

Avian senescence: underlying mechanisms

Carol M. Vleck · Mark F. Haussmann ·
David Vleck

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Abstract Candidate mechanisms for physiological aging include free radical production and resulting oxidative damage, progressive erosion of telomeres and cellular senescence, age-dependent trade-offs in hormone signaling pathways, and immunosenescence, leading to an increased risk of infection, autoimmune disease, and cancer. These mechanisms are inter-related, not mutually exclusive, and probably all contribute to the aging phenotype. To date, most studies on mechanisms of aging are based on cell culture or lab animals, but interest in comparative studies is growing rapidly. Compared to mammals, birds have long life spans for their body sizes. Birds also appear to have lower rates of free radical production and oxidative damage than mammals, despite higher levels of oxidative metabolism. High levels of the antioxidant, uric acid, in birds may help protect against oxidative damage. Cultured bird cells are more resistant to oxidative damage than mammal cells, and membrane phospholipids of birds are less susceptible to peroxidation than those of mammals of the same size, but show a similar susceptibility as those of mammals with the same life span. In birds, telomeres shorten with age, and the rate of shortening is proportional to life span. Telomerase

has a higher activity in long-lived than in short-lived species. Within a species, short telomeres correlate with reduced survival. Birds have higher plasma glucose than mammals, but lower levels of protein glycation, which contributes to aging damage. Immunosenescence is linked to both oxidative damage and telomere shortening. Patterns of cellular and humoral immunosenescence differ among species in birds. The rate of decline in cell-mediated immune function is inversely correlated with life span. Comparative studies on mechanisms underlying senescence in birds will continue to provide us with valuable information on how aging mechanisms have evolved.

Keywords Aging · Antioxidants · Immunosenescence · Oxidative damage · Telomeres

Introduction

Senescence refers to the progressive deterioration of structure and function over time. It manifests as an age-specific increase in mortality and/or decrease in fertility. There has been some debate about whether free-living birds show senescence, and birds have been characterized as the only large group of organisms having a Type II survivorship curve in which the probability of adult survival is more or less independent of age (Campbell and Reece 2002). That view is changing, however, as long-term field studies have now documented age-dependent declines in both the prospects of survival and breeding productivity in free-living bird populations (Newton and Rothery 1997; Møller and De Lope 1999; Robertson and Rendell 2001; Tavecchia et al. 2001; Saino et al. 2002).

Understanding senescence demands both insight into its evolutionary basis and the identification of its molecular

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C. M. Vleck (✉) · D. Vleck
Department of Ecology, Evolution and Organismal Biology,
Iowa State University, Ames, IA 50011-1020, USA
e-mail: cvleck@iastate.edu

D. Vleck
e-mail: dvleck@iastate.edu

M. F. Haussmann
Department of Biology, Kenyon College,
Gambier, OH 43022, USA
e-mail: haussmannm@kenyon.edu

and physiological mechanisms. Here we focus on the latter and review current knowledge of the physiology and biochemistry of aging in birds. Questions of interest include, which systems, cells, and molecules within an organism show the marks of senescence, how variation in these characters affects fitness-related variables like survival and reproduction both within and between species, and what mechanisms account for differences in life span. Most of our understanding of biochemical and physiological mechanisms that result in or defend against aging is based on cell culture or studies of laboratory model organisms. Such studies have identified an array of genes that affect life span and aging (Guarente and Kenyon 2000; Nyström and Osiewacz 2004). As yet, however, we know little about the relevance of many of these laboratory results to variations in life span in nature (Miller et al. 2002). Selection for rapid growth and early reproduction under laboratory conditions is likely to have been accompanied by the accumulation of genes causing early aging that are easily identified in laboratory screens but of little importance in natural populations. Similarly, the interpretation of cell culture data can be difficult to relate to in vivo conditions (Patil et al. 2005). To understand the mechanisms responsible for the evolution of aging, insights from these laboratory models must be evaluated in free-living populations. Birds constitute an attractive system for comparative studies because they are relatively long-lived (Holmes and Austad 1995) and because a large amount of demographic and population data are available for free-living birds that differ greatly in life span.

Among (but not within) species, life span varies with body mass; larger organisms generally live longer than smaller organisms. Metabolic rate also scales with body size, such that mass-specific metabolic rates and cellular rates of O₂ consumption increase with decreasing size (Calder 1984). This observation has led to the “rate of living” hypothesis, which suggests that smaller animals “live fast and die young” (Pearl 1928; Sohal and Weindruch 1996; Speakman et al. 2002). This pattern, which was developed from comparative biology studies, is also consistent with the idea that a principal mechanism of senescence is the accumulation of oxidative damage. Within many species, manipulations to increase daily energy expenditure generally decrease survival and vice versa (reviewed in Speakman et al. 2002). For example, birds that delay reproduction produce fewer offspring, but live longer (Alonso-Alvarez et al. 2006). This rate of living hypothesis is, however, clearly incomplete. Birds have higher metabolic rates than mammals of comparable size, yet they also live longer (Speakman 2005b). Relative to their maximum life span, the basal metabolic rates of birds are more than threefold higher than those of mammals (Fig. 1). Analysis of how birds manage to achieve low

