Functional Ecology

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Article type : Review

Section: Evolutionary Ecology

Editor: Dr Jean-François Lemaître

The untapped potential of reptile biodiversity for understanding how and why animals age

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Funding Information

USA NIH RO1-AG049416 to AMB and DAWM

USA NSF IOS-1558071 to AMB, DAWM, and AMS

Abstract

1. The field of comparative aging biology has greatly expanded in the past 20 years. Longitudinal studies of populations of reptiles with a range of maximum lifespans have accumulated and been analyzed for evidence of mortality senescence and reproductive decline. While not as well represented in studies of amniote senescence, reptiles have been the subjects of many recent demographic and mechanistic studies of the biology of aging.
2. We review recent literature on reptile demographic senescence, mechanisms of senescence, and identify unanswered questions. Given the ecophysiological and demographic diversity of reptiles, what is the expected range of reptile senescence rates? Are known mechanisms of aging in reptiles consistent with canonical hallmarks of aging in model systems? What are the knowledge gaps in our understanding of reptile aging?
3. We find ample evidence of increasing mortality with advancing age in many reptiles. Testudines stand out as slower aging than other orders, but data on crocodilians and tuatara are sparse. Sex-specific analyses are generally not available. Studies of female reproduction suggest that reptiles are less likely to have reproductive decline with advancing age than mammals.

4. Reptiles share many physiological and molecular pathways of aging with mammals, birds, and laboratory model organisms. Adaptations related to stress physiology coupled with reptilian ectothermy suggest novel comparisons and contrasts that can be made with canonical aging phenotypes in mammals. These include stem cell and regeneration biology, homeostatic mechanisms, IIS/TOR signaling, and DNA repair.
5. To overcome challenges to the study of reptile aging, we recommend extending and expanding long-term monitoring of reptile populations, developing reptile cell lines to aid cellular biology, conducting more comparative studies of reptile morphology and physiology sampled along relevant life-history axes, and sequencing more reptile genomes for comparative genomics. Given the diversity of reptile life histories and adaptations, achieving these directives will likely greatly benefit all aging biology.

Keywords: senescence, physiology, demography, life history, ectotherm, vertebrate

1 | INTRODUCTION

Senescence is the complex biological process of disintegrating physiological and biochemical function with increasing age, leading to age-related frailty and functional decline. At the cellular level, senescence involves gene regulatory networks, cell signaling pathways, and cellular functional changes (Lopez-Otin et al., 2013; Kennedy et al., 2014). At the organismal level, senescence affects individual fitness by increasing the risk of mortality and reducing reproductive contributions with increasing age (Charlesworth, 2000). At the population-level, senescence leaves a signature in the vital rates of accelerating age-specific mortality with advancing age, often accompanied by declining age-specific fecundity rates (Promislow & Bronikowski, 2006). Thus, the comprehensive study of senescence requires an integrative approach across many scales of biological organization, from the level of

individual genes and single molecules upwards through cellular, organismal, and population-level approaches (e.g., Massot et al., 2011). In addition, a complete understanding of senescence requires a comparative approach. Comparisons among different species can yield insights into those mechanisms of aging that are fixed or constrained versus those that have evolved independently in different lineages, providing insights into the raw materials on which natural selection and evolution operated (Austad, 2010). Sauropsids (including ectothermic and endothermic lineages—hereafter "reptiles" and "birds") as the sister clade to mammals are well-positioned phylogenetically for comparative studies of aging, altogether forming the monophyletic amniotes. Within amniotes, studies of aging in ectothermic reptiles are far fewer in number than those of mammals and birds. This is due in part to the fact that the ecology and life history of many reptiles makes them difficult to monitor (McDiarmid et al. 2012) and, in concert with the increasingly imperiled conservation status of many reptile species (e.g., Rhodin et al., 2018), poses significant logistical and ethical constraints on research. Regardless, the actual contribution of existing reptile research to our understanding of the evolution of aging in its demographic and mechanistic dimensions may also be underestimated due to a lack of substantive reviews of key, up-and-coming research on this topic—an issue this contribution seeks to address.

Evolutionary theories of senescence predict that the rate of senescence is determined by the strength of selection against mutations with age-specific effects, i.e., selection varies with age class giving rise to age-specific mutation-selection balance (Medawar, 1952; Williams, 1957; Hamilton, 1966). The strength of selection on age-specific mutations is shaped by demography; indeed skewed age-structure (with few older individuals in populations than younger) is sufficient for senescence to evolve (Hamilton, 1966). Importantly, evolution molds mortality and fertility trajectories within specific ecological contexts (e.g., environmentally-driven mortality, resource availability, and pathogens;

Williams & Day, 2003) subject to physiological and genetic constraints. At the same time, life histories, realized within an ecological context, determine the relative selection for early- versus late- life fitness, creating a feedback loop that shapes the timing and rate of senescent processes. Selection for reduced rates of senescence (and increased reproductive lifespan) should occur when environmentally-driven mortality is lowered and reproduction increases beyond the age of first reproduction (Medawar, 1952; Hamilton, 1966). Indeed, such an early- versus late-life fitness balance should shape senescence rates; and senescence rates should evolve if this balance changes and there is genetic variation underlying the balance. We discuss variation in mortality and reproductive senescence of reptiles in Section 2 below. Of note, while we might expect longevity and senescence to coevolve, similar rates of senescence do not necessarily equal similar longevity (e.g., within species: Bronikowski et al. 2002, Miller et al. 2014; across species: e.g., Bronikowski et al. 2011, Ruby et al. 2018). Nevertheless, in understudied taxa such as reptiles, longevity is often used as a proxy for senescence rate. We take care throughout this review to acknowledge and distinguish the difference between longevity and senescence.

The preceding mutation-selection balance theory of the evolution of senescence tends to make inference from age-specific demographic patterns. In addition, several theories have emerged that attempt to explain the evolution of longevity and senescence rates as a response to correlated selection on functional traits (e.g., metabolism). These include the oxidative stress theory (formerly free-radical theory) (reviewed in Speakman et al., 2015), the pace-of-life theory (formerly the rate-of-living theory) (reviewed in Dammhan et al., 2018), and related thermal theories (e.g., cold-living, brumation) (Stark et al., 2018; Garbarino et al., 2015) for the evolution of variable rates of senescence and concomitant longevity. Although these can be thought of as evolutionary hypotheses in the sense that they involve correlated evolution to adaptations in physiological and molecular traits, their relationship to the

evolution of senescence would still be viz. age-specific mutation-selection balance. Thus, we discuss these further in Section 3 below on mechanisms of senescence.

