in later life. However, the relationship between TL and survival following recruitment does not differ between the urban and rural environments, as it does in early life. This could, in part, be explained by the elimination of poor quality individuals owing to selective disappearance of those with short telomeres early in life. It is also possible that the urban environment may not present the same level of stress to adult birds, as it does to young inexperienced birds. However, the two mechanisms are not necessarily mutually exclusive.

Longitudinal sampling is essential to investigate within-individual telomere dynamics, because cross-sectional data can easily be confounded by selective disappearance and cohort effects. Indeed, we have demonstrated strong evidence for selective disappearance in our great tit populations. Although many longitudinal studies have revealed within-individual declines in TL with advancing age (e.g. [25,57]), this study demonstrates no age-dependent change in TL. Previous studies have shown a similar lack of relationship between TL and age in longitudinal analysis, suggesting that telomeres can be well maintained through life in some species [58,59]. However, these studies were carried out in long-lived organisms in which telomerase activity has been shown to be maintained in adult somatic cells (e.g. [59,60]). The role of telomerase in maintaining TL is still very poorly studied in non-human organisms. It is also important to note that the technique employed to measure TL does not distinguish between different telomere classes [61] and a higher presence of ultra-long telomeres (Class III) may obscure the relationship between TL and age in our dataset. Nonetheless, more research is needed in order to understand the role of the different telomere classes with age.

In nature, stressors rarely act in isolation, and it is likely that the observed accelerated telomere shortening early in life [23] and lower survival probability for a given TL (demonstrated in this study) in the urban habitat are the result of exposure to multiple stressors [62]. Crucially, this study demonstrates that exposure to urban stressors is more critical in early life, with no evident effects on TL or dynamics in adulthood. Recent studies have shown that great tit nestlings reared in urban habitats have a lower constitutive immunity response [36] and also shorter TL than those reared in rural ones [23]. Both results suggest that urban nestlings might been constrained by resource limitation during development as nutritional status plays an important role in immune response [63] and TL maintenance [64]. Indeed, previous research indicates that nutrition for developing chicks may differ between urban and rural habitats, e.g. differences have been found in the relative proportion of ω-3 and ω-6 polyunsaturated fatty acids in egg yolks [56] and the concentration of dietary antioxidants such as carotenoids in caterpillars [65].

It is well understood that early-life conditions can have marked effects on phenotypic development and later-life performance, such as survival and reproductive success [53]. However, we do not find any differences in survival *per se* between urban and rural habitats, as other authors have reported [30]. While adult individuals are presumably still exposed to the same stressors, the results suggest that they are better able to cope with them and/or they are buffered in some way to the effects. For example, feeding birds in urban areas is a common practice (e.g. [66–68]) and may increase adult winter survival (reviewed in [69]). This bottom-up control could be an explanation for the absence of differences in how TL links to adult survival between our urban and rural populations. The suggestion that the urban environment may, in fact, be relatively benign for adult birds is perhaps in contrast to many studies reporting phenotypic effects associated with living in an urban environment [5,70,71]. However, relatively few studies have linked such phenotypic changes with fitness parameters.

Urban habitats impose both challenges and opportunities for wild organisms. The present study demonstrates clear effects of the urban environment on telomere dynamics and survival in early life, but not adult life. This indicates adverse consequences of exposure to urban-related stressors during early life, while suggesting that individuals are more resilient in later life and/or the benefits of the urban environment outweigh the costs. Nonetheless, long-term studies are still needed to corroborate if the effect is sensitive to environmental variation, e.g. cohort effects [55], and if there are long-term effects on the population structure. Furthermore, we should be cautious to generalize the observed pattern, as the consequences of urbanization, even though it is a global phenomenon, can depend on regional characteristics [2]. Further research is also needed to understand the physiological mechanism(s) underlying the observed differences in telomere dynamics, and particularly the relative role of individual urban stressors (e.g. air, light at night or noise pollution).

Ethics. All applicable national guidelines and regulations for the care and use of animals were followed, and all experimental procedures were approved by the Malmö/Lund Ethical Committee (ref. no. M454 12:1).

Data accessibility. Data available from the Dryad Digital Repository (http://dx.doi.org/10.5061/dryad.rn6k1) [72].

Authors' contributions. P.S. conceived the study with input from C.I., J.F.N. and H.W. C.I., P.S. and H.W. performed the fieldwork and sample collection. J.F.N. and P.S. carried out the laboratory analysis. P.S. analysed the data with input from J.F.N. and H.W. S.B., C.I., J.F.N., P.S. and H.W. drafted the initial manuscript. All authors gave approval for publication.