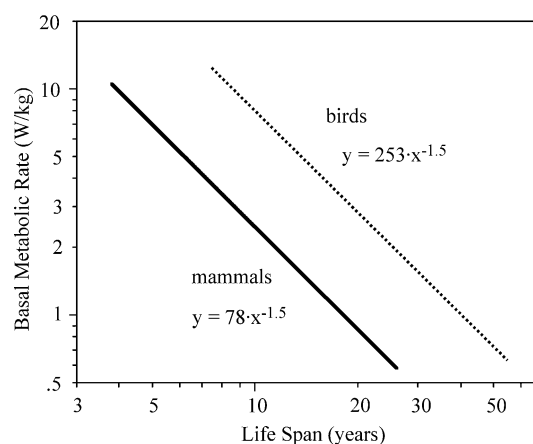


Fig. 1 Basal metabolic rate and life span in birds (*dashed line*) and mammals (*solid line*) calculated from equations relating metabolic rates (McKechnie and Wolf 2004; White and Seymour 2005) and life spans (Speakman 2005b) to body mass. For birds, we converted rates of oxygen consumption to power, assuming 19.64 J/ml O₂

rates of senescence and long life spans compared to mammals with the same “rate of living” may direct our attention to effective targets for intervention to slow senescence.

Candidate mechanisms for physiological aging with broad experimental support include oxidative damage to macromolecules, progressive erosion of telomeres and cellular replicative senescence, age-dependent trade-offs in signaling pathways involving insulin and insulin-like growth factors, and immunosenescence with increasing risk of infection, autoimmune disease, and cancer. These mechanisms are inter-related—not mutually exclusive—and probably all contribute to the aging phenotype. We will examine each of these, drawing selectively from work on birds and other taxa to identify current insight and future opportunities to advance our understanding of aging using birds as a model system.

Mechanisms of aging: oxidative damage

Harman’s (1956) hypothesis that cumulative oxidative damage leads to the progressive loss of physiological function over time is the leading candidate mechanism for aging (Beckman and Ames 1998; Grune and Davies 2001). The production of reactive oxygen species (ROS) is a routine consequence of cellular metabolism; consequently, oxidative damage can account for the ubiquity of aging. ROS molecules are produced primarily at the membranes of the mitochondria in association with the electron transport chain of cellular respiration and include the superoxide radical, hydroxyl radical, and hydrogen peroxide. The latter is often a focus of study because it is relatively stable and

can diffuse out of the cell. These molecules are all highly reactive and can generate substantial damage to macromolecules. The production of ROS is also associated with the immune system, where they play a role in defense against toxins and invading microorganisms, and in cellular apoptosis; thus, a certain level of ROS production is necessary for homeostasis (Finkel and Holbrook 2000). The production of ROS is tempered by a variety of antioxidant molecules that scavenge ROS, including enzymatic antioxidants (e.g., superoxide dismutase, catalase, and glutathione peroxidases), endogenous non-enzymatic antioxidants (e.g., glutathione, ascorbate, uric acid) and diet-derived molecules such as vitamin E and carotenoids. The balance between ROS production and activity of the antioxidant systems determines the level of ROS in the cells and the rate of damage to macromolecules, including DNA, proteins, carbohydrates, and lipids. The extent of damage at any point in time is also a function of the susceptibility of different macromolecules to damage based on their chemistry and on a battery of repair processes, particularly excision repair of damaged DNA (Lombard et al. 2005). A review of methods for analyzing oxidative stress can be found in Han et al. (2000).

ROS production

Mitochondria are thought to be the most important source of ROS in animal cells, although much remains to be learned about the mechanisms and control of ROS generation (Balaban et al. 2005). In limited comparative studies, animal species with longer life spans appear to have lower rates of ROS production (Sohal et al. 1989; Sohal et al. 1990; Barja et al. 1994; Perez-Campo et al. 1998; Barja and Herrero 2000). In mammals, metabolic rate and rate of mitochondrial ROS production in the heart, kidney, and liver are inversely related to maximum life span (Sohal and Weindruch 1996). Such observations suggest a biochemical link between the concept of rate of living and oxidative stress, although as Speakman (2005a) has pointed out, such correlations may reflect covariation of both traits with body mass rather than any functional relationship. Differences in rates of ROS production have been proposed to account for the comparatively long life spans of birds compared to mammals. The estimated production of hydrogen peroxide per unit of oxygen consumed in pigeons (*Columba livia*) is lower than that in similar-sized rats (*Rattus norvegicus*) (Ku and Sohal 1993; Barja et al. 1994). Relatively low mitochondrial ROS production was subsequently confirmed in several pigeon tissues and in canaries (*Serinus canarius*) and budgerigars (*Melopsittacus undulates*) (Herrero and Barja 1997; Barja 1998). The differences between species in terms of mitochondrial ROS production are, however, generally smaller than the differences in life

span (Barja 2004c), and St-Pierre et al. (2002) suggested that technique artifacts may have exaggerated the magnitude of reported differences between birds and mammals. If birds do have lower rates of ROS production than mammals, uncoupling proteins, which are molecules that can prevent ROS accumulation in mitochondria (Criscuolo et al. 2005a), are one candidate mechanism. Uncoupling proteins are present in birds and can modulate ROS production (Criscuolo et al. 2005b).