Our review of senescence in reptiles is structured into two parts generally aligned with biological levels of organization (see Figure 1). In the first section, we review population-level, demographic senescence—where selection and evolution occur (Fig. 1A). And, in the second section, we review mechanisms of individual senescence. In reviewing the demographic senescence of reptiles, we start by considering how indeterminate growth and the evolution of 'protective phenotypes' that lead to low environmentally-driven mortality rates are expected to shape the evolution of senescence (Fig. 1B). We then review patterns of senescence within reptiles, focusing on longevity and rates of demographic decline across reptilian lineages. In reviewing the mechanisms of individual senescence, we organize the evidence around seven pillars of (human) aging put forth by the Geroscience Initiative at the National Institutes of Health (USA; Kennedy et al., 2014; Fig 1C), and highlight areas where reptiles age similarly and contrastingly with mammals. We end our review with suggestions for needed research on reptile senescence and their potential for bringing new insights to the field of aging biology.

2 | POPULATION SENESCENCE: DEMOGRAPHY, LIFE HISTORY, AND SELECTION

2.1 Indeterminate growth

Indeterminate growth—defined as extended growth past the age of first reproduction that may continue throughout life—has important implications for the evolution of senescence. Increasing fecundity with size reduces the relative contribution of early age classes to overall fitness, which at least in part counters the lower probability that animals survive and contribute at later ages. Consequently, indeterminate growth coupled with size-

dependent fecundity can increase reproductive output and fitness contributions of older individuals, thus increasing selection against senescence. The extent to which this occurs depends on the proportion of overall growth that remains after reproduction begins and the degree to which size determines the quantity and quality of offspring (Vaupel et al. 2004).

Debate continues about the extent to which reptile growth is indeterminate (e.g., Congdon et al., 2013). For example, recent reports on American alligators (*Alligator mississippiensis*) suggest that they are determinate growers with growth cessation sometime after maturation (e.g., Wilkinson et al., 2016). Similarly, a recent study of green sea turtles and loggerhead turtles argues that marine turtles exhibit determinate growth, adding to the growing evidence for determinate growth in turtles (Omeyer et al., 2018). However, even if growth is not truly indeterminate, continued growth past the age of first reproduction is the norm for reptiles and distinguishes them from mammals and birds (Hariharan, Wake, & Wake, 2016). Birds generally reach asymptotic size by the time they gain flight (Gill 2007). Similarly, mammals are generally near their full size by the time they reach the age of first reproduction. For instance, the ratio between length-at-maturity versus length-at-maximum adult body size is 0.95 for elephants (*Elaphus maximus*), 0.9 for New Zealand fur seals (*Arctocephalus forsteri*), and 0.97 for polar bears (*Ursus maritimus*) (Derocher and Wiig 2002; McKenzie et al. 2007; Mumby et al. 2015). By contrast, the proportion of remaining growth after reproduction among snakes, turtles, and lizards averages 0.68, 0.70, and 0.74, respectively (Shine & Charnov, 1992; Shine & Iverson, 1995). This suggests that there is ample opportunity for increased strength of selection against senescence at later ages in reptiles relative to birds or mammals, at least in so far as indeterminate growth is coupled with indeterminate fecundity.

The relationship between size and fecundity is complex. Although in many species fecundity increases as individuals grow, the pattern is by no means ubiquitous within reptiles

(reviewed in Sauer & Slade, 1987; Thorbjarnarson, 1996). For example, Congdon and van Loben Sels (1993) show that although clutch size is positively related to maternal body size for Blanding's turtles (*Emydoidea blandingi*), clutch size is not related to age. In cases such as this, the size-fecundity relationship is likely attributable to individuals who grow faster and larger having larger clutches rather than individuals increasing reproductive output as they age. Even so, numerous studies have shown a positive relationship between reproduction and size or age. For instance, Sparkman et al. (2007) found increasing reproduction with size among populations of the western garter snake with individuals in populations of fast-growing snakes increasing litter size with age at a faster rate than individuals in populations of slow-growing snakes. Similarly, Congdon et al. (2013) found clutch size increased by an average of 1 egg for every 8.6 years of age for 13 populations of 9 species of North American freshwater turtles, with a range of 2.3 to 18.5 years/egg.

2.2 Protective phenotypes

Traits that are associated with low annual mortality have the potential to also be associated with reduced rates of senescence. If reduced senescence rates increase fitness, then a reduction in environmentally-driven mortality risk should increase the efficacy of selection on senescence rate, particularly in populations with density-dependent regulation (Williams, 1957; Abrams, 1993, but see Moorad et al. 2019). Protective phenotypes (reviewed in Schwartz & Bronikowski, 2011) are putative adaptations that directly impact environmentally-driven mortality–possibly in an ontogenetic manner if the protections develop or wane over the course of life. Such protective phenotypes in reptiles include skin armor (i.e. scutes) in crocodilians, external ribcages (i.e. shells) in turtles, and venom or toxicity in some lizards and snakes. For example, adult crocodilians are apex predators with few natural predators and high annual survival probabilities of young and old adults (ca. 0.80 – 0.89, American alligators, Garner 2016). Even if a trait did not evolve for protective

purposes, protective phenotypes can still have demographic consequences. For instance, while conventional wisdom acknowledges that turtle shells are protective and substantially reduce adult predation (e.g., Spencer & Thompson, 2005), the original fossorial turtle shell is thought to be an adaptation for digging (Lyson et al., 2016). Regardless, among all reptiles, turtles and tortoises are associated with low mortality and long lifespan (Gibbons 1987; Shine and Iverson 1995). Still, a mortality comparison of hard-shelled and soft-shelled turtles, controlling for body size, has not been undertaken, and would provide a welcome test of the importance of protective phenotypes for the evolution of senescence.

