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SHORT NOTE

Age-specific variation of resistance to oxidative stress in the greater flamingo (*Phoenicopterus ruber roseus*)

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Abstract Birds exhibit exceptional longevity and are thus regarded as a convenient model to study the intrinsic mechanisms of aging. The oxidative stress theory of aging suggests that individuals age because molecules, cells, tissues, organs, and, ultimately, animals accumulate oxidative damage over time. Accumulation of damage progressively reduces the level of antioxidant defences that are expected to decline with age. To test this theory, we measured the resistance of red blood cells to free radical attack in a captive population of greater flamingo (*Phoenicopterus ruber roseus*) of known age ranging from 0.3 to 45 years. We observed a convex relationship with young adults (12–20 years old) having greater resistance to oxidative stress than immature flamingos (5 months old) and old flamingos (30–45 years old). Our results suggest that the antioxidant detoxifying system must go through a maturation process before being completely functional. It then declines in older adults, supporting the oxidative theory of aging. Oxidative stress could hence play a significant role in shaping the pattern of senescence in a very long-lived bird species.

Keywords Antioxidant defences · Oxidative stress · *Phoenicopterus ruber roseus* · Senescence

Introduction

Aging is the natural process of decline of vital functions during life and affects almost all living beings. This issue is central when studying evolution of life-history traits, because alteration of reproductive function and longevity mostly determines individual fitness (Stearns 1992; Monaghan et al. 2008). The intrinsic complexity responsible for aging processes led to a proliferation of hypotheses (more than 300 "theories" reviewed by Medvedev 1990). Empirical testing of these hypotheses is extremely difficult and hypothetical mechanisms involved in senescence still remain unresolved (Kirkwood 2005), most likely because they are inter-related and/or non-exclusive (Vleck et al. 2006). Among these hypotheses, the oxidative theory of aging (Harman 1956) postulates that reactive oxygen species (ROS) produced during aerobic respiration inflict oxidative damage on cell constituents; cumulative damage is thus considered the main proximate cause of aging (Harman 1956). Resistance to oxidative stress occurs through the mobilization of antioxidant enzymes and antioxidant molecules, for example carotenoids or vitamins C and E (Beckman and Ames 1998). Oxidative damage occurs when production of ROS exceeds antioxidant defence and damages the repair system itself. Accumulation of damage progressively reduces resistance to oxidative stress and leads gradually to functional alterations, pathophysiology of aging, and, finally, death.

Although some studies have not found evidence of a relationship between antioxidant levels and longevity (Andziak et al. 2005; Andziak et al. 2006), the oxidative

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theory of aging has received much theoretical (Finkel and Holbrook 2000; Kregel and Zhang 2007) and empirical (Harmann and Mattick 1976; Ku et al. 1993; Kapahi et al. 1999; Brunet-Rossinni 2004; Bize et al. 2008) support. Moreover, at the intra-specific level, several studies showed a decline in antioxidant defence with advancing age, either by tracking single antioxidant components (reviewed by Beckman and Ames 1998; Hagen 2003) or by using global measures of resistance to oxidative stress (Alonso-Alvarez et al. 2006; reviewed by Costantini 2008).

Resistance to oxidative stress during the juvenile phase has been poorly investigated and few studies have approached this topic. Blount et al. (2003) showed that juvenile zebra finches (*Taenopygia guttata*) have less non-enzymatic antioxidants than adults. Similarly, Kim et al. (1996) showed that transfer proteins carrying antioxidant vitamins rapidly increase during the first weeks of life in young rat liver. They suggested that this increase might result from inadequate maturation of the liver and, therefore, in a general enhancement of antioxidant capacity from birth to maturity.

Many birds exhibit exceptional longevity (Ricklefs and Scheuerlein 2001) and are thus regarded as convenient models to study the intrinsic mechanisms of aging (Holmes et al. 2001; Holmes and Ottinger 2003). Indeed, mechanisms of somatic maintenance are expected to evolve preferentially in species in which extrinsic factors of mortality (adult predation, extreme fluctuations of food, epidemics, etc.) are low (Kirkwood 2005). The annual survival rate of the greater flamingo (*Phoenicopterus ruber roseus*) in the wild has been estimated at 93.3 and 97.3% (Cézilly et al. 1996; Lebreton et al. 1992). As such this species is regarded as an extremely long-lived species. For instance, Basel Zoo received a greater flamingo as an adult, which then survived another 69 years (personal communication A. S.-T.). The well-surveyed population of greater flamingos of Basel Zoo provided a unique opportunity to test the effect of age on the ability to resist oxidative stress on an extensive range of individuals of known age. Moreover, this captive population lives in controlled conditions that enable minimization of inter-individual variation as a result of environmental effects (e.g. food, sanitary status).

