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Telomere damage and redox status alterations in free-living passerines exposed to metals

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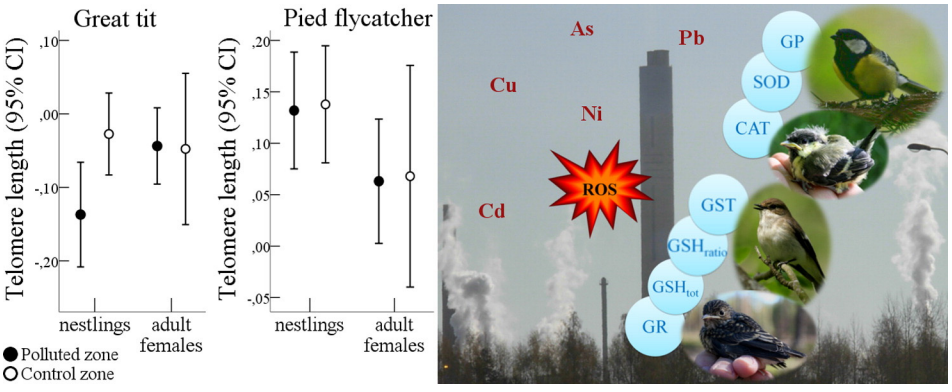
HIGHLIGHTS

- We studied associations between metals, TL and redox status in two bird species.
- We measured several components to get a broad assessment of the redox status.
- Pollution and growth stress were associated with short TL in great tit nestlings.
- The stress effects on biological aging varied depending on the species and age.

GRAPHICAL ABSTRACT



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ABSTRACT

Telomere length may reflect the expected life span and possibly individual quality. Environmental stressors are known to increase oxidative stress and accelerate telomere attrition: however the interactions between redox status and telomere dynamics are not fully understood. We investigated whether exposure to heavy metal pollution is associated with oxidative stress and telomere damage in two insectivorous passerines, the Great tit (*Parus major*) and the Pied flycatcher (*Ficedula hypoleuca*). We were also interested to know whether within-brood competition could influence the nestling redox status or telomere length. Breeding females and nestlings were sampled near the point pollution source and compared to birds in non-polluted control zone. We measured heavy metal concentrations, calcium, metallothioneins, telomere lengths and redox status (oxidative damage, and enzymatic and non-enzymatic antioxidants) in liver samples. Great tit nestlings in the polluted zone had significantly shorter telomeres compared to those in the unpolluted control zone. In addition, those great tit nestlings that were lighter than their average siblings, had shorter telomeres compared to the heavier ones. In pied flycatchers neither pollution nor growth stress were associated with telomere length, but adult females had significantly shorter telomeres compared to the nestlings. All the results related to redox status varied remarkably among the species and the age groups. In both species antioxidants were related to pollution. There were no significant associations between redox status and telomere length. Our results suggest that wild birds at a young age are vulnerable to pollution and growth stress induced telomere damage. Redox status seems to interact with pollution and growth, but more studies are needed to clarify the underlying physiological mechanisms of telomere attrition. Our study highlights that all the observed associations and differences between the sampling zones varied depending on the species, age, and degree of exposure to pollution.

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1. Introduction

Telomeres are repetitive non-coding DNA sequences (TTAGGG) at the end of chromosomes and their main function is to stabilize the genome during mitosis (Blackburn, 2005). Short telomere length (TL) is strongly associated with increased mortality and age-related disease risk (von Zglinicki and Martin-Ruiz, 2005). Individuals with shorter TLs have a shorter life time expectancy than individuals with longer TLs (Haussmann et al., 2005; Pauliny et al., 2006; Salomons et al., 2009; Vera et al., 2012). Several studies show that the TL in different organisms declines with advancing age (e.g. Haussmann et al., 2005; Iwama et al., 1998; Jemielity et al., 2007), and the attrition rate is highest at early age when the growth is more rapid (Heidinger et al., 2012; Salomons et al., 2009). TL could also reflect a more general aspect of individual quality (Pauliny et al., 2006) or ability to cope with stress (Epel et al., 2004).

Chronic stress has been shown to cause telomere attrition and interfere with repair mechanisms (Epel et al., 2004; Kotrschal et al., 2007); this is likely to be caused by oxidative stress (Epel et al., 2004). The sequence of multiple adjacent guanines in the telomeres makes them highly sensitive to oxidative stress and single-strand breaks (Henle et al., 1999; Kawanishi and Oikawa, 2004; Kurz et al., 2004; Sitte et al., 1998). The repair of single-strand breaks is less efficient in telomeric DNA than in non-telomeric sequences (Petersen et al., 1998). Most of the studies have found high variation in TLs between individuals of the same age class. This inter-individual variation is known to be, at least partly, heritable, but it has also been suggested to be due to differential exposure to environmental stressors and oxidative stress (Houben et al., 2008). As reactive oxygen species (ROS) have a negative influence on telomeres, antioxidants (AO) prevent telomere damage (Houben et al., 2008). Antioxidants such as glutathione (GSH) have been reported to increase telomerase (a reverse transcriptase enzyme) activity (Borras et al., 2004) and an increase in antioxidants, such as vitamin C, have been shown to delay telomere shortening and senescence (Furumoto et al., 1998; Kashino et al., 2003). Bize et al. (2008) found that oxidative stress affects the survival of wild birds: alpine swift (*Apus melba*) males with a high resistance to oxidative stress show a higher annual survival rate than less resistant males.