The protective role of venom or poison is perhaps even more equivocal and deserves further study. Chemical protection (venom or poison) is positively correlated with maximum lifespan after controlling for body size in fishes, snakes, caudatans, and anurans (Blanco & Sherman, 2005). And relative to non-venomous colubrids, timber rattlesnakes (*Crotalus horridus*) and cottonmouths (*Agkistrodon piscivorus*) have some of the highest annual survival probabilities of all snakes (0.80 – 0.90) (Koons et al., 2009; Brown & Simon, 2018). But these high annual survivals are also not unheard of in non-venomous snakes living in populations with low predation (western garter snakes: Miller et al., 2011). Furthermore, a recent phylogenetic analysis of amphibian and snake species that are highly toxic or venomous suggests that venomous snakes, which use their venom primarily to acquire prey, are not more longevous than non-venomous snakes (Hossie et al., 2013).

2.3 Longevity and demographic senescence in reptiles

Longevity in reptiles ranges from one year (Labord's chameleon, *Furcifer labordi*) to ~150 years (Galapagos tortoise, *Chelonoidis nigra*) (Eckhardt, Kappeler, & Kraus, 2017). Although not a measure of change or decline with age, which is the definition of senescence, longevity provides important insights into aging processes. Longevity requires that survival

remain high into late ages and may be associated with increased fitness contributions from later-age phenotypes. The evolution of extreme longevity likely requires low environmentally-driven mortality and strong selective pressure to reduce rates of senescence (Finch, 2009), resulting in negligible senescence or even negative senescence (i.e., a decline in mortality with age, Vaupel et al., 2004). Practically speaking, records of longevity are more easily obtained than measures of actuarial senescence. While estimates of longevity are still dependent on the number of observations for a species and ease of determining age, observations of maximum lifespan do provide insights into general patterns. Among the chordates, current records of animals living longer than 100 years are restricted to a handful of (mostly) marine fishes, a few large marine mammals, humans, and five turtle species (de Magalhães & Costa 2009; Table 1). Within reptiles, turtles comprise the majority of species with the longest lifespan records (Gibbons 1987), with 23 species documented to be capable of reaching at least 40 years (Table 1). Crocodilians are also relatively long-lived with 18 of 21 species in the AnAge database having longevity records > 40 years, albeit mostly based on records of captive animals (Weigl, 2014). Similarly, tuatara, the only extant species in the order Rhynchocephalia, are reported to live as long as 90 or more years in captivity (Castanet 1994). In contrast, only seven of the 401 squamate (snakes and lizards) species in the AnAge database have maximum recorded lifespans of >40 years. These patterns are generally consistent with adult survival rates, with turtles having consistently higher annual survival than squamates (Shine & Charnov, 1992; Shine & Iverson, 1995).

Terrestrial and fresh-water turtles have long been models of longevity (e.g., Gibbons, 1987; Congdon et al., 2003), but recent studies suggest that turtles may exhibit significantly more variation in demography than previously appreciated (Warner et al., 2016, see also Spencer & Janzen, 2010). While the representative turtle (*Gopherus agassizii*) used in a recent meta-analysis of aging is notable for declining mortality with age (Jones et al., 2014),

comparative analyses of aging rates in painted turtles (*Chrysemys picta*) and western garter snakes found that mortality accelerated across the adult lifespan (Miller et al., 2014).

Unfortunately, despite considerable research on marine turtles, our understanding of sea turtle aging is primarily limited to demographic studies and skeletochronological estimates of age (reviewed in Chaloupka & Musick, 1997). Estimates of annual survival in adult sea turtles, including age-class specific estimates for populations of loggerhead sea turtles (*Caretta caretta*, Chaloupka & Limpus, 2002) and green sea turtles (*Chelonia mydas*, Chaloupka & Limpus, 2005) do not yet allow estimation of actuarial senescence. Demographic studies are rare in general for reptiles, resulting in a lack of knowledge regarding demographic senescence relative to endothermic vertebrates (e.g., see review Ricklefs, 2010; also Jones et al., 2014). Studies that do exist indicate a wide range of patterns in the demographic senescence of reptile mortality and fecundity—a range which largely overlaps estimates from other animal lineages (e.g., Warner et al., 2016; Colchero et al., 2019). Ricklefs (2010) suggested that patterns of actuarial senescence in reptiles are similar to birds. However, all reptile studies included in the comparative analysis were of captive populations. Captivity can impact animal physiology and behavior in complex ways with significant implications for animal health and longevity (e.g. Lahdenperä et al., 2018). In contrast, studies by Congdon et al. (2001, 2003) in two species of wild turtles showed no increases in mortality with age. More recently, Warner et al. (2016) reported evidence for increased mortality with age in a wild population of painted turtles, but the rate of increase was slow relative to western garter snakes, yellow-legged frogs (*Rana sierrae*), and other well-studied taxa (Miller et al. 2014). In fact, some of the best candidates for negligible senescence and extreme longevity are reptiles (Gibbons, 1987; de Magalhães & Costa, 2009). A recent comparative study by Jones et al. (2014) demonstrated that three reptile species (freshwater crocodile (*Crocodylus johnstoni*), common lizard (*Zootoca vivipara*), and desert

tortoise (*Gopherus agassizi*), showed slow or negative rates of senescence with respect to mortality. In addition, two of the three showed no decline in reproduction with advancing age (crocodile and desert tortoise). Thus, while limited, the majority of studies in the wild suggest that slower aging may in fact be widespread among reptiles.

While the limited availability of long-term demographic studies in reptiles has often precluded robust tests of life-history theories for aging, several single-species estimates of longevity and somatic growth rate give some indication that sea turtle longevity may be governed by life-history tradeoffs and possibly an allometric relationship with body size. The smaller Kemp's ridley sea turtle (*Lepidochelys kempii*) has a maximum estimated age of 30.25 yr with reproductive longevity estimated to be less than or equal to 10 yr post-maturation (mean = 4.5 yr) (Avens et al., 2017). The larger North Atlantic loggerhead sea turtles has a maximum estimated age of 70-77 yr with reproductive longevity less than 46 yr (but mean = 19 yr) (Avens et al., 2015). It is possible that the relatively small size and shorter interbirth interval of the Kemp's ridley turtle contributes to its relatively shorter lifespan.