Understanding the role of ROS production in aging is challenging as much of this research must be carried out with cell cultures, isolated mitochondria, or cell constructs such as yeast expressing avian proteins (see Criscuolo et al. 2005b), which makes the interpretation of its importance to aging in free-living birds difficult at best. It seems likely that birds have relatively lower ROS production per unit of oxygen consumed than mammals, but the magnitude of that reduction, its root cause, and its overall contribution to determining rates of senescence are still active areas of research. Birds remain an attractive model for understanding the role of ROS production in senescence because life spans (and presumably rates of senescence) vary tremendously among species, providing the raw material for critical comparative studies.

Antioxidants

Elevated antioxidant levels have been correlated with increases in life span both within and among species (Cutler 1991; Arking 1995; Packer 1995; Sohal et al. 2002; Serra et al. 2003), although the evidence is not always compelling (Finkel and Holbrook 2000; Sohal et al. 2002; Barja 2004b) and there are clear exceptions (Lopez-Torres et al. 1993; Andziak et al. 2005). Antioxidant supplements usually do not increase longevity, and are even harmful in some cases, but they may protect against some pathologies and early death (Barja 2004a). Ku and Sohal (1993) compared superoxide dismutase and glutathione peroxidase activity between similarly sized pigeons and rats and found higher levels of these enzymatic antioxidants in pigeons for most tissues. In a larger comparative study (Perez-Campo et al. 1998), levels of glutathione peroxidase and reductase were lower in long-lived pigeons and canaries than in shorter-lived guinea pigs, rats, trout, and frogs, which is contrary to the expected result if enzymatic antioxidants are a major contributor to the regulation of life span.

In humans, total antioxidant capacity and superoxide dismutase decline with age (Mecocci et al. 2000; Rao et al. 2003). We recently measured total antioxidant capacity in zebra finch (*Taeniopygia guttata*) plasma using a modified oxygen radical absorbing capacity procedure (Huang et al. 2002; Prior et al. 2003). We found no change in plasma antioxidant capacity in birds ranging in age from 0 to

7 years ($F = 0.74$, $n = 32$, $p = 0.40$) (Conrad and Vleck, unpublished data). Such maintenance of antioxidant capacity, if characteristic of birds in general, could reduce oxidative damage and decrease rates of senescence. Red blood cell resistance to oxidative stress, however, did decline in zebra finches between 200 and 400 days of age (Alonso-Alvarez et al. 2006).

The ubiquity of senescence suggests that mechanisms that reduce the accrual of age-related damage and increase life span are expensive, so we expect to see trade-offs between the investment in such self-maintenance and the investment in current reproduction. In zebra finches whose reproductive effort was increased by brood manipulation, the effective levels of superoxide dismutase and glutathione peroxidase decreased, either because enzyme activity decreased (males) or because estimated daily energy expenditure and presumably ROS production increased (females) (Wiersma et al. 2004). In male zebra finches (but not females), resistance of the red blood cells to free radical attack and short-term survival declined with increased reproductive effort, although individual life span was not correlated with resistance (Alonso-Alvarez et al. 2006). Reduced protection against oxidative damage when reproductive effort increases and compromised survival when oxidative protection is reduced suggest that in birds, oxidative protection may be an important variable in the evolution of life span and reproductive schedules.

Carotenoids are antioxidants that have been the focus of considerable research because their dietary origin (for vertebrate animals) facilitates manipulation and because they serve multiple functions. In many bird species, carotenoids are important for plumage, beak color, and other sexual ornaments (von Schantz et al. 1999; Blount et al. 2003a; McGraw et al. 2004). Carotenoids in egg yolk affect embryonic physiology (Royle et al. 2001) and in adults, they affect immunocompetence (McGraw and Ardia 2003). Nutritional state early in life has long-term effects on carotenoid levels (Blount et al. 2003b; Alonso-Alvarez et al. 2004), so there are multiple reasons to suspect that trade-offs in carotenoid allocation could affect survival. However, carotenoid supplementation does not increase resistance to oxidative stress in nestling kestrels (*Falco tinnunculus*) (Costantini et al. 2007) or in greenfinches (*Carduelis chloris*) (Horak et al. 2006). Barja (2004a) suggested that dietary compounds are unlikely to be determinants of endogenous aging and species-specific life spans, but this suggestion may not hold for dietary factors involved in life-history tradeoffs, such as carotenoids.