Competing interests. We have no competing or financial interests.

Funding. Funding was provided by the Swedish Research Council C0361301 and Marie Curie Career Integration Grant FP7-CIG ID:322217 (to C.I.) and the Royal Physiographic Society in Lund, Lund Djurskyddsfonden and the Lars Hierta Memorial Foundation (to P.S.). H.W. was supported by European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie (grant no. 659643).

Acknowledgements. We are grateful to Andreas Nord for assistance with fieldwork in 2013 and Alejandra Toledo in 2014. We also thank Mariana Lapa for help in the laboratory, and Dennis Hasselquist and Jan-Åke Nilsson for feedback on the results. We also acknowledge Sydvatten AB and the city of Malmö for access to the field sites.

References

1. Hendry AP, Gotanda KM, Svensson EI. 2016 Human influences on evolution, and the ecological and societal consequences. *Phil. Trans. R. Soc. B* **372**, 20160028. (doi:10.1098/rstb.2016.0028)
2. McKinney ML. 2002 Urbanization, biodiversity, and conservation: the impacts of urbanization on native species are poorly studied, but educating a highly urbanized human population about these impacts can greatly improve species conservation in all ecosystems. *Bioscience* **52**, 883–890. (doi:10.1641/0006-3568(2002)052[0883:ubac]2.0.co;2)
3. Evans KL, Gaston KJ, Sharp SP, McGowan A, Simeoni M, Hatchwell BJ. 2009 Effects of urbanisation on disease prevalence and age structure in blackbird *Turdus merula* populations. *Oikos* **118**, 774–782. (doi:10.1111/j.1600-0706.2008.17226.x)
4. Shochat E, Warren PS, Faeth SH, McIntyre NE, Hope D. 2006 From patterns to emerging processes in mechanistic urban ecology. *Trends Ecol. Evol.* **21**, 186–191. (doi:10.1016/j.tree.2005.11.019)
5. Isaksson C, Örnborg J, Stephensen E, Andersson S. 2005 Plasma glutathione and carotenoid coloration as potential biomarkers of environmental stress in great tits. *EcoHealth* **2**, 138–146. (doi:10.1007/s10393-005-3869-5)
6. Partecke J, Schwabl I, Gwinner E. 2006 Stress and the city: urbanization and its effects on the stress physiology in European blackbirds. *Ecology* **87**, 1945–1952. (doi:10.1890/0012-9658(2006)87[1945:satcua]2.0.co;2)
7. Slabbekoorn H, den Boer-Visser A. 2006 Cities change the songs of birds. *Curr. Biol.* **16**, 2326–2331. (doi:10.1016/j.cub.2006.10.008)
8. Moller AP. 2010 Interspecific variation in fear responses predicts urbanization in birds. *Behav. Ecol.* **21**, 365–371. (doi:10.1093/beheco/arp199)
9. Bonier F. 2012 Hormones in the city: endocrine ecology of urban birds. *Horm. Behav.* **61**, 763–772. (doi:10.1016/j.yhbeh.2012.03.016)
10. Dominoni D, Quetting M, Partecke J. 2013 Artificial light at night advances avian reproductive physiology. *Proc. R. Soc. B* **280**, 20123017. (doi:10.1098/rspb.2012.3017)
11. Isaksson C, Andersson MN, Nord A, von Post M, Wang H.-L. 2017 Species-dependent effects of the urban environment on fatty acid composition and oxidative stress in birds. *Front. Ecol. Evol.* **5**, 44. (doi:10.3389/fevo.2017.00044)
12. Blackburn EH. 1991 Structure and function of telomeres. *Nature* **350**, 569–573. (doi:10.1038/350569a0)
13. O'Sullivan RJ, Karlseder J. 2010 Telomeres: protecting chromosomes against genome instability. *Nat. Rev. Mol. Cell Biol.* **11**, 171–181. (doi:10.1038/nrm2848)
14. Blackburn EH. 2005 Telomeres and telomerase: their mechanisms of action and the effects of altering their functions. *FEBS Lett.* **579**, 859–862. (doi:10.1016/j.febslet.2004.11.036)
15. Dantzer B, Fletcher QE. 2015 Telomeres shorten more slowly in slow-aging wild animals than in fast-aging ones. *Exp. Gerontol.* **71**, 38–47. (doi:10.1016/j.exger.2015.08.012)