Studies of reproductive senescence in reptiles are even more rare than studies of actuarial senescence. We know that female three-toed box turtles can live >60 yr with no apparent decline in reproductive performance (Miller, 2001). Furthermore, considerable increases in fecundity with age can occur in reptiles even in the oldest documented wild individuals (e.g., Sparkman, Arnold, & Bronikowski, 2007; Warner et al., 2016, but see Brown, 2016)—although this does not preclude reproductive senescence at the end of life (Warner et al., 2016, see also Massot et al., 2011). Similarly, long-term studies of sea turtles generally find increasing reproductive output with age (Garner, 2012). Empirical data for this pattern comes primarily from females, due to the inherent bias of sampling different sexes of some species (e.g., semi-aquatic turtles; Reinke et al., 2019). It is possible that sex-specific growth promotes sex-specific selective environments (e.g., Hoekstra et al., 2018), and results

in sex-specific aging and longevity (Austad & Fisher, 2016), but this question remains elusive at this time for reptiles.

Overall, great strides have been made in documenting variation in longevity and rates of aging among reptiles. The intriguing phenomenon of extreme longevity coupled with evidence for slower aging relative to birds and mammals suggests that the field of comparative aging would be greatly enriched by more in-depth, long-term demographic studies on a wider range of reptiles.

3 | INDIVIDUAL SENESCENCE: MECHANISMS

We present this section in the framework of the seven pillars of aging (Kennedy et al., 2014; see also Lopez-Otin et al., 2013), which represent common drivers of the aging process contributing to aging phenotypes and age-related disease across animals, but have typically focused on humans and other mammals. We choose this framework to (i) emphasize evidence for these aging processes in reptiles, (ii) contrast reptiles with mammals, and (iii) highlight fruitful future research directions using reptiles as models to understand aging biology. Direct evidence for the pillars of aging in reptiles is summarized in Table 2 and detailed below.

3.1 Macromolecular damage

Despite the existence of cellular protection and repair mechanisms, accumulating damage to proteins, lipids, and DNA has long been a hallmark of aging (Maynard et al., 2015). Whether due to imperfect, age-independent repair mechanisms *per se*, or whether due to decreasing efficiency of repair mechanisms with age, studies across broad comparative landscapes show that accumulating damage has myriad negative downstream effects (e.g., MacRae et al., 2015). In reptiles, understanding of DNA-damage-repair mechanisms is limited, but does include some studies on lipid membrane damage (Manibabu & Patnaik,

1991; Jena, Das, & Patnaik, 1995). Less research has focused on damaged proteins. In this section, we highlight what is known about damage and repair in reptiles; in other sections, we focus on the degradation of protective mechanisms (e.g., telomeres, epigenetics, protein chaperones) with age.

DNA damage

The structure and function of DNA is 100% conserved among all living organisms. As a consequence, impairment resulting from DNA-damaging agents is also conserved, as are the mechanisms to repair damage (Uphoff et al. 2013). Because deficiencies in DNA repair capacity have been linked to the induction of replicative senescence and progeroid syndromes, it is reasonable to suppose that functional DNA repair pathways are an essential component of longevity, both within and among species. Early studies on mammals supported this hypothesis, but have been criticized for failing to control for phylogeny and body size (Promislow, 1994). More recently, Page and Stuart (2012) demonstrated that body mass accounted for most of the variation seen in DNA repair capacity in an array of endothermic vertebrates, particularly in the context of oxidative DNA damage. Furthermore, early studies on DNA repair and longevity in some ectothermic vertebrates (turtles and fish) found no association; individual species had approximately equal amounts of repair after insult (Woodhead, Setlow & Grist, 1980; Regan et al., 1982). Unfortunately, interpretation of these data are confounded by the small number of species and individuals tested, as well as technical issues—most notably heavy use of domesticated species, differences in euthanasia and sampling techniques, as well as differences in the specific methods used to quantify DNA repair. Clearly, more research is needed to test whether age-related DNA repair deteriorates similarly between mammals and reptiles, as well as to clarify the significance of these processes as they relate to organismal aging.

Central to this mechanistic damage-accumulation hypothesis for senescence is the assumption that declining ability to repair DNA damage with age causes an accumulation of damaged cells as well as somatic mutations (Gladyshev, 2013). In reptiles, in the absence of age-specific repair capability, we can look to studies of associations between DNA-repair capacity and longevity for a first approximation. In painted turtles, juveniles and adults did not differ in their DNA repair capacity (Schwanz et al., 2011). In snakes, long-lived species had more efficient DNA repair than short-lived species (Bronikowski, 2008), and slow-aging ecotypes of garter snakes had higher capacity to repair DNA relative to fast-aging ecotypes (Robert and Bronikowski, 2010; Schwartz and Bronikowski, 2015). In the same slow- and fast-aging ecotypes of garter snakes, cells grown from larger/older females were more resistant to the lethal effects of the DNA-alkylating reagent methyl methanesulfonate (MMS) than were cell lines grown from smaller/younger females irrespective of ecotype (Alper et al. 2015). This result showed the opposite pattern than predicted if DNA repair capacity declines with age. Moreover, using dermal fibroblast cells, reptile cells were more resistant to a battery of cytotoxins, including MMS, than birds and mammals (Alper, Bronikowski, & Harper, 2015).

Lipid & Protein Damage

Membrane fatty acid composition changes over the lifespan, and profiles of damaged membrane fatty acids change with age in mammals (Hulbert et al., 2007; Hulbert 2010). Changing fatty acids give rise to variation with age in lipid peroxidation, which is both damaging to lipids and other molecules. While a vast literature exists on mammal and bird age-specific membrane lipid profiles and their peroxidation index, data are sparse at best in reptiles. In garden lizards (*Calotes versicolor*), lipid composition changes with age in agreement with mammals, and lipid peroxidation increases with age in brain, kidney, and

liver (Manibabu & Patnaik, 1991; Jena, Das, & Patnaik, 1995). Accumulating protein carbonyls, which occurs when carbonyl groups are attached to proteins through interactions with free radicals or damaged lipids, is a stable hallmark of aging. One study in asp vipers (*Vipera aspis*) linked protein carbonylization to the stress response (Stier et al., 2017) and another study in garden lizards correlated carbonyl accumulation with age (Jena, Das, & Patnaik, 1996), but no studies in reptiles have directly linked stress to aging via increased carbonylization.