In most birds, the major nitrogen excretory product is uric acid (Braun and Dantzler 1984), and plasma uric acid levels are routinely in the 0.2–0.5 mM range (see Vleck and Vleck 2002). In humans, chronic levels of uric acid greater than about 0.4 mM cause gout (Pittman and Bross

1999), and values in most mammals (<0.01 mM) are much lower than in birds (Johnson and Rideout 2004). Uric acid is a potent scavenger of oxidants, and it has been proposed that uricotelism in birds could contribute to low ROS levels (Klandorf et al. 1999). Diet manipulation studies in chickens have shown that plasma uric acid concentration is inversely correlated with cellular oxidation activity (Simoyi et al. 2002), leading the authors to suggest that uric acid could have an important anti-aging role in birds. Similarly, the approximately threefold higher level of uric acid in humans compared to other primates may account for their longer life span (Short et al. 1997). The role of uric acid as an antioxidant system accounting for greater life spans in birds compared to mammals deserves further investigation.

Cumulative damage to macromolecules

Oxidative damage to lipids and proteins increases with age in many animals (Stadtman 1992; Cini and Moretti 1995; Dubey et al. 1996; Yan et al. 1997; Pamplona et al. 1999; Tahara et al. 2001), although we are aware of no published data for birds. Oxidative damage to DNA as a function of age has attracted more attention because of its central role in determining the structure of other macromolecules. DNA damage can be estimated by measuring the oxidation product, 8-oxo-deoxyguanosine (8-oxo-dG) (Fraga et al. 1990; Hamilton et al. 2001a, b). Such oxidative damage to both nuclear and mitochondrial DNA increases with age in several mammals (Sohal and Weindruch 1996; Stedman and Levine 2000; Hamilton et al. 2001b). Oxidative damage to heart and brain is inversely related to maximum life span in six species of mammals (Barja and Herrero 2000), although this relation disappears when the effects of body mass on life span are included in the analysis (Speakman 2005a). Herrero and Barja (1999) reported that 8-oxo-dG levels are generally lower in the mitochondrial DNA of the heart and brain of birds than in those of mammals, but in these studies they did not account for age-related changes. We recently found that the level of 8-oxo-dG increases significantly with age in zebra finch skeletal muscle ($p = 0.002$) and brain ($p = 0.01$), but not in the liver ($p = 0.54$), which includes actively dividing cells (Liu 2004). These tissue differences presumably reflect the failure of cells with damaged DNA to replicate and the accumulation of damage in post-mitotic tissues such as muscle and brain. Surprisingly, in free-living birds of three other species, we did not find an increase in oxidative damage to muscle tissue with age (Liu 2004). In tree swallows (*Tachycineta bicolor*), common terns (*Sterna hirundo*), and Leach's storm-petrels (*Oceanodroma leucorhoa*), which were sampled at about 60% of their maximum life span, the levels of 8-oxo-dG were the same or

even lower than those in nestlings, although the sample sizes were small. The difference between captive (zebra finch) and free-living (swallows, terns, and storm-petrels) populations could indicate that in the wild, only those individuals with the lowest exposure to damage or greatest capacity to resist oxidative stress live to old age. Testing this hypothesis would require a longitudinal study—not a cross-sectional study of age-related changes—because the pattern of change in age-dependent traits can differ within- and between-individuals (van de Pol and Verhulst 2006).

Susceptibility to damage

Birds have been reported to have superior defenses against cellular damage by ROS. Cultures of avian kidney cells and embryonic fibroblasts of birds survive exposure to ROS and hyperoxic conditions better than mammalian cells (Ogburn et al. 1998; Ogburn et al. 2001). Within birds, budgerigars with a maximum life span of about 20 years show greater cellular resistance to oxidative damage than Japanese quail (*coturnix coturnix japonica*) with a maximum life span of only about 5 years (Ogburn et al. 2001). The resistance of fibroblasts to a variety of stressors is positively correlated with maximum life span in eight species of mammals (Kapahi et al. 1999).

One trait that affects susceptibility to oxidative damage is the level of saturation of membrane phospholipids. Susceptibility to lipid peroxidation (the peroxidizability index) (Holman 1954) increases with the polyunsaturation of membrane phospholipids and is associated with elevated oxidative damage to lipids, proteins, and DNA (Pamplona et al. 2004). Animals with high cellular metabolism tend to have polyunsaturated phospholipid membranes; functionally, this accelerates many processes catalyzed by membrane proteins because of the greater fluidity of such membranes (Hulbert 2005). The degree of unsaturation tends to be greater in small species than in large species in both birds and mammals, although the unsaturation is generally lower in birds than in mammals of the same body mass (Hulbert et al. 2002). Oxidation of membrane phospholipids is particularly insidious because lipid peroxidation is an autocatalytic chain reaction, causing further oxidative damage. Thus, adjustments in membrane lipid chemistry could mediate the rates of oxidative damage and contribute to variations in life span. Pamplona et al. (1999) reported that the peroxidizability index was lower in pigeons than in rats, as were the products of lipid peroxidation. The peroxidizability index is negatively correlated with life span in mammals and birds (Pamplona et al. 1998, 2000; Hulbert et al. 2002). Remarkably, the relationship between peroxidizability index and life span appears to be identical for both mammals and birds (Hulbert 2003, 2005), even though the metabolic rate for a given life span

is much higher in birds than in mammals (see Fig. 1). The identical scaling of the peroxidizability index and life span in birds and mammals suggests that we may be able to account for much of the variation in maximum life span among taxa based on membrane lipid composition.