Some metals generate ROS (redox-active metals), and others reduce the AOs (redox-inactive metals) (Koivula and Eeva, 2010). Several heavy metals also have negative effects on DNA (Ercal et al., 2001; Warchalowska-Sliwa et al., 2005). In general, some pollutants seem to cause telomere shortening and others telomere elongation (Li et al., 2012; Zhang et al., 2013). These inconsistent results underline the importance of investigating the underlying mechanisms, such as oxidative stress, telomerase activity and inflammation. Moreover, other stressors such as growth stress, e.g. due to high sibling competition, could disturb antioxidant defence and/or induce telomere attrition. There are few studies on the growth-dependent modulation of aging mechanisms in wild animals. Geiger et al. (2012) have shown in King penguin (*Aptenodytes patagonicus*) chicks a trade-off between fast growth, TL, and oxidative damage and similar results have been reported with free-living (Debes et al., *in press*) and farmed Salmonid (Almroth et al., 2010) and transgenic (growth-manipulated) fish (Pauliny et al., 2015). Furthermore, three recent studies on passerines have shown that experimental manipulation of within-brood competition results in accelerated telomere attrition in subdominant chicks (Nettle et al., 2013; Nettle et al., 2015; Stier et al., 2015). Stier et al. (2015) found that TL erosion was accompanied by increased oxidative damage, however Nettle et al. (2015) did not.

Since most of the studies on how pollution affects TL are laboratory experiments or correlative human studies, their results may not be directly applicable to natural populations. In natural populations, the dose and exposure time might vary and cocktail-effects from different pollutants are probable. To our knowledge, only two previous studies have investigated how pollution affects on TLs in wild birds (Blévin et al., 2016; Sletten et al., 2016), but both studies focused on persistent organic pollutants (POPs). Additionally, the age and the phase of development need to be taken into account when comparing different effects. Wild bird populations provide a good opportunity to study whether environmentally relevant doses of several heavy metal pollutants together with other environmental stressors are associated with the redox status and TL of individuals.

We sampled the metal exposure of Pied flycatchers (*Ficedula hypoleuca*) and Great tits (*Parus major*) near to an Ni/Cu-smelter (Harjavalta, Finland) and also further away in less polluted control zone. Liver samples were collected from breeding females and nestlings. We measured the heavy metal concentrations [arsenic (As), lead (Pb), cadmium (Cd), copper (Cu) and nickel (Ni)], calcium (Ca), telomere lengths (TLs) and multiple biomarkers [superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GP), glutathione reductase (GR), glutathione S-transferase (GST), ratio between reduced and oxidized glutathione (GSH_{ratio}), total amount of glutathione (GSH_{tot}), lipid peroxidation (LP)] to achieve a comprehensive assessment of redox status. In addition we measured the metallothioneins (MT), which are a group of metal-binding proteins that can be used as biomarkers for metal exposure (Barjaktarovic et al., 2002; Elliott and Scheuhammer, 1997; Koivula and Eeva, 2010; Nath et al., 2000; Roesijadi, 1996). MTs can act as a H_2O_2 scavenger (Anderson et al., 1999; Andrews, 2000; Sato and Bremner, 1993), but on the other hand they might also increase ROS concentrations, and therefore it is not fully understood how MTs, ROS and AOs interact with each other (Koivula and Eeva, 2010).

Our intention was to evaluate the associations of heavy metals with TLs and redox status in nestlings and adult birds depending on the species. In addition to pollution, we investigated the potential impacts of within-brood competition and growth stress on nestling redox status and TLs by comparing subdominant and dominant nestlings, as judged by their relative body mass. Our *a priori* hypothesis, based on previous results, was that subdominant nestlings would show TL attrition as a result of growth stress induced within-brood competition.

2. Materials and methods

Data was collected in 2009 in the nest box sites surrounding the Cu/Ni smelter in Harjavalta (61°20′ N, 22°10′ E), SW Finland. Due to the exponentially decreasing metal concentrations with an increasing distance from the factory complex (Kiikkilä, 2003), the study area was divided into a “polluted zone” (5 nest-box sites within 2 km from the smelter) and a “control zone” (5 nest-box sites further than 9 km from the smelter). Females were collected while incubating and one nestling per brood was sampled at the age of 8–11 days old for the pied flycatchers and 8–15 days old (one 8 days old, others 11–15 days old) for the great tits. All the nestlings were measured for their body mass with a spring balance with a precision of 0.1 g. Liver samples from females and nestlings were dissected immediately after decapitation, and preserved frozen in liquid nitrogen in sealed plastic tubes. The data were collected under the license of the Regional Environmental Centre (LOS-2008-L-224-254).

Heavy metal (As, Pb, Cd, Cu, Ni) and calcium (Ca) concentrations (mg/kg, dry mass) in the liver were determined by ICP-MS (Elan 6100 DCR+ from PerkinElmer-Sciex; details reported in (Berglund et al., 2011)). The amount of MTs was measured spectrophotometrically (412 nm) using Ellman's reaction and a calibration curve of reduced glutathione (GSH) was utilized to quantify the MT content (Viarengo et al., 1997).

The total genomic DNA was extracted with the salt extraction method (Aljanabi and Martinez, 1997). Relative TL (the amount of telomere sequence DNA in a sample relative to the amount of a single-copy gene DNA) was measured using a quantitative real-time PCR (qPCR) method (Cawthon, 2002; Criscuolo et al., 2009), which was adapted for our study species. qPCR was performed on a 7900HT fast real-time

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