3.2 Epigenetics

Because the epigenome integrates environmental cues and changes over the lifespan, epigenetics has been a fast-growing area of research in aging biology (Booth & Brunet, 2016; Pal & Tyler, 2016). Not only may epigenetic changes correlate with advancing age (i.e., the "epigenetic clock", Horvath & Raj, 2018), but these age-related changes in the epigenome have been found to vary across species (DePaoli-Iseppi et al. 2017). Thus, they may be predictive of stress and aging rates in other species as well (see Parrott & Bertucci, 2019).

Although few researchers have queried age-related changes in the epigenome of reptiles, some progress has been made in quantifying methylation. For example, alligators, like other vertebrates, show decreasing global methylation with advancing age (Nilsen et al. 2016). In general, reptiles (including crocodilians, lizards, snakes, and turtles) have global methylation levels that span the range observed in mammals and fishes (i.e., relatively lower to higher levels). This greater range than expected global methylation occurs despite similar GC content and CpG motifs as other vertebrates (Varriale & Bernardi, 2006; Head, Mittal, & Basu, 2014). How methylation and other epigenetic modifications change with advancing age in reptiles, and whether this affects transcriptional networks and age-related phenotypic decline in reptiles is an area that should be a future avenue for research.

3.3 Inflammation

The ubiquitous nature of increasing inflammation with age has been a long-appreciated aspect of aging. Under this category, reports on age-related innate immune function are relatively plentiful in reptiles, while fewer studies have tested for changes in adaptive immunity with age (e.g., lymphocyte proliferation). No studies, to our knowledge, have sought to test whether senescent cells of reptiles secrete pro-inflammatory signals to neighboring cells as is seen in model systems (Campisi, 2018).

Central to the immunosenescence hypothesis for organismal aging is the assumption that declining immune function, or immunocompetence, causes organismal senescence (Maue & Haynes, 2009; Brand & Nicholls, 2011). For innate immunity, although reptile studies have demonstrated significant plasticity in innate immune function in relation to biotic and abiotic factors (Refsnider et al. 2015; Palacios, Cunnick, & Bronikowski, 2013; Zimmerman et al. 2017), there has been no consistent finding of an effect of age on innate immunity (Palacios et al. 2011; Sparkman & Palacios 2009; Schwanz et al. 2011) in reptiles. Regarding acquired immune proliferative ability in reptiles, Zimmerman et al. (2017) found no decline with age in B-cell proliferative ability in red-eared slider turtles, a species that also exhibits plasticity in B-cell function with temperature (Palackdharry et al., 2017). Similarly, while we have found differences in T-cell proliferative ability between pregnant and non-pregnant females in garter snakes, we have not found a decline in immunocompetence with advancing age (Palacios & Bronikowski, 2017), despite differences between fast- and slow-aging ecotypes (Palacios, Cunnick, & Bronikowski, 2013).

The immunosenescence mechanism of aging has been examined in the common lizard (*Zootoca vivipara*), where polyandrous and monandrous females coexist and mating strategy is condition- and context-dependent. Polyandrous females have higher reaction to inoculation

with phytohaemagglutinin, a reaction that involves both T-cell responses and innate immunity. Polyandrous females also have declining reproductive success (litter size and offspring quality) with age. Thus, there is a positive correlation between increased immunocompetence in an ecotype that has reproductive senescence, contrary to what one might predict under the immunosenescence hypothesis (Richard et al., 2012).

Interestingly, Quesada et al. (2019) reported lineage-specific duplication of immunity-related genes in the long-lived giant tortoise and other turtles. These included gene duplications thought to be involved with the pro-apoptotic activity of granzymes and cytoxic T-lymphocytes (e.g. PRF1, chymase), as well as the expansion of some gene families related to viral, microbial, fungal, and parasitic innate immune defense. They argue that the expansion/diversification of innate immunity genes in turtles suggests that innate immunity may be relatively more important for turtles than mammals, but functional studies are required.

Important to both innate and adaptive immunity are the various cytokines. Cytokines are small proteins secreted by immune system cells, typically implicated in cell signaling and the inflammation response. While cytokine research in reptiles has lagged behind other aspects of immunity (reviewed in Zimmerman, Bowden, & Vogel, 2014), recent advances in reptile cytokines suggest that understanding their influence over senescence in reptile immunocompetence may be a reasonable near-term goal (e.g. Zhou, Guo, & Dai, 2009; Zhang et al., 2015; Rayl et al., 2019).

3.4 Adaptation to Stress

Reptiles possess many morphological and physiological adaptations that may lend protection to individuals (Schwartz & Bronikowski 2011). These may fundamentally alter the trajectory of age-specific mutation-selection balance in populations—skewing populations

towards older individuals with more right-skewed age structures relative to mammals and birds. Examples include extended metabolic shutdown, starvation resistance, freeze and heat tolerance, and hypoxia resistance. Clade-specific adaptations also include shells, super-cooling, and anoxia tolerance in some turtles; venom and intermittent feeding and gut remodeling in some snakes (reviewed in Schwartz & Bronikowski 2011). Many of these adaptations require protection and repair of cellular components that typically accumulate damage with age. One of the requirements for an effective response to environmental stressors is a set of robust mechanisms for proteostasis, including heat shock proteins (HSPs) for chaperoning unfolded proteins (e.g., Krivoruchko & Storey, 2010) and targeting denatured proteins for autophagy and proteosomal degradation. It is worth considering how the adaptive mechanisms for extreme-stress response may alter senescent parameters, and perhaps even alter how different species age. For example, reptile species that have evolved a highly adapted HSP response may have enhanced aging resistance via effective triage of damaged proteins, but still perhaps senesce through alternative mechanisms. We engage the state of the field with regard to the relationships between aging and both thermal and oxidative stress in more detail below.

Thermal Stress

Resilience to extreme temperature fluctuations, with ability to adjust metabolic rate from low to high with temperature (e.g. 4 – 40°C, e.g. Gangloff et al., 2016), raises the interesting prospect of correlated evolutionary responses of stress resistance. If present, these evolved stress responses should utilize molecular networks and cell-signaling pathways common to those found in animal models of aging, and could subsequently reveal nodes in these pathways that have been free to evolve (see the seventh pillar below on Metabolism for empirical data supporting this contention).