Repair mechanisms

Numerous mechanisms to repair nuclear DNA have evolved, reflecting the selective importance of the accurate maintenance of DNA to cell and organismal function. The efficiency of DNA repair mechanisms within an individual may decline over time and contribute to a progressive increase in oxidative damage (see review in Lombard et al. 2005), although this has not been examined in birds. As yet, comparative data on DNA repair capability are not available, but data from mouse mutants (Nijnik et al. 2007) demonstrate that variation in repair affects the decline in hematopoietic stem cells and, thus, one symptom of aging and immunosenescence.

Genome size and structure may also affect senescence because it affects mechanisms for the repair of double strand-breaks in DNA. Non-homologous DNA end-joining is an efficient—but error-prone—repair mechanism that should be favored if genome size (and thus the frequency of double-stranded breaks) is large or if DNA contains a substantial fraction of repetitive DNA. Homologous recombination is a less efficient—but a more precise—mechanism for repair. The avian genome is, on average, two- to three-fold smaller than other multicellular eukaryotes because of a low fraction of repetitive DNA (Burt 2002). If bird cells favor homologous recombination over non-homologous DNA end-joining to repair double-stranded DNA breaks (see Takata et al. 1998), then this could contribute to reduced damage to chromosomes after oxidative challenge and also to birds' relatively long life spans (Lieber and Karanjawala 2004). Interestingly, despite the generally small genome sizes in birds, there appears to be an inverse relationship between life span and genome size within avian families (Monaghan and Metcalfe 2000; but see Ricklefs and Scheuerlein 2001).

Caloric restriction

Within a species, it is well known that caloric restriction can increase longevity (Masoro 2003). Restricting caloric intake by 20–40% while maintaining essential nutrients appears to slow the intrinsic rate of aging and onset of age-associated diseases by attenuating oxidative damage and/or altering the insulin/insulin-like growth factor systems (see below) (Masoro 2005). In domestic poultry, caloric restriction is routinely used to delay maturation and extend the reproductive life span, and similar results can be

produced in Japanese quail (Ottinger et al. 2005). Such a response, however, is probably best viewed as an adaptive mechanism to prolong life in short-term stressful situations by diverting energy from reproduction to self-maintenance (Kirkwood 2002). Caloric restriction per se is unlikely to underlie mechanisms accounting for differences in the rates of senescence among species since reduction in reproductive value of an individual would inherently be selected against.

Mechanisms of aging: telomeres and cellular senescence

Telomeres are protective structures at the ends of eukaryotic chromosomes, whose sequence (TTAGGG)_n is highly conserved among vertebrates (Meyne et al. 1989). Telomeres function during cell replication and in maintaining genomic stability and normal physiology (McClintock 1941; Watson 1972; Prowse and Greider 1995). They shorten when cells divide, in part because DNA polymerase is unable to completely replicate the 3' end of linear DNA, leaving a single-stranded overhang (Sedivy 1998). Thus, aging is normally accompanied by telomere shortening (reviewed in Haussmann et al. 2003). Oxidative stress, which preferentially causes breaks in the G-rich, single-strand overhang, also contributes to telomere shortening (von Zglinicki 2000; von Zglinicki et al. 2000). In cell culture, when telomeres shorten to a critical length, cells enter a terminal non-dividing state known as replicative senescence (Hornsby 2003). Whether or not replicative senescence is a causal agent of aging in vivo is controversial, because cells in culture often suffer abnormal stress, and most cells in vivo probably do not divide often enough to erode telomeres significantly (Rubin 2002; Wright and Shay 2002; Campisi 2003; Patil et al. 2005). Although telomeres are not strict “aging” clocks (Harley 1991), substantial evidence suggests that telomeres are important to the aging phenotype (Campisi 2003; Patil et al. 2005). Senescent cells in vivo secrete degradative enzymes and inflammatory cytokines that disrupt nearby cells, contributing to aging and the threat of cancer (Campisi 2005). Telomere erosion in some cells (perhaps most importantly in the immune system) is thought to eventually limit their replicative capacity (Weng et al. 1995; Akbar et al. 2004; Reed et al. 2004). Furthermore, telomere erosion, which occurs more rapidly in the face of various types of stressors (Epel et al. 2004; Valdes et al. 2005), is associated with several age-related diseases, including cancer and cardiovascular disease (Klapper et al. 2001; Wong et al. 2003; Wu et al. 2003; Serrano and Andres 2004; Campisi 2005).