16. Monaghan P. 2010 Telomeres and life histories: the long and the short of it. *Ann. NY Acad. Sci.* **1206**, 130–142. (doi:10.1111/j.1749-6632.2010.05705.x)
17. Simons MJP. 2015 Questioning causal involvement of telomeres in aging. *Ageing Res. Rev.* **24**, 191–196. (doi:10.1016/j.arr.2015.08.002)
18. Young RC, Kitaysky AS, Haussmann MF, Descamps S, Orben RA, Elliott KH, Gaston AJ. 2013 Age, sex, and telomere dynamics in a long-lived seabird with male-biased parental care. *PLoS ONE* **8**, e74931. (doi:10.1371/journal.pone.0074931)
19. McLennan D, Armstrong JD, Stewart DC, McKelvey S, Boner W, Monaghan P, Metcalfe NB. 2016 Interactions between parental traits, environmental harshness and growth rate in determining telomere length in wild juvenile salmon. *Mol. Ecol.* **25**, 5425–5438. (doi:10.1111/mec.13857)
20. Herborn KA, Heidinger BJ, Boner W, Noguera JC, Adam A, Daunt F, Monaghan P. 2014 Stress exposure in early post-natal life reduces telomere length: an experimental demonstration in a long-lived seabird. *Proc. R. Soc. B* **281**, 20133151. (doi:10.1098/rspb.2013.3151)
21. Asghar M, Palinauskas V, Zaghdoudi-Allan N, Valkiūnas G, Mukhin A, Platonova E, Färnert A, Bensch S, Hasselquist D. 2016 Parallel telomere shortening in multiple body tissues owing to malaria infection. *Proc. R. Soc. B* **283**, 20161184. (doi:10.1098/rspb.2016.1184)
22. von Zglinicki T. 2002 Oxidative stress shortens telomeres. *Trends Biochem. Sci.* **27**, 339–344. (doi:10.1016/S0968-0004(02)02110-2)
23. Salmón P, Nilsson JF, Nord A, Bensch S, Isaksson C. 2016 Urban environment shortens telomere length in nestling great tits, *Parus major*. *Biol. Lett.* **12**, 20160155. (doi:10.1098/rsbl.2016.0155)
24. Meillère A, Brischoux F, Ribout C, Angelier F. 2015 Traffic noise exposure affects telomere length in nestling house sparrows. *Biol. Lett.* **11**, 20150559. (doi:10.1098/rsbl.2015.0559)
25. Heidinger BJ, Blount JD, Boner W, Griffiths K, Metcalfe NB, Monaghan P. 2012 Telomere length in early life predicts lifespan. *Proc. Natl Acad. Sci. USA* **109**, 1743–1748. (doi:10.1073/pnas.1113306109).
26. Pauliny A, Wagner RH, Augustin J, Szép T, Blomqvist D. 2006 Age-independent telomere length predicts fitness in two bird species. *Mol. Ecol.* **15**, 1681–1687. (doi:10.1111/j.1365-294X.2006.02862.x)
27. Olsson M, Pauliny A, Wapstra E, Uller T, Schwartz T, Miller E, Blomqvist D. 2011 Sexual differences in telomere selection in the wild. *Mol. Ecol.* **20**, 2085–2099. (doi:10.1111/j.1365-294X.2011.05085.x)
28. Boonekamp JJ, Mulder GA, Salomons HM, Dijkstra C, Verhulst S. 2014 Nestling telomere shortening, but not telomere length, reflects developmental stress and predicts survival in wild birds. *Proc. R. Soc. B* **281**, 20133287. (doi:10.1098/rspb.2013.3287)
29. Fairlie J, Holland R, Pilkington JG, Pemberton JM, Harrington L, Nussey DH. 2016 Lifelong leukocyte telomere dynamics and survival in a free-living mammal. *Aging Cell* **15**, 140–148. (doi:10.1111/acel.12417)
30. Hõrak P, Lebreton J.-D. 1998 Survival of adult great tits *Parus major* in relation to sex and habitat; a comparison of urban and rural populations. *Ibis* **140**, 205–209. (doi:10.1111/j.1474-919X.1998.tb04380.x)
31. Evans BS, Ryder TB, Reitsma R, Hurlbert AH, Marra PP. 2015 Characterizing avian survival along a rural-to-urban land use gradient. *Ecology* **96**, 1631–1640. (doi:10.1890/14-0171.1)
32. Whittaker KA, Marzluff JM. 2009 Species-specific survival and relative habitat use in an urban landscape during the postfledging period. *The Auk* **126**, 288–299. (doi:10.1525/auk.2009.07136)