At the cold end of the thermal spectrum, brumation, like hibernation, is a metabolic adaptation that allows animals to persist in seasonally frigid environments. Both brumation and hibernation are associated with increased survival and the evolution of slower senescence in reptiles (brumation) and mammals (hibernation) (Ruf, Bieber, & Turbill, 2012). However, brumation is a very different metabolic state than hibernation, and is characterized by dormancy (very low metabolism and heart rate) with periods of activity, and is controlled by temperature. Both hibernating mammals and brumating reptiles would be expected to face the same oxidative stress upon increasing metabolism and reperfusion of oxygenated blood. Other theories of senescence arise from the observation that cold thermal environments are often associated with longevous species or representative populations (e.g. Stark et al., 2018). Lower body temperatures could simply delay the attritive effects of metabolic byproducts, but the mechanisms behind the preservative effects of cold temperature are still elusive (Flouris & Pantoni, 2015).

At the warm end of the thermal spectrum, protective mechanisms consistent with mammals have been observed, such as upregulation of heat shock proteins, increased activity of the Hypothalamic-Pituitary-Adrenal axis, and increased oxidative stress resistance (Schwartz & Bronikowski, 2013). This last factor, oxidative stress resistance, has been a long-studied protective mechanism for damage accumulation and has direct relation to the proposed mechanism of mitochondrial dysfunction with age.

Oxidative Stress

As noted above (3.1 Damage), one of the most cited causes of aging is damage to macromolecules. One specific type of damage is oxidative damage that is a by-product of aerobic respiration (Sena & Chandel, 2012) and oxidative phosphorylation by mitochondria, especially in the context of chronic stress conditions faced by free-living organisms (Salmon,

Richardson, & Perez, 2010). More specifically, damage to macromolecules, such as mitochondrial DNA (Barja & Herrero, 2000) and membrane lipids (Hulbert et al., 2007), as a consequence of mitochondrial-derived reactive oxygen species (ROS), is thought to lead to age-related pathology and functional decline (Wallace, 2005). Both within and among mammals and birds, many studies have supported the notion that longer-lived species tend to have more efficient mitochondria, less ROS production, and/or increased antioxidant capacities (e.g. Ku, Brunk, & Sohal, 1993; Barja & Herrera, 2000; Lambert et al., 2007). At the same time, many exceptions to this pattern exist, with perhaps the most famous being naked mole rats that demonstrate high levels of ROS and oxidative damage (Perez et al., 2009), with little impact on longevity (Ruby, Smith, & Buffenstein, 2018). Notwithstanding, life-extending dietary interventions, such as caloric restriction, methionine restriction, and every-other-day feeding, are also associated with a decline in mitochondrial ROS (e.g. Sohal et al., 1994; Gomez et al., 2007; Caro et al., 2008; Sanchez-Roman et al., 2012).

There is much interest in the role of oxidative stress, primarily through ROS, in shaping variation in life histories, including aging rates and longevity in reptiles. For example in painted lizards, Olsson et al. (2008; 2009) found that longevity-associated life-history traits are predicted by the degree of ROS production. In colubrid snakes, Robert et al. (2007) found a positive correlation between ROS production and longevity. Studies in painted and slider turtles have shown that they are well-suited to resist the effects of oxidative stress during cooling, with increased neuronal antioxidants during cooling (reviewed in Rice et al., 2002). Slow-aging neonatal garter snakes have more efficient mitochondria and cellular antioxidant defenses than fast-aging neonates, despite equivalent mass-independent metabolic rates (Robert and Bronikowski, 2010). Not surprisingly, however, results that contest oxidative stress as a mechanism of aging have been observed in reptiles as they have in mammals. For example, in a subsequent study on these same garter snakes populations, slow-aging garter

snakes produced higher baseline levels of ROS in the blood that decreased in response to acute heat stress, relative to fast-aging populations (Schwartz and Bronikowski, 2013). Interestingly, in contrast to mammals, reptiles and birds have nucleated red blood cells with mitochondria and gene expression in these cells includes both antioxidants and heat shock proteins (Waits et al., 2019). Despite a lack of consensus with respect to the role of oxidative stress in reptile aging, the combination of these intriguing findings and potential novel protective mechanisms in non-mammalian amniotes warrants more research to elucidate the similarities and differences in mitochondrial energetics between reptiles and endothermic vertebrates.

3.5 Proteostasis

Maintaining cellular function requires accurate protein synthesis, folding, maintenance, and removal of degraded proteins. The cellular pathways responsible for maintaining protein homeostasis, or proteostasis, in humans are themselves comprised of at least 2,000 components (Klaips, Jayaraj, & Hartl, 2018). Failures at various steps along the proteostasis network cause disease states, including neurodegenerative disorders Alzheimer's and Parkinson's disease (Ross & Poirier, 2004). More generally, decline in the function of the proteostasis network and increased protein aggregation has been associated with aging in model organisms (e.g. Labbadia & Morimoto, 2015). For non-avian reptiles, although we lack a comprehensive comparison of reptile proteome evolution, genome sequencing has generally revealed conservation of core components of the proteostasis network in reptiles (e.g. Shaffer et al., 2013; Quesada et al., 2018, Schield et al., 2019). There is some evidence for expansion of gene families associated with protein homeostasis in reptile lineages (e.g. prion proteins, immunoglobulins; Harrison, Khachane, & Kumar, 2010; Shaffer et al., 2013), but the functional or adaptive role of these gene expansions is still unknown. Given the

documented role of pseudogenization and gene loss across multiple reptile genomes (e.g. Shaffer et al., 2013; Liu et al., 2015; McGaugh & Schwartz, 2017), we anticipate that loss or change in function of proteostatic genes will also be a key feature of diversification of proteostasis in non-avian reptiles. Most functional work on proteostasis in reptiles has focused on the environmental stress response and molecular chaperone function (Chang, Knowlton, & Wasser, 2000; Heikkila, 2017; Takii et al., 2017). These studies provide basic knowledge of the composition and patterns of expression of the molecular stress response in reptiles. Notwithstanding, nothing is specifically known about age-related declines in molecular chaperone function and about other components of the proteostasis network, such as protein removal, in any context.