The enzyme telomerase, a ribonucleoprotein capable of elongating telomeres (Greider 1995), can maintain telomere

length in some cells, most notably the germ cells. Telomerase activity in most cell lines is, however, too low to maintain constant telomere length (Engelhardt et al. 1997; Lansdorp 2005), and thus telomeres in tissues like blood cells shorten with age. Telomerase activity is repressed in most normal adult somatic tissues, probably as a mechanism to prevent tumor growth (Taylor and Delany 2000; Grupp and Parwaresch 2002). Blood cell telomeres shorten with age in most bird species (Vleck et al. 2003; Monaghan and Haussmann 2006), and we have shown that the rate of this shortening is inversely related to maximum life span in both birds and mammals—shorter-lived species show greater telomere loss per year than longer-lived species (Haussmann et al. 2003). This result remains when the comparative method (Felsenstein 1985) is used to account for phylogenetic relatedness between the species sampled (Haussmann 2005). Interestingly, in three long-lived seabirds, two procellariids—Leach's storm petrel and wandering albatross (*Diomedea exulans*)—and the European shag (*Phalacrocorax aristotelis*), telomeres do not appear to shorten with age in adults and may even lengthen (Haussmann et al. 2003; Hall et al. 2004). The rate of shortening is not constant over the lifetime of an individual; greater shortening occurs early in life when growth and cell turnover would be the highest (Brümmendorf et al. 2002; Pauliny et al. 2006). In shags, the telomere shortening rate is greater for chicks heavy for their size or in those produced late in the season, both of which may be associated with increased levels of oxidative stress that could increase telomere shortening (Hall et al. 2004).

Patterns of telomerase activity are consistent with patterns in rates of change in telomere length among bird species (Haussmann et al. 2004). Telomerase profiles in bone marrow (stem cells for red blood cells) are high in hatchlings and down-regulated in adults in relatively short-lived zebra finches and tree swallows, but remain high throughout life in two long-lived seabirds, common terns and Leach's storm-petrel (Haussmann et al. 2007). Given the prevailing view that down-regulation of telomerase after embryonic development is important as a deterrent against tumor formation, how these long-lived bird species avoid the tendency of telomerase to promote the formation of tumors is of special interest.

Emerging evidence suggests that telomere length is correlated with fitness in a variety of species. In humans, short telomere length at a given age is associated with increased risk of mortality (Cawthon et al. 2003), and long telomeres confer a survival advantage in the face of stress in the roundworm, *C. elegans* (Joeng et al. 2004). Within birds, 1-year-old tree swallows with long telomeres are more likely to survive and return to the breeding colony than individuals of the same age but with shorter telomeres (Haussmann et al. 2005b). Indeed, the predicted life span

based on survival data is nearly threefold greater for swallows in the top quartile for telomere length than for birds in the bottom quartile. Consequently, cross-sectional comparisons may actually underestimate the rate of telomere shortening within individuals. Pauliny et al. (2006) also reported evidence that birds with longer than expected telomeres were of higher quality, which these researchers defined as greater longevity (sand martins, *Riparia riparia*) or higher reproductive success (male, but not female, dunlin, *Calidris alpina*).

Aviv et al. (2003) and Monaghan and Haussmann (2006) have suggested that telomere length may provide a measure of biological life expectancy rather than chronological age because it indexes individual history, such as early growth conditions or the pace of deterioration. We know that various types of stressors can accelerate telomere shortening (Zeichner et al. 1999; Epel et al. 2004; Hall et al. 2004; Valdes et al. 2005), and telomere shortening could simply be a covariate of other changes, such as oxidative damage (von Zglinicki 2002), that may directly affect function and fitness. Simultaneous analysis of fitness components, oxidative stress, and telomere dynamics are required to determine whether or not in vivo cellular senescence can contribute directly to organismal level senescence and decreased survival.

Mechanisms of aging: hormonal signaling

Endocrine regulation plays a central role in metabolism, reproduction, and behavior and probably has an integral role in affecting life span (Wise 2000). Signaling pathways involving insulin and insulin-like growth factor-1 (IGF-1) have received considerable attention because they are implicated in the regulation of life span in organisms varying from yeast (Fabrizio et al. 2001) to mice (Bartke et al. 2001) although, clearly, it is the processes which these signaling molecules regulate that may affect life span, not simply their levels. Chronically elevated insulin may negatively affect survival and life span in vertebrates via its effects on mitochondrial ROS production (Lambert and Merry 2004), but normal levels of insulin are necessary for the regulation of glucose metabolism. In vertebrates, growth hormone elevates IGF-1 production, which works through a signaling pathway that overlaps that of insulin to affect growth, development, and metabolism. Silencing the IGF-1 pathway lengthens life span in a variety of laboratory model organisms, possibly by increasing resistance to oxidative stress (Migliaccio et al. 1999; Walker and Lithgow 2003) and/or reducing cancer risk (Pollak et al. 2004). Effects of reduced IGF-1 usually also include reduced metabolic rate (and body temperature in homeotherms), hypoglycemia, small body size, hypogonadism, and

reduced fertility (but see Holzenberger et al. 2003), all of which should be strongly selected against in nature (Harley 1991; Carter et al. 2002). Consequently, it remains unclear whether or not the modulation of IGF-1 signaling contributes to variations in life span between species.

