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Effects of breeding habitat (woodland versus urban) and metal pollution on the egg characteristics of great tits (*Parus major*)

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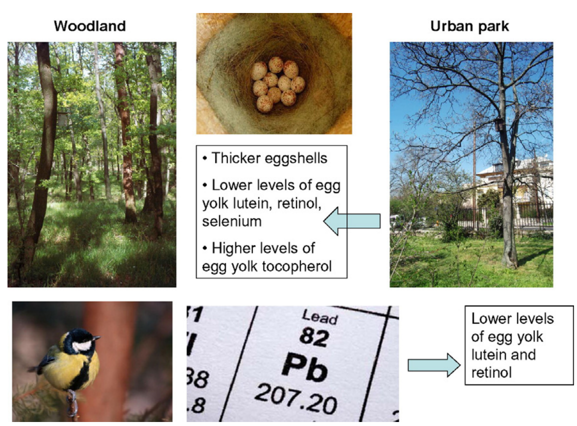
HIGHLIGHTS

- Eggshell spotting and egg size did not differ between the woodland and urban park.
- Eggs with a more aggregated spotting pattern had higher Cu level in the eggshell.
- Eggshells were thinner in the woodland, which could be due to low Ca availability.
- Urban great tits laid eggs with lower yolk lutein, retinol and Se levels.
- Yolk lutein and retinol levels were negatively related to eggshell Pb level.

GRAPHICAL ABSTRACT



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ABSTRACT

In an urban environment, birds are exposed to metals, which may accumulate in their tissues and cause oxidative stress. Female birds may eliminate these pollutants through depositing them into eggs, thus eggs become suitable bioindicators of pollution. In this study, we aimed to analyse whether eggshell spotting pattern, egg volume, eggshell thickness and egg yolk antioxidant (lutein, tocopherol, retinol and selenium) levels were related to the breeding area (woodland versus urban) and the metal levels in the eggshell of a small passerine species, the great tit (*Parus major*). In the urban habitat, soil and eggshells contained higher concentrations of metals, and soil calcium level was also higher than that in the woodland. Eggshell spotting intensity and egg volume did not differ between eggs laid in the woodland and the urban park, and these traits were not related to the metal levels of the eggshell, suggesting that these egg characteristics are not sensitive indicators of metal pollution. A more aggregated eggshell spotting distribution indicated a higher Cu concentration of the eggshell. We found that eggshells were thinner in the less polluted woodland habitat, which is likely due to the limited Ca availability of the woodland area. Great tit eggs laid in the urban environment had lower yolk lutein, retinol and selenium concentrations, however, as a possible compensation for these lower antioxidant levels, urban females deposited more tocopherol into the egg yolk. It appears that females from different breeding habitats may provide similar antioxidant protection for their offspring against oxidative damage by depositing different

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specific dietary antioxidants. Egg yolk lutein and retinol levels showed a negative relationship with lead concentration of the eggshell, which may suggest that lead had a negative impact on the amount of antioxidants available for embryos during development in great tits.

1. Introduction

The impact of urban environment on the physiology of wild birds is poorly investigated. In an urban or industrialized environment, birds are exposed to metals and other anthropogenic pollutants, which may accumulate in their tissues. Soil contamination may represent a significant hazard for birds, as birds may ingest toxicants through food and/or water (Sawicka-Kapusta et al., 1986; Dauwe et al., 2005). Pollution may cause negative effects on their immunocompetence, oxidative status and reproductive performance (Scheuhammer, 1987; Burger, 1995; Eeva and Lehikoinen, 1995; Janssens et al., 2003; Snoeijs et al., 2004, Vermeulen et al., 2015). Metals can be excreted through faeces and gland excreta or by depositing them into feathers (Burger and Gochfeld, 1985; Burger, 1993; Eens et al., 1999). Additionally, female birds may eliminate pollutants through depositing them into the eggs, thus making the eggs suitable bioindicators of pollution (Burger, 1994; Dauwe et al., 1999, 2005; Ruuskanen et al., 2014). The amount of toxic metals in the eggs is supposed to be related to the extent of pollution in the environment and of the female bird (Dauwe et al., 2005; Ayaş, 2007; Ruuskanen et al., 2014).

Metal exposure has been shown to induce oxidative stress in animals, as indicated by the increase in lipid peroxidation and upregulated antioxidant enzyme activities (Mateo and Hoffman, 2001; Berglund et al., 2007; Isaksson et al., 2009; Koivula and Eeva, 2010). Accumulated metals may cause oxidative stress by increasing the production of reactive oxygen species, which may render the antioxidant defence system incapable of neutralizing the elevated amount of free radicals (Ercal et al., 2001; Isaksson, 2010; Koivula and Eeva, 2010). Some metals, such as lead (Pb) and cadmium (Cd), for which no biological function is known, can be highly toxic even at low concentrations, and may cause oxidative stress (Ercal et al., 2001). Other metals, such as copper (Cu) and zinc (Zn), are essentials, but at high concentrations can be toxic and also generate oxidative stress (Ercal et al., 2001).

