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Evolutionary biology

Urban environment shortens telomere length in nestling great tits, *Parus major*

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Urban environments are expanding rapidly, and with urbanization come both challenges and opportunities for wildlife. Challenges include combating the anthropogenic disturbances such as light, noise and air pollution and lower availability of natural food sources. The benefits are many, including the availability of anthropogenic food sources, breeding boxes and warmer temperatures. Thus, depending on the context, urbanization can have both positive and negative effects on fitness related traits. It is well known that early-life conditions can have lifelong implications on fitness; little is however known about development in urban environments. We reciprocally cross-fostered urban and rural nestling great tits (*Parus major* L.) to study how growing up in an urban versus rural habitat affected telomere length (TL)—a suggested biomarker of longevity. We show, for the first time, that growing up in an urban environment significantly shortens TL, independently of natal origin (i.e. urban or rural). This implies that the urban environment imposes a challenge to developing birds, with potentially irreversible effects on lifespan.

1. Introduction

It is well established that early-life conditions can have long-term consequences for phenotypic development and fitness [1,2]. Some of these phenotypic effects are not evident until later in life, when individuals show signs of accelerated ageing. Telomere attrition could be one of the mechanisms involved in these processes [3]. Telomeres are highly conserved tandem repeats of DNA at the ends of eukaryotic chromosomes, which play an important role in genome stability and replication [4]. They shorten with each cell division, to eventually pass a threshold length, after which the cell starts to senesce. While telomere length (TL) has a relatively large heritable component [5], shorter TL and increased telomere attrition have been linked to stressful conditions during development and disease [6,7]. The mechanistic pathway of this remains unclear, but it has been proposed that the glucocorticoid-mediated stress response, inflammatory responses, oxidative stress and starvation could play important roles [8].

In wild populations, TL correlates with life-history traits such as lifespan and survival [9,10]. However, we still know very little about how an animal's environment *per se* affects TL and telomere attrition [11]. This is an unfortunate lack of information in the face of present-day rapid increase in urbanization and consequent deterioration of natural environments. Urban environments differ from natural habitats in many regards and are for instance associated with higher levels of anthropogenic pollution, such as traffic noise, artificial light pollution and air pollution, which enhance oxidative stress and inflammatory responses. Moreover, food availability is generally higher but of lower quality owing to relatively more anthropogenic to natural food sources (reviewed in [12]). All these factors can have profound effects not only on development, but also on the health of adults. For instance, it has been shown that artificial light during the night suppresses immune function [13] and advances timing of reproductive

physiology [14], air pollution increases antioxidant activity [15] and traffic noise shortens early-life TL [16].

Here, we used a reciprocal cross-fostering design to test whether being reared in an urban versus a rural habitat influences early-life TL in great tits (*Parus major* L.). We predicted that urban-reared nestlings, independent of their origin, would have shorter TL than rural-reared nestlings.

2. Material and methods

The experiment was carried out between April and June 2013 using urban and rural nest-box populations of great tits located in southern Sweden (see [17] and electronic supplementary material for details).

The start of egg laying, clutch size and onset of incubation were determined by weekly visits to the nest-boxes. When nestlings were 2 days old (day of hatching = 0), we recprocally cross-fostered half of a brood in the urban habitat with the same number of nestlings from a nest with identical age and similar brood size (± 1 nestling) from the rural habitat ($n = 16$ nest pairs). In addition, nestlings were cross-fostered within each habitat in order to assess the potential effects of cross-fostering on TL (urban: $n = 8$ nest pairs; rural: $n = 11$ nest pairs). These results are presented in the electronic supplementary material. When nestlings were 15 days old, body mass and tarsus length were measured, and a 100 µl blood sample was collected from the jugular vein.

TL was quantified in red blood cells by qPCR and sex determination followed standard molecular methods (see the electronic supplementary material). Body condition index (SMI) was calculated using individual body mass and tarsus length measures (see the electronic supplementary material).

All statistical models were performed in R 3.0.2 using linear-mixed models fitted with maximum restricted likelihood and normal error structures using the lme4 package. TL (log-transformed) was set as the dependent variable, rearing habitat (urban or rural), habitat of origin (urban or rural), sex, hatching date and SMI as independent variables/factors. The nest of origin (to account for genetic and maternal effects) and the nest of rearing (to account for effects of the common environment) were included as random effects. The full model also included all two-way interactions of interest. Non-significant factors/variables were dropped from the full model using a backward elimination approach, starting with the least significant interactions followed by the least significant main effect terms. Denominator degrees of freedom for fixed effects were calculated using the Satterthwaite approximation. The significance of random effects was assessed using likelihood ratio tests. The cross-fostering experiments, both between- and within-habitat, were also used to test the effect of rearing habitat on the SMI. These results are presented in the electronic supplementary material.

3. Results

Rearing environment significantly influenced the TL of great tit nestlings ($p = 0.03$, figure 1 and table 1). Overall, TL differed by 10.7% between habitats, independent of nestling origin. Urban nestlings reared in the rural habitat had 11% longer TL than their non-fostered siblings in the urban habitat and rural nestlings reared in the urban habitat had 10.4% shorter TL than their non-fostered siblings reared in the rural habitat. There was no difference in TL between cross-fostered and non-fostered nestlings when reared in the same habitat (rearing habitat × habitat of origin: $p = 0.79$, figure 1 and table 1). There was no difference in TL between the sexes, and neither body condition nor hatching date affected TL