In this study we compared oxidative stress resistance levels among greater flamingos of known age. The oxidative theory of aging predicts that young adult flamingos should have higher resistance to oxidative stress than aged adults. Moreover, immature individuals should have lower resistance to oxidative stress than young adults. Overall, antioxidant defences should follow a convex profile over individual age, increasing in the first stages of life, reaching a maximum in adulthood and finally decreasing in older individuals.

Methods

The greater flamingo population of Basel Zoo, Switzerland, is composed of 20–30 juveniles less than one year old and approximately 100 adults, some of known age ranging from 1–2 up to 65 years. Birds stay outside during the year and can stay in a resting room during the colder nights of winter. Food is composed of a standard special zoo-made mixture for flamingos and is provided ad libitum. Because flamingos are extremely stress–sensitive, sample size was reduced and manipulations were not repeated. Fifteen three-month-old juveniles, and six males and nine females ranging from 12 to 45 years old were sampled. Birds were caught early in the morning and blood was sampled from the jugular vein by the veterinarian of the zoo. Samples of heparinised whole blood were stored at 4°C before analysis, which occurred within 24 h of blood collection. Resistance to oxidative stress was estimated using the KRL diagnostic test derived from human medicine and adapted to bird physiology (temperature and osmolarity). The principle of the test is to submit a solution of whole blood to a thermo-controlled oxidative attack in which all antioxidants present in blood interact to slow down red blood cell haemolysis. The time needed to haemolyse 50% of red blood cells is used to assess resistance to oxidative stress. The procedure is described in detail in Alonso-Alvarez et al. (2006). Statistical analyses were performed by use of the statistical software JMP 6.0.0.

Results

Resistance to oxidative stress and age were linked by a convex relationship (age: $t = 2.26$, $df = 28$, $p = 0.032$, slope ± SE = 0.71 ± 0.31; squared age: $t = -2.34$, $df = 26$, $p = 0.027$, slope ± SE = − 0.02 ± 0.01; Fig. 1). In this quadratic model, resistance to oxidative stress increased between 0 and 15 years old, reached an asymptote between 16 and 25 years old, and finally slightly decreased in older age. Among adult flamingos, older individuals tended to have lower levels of antioxidant defence than their younger counterparts; no difference was observed between males and females (ANCOVA: age $F_{1,12} = 4.05$, $p = 0.067$; sexes $F_{1,12} = 0.32$, $p = 0.58$).

Discussion

In this study we investigated the relationship between age and resistance to oxidative stress in a population of greater flamingos of known age. We found a convex relationship between resistance and oxidative stress and age, with a peak at intermediate age.

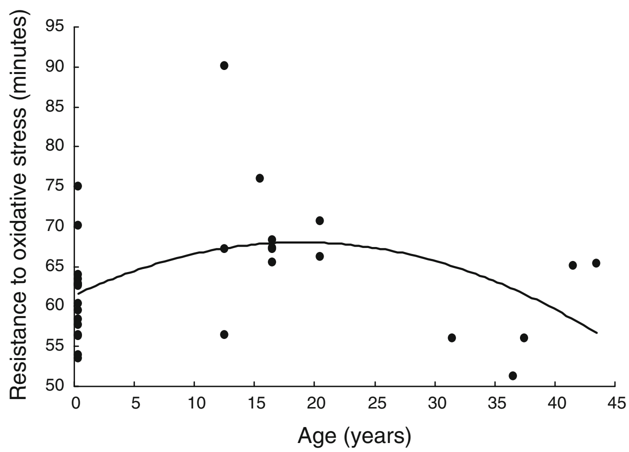

Fig. 1 Age-related variation in resistance to oxidative stress in the great flamingos of Basel Zoo, Switzerland. Resistance to oxidative stress is assessed as the time needed to haemolyse 50% of the red blood cells exposed to a controlled attack by reactive oxygen species

