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Exposure to oxychlordane is associated with shorter telomeres in arctic breeding kittiwakes

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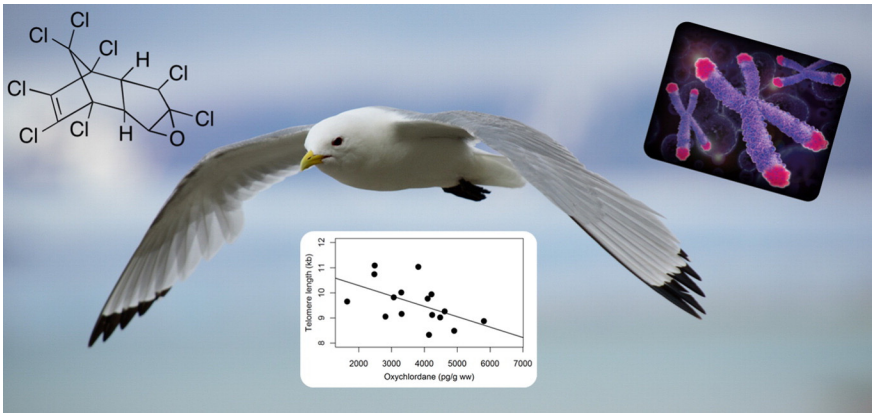
HIGHLIGHTS

- Potential impacts of POPs on telomeres were studied in an arctic seabird.
- No relationship was found between PCBs and telomere length.
- Oxychlordane concentration was associated with shorter telomeres in females.
- This study highlights sex-related sensitivity to banned organochlorine pesticides.

GRAPHICAL ABSTRACT



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ABSTRACT

Telomeres are DNA-protein complexes located at the end of chromosomes, which play an important role in maintaining the genomic integrity. Telomeres shorten at each cell division and previous studies have shown that telomere length is related to health and lifespan and can be affected by a wide range of environmental factors. Among them, some persistent organic pollutants (POPs) have the potential to damage DNA. However, the effect of POPs on telomeres is poorly known for wildlife. Here, we investigated the relationships between some legacy POPs (organochlorine pesticides and polychlorobiphenyls) and telomere length in breeding adult black-legged kittiwakes (*Rissa tridactyla*), an arctic seabird species. Our results show that among legacy POPs, only blood concentration of oxychlordane, the major metabolite of chlordane mixture, is associated with shorter telomere length in females but not in males. This suggests that female kittiwakes could be more sensitive to oxychlordane, potentially explaining the previously reported lower survival rate in most oxychlordane-contaminated kittiwakes from the same population. This study is the first to report a significant and negative

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Organochlorines
PCBs
DNA

relationship between POPs and telomere length in a free-living bird and highlights sex-related susceptibility to banned pesticides.

1. Introduction

Telomeres are DNA-protein complexes located at the end of linear chromosomes which play a critical role in maintaining the genomic integrity (Blackburn, 1991; Monaghan and Haussmann, 2006). The DNA polymerase protein complex cannot replicate the very ends of chromosomes during mitosis, and, consequently telomeres shorten at each cell division (Olovnikov, 1996). When telomeres reach a critical lower threshold, cell division can damage coding DNA leading to apoptosis or replicative senescence (Olovnikov, 1996; Campisi et al., 2001). It was originally thought that telomere loss occurred at a constant rate in individuals through their life, and telomere length could therefore act as an internal 'mitotic clock' to measure the chronological age of organisms into the wild (Haussmann and Vleck, 2002). However, recent studies have shown that telomere length predicts survival (Haussmann et al., 2005; Bize et al., 2009; Salomons et al., 2009; Heidinger et al., 2012; Angelier et al., 2013; Barrett et al., 2013) and is related to a wide range of environmental stressors (Mizutani et al., 2013; Meillère et al., 2015). Consequently, telomere length is considered as more related to biological age than chronological age *per se* (Monaghan and Haussmann, 2006; Barrett et al., 2013).

In humans, telomere erosion can be accelerated by different environmental factors such as exposure to pollutants (Zhang et al., 2013). For instance, it has been reported that outdoor workers exposed to traffic pollution have shorter telomeres than indoor office workers (Hoxha et al., 2009). Similarly, women telomere length decreases as exposure to pollution caused by hazardous wastes increases (De Felice et al., 2012). One underlying mechanism that could potentially explain accelerated telomere shortening is oxidative stress (Von Zglinicki et al., 2000; Zhang et al., 2013). This corresponds to the imbalance between the production of reactive oxygen species (ROS) and the antioxidant capacity of an organism (Finkel and Holbrook, 2000). When metabolic by-products, such as ROS, are not fully neutralized by anti-oxidant defenses, they may oxidize cellular macromolecules such as DNA (Houben et al., 2008). Hence, telomere length may partly reflect oxidative stress history of an individual (Houben et al., 2008). Among contaminants, some persistent organic pollutants (POPs) have the potential to damage DNA by triggering oxidative stress and even decrease survival (Fernie et al., 2005; Isaksson, 2010; Letcher et al., 2010; Erikstad et al., 2013; Costantini et al., 2014; Sletten et al., 2016). However, the effect of POPs on telomere length is poorly known for wildlife. To the best of our knowledge, only one study has addressed this topic in a free-living animal with low contamination levels but failed to find any significant relationships (Sletten et al., 2016).