There should be balancing selection on insulin and IGF-1 levels within a species to optimize growth, metabolism, and reproduction (facilitated by elevated levels) while limiting damage from elevated levels. Altered insulin levels have profound pathological effects in mammals. In particular, chronically elevated plasma glucose and insulin is associated with an increased risk of type II diabetes in humans, but insulin levels have not yet been linked directly to life span (Rincon et al. 2005). In birds, insulin is more potent than mammalian insulin, and both insulin and glucagon are consistently found at higher concentrations in birds than in mammals (Hazelwood 2000). To date, the contribution of insulin and IGF-1 and their signaling pathways to variations in life span among free-living animals is unknown, although there are a few hints based on rodent work (Miller et al. 2002; Harper et al. 2003). If these signaling pathways play a role in governing functional decline with aging, contrasting birds and mammals should provide a powerful tool for identifying that role.

Birds have extremely high concentrations of blood glucose relative to those found in mammals. Plasma glucose concentrations in hummingbirds feeding on high carbohydrate solutions can reach 41 mM (vs. a typical level of about 5 mM in humans) (Beuchat and Chong 1998), yet hummingbirds can live as long as 12 years (Calder 1990). Plasma glucose in fasting penguins averages over 16 mM (Vleck and Vleck 2002). Hyperglycemia is thought to contribute to aging in part due to the non-enzymatic glycation of proteins, yet levels of glycated hemoglobin in birds are lower than those in many mammals (reviewed in Beuchat and Chong 1998). Glycated proteins can react further to form advanced glycosylation end products (AGEs), which then cause cross-linking in long-lived protein such as collagen and lens crystallins (Holmes et al. 2001). Pentosidine is one such AGE that increases with age in the collagen of skin. Pentosidine in skin and foot webbing in a variety of bird species has been shown to increase linearly with age, leading to the suggestion that its level in skin could be used as a measure of age in wild birds (Chaney et al. 2003). To date, we do not know if rates of AGE accumulation are related to life span in birds.

Mechanisms of aging: immunosenescence

Immunosenescence refers to the deterioration of immune function with age. It is thought to increase susceptibility to infection, autoimmune disease, and cancer (Miller 1996).

Immunological deterioration has been linked to both oxidative damage (de la Fuente 2002) and telomere shortening (Mariani et al. 2003), so it is probably not so much a *cause* of aging as a *symptom*. The rate of immunosenescence is correlated with individual longevity in humans as well as with differences in life expectancy among species (Pawelec et al. 1999).

Immune function in vertebrates includes both innate (or natural) immunity and acquired (or adaptive) immunity. Both types can be subdivided into humoral and cell-mediated immunity, although there is much interaction among these systems. Immunosenescence in mammals is characterized by decreases in both cellular (T-cell mediated) and humoral (B-cell mediated) responses, with the most prominent changes involving a decline in T-cell-mediated immunity (Miller 1996). B-cell function may not change with age (Son et al. 2003), or observed changes may be largely the result of age-related changes in helper T-cells (Pawelec et al. 1999). Acquired immunity is guided by T-cells that must undergo extensive clonal proliferation on contact with an antigen; hence, unlike most tissues, these lymphocytes are under enormous replicative stress, leading to the potential for critical telomere shortening (Akbar et al. 2004). Consequently, replicative senescence of lymphocytes and the consequent reduction of their diversity are likely candidates for causing immunosenescence (Romanukha and Yashin 2003; Effros 2004). Essentially, both lymphocyte specificity and replicative capacity decrease with age. In elderly humans, T-cell distribution changes in favor of memory cells, with the lower production of new naïve cells (Akbar et al. 2004), a condition which in mice leads to relatively short life spans (Miller 2001). Unlike adaptive immunity, components of innate defense may actually increase in old age (Ortega et al. 2000), which may not be completely desirable because it increases the potential for autoimmune disease.

Immune function has received considerable attention in behavioral ecology of birds because the cost of mounting an immune response affects life-history traits such as breeding effort and reproductive success (see Norris and Evans 2000; Martin et al. 2001; Kilpimaa et al. 2003; Ardia 2005). In birds, the strength of cell-mediated immunity increases with maximum life span (Tella et al. 2001), suggesting that long-lived species invest more in immune function than shorter-lived species. This is consistent with evolutionary theory that predicts a greater investment in self-maintenance should be associated with longer life span (Kirkwood and Holliday 1979).

Information on how immune function changes as birds age is just beginning to emerge. Lozano and Lank (2002) found that cell-mediated immunity was lower in older birds than younger ones in a captive breeding flock of ruffs (*Philomachus pugnax*). Humoral immunity decreases with

age in barn swallows (*Hirundo rustica*) (Saino et al. 2003) and in collared flycatchers (*Ficedula albicollis*) (Cichon et al. 2003). We found that cell-mediated immunity decreases with age in zebra finches, tree swallows, and Leach's storm-petrels and that the rate of decrease is inversely correlated with life span (Haussmann et al. 2005a). About 50% of the response is gone after about 80% of the maximum life span, but in zebra finches this occurs over about 4 years, whereas in the long-lived storm-petrel it occurs over about 30 years. Most recently, we have shown that acquired T-cell mediated immunity declines with age in tree swallows but that acquired and innate humoral immunity do not change (Palacios et al. 2007); thus, in this species, immunosenescence is not a feature of all aspects of the immune system. Since humoral immunity does show age-related decline in barn swallow and collared flycatchers, there is clearly variation in the pattern of immunosenescence among species. Whether this variation is adaptive, perhaps responding to prevalence of parasites or risk of infection, or allows for trade-offs among the different branches of the immune system is not yet known. Likewise, how a compromise of the immune system affects survival in nature is not straightforward (Adamo 2004), although this information is critical to our understanding of the role that immunosenescence may play in the evolution of differences in life span between species.