Variation of resistance to oxidative stress may be affected by a variety of factors, for example reproductive effort and environment (e.g. stress, diet). Indeed, zebra finches showed a decrease in resistance to oxidative stress linked to the intensity of reproductive effort (Wiersma et al. 2004; Alonso-Alvarez et al. 2004, 2006) and females of the alpine swift (*Apus melba*) with higher resistance to oxidative stress laid larger clutches (Bize et al. 2008). Our results suggest that aging is linked to a decrease in antioxidant defence and give some support to the oxidative stress theory of aging. A similar pattern has been described in zebra finches (Alonso-Alvarez et al. 2006), a short-lived species living up to five years only (Zann 1996).

Considering both adults and juveniles, we found that resistance to oxidative stress increases from juveniles to young adults and subsequently decreases in older age. Low levels of specific antioxidants have already been observed in juvenile zebra finches and rats (Blount et al. 2003; Kim et al. 1996). As the immune system is not fully developed at birth (Apanius 1998; Haussmann et al. 2005), we can hypothesize that the antioxidant detoxifying system needs to mature during the juvenile phase of growth. However, the management constraints of this flamingo population induce a gap in our sample (from the immature stage to the adult stage) and this hypothesis should be interpreted with caution. Surprisingly, antioxidant defences in early age have been rarely studied while that stage appears determinant in the variation observed in adult antioxidant defences (Blount et al. 2003; Alonso-Alvarez et al. 2006). Therefore, it would be interesting to study the evolution of resistance to oxidative stress from the early stages of life until adulthood at regular time intervals to more precisely describe this temporal pattern and to empirically confirm the hypothesis of the maturation of antioxidant detoxifying system.

To summarize, our results suggest that the antioxidant defence system first needs to mature to be completely functional, and then declines in older adults, supporting the oxidative stress theory of aging. In an evolutionary and ecological perspective, birds are a very appropriate model for testing aging theories (Kirkwood and Austad 2000). Indeed flight is supposed to reduce extrinsic mortality by reducing the risk of predation, starvation, or diseases, which is a prerequisite to selecting mechanisms which slow intrinsic mortality (Holmes and Ottinger 2003; Kirkwood and Austad 2000). Captive populations of birds are ideal for understanding intrinsic mechanisms of aging in controlled conditions (Scheuerlein and Ricklefs 2006). Thus, ornithology can offer the rare chance of combining zoo experiments with complementary field work on long-term data sets in natural populations and along the way answering the topical question of senescence in the wild.

Zusammenfassung

Altersabhängige variation von resistenz gegenüber oxidativem stress beim rosaflamingo (*Phoenicopterus ruber roseus*)

Vögel können sehr alt werden und stellen deshalb ein geeignetes Model dar, die wesentlichen Mechanismen des Alterns zu untersuchen. Die oxidative Stress Theorie des Alterns suggeriert, dass Individuen altern, da Moleküle, Zellen, Gewebe, Organe und letztlich der gesamte Organismus oxidative Schäden über die Zeit ansammelt. Die Ansammlung dieser Schäden reduziert zunehmend den Level des antioxidativen Schutzmechanismus, welcher erwartungsgemäss mit dem Alter abzunehmen scheint. Um diese Theorie zu untersuchen, wurde die Widerstandskraft roter Blutkörperchen gegen den Angriff freier Radikale im Blut einer Gruppe von Rosaflamingos (*Phoenicopterus ruber roseus*) gemessen. Die Tiere stammen aus dem Zoo Basel und ihr Alter lag zwischen 3 Monaten und 45 Jahren. Ein Anstieg des Widerstandes gegenüber oxidativem Stress konnte bei den Jungvögeln beobachtet werden, wohingegen ältere Flamingos eine geringere Resistenz aufwiesen als jüngere. Unsere Ergebnisse legen die Vermutung nahe, dass das antioxidative Entgiftungssystem eine gewisse Zeit zum Reifen benötigt, um vollumfänglich funktionieren zu können. Im Alter nimmt es wieder ab. Diese Daten unterstützen somit die oxidative Theorie des Alterns.

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