Due to their high volatility and persistence in time, POPs reach remote areas such as the Arctic (Gabrielsen and Henriksen, 2001). Once deposited in marine ecosystems, living organisms assimilate the POPs *via* food intake. The POP concentrations then increase from the marine environment into the organisms and throughout food webs due to bioaccumulation and biomagnification (Letcher et al., 2010). Seabirds are top predators; consequently, they are particularly exposed to POPs contamination. They therefore appear as highly relevant biological models to investigate the influence of POPs on telomere length. A previous study in a Svalbard population of black-legged kittiwakes *Rissa tridactyla* ("kittiwakes" hereafter) has reported high oxidative stress levels in most POPs contaminated individuals (POPs included polychlorobiphenyls: PCBs and one organochlorine pesticide: OCP; Lindsøe, 2012). Additionally, adult survival rate in the same population of kittiwakes was negatively linked to some OCPs (Goutte et al., 2015). In this study, we investigated the relationships between some legacy POPs (OCPs and PCBs) and telomere length in Svalbard kittiwakes. Because telomere length is classically reduced in response to oxidative stress (Von Zglinicki et al., 2000; Zhang et al., 2013) and often tightly linked to survival (Haussmann et al., 2005; Bize et al., 2009; Salomons et al., 2009; Heidinger et al., 2012; Angelier et al., 2013; Barrett et al., 2013), we predicted that POP levels would be negatively related to telomere length.

2. Materials and methods

2.1. Study area and sampling collection

Fieldwork was carried out in 2012 from July 12th to July 27th in Krykkjefjellet colony of Kongsfjorden, Svalbard (78°54′N, 12°13′E). A total of 38 individuals (22 males and 16 females) were caught on their nest with a noose at the end of a 5 m fishing rod during the chick rearing period. At capture, a 2 mL blood sample was collected from the alar vein using a heparinized syringe and a 25G needle to determine legacy POP levels, telomere length and the sex of individuals. Blood samples were stored at −20 °C until subsequent analyses. The sex of individuals was determined from red blood cells by polymerase chain reaction (PCR) at the Centre d'Etudes Biologiques de Chizé (CEBC) as previously described (Weimerskirch et al., 2005).

2.2. Telomere assay

Telomere length was determined at the CEBC by Southern blot using the TeloTAGGG Telomere Length Assay (Roche, Mannheim, Germany) as previously described and with minor modifications (Foote et al., 2010; Kimura et al., 2010). Telomere length analysis has already been successfully achieved on the same population of Svalbard kittiwakes (Schultner et al., 2014a). Briefly, samples were digested with proteinase K, and DNA was extracted from red blood cells by using the DNeasy blood and tissue kit (Qiagen). DNA quality was checked by gel electrophoresis and optical density spectrophotometry. Preliminary tests have been conducted to determine the optimal amount of DNA to be used and, for each sample, 0.7 μg of DNA was digested with the restriction enzymes *HinfI* and *RsaI* for 16 h at 37 °C. Digested DNA samples were then separated using a pulse-field gel electrophoresis (Bio-Rad) on a 0.8% agarose gel. All samples were run in four gels. Samples were randomly assigned to a gel. Internal controls were run on each gel to measure inter-gel variations. The gels were run at 3.0 V/cm with an initial switch time of 0.5 s to a final switch time of 7 s for 14 h. Following that step, the gel was depurinated and denaturated in an alkaline solution. The gel was then neutralized and DNA was transferred onto a nitrocellulose membrane by Southern blot (Hybond N+, Amersham Life Science, Amersham, UK). The membrane was incubated at 120 °C for 20 min in order to fix the DNA. The DNA was then hybridized with a digoxigenin-labeled probe specific for telomeric sequences and incubated with antidigoxigenin-specific antibody before visualization with a Chemidoc (Bio Rad). Telomere length was then analyzed using ImageJ to extract telomere smear densities. Lane-specific background was subtracted from each density value and telomere length (mean value) was then calculated using a window of 5–30 kb that includes the whole smear (Nussey et al., 2014). Inter-gel CV was 1.40%.

2.3. POPs analyses

POPs were analyzed from whole blood at the Norwegian Institute for Air Research (NILU) in Tromsø, Norway. Only compounds that