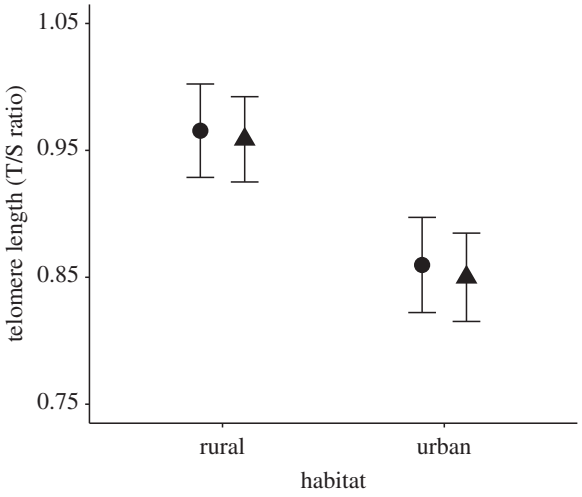

Figure 1. Mean ($\pm$s.e.) telomere length (T/S ratio, telomere length relative to a reference single-copy gene) in 15 day old great tit nestlings, reared in an urban or a rural habitat ($n = 16$ nest pairs; 156 nestlings). Circles represent cross-fostered nestlings, and triangles represent non-fostered nestlings.

($p > 0.07$, table 1). The random effect revealed that siblings originated from the same nest showed a similar TL when 15 days old (nest of origin: $p < 0.01$, table 1). The within-habitat cross-fostering (control) did not affect TL, regardless of the rearing habitat ($p > 0.50$, electronic supplementary material, table S1).

4. Discussion

Here we show that being raised in an urban environment shortens the TL of great tit nestlings. Thus, growing up in an urban environment may have consequences for lifespan, as TL is a suggested biomarker of cellular senescence. Even though we can only speculate about the underlying mechanisms, it is likely that both urban pollution and dietary differences play significant roles. Previous studies have reported higher stress-induced corticosterone levels and higher oxidative stress in urban bird populations [15,18], likely as a consequence of increased exposure to anthropogenic pollution. As both these factors have previously been linked to telomere attrition [8,19], we propose that similar mechanisms could be driving the observed differences in TL in our experiment. This can be further exacerbated by dietary differences between habitats. For instance, caterpillars, which are the main food for great tit nestlings, differ in carotenoid content between urban and rural habitats [17,20], and recent experimental studies have shown that dietary antioxidants, such as carotenoids, can influence the maintenance of TL even early in life [21]. Moreover, we found significant differences in body condition—a proxy of rearing conditions—between urban- and rural-reared nestlings (see the electronic supplementary material). This reinforces the idea of poorer developmental and/or environmental conditions in the urban environment.

We found that the nest of origin affected TL, whereas rearing nest *per se* did not. This is in line with previous studies, which have found that TL is to some degree genetically or epigenetically (via maternal effects) inherited [5]. This shows the importance of both genetics and habitat quality (i.e. environmental stress) as determinants of nestling TL. However, according to our results, the differences between

Table 1. Summary of the full and final model for between-habitat cross-fostering investigating the effect of urbanization on telomere length (TL) in nestling great tits. Rearing habitat (urban or rural), habitat of origin (urban or rural) and sex were included as fixed factors, and hatching date and body condition (SMI) were included as covariates in the original model ($n = 16$ nest pairs, 156 nestlings). s.e. standard error, d.f. degrees of freedom.

		full model				final model			
dependent variable	source of variation	estimate (s.e.)	*F*	d.f.	*p*-value	estimate (s.e.)	*F*	d.f.	*p*-value
telomere length (TL)	intercept	1.06 (0.985)				−0.098 (0.041)			
	rearing habitat (urban)	−2.646 (1.285)	4.27	1, 137.44	0.04	−0.10 (0.048)	4.60	1, 150.82	0.033
	habitat of origin (urban)	0.013 (0.085)	0.0003	1, 28.75	0.98				
	sex (female)	−0.082 (0.062)	1.08	1, 131.72	0.29				
	hatching date	−0.012 (0.015)	0.015	1, 31.48	0.90				
	SMI	−0.024 (0.021)	0.068	1, 131.65	0.79				
	rearing habitat × habitat of origin	−0.029 (0.098)	0.089	1, 143.90	0.79				
	rearing habitat × sex	0.066 (0.097)	0.47	1, 136.96	0.49				
	rearing habitat × hatching date	0.029 (0.018)	2.59	1, 133.38	0.10				
	rearing habitat × SMI	0.057 (0.031)	3.398	1, 145.90	0.067				
	random factors					χ^2			
	nest of rearing					0.00			1.00
	nest of origin					8.90			0.003

the urban and rural habitats have a larger impact than the inherited effects.

In conclusion, our results show for the first time, we believe, that even a relatively brief exposure (less than two weeks) to an urban environment during early development can have detrimental effects on TL, with potential carryover effects on lifespan and fitness. The mechanisms behind these effects are still unknown, but diet deficiency and stress-induced glucocorticoid response may be likely drivers of the observed differences in TL between our urban and rural populations. Therefore, further research should be carried out in order to disentangle the underlying mechanisms. Our findings highlight the need to study the physiological mechanisms underlying the impact of urbanization on wildlife in order to better understand present and future threats to populations and the adaptations animals make to counter these.

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. The dataset supporting this article was uploaded to the Dryad digital repository: http://dx.doi.org/10.5061/dryad.4vp41.

Authors' contributions. P.S., A.N., S.B. and C.I. planned and conceived the study, P.S., A.N. and C.I. performed the fieldwork, and P.S. and J.F.N. performed the molecular work. P.S. analysed the data and P.S., J.F.N. and C.I wrote the paper, with help from A.N. and S.B. All authors agree to be held accountable for the content therein and approve the final version of the manuscript.

Competing interests. The authors have no competing or financial interests.

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