33. Ryder TB, Reitsma R, Evans B, Marra PP. 2010 Quantifying avian nest survival along an urbanization gradient using citizen- and scientist-generated data. *Ecol. Appl.* **20**, 419–426. (doi:10.1890/09-0040.1)
34. Capilla-Lasheras P *et al.* 2017 Elevated immune gene expression is associated with poor reproductive success of urban blue tits. *Front. Ecol. Evol.* **5**, 64. (doi:10.3389/fevo.2017.00064)
35. Senar JC, Conroy MJ, Quesada J, Mateos-Gonzalez F. 2014 Selection based on the size of the black tie of the great tit may be reversed in urban habitats. *Ecol. Evol.* **4**, 2625–2632. (doi:10.1002/ece3.999)
36. Bailly J *et al.* 2016 Negative impact of urban habitat on immunity in the great tit *Parus major*. *Oecologia* **182**, 1053–1062. (doi:10.1007/s00442-016-3730-2)
37. Watson H, Videvall E, Andersson MN, Isaksson C. 2017 Transcriptome analysis of a wild bird reveals physiological responses to the urban environment. *Sci. Rep.* **7**, 44180. (doi:10.1038/srep44180)
38. Seress G, Lipovits Á, Bókony V, Czúni L. 2014 Quantifying the urban gradient: a practical method for broad measurements. *Landsc. Urban Plann.* **131**, 42–50. (doi:10.1016/j.landurbplan.2014.07.010)
39. Andersson MN, Wang H.-L., Nord A, Salmón P, Isaksson C. 2015 Composition of physiologically important fatty acids in great tits differs between urban and rural populations on a seasonal basis. *Front. Ecol. Evol.* **3**, 93. (doi:10.3389/fevo.2015.00093)
40. Griffiths R, Double M, Orr K, Dawson R. 1998 A DNA test to sex most birds. *Mol. Ecol. Notes* **7**, 1071–1075. (doi:10.1046/j.1365-294x.1998.00389.x)
41. Svensson L. 1992 *Identification guide to European passerines*. Stockholm, Sweden: Märstatryck.
42. Cawthon RM. 2002 Telomere measurement by quantitative PCR. *Nucleic Acids Res.* **30**, e47. (doi:10.1093/nar/30.10.e47)
43. Criscuolo F, Bize P, Nasir L, Metcalfe NB, Foote CG, Griffiths K, Gault EA, Monaghan P. 2009 Real-time quantitative PCR assay for measurement of avian telomeres. *J. Avian Biol.* **40**, 342–347. (doi:10.1111/j.1600-048X.2008.04623.x)
44. Nussey DH *et al.* 2014 Measuring telomere length and telomere dynamics in evolutionary biology and ecology. *Methods Ecol. Evol.* **5**, 299–310. (doi:10.1111/2041-210X.12161)
45. Atema E, van Oers K, Verhulst S. 2013 GAPDH as a control gene to estimate genome copy number in great tits, with cross-amplification in blue tits. *Ardea* **101**, 49–54. (doi:10.5253/078.101.0107)
46. Bustin SA *et al.* 2009 The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clin. Chem.* **55**, 611–622. (doi:10.1373/clinchem.2008.112797)
47. Pfaffl MW. 2001 A new mathematical model for relative quantification in real-time RT–PCR. *Nucleic Acids Res.* **29**, e45. (doi:10.1093/nar/29.9.e45)
48. R Core Team. 2015 *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
49. Bates D, Mächler M, Bolker B, Walker S. 2015 Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* **67**, 48. (doi:10.18637/jss.v067.i01)
50. Clobert J, Perrins CM, McCleery RH, Gosler AG. 1988 Survival rate in the great tit *Parus major* in relation to sex, age, and immigration status. *J. Anim. Ecol.* **57**, 287–306. (doi:10.2307/4779)
51. Verhulst S, Aviv A, Benetos A, Berenson G, Kark J. 2013 Do leukocyte telomere length dynamics depend on baseline telomere length? An analysis that corrects for 'regression to the mean'. *Eur. J. Epidemiol.* **28**, 859–866. (doi:10.1007/s10654-013-9845-4)
52. van de Pol M, Wright J. 2009 A simple method for distinguishing within- versus between-subject effects using mixed models. *Anim. Behav.* **77**, 753–758. (doi:10.1016/j.anbehav.2008.11.006)
53. Metcalfe NB, Monaghan P. 2001 Compensation for a bad start: grow now, pay later? *Trends Ecol. Evol.* **16**, 254–260. (doi:10.1016/S0169-5347(01)02124-3)