Conclusions and future research

The complexity of aging makes it highly unlikely that it can be attributed to a single cause (Weinert and Timiras 2003). It has been suggested that somewhere between 25 and 33% of the determination of life span is heritable (Kirkwood 2002), and life span is undoubtedly a complex polygenic trait. Recent insight that homologous genes, such as those associated with the insulin and IGF-1 signaling pathways, contribute to life span in diverse organisms (Longo and Finch 2003) suggests that such genes may be important in all animals, although this line of investigation would profit from working with animals in their natural environment to avoid the biases that result from using laboratory lines selected for rapid population growth in a favorable environment. Now that the chicken genome has been sequenced (Wallis 2004) and work is proceeding on several other avian species (e.g., zebra finches), it is only a matter of time until the genomics of aging will be extended to include birds.

Identifying the evolutionary importance of different mechanisms as causes of aging requires comparative studies and statistical approaches that can evaluate the direct and indirect effects of multiple interacting factors. Figure 2 indicates how the various factors we have discussed here

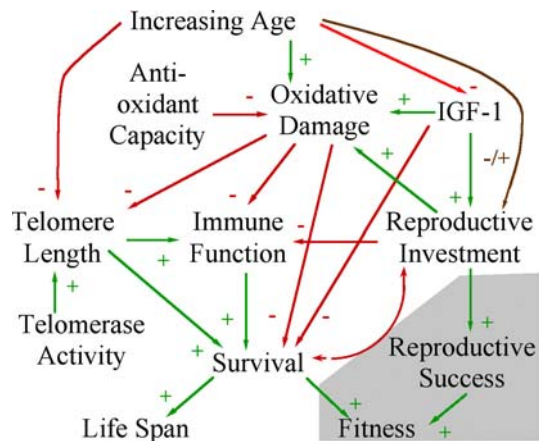


Fig. 2 Diagram of hypothesized causes of aging and their interactions in birds. Effects are either positive (green, +), negative (red, -), or change with age (brown, +/-). Interactions among these factors determine reproductive success and fitness differences among individuals within a species (shaded portion) and are hypothesized to be the underlying mechanism for the evolution of different life spans among species (unshaded portion)

could interact to affect survival and reproductive success, the critical components of fitness. Physiological studies that aim to identify mechanisms must always be mindful of the pitfalls of assigning cause and effect based on correlation, or of attributing function to a particular process that may only be correlated with yet a third variable. For example, because life span scales with body mass, any claim that a particular process governs life span must take body mass into account (Speakman 2005a).

Longitudinal studies of aging in free-living birds are rare, but need to increase because longitudinal studies have greater power to identify age-related changes than do cross-sectional studies (Aviv et al. 2006). In addition, longitudinal studies allow us to distinguish between differences that result from intrinsic processes in all individuals within a population and differences (or similarities) between age groups that result from selection that preferentially removes some phenotypes earlier in life than others (van de Pol and Verhulst 2006). That is, they may allow us to identify traits of current selective importance in the evolution of life span that could otherwise be overlooked.

Comparative studies have a special power for identifying which of the candidate mechanisms of aging have been of evolutionary importance in vertebrate animals because they can take advantage of the long-term natural experiments that have produced an array of species that differ in life span. However, conclusions based on comparative data require special methods of comparative analysis. Such analyses (Speakman et al. 2002; Haussmann 2005; Lorenzini et al. 2005; Speakman 2005a) are surprisingly rare in the literature on aging. Different species may share traits because of shared ancestry, or they may exhibit

differences that are not, in fact, related to differences in their biology of aging. A comparison of two species (e.g. pigeons vs. rats) provides little basis for generalizations about adaptation (Garland and Adolph 1994), but they are common in the literature on aging.

Birds are diverse and taxonomically well known and show considerable variation in their maximum life span; in addition, many populations are well studied both physiologically and demographically. Some well-studied populations provide access to large numbers of known-aged individuals. It is relatively easy to observe functionally and selectively important traits such as growth rate, foraging behavior, reproductive effort, and reproductive success in birds. To date, the study of birds has provided a wealth of information about life history evolution and the trade-off between survival and reproductive effort. Birds hold considerable promise as candidate organisms enabling critical tests of the mechanisms underlying senescence, in part because measurements can be made across the life spans of individuals to track cellular and molecular changes with age, to determine which factors affect rate of change, and to assess how these changes correlate with individual survival schedules. Continued study of birds will substantially strengthen our understanding of the evolution of the mechanisms involved in fundamental life history trade-offs.

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