3.6 Stem Cells and Regeneration

Germline Stem Cell Continuity

One aspect of reproductive biology that distinguishes reptiles among amniotes is the presence of oogenesis from stem cells throughout the reproductive lifespan of females (reviewed in Guraya, 1989). The germinal bed of reptilian ovaries is a unique morphological feature supplying the abundant supply of oogonia–the germ stem cells. Our understanding of reproductive senescence in reptiles is data deficient, particularly for longer-lived species whose longevity requires repeated captures of females to document reproduction. As mentioned earlier, the striking reproductive senescence seen in mammals is absent or nearly so in many reptiles (e.g., Sparkman et al., 2007, Warner et al., 2016), is perhaps in part due to the "unlimited" supply of gametes without an expiration date, though more work needs to be done to investigate this possibility further,

Cellular Senescence

The relationship between cellular replicative senescence and whole-organism senescence has been a topic of debate for several decades. Recent clarity has been brought to this question by the discovery that senescent cells are not simply accumulated non-functioning cells, but are actually cells that dramatically alter their near-environment by the release of pro-inflammatory signals (Campisi, 2018). In the field of human aging, this has spurred research into chemicals (i.e. senolytics) that can selectively destroy senescent cells. Notwithstanding, normal cells cannot divide indefinitely due to the shortening of telomere ends of chromosomes with each cell division. Although whether the cell division upper limit of reptile cells is equal to that of mammals (i.e., the "Hayflick limit", Hayflick & Moorhead, 1961) has not been well-studied, several turtle species indicate that the maximum number of cell divisions may be correlated with species' longevity (Christiansen et al., 2001).

Telomere dynamics

Telomeres are repetitive nucleotides at the ends of eukaryotic chromosomes that potentially protect DNA from replication errors. Telomere length is commonly associated with longevity in humans and telomerase-mutant mice have reduced longevity (reviewed in Gomes, Shay, & Wright. 2010). Telomere shortening with age is thought to be due to (1) telomeres shortening during replication (Blackburn, 2005) and (2) telomerase shutting off in adult somatic tissues as an anti-tumor mechanism (Tanaka et al., 2005). In contrast to mammals, some studies in reptiles indicate that telomerase may not be turned off in adult somatic tissues. A meta-analysis of wild-animal telomere dynamics, including 14 birds and 3 non-avian reptiles, found general support for the correlation between telomere maintenance and longevity (Dantzer & Fletcher, 2015). However, in the three non-avian reptiles used in the meta-analysis the relationship between telomere length and age is complex (see Table 2).

One study reported an association between telomere shortening and longevity in males (western terrestrial garter snake; Bronikowski, 2008). A second study also found evidence of age-related decline in telomere length of males, but not females, even though females with longer telomeres lived longer (sand lizard; Olsson et al., 2011b). The third study found no evidence for telomere-related cellular aging in either sex (water python (*Liasis fuscus*); Ujvari & Madsen, 2009). A study on the loggerhead sea turtle, which was not included in the meta-analysis, failed to detect a significant age-related decline in telomere length (Hatase et al., 2008), yet age-related decline was observed in crocodilians (Scott et al., 2006, Xu et al., 2009). Even more complicating, a comprehensive study of telomere dynamics with age in frillneck lizards (*Chlamydosaurus kingii*) found that telomere length initially increases with age in the first few years of life followed by a decline, but that telomere length was unrelated to survival (Ujvari et al., 2017). Recent work in red-sided garter snakes found age-related telomere shortening in males, but not females, confirming that selection on telomere length is, at best, context-dependent (Rollings et al., 2017). An earlier review across Metazoans (Gomes et al., 2010, and references therein) found that many fishes, amphibians, and reptiles have active telomerase activity in somatic tissue, whereas telomerase is repressed in most birds and mammals. Active telomerase has thus been hypothesized as a mechanism that allows for the regeneration capabilities seen in some species of reptiles (reviewed by Olsson, Wapstra, & Friesen, 2018). Comparability among studies is hampered by varying techniques used to measure telomere lengths (e.g., qPCR, telomere restriction fragments). Nonetheless, reptiles lend themselves to straightforward assessment of telomeres due to their nucleated red blood cells, and thus the feasibility of sampling adequate blood volumes from even the smallest species.

Regeneration

Stem cells and progenitor cells may generally act as a somatic repair service capable of countering cellular senescence, but stem cells are also susceptible to the accumulation of deleterious changes that cause cellular senescence and may contribute to stem cell exhaustion. Senescence or exhaustion of various stem cell populations has been correlated with aging in humans and other mammals (reviewed in Lopez-Otin et al., 2013). There is considerable interest in stem cell revitalization, perhaps through epigenetic mechanisms, to reverse aging phenotypes (Rando & Change, 2012). Within the animal kingdom, regenerative capacities correlate with ectothermy (Alvarado, 2000). Reptiles such as snakes and lizards, and amphibians such as salamanders have tissue-regenerative capacities that far surpass that of mammals. Many salamanders and lizards can atomize their tails and regenerate the tail tissue, the extent of which is dependent upon the types of stem cells that remain active (Sun et al., 2018). The gut tissue and many organs in pythons and boas that have infrequent meals degenerate in the weeks/months between feedings and then can fully regenerate within days of taking a meal (Secor & Diamond, 1998) utilizing molecular pathways known to regulate aging (mTOR, NRF2; Andrew et al., 2017). These regenerative capacities of reptiles have brought considerable interest in the wound healing field (e.g. Lozito & Tuan, 2017; Jacyniak, McDonald & Vickaryous, 2017), but there has been little consideration of how these regenerative capacities affect the rate of aging in these species, or if these capacities themselves senesce. This is partly because we lack tools, such as reptilian cell lines, necessary to study the cellular biology of senescence in reptiles (see Alper, Bronikowski & Harper, 2015). While evidence for cellular senescence and age-related decline of tail regeneration are still lacking, there are tantalizing examples of age-related patterns in the costs of tail regeneration (Vitt & Cooper, 1986) as well as condition- or environment-dependent effects on regenerative capacity such as decreased tail regeneration ability with

increased parasite load (Oppliger & Clobert, 1997). Furthermore, there is also evidence for positive selection in geckos and anoles for genes potentially underlying tail regeneration, including genes related to cell proliferation and prostaglandin biosynthetic processing, which are known to be expressed during tail regeneration (Liu et al., 2015). All of these aspects could represent novel targets for aging research.