Birds allocate dietary lipid-soluble antioxidants (carotenoids, tocopherol, retinol) into the egg yolk in order to help protect the vulnerable, lipid-rich tissues of embryos from the damaging effects of free radicals and other reactive oxygen species (Olson, 1993; Woodall et al., 1997; Surai et al., 1999; Surai, 2002). Birds cannot synthesize these compounds, they must obtain them from their diet (Goodwin, 1984), and their environmental availability may vary substantially between habitats and seasons (Hõrak et al., 2002; Hargitai et al., 2006; Isaksson et al., 2008). Furthermore, birds deposit selenium (Se) into their eggs (Pappas et al., 2006; Surai et al., 2006), as this trace element is an essential component of glutathione peroxidase, an antioxidant enzyme, which functions to reduce harmful peroxides that are generated during metabolism (Omaye and Tappel, 1974; Surai, 2000; Papazyan et al., 2006; Pappas et al., 2008). Intensive environmental pollution may affect the ability of birds to deposit dietary antioxidants into their eggs. As a consequence, in a polluted area birds may allocate lower amounts of antioxidants into their eggs, which may jeopardize embryonic development and the survival prospects of the offspring.

Toxic metals may also impair eggshell structure (Grandjean, 1976; Eeva and Lehikoinen, 1995), as they may cause disturbance in the absorption of Ca from the gut, and disturb the mobilization of Ca from bones (Hamilton and Smith, 1978; Scheuhammer, 1991; Graveland, 1998; Dauwe et al., 2005). Eggshell spotting pattern may also be affected by environmental pollution. The brown and black eggshell spots are caused by protoporphyrin pigment (Kennedy and Vevers, 1976; Gorchein et al., 2009), which possesses pro-oxidant properties (Afonso et al., 1999; Pimentel et al., 2013). Metal pollution may alter the synthesis and excretion of protoporphyrin (Bernard and Lauwerys, 1987; Casini et al., 2003; Mateo et al., 2006), elevating erythrocyte protoporphyrin concentration (Marks, 1985; Pain, 1989; Hoffman et al., 2000). In addition, the poor physiological condition and high level of oxidative stress of female birds exposed to pollution may induce them to remove larger amounts of this potentially harmful pro-oxidant from their body (Moreno and Osorno, 2003). Therefore, birds exposed to pollution may lay eggs with higher concentration of protoporphyrin pigment in the eggshell, and thus, eggshell spotting pattern may be used as a non-destructive method for estimation of metal pollution.

In this study, we aimed to analyse whether maternal prenatal reproductive investment of great tits (*Parus major*) differs between a supposedly more polluted and more oxidatively stressful urban environment and a woodland area, and whether it is related to the metal concentration of the eggshell, which could indicate the metal concentration in the breeding area (Burger, 1994; Dauwe et al., 1999, 2005). We predicted that females breeding in an urban environment and those laying eggs with higher metal concentration would deposit lower amounts of antioxidants (carotenoid, tocopherol, retinol and Se) into the egg yolk. In addition, we hypothesised that females would lay smaller eggs with thinner and more spotted eggshells in a more polluted environment.

2. Materials and methods

2.1. Field methods

This study was carried out on a nestbox-breeding population of great tits 1) in a sessile oak dominated woodland in the Pilis Mountains (47° 43′N, 19° 01′E), Hungary, in 2011, and 2) in a 7.5 ha urban park (Arboretum of Buda) in Budapest (47° 28′N, 19° 02′E), Hungary, in 2012. The woodland has clayed brown forest soil on volcanic rock (andesite), and land snails are rare in the area (Hargitai et al., 2013). Nestboxes were situated about 700–1500 m from the nearest road and 1500–3000 m from the nearest village. The urban park has calcareous clay-based soil on sedimentary rock (marl). The soil in the urban park was not limed or manured. In the urban area, nestboxes were approximately 10–70 m from a road, and within the city of Budapest. 15–20 g soil samples were collected from the upper soil layer of the two study areas (woodland: $N = 9$; urban park: $N = 12$) for element concentration analysis and pH measurements. Soil samples were stored at -20 °C until analysis.

In the forest, nestboxes were visited daily to determine the initiation of egg laying and the exact laying order of the eggs. Eggs were numbered with a waterproof marker according to laying order, and first-laid eggs were collected for these analyses ($N = 25$). Collected eggs were replaced by dummy eggs. In the urban park, clutches were weekly observed, therefore, the exact laying order of the eggs was not known. Here, the smallest egg present at the time of observing eggs in the nest was collected ($N = 17$), supposing that it was the first-laid egg (Hargitai et al., 2013). Clutch size at the time of egg collection at the urban site ranged from two to ten eggs. It has been shown that metal concentration does not change with laying order of eggs of great tits (Dauwe et al., 2005), similarly to organohalogenated contaminants (Van den Steen et al., 2006). In rural great tits, lutein concentration declines with laying order (Hõrak et al., 2002; Hargitai et al., 2015), but in urban great tits, no decline was observed between the first and last eggs (Hõrak et al., 2002). Yolk tocopherol concentration also declines with egg laying order, but retinol concentration does not vary significantly with laying order (Hõrak et al., 2002; Hargitai et al., 2015). In addition, eggshell thickness also declines with laying order (Hargitai et al.,