54. Asghar M, Hasselquist D, Hansson B, Zehtindjiev P, Westerdahl H, Bensch S. 2015 Hidden costs of infection: chronic malaria accelerates telomere degradation and senescence in wild birds. *Science* **347**, 436–438. (doi:10.1126/science.1261121)
55. Watson H, Bolton M, Monaghan P. 2015 Variation in early-life telomere dynamics in a long-lived bird: links to environmental conditions and survival. *J. Exp. Biol.* **218**, 668–674. (doi:10.1242/jeb.104265)
56. Toledo A, Andersson MN, Wang H.-L., Salmón P, Watson H, Burdge GC, Isaksson C. 2016 Fatty acid profiles of great tit (*Parus major*) eggs differ between urban and rural habitats, but not between coniferous and deciduous forests. *Naturwissenschaften* **103**, 55. (doi:10.1007/s00114-016-1381-0)
57. Barrett ELB, Burke TA, Hammers M, Komdeur J, Richardson DS. 2013 Telomere length and dynamics predict mortality in a wild longitudinal study. *Mol. Ecol.* **22**, 249–259. (doi:10.1111/mec.12110)
58. Pauliny A, Larsson K, Blomqvist D. 2012 Telomere dynamics in a long-lived bird, the barnacle goose. *BMC Evol. Biol.* **12**, 257. (doi:10.1186/1471-2148-12-257)
59. Ujvari B, Biro PA, Charters JE, Brown G, Heasman K, Beckmann C, Madsen T. 2016 Curvilinear telomere length dynamics in a squamate reptile. *Funct. Ecol.* **31**, 753–759. (doi:10.1111/1365-2435.12764)
60. Haussmann MF, Winkler DW, Huntington CE, Nisbet ICT, Vleck CM. 2007 Telomerase activity is maintained throughout the lifespan of long-lived birds. *Exp. Gerontol.* **42**, 610–618. (doi:10.1016/j.exger.2007.03.004)
61. Delany ME, Krupkin AB, Miller MM. 2000 Organization of telomere sequences in birds: evidence for arrays of extreme length and for *in vivo* shortening. *Cytogenet Genome Res.* **90**, 139–145. (doi:10.1159/000015649)
62. Haussmann MF, Heidinger BJ. 2015 Telomere dynamics may link stress exposure and ageing across generations. *Biol. Lett.* **11**, 20150396. (doi:10.1098/rsbl.2015.0396)
63. Houston AI, McNamara JM, Barta Z, Klasing KC. 2007 The effect of energy reserves and food availability on optimal immune defence. *Proc. R. Soc. B* **274**, 2835–2842. (doi:10.1098/rspb.2007.0934)
64. Paul L. 2011 Diet, nutrition and telomere length. *J. Nutr. Biochem.* **22**, 895–901. (doi:10.1016/j.jnutbio.2010.12.001)
65. Isaksson C, Andersson S. 2007 Carotenoid diet and nestling provisioning in urban and rural great tits *Parus major*. *J. Avian Biol.* **38**, 564–572. (doi:10.1111/j.2007.0908-8857.04030.x)
66. Jones DN, James Reynolds S. 2008 Feeding birds in our towns and cities: a global research opportunity. *J. Avian Biol.* **39**, 265–271. (doi:10.1111/j.0908-8857.2008.04271.x)
67. Davies ZG, Fuller RA, Loram A, Irvine KN, Sims V, Gaston KJ. 2009 A national scale inventory of resource provision for biodiversity within domestic gardens. *Biol. Conserv.* **142**, 761–771. (doi:10.1016/j.biocon.2008.12.016)
68. Reynolds SJ, Galbraith JA, Smith JA, Jones DN. 2017 Garden bird feeding: insights and prospects from a north-south comparison of this global urban phenomenon. *Front. Ecol. Evol.* **5**, 24. (doi:10.3389/fevo.2017.00024)
69. Robb GN, McDonald RA, Chamberlain DE, Bearhop S. 2008 Food for thought: supplementary feeding as a driver of ecological change in avian populations. *Front. Ecol. Environ.* **6**, 476–484. (doi:10.1890/060152)
70. Fokidis HB, Orchinik M, Deviche P. 2009 Corticosterone and corticosteroid binding globulin in birds: relation to urbanization in a desert city. *Gen. Comp. Endocrinol.* **160**, 259–270. (doi:10.1016/j.ygcen.2008.12.005)
71. Meillère A, Brischoux F, Parenteau C, Angelier F. 2015 Influence of urbanization on body size, condition, and physiology in an urban exploiter: a multi-component approach. *PLoS ONE* **10**, e0135685. (doi:10.1371/journal.pone.0135685)
72. Salmón P, Nilsson JF, Watson H, Bensch S, Isaksson C. 2017 Data from: Selective disappearance of great tits with short telomeres in urban areas. Dryad Digital Repository. (http://dx.doi.org/10.5061/dryad.rn6k1)