Molecular Network for Cellular Senescence: p53/p16

Cellular senescence can occur through mechanisms other than the replicative lifespan of cells. One of the primary drivers of cellular senesce is the p53 molecular network. Upon experiencing environmental stress that causes DNA damage, the p53 molecular network determines a cell's fate (i.e., death, senesce, repair), balancing anti-cancer and anti-aging mechanisms. Recent work on the comparative genomics of the p53 molecular network provided evidence of positive selection on genes within p53 networks of reptile (both ecto- and endothermic) relative to mammals, with reptiles having more positive selection on upstream genes (*p53* and its regulators), especially in lizards and snakes (Passow et al., 2019). This is in contrast to the purifying selection on p53 across mammals (Passow et al., 2019; Guar et al., 2017), with the exception of the branch leading to elephants (Passow et al., 2019). We also found that species with longer lifespans experienced less variation in selection across the p53 network, whereas those with shorter lifespans were equally likely to have both positive and purifying selection (Passow et al., 2019). A recent publication on Galapagos tortoise genomes (Quesada et al., 2019) identified variants in *p53* that are associated with hypoxia tolerance in other species (Zhao, 2013), providing an interesting link between this network and stress resistance in an exceptionally long-live species. Future work is needed to test functional consequences of the observed amino acid changes on rates of senescence and cancer.

3.7 Metabolism and Deregulated Nutrient Sensing

In the last 25 years, molecular networks have been identified in laboratory model organisms that govern stress responses and senescence at the cellular level, and ultimately govern stress responses and senescence at the organismal level (Greer & Brunet, 2008; Riera et al., 2016). These networks are antagonistically pleiotropic in the sense that increasing or decreasing signaling through their pathways shifts resources between growth and reproduction on the one hand, or maintenance and survival on the other (Barzilai et al., 2012). This has led to a general hypothesis that the fundamental life-history trade-off between growth and reproduction versus maintenance and survival may be controlled by variation in signaling through these networks – both within and among species – and may underlie intra- and interspecific variation in longevity (Schwartz & Bronikowski, 2011; Bartke 2017, Bartke, Sun, & Longo, 2013). Specifically, these homologous signaling networks include nutrient sensing pathways, namely the Insulin/Insulin-like signaling pathway (IIS), TOR, AMPK, and Sirtuins. Across model species, dietary restriction extends health and/or longevity through these nutrient-signaling networks (reviewed in Allison et al., 2016). Given the unique characteristics and plasticity of many reptiles in terms of nutrient signaling and metabolic plasticity, infrequent feeding in many species of snakes, and downregulation of metabolism with low temperatures and hibernation, reptiles provide unique avenues to understand these networks and their regulation of aging.

Across animals, reduced signaling through the IIS and TOR pathways across the lifespan, involving low circulating levels of IGF1 or mutations in the IGF1 receptor, has been associated with increased longevity (e.g., Fontana, Partridge, & Longo, 2010). Paradoxically, in adults a decline in the level of circulating IGF1 is also a biomarker of aging (e.g., Sanders et al., 2018). While there is currently no information on how dietary restriction or other

manipulation of the IIS and TOR pathways alters aging in reptiles, these networks have been examined in an evolutionarily comparative framework within amniotes including reptile, bird, and mammalian clades (McGaugh et al., 2015, Sparkman et al., 2012). While the overall structure of these molecular signaling networks is highly conserved, there is considerable evidence of positive selection on many components of this network between the reptile and mammal clades and specifically within the squamate (snakes and lizards) lineage. Included in this positive selection are top extracellular regulators (IGF1, insulin receptor, and IGF1 receptor), and key intracellular nodes, insulin receptor substrate, PI3K and mTOR (McGaugh et al., 2015). Genetic variation found in humans and mice models in the same nodes are associated with shifts in longevity. This suggests that natural selection is working on these networks to alter their function across these taxonomic groups. Future work is needed to test functional consequences of this diversifying selection on life history and rates of senescence.

In the context of the process of senescence within a lifetime we have essentially no knowledge of how these networks are functioning in reptiles and if their function varies across reptile and mammal species. As with most aspects of aging biology, the majority of study has been on lab rodent models. Mammals have relatively high levels of IGF1 (protein and gene expression) compared to reptiles. As well, lab rodents, in contrast to humans, do not express IGF2 as adults (Brown et al., 1986). Reptiles are more similar to humans in that they express IGF2 throughout their lifespan (Reding et al., 2016), and could be used to study IGF2 in the context of natural aging. Biomarkers of senescence related to these pathways from mammalian studies, such as the decrease in IGF1 with age, have yet to be investigated in reptiles in longitudinal or even cross-sectional studies.

In addition to the IIS/TOR pathway, increased signaling in AMPK and sirtuins is associated with longevity in lab models. Genes in the NAD+/sirtuin pathway, of interest due to connections to FOXO and mitochondrial signaling, are conserved in reptiles and appear

relatively evolutionarily constrained, particularly with respect to fishes (Gaur et al., 2017). Of note, SIRT2, a member of the sirtuin family of protein deacetylases and involved in cellular stress responses and tumor suppression, demonstrates some positively selected sites in reptiles (Gaur et al., 2017). The functional significance of positive selection in SIRT2, the positive selection across p53 and IIS/TOR networks for reptiles remains unknown at this time.

4 | CONCLUSIONS

Ectothermic reptiles possess several features ideally suited for increasing our understanding of the evolution of aging—including many with the life-history patterns of indeterminate growth and increasing fecundity with age, and many with plastic physiologies adapted to coping with stressful environments throughout the course of life. We believe that the ultimate cause of variation in longevity is its dependency on the evolution of correlated life-history traits. Reptiles have three times more variation in life history traits than mammals (e.g. Babich Morrow et al. 2019), representing vast evolutionary potential for innovative solutions to senescence.

This review has uncovered key aspects of biology where an understanding in reptiles would facilitate a broad understanding of the biology of aging. Foremost is further characterization of senescence in reptiles, and whether biomarkers of aging in mammals are robust across reptile species. For example, we have yet to reach a conclusion on telomere dynamics with age in reptiles. And, while increasing levels of *p16* (CDKN2A) and decreasing IGF1 are the best-known biomarkers of senescence in organismal and cell culture studies (Krishnamurthy et al. 2004; Ressler et al. 2006; Sanders et al. 2018), we have no information on how or if this translates to reptiles. Even so, from rapidly evolving molecular networks that regulate aging, to regenerative capacities, and to adaptations to extreme

environmental stress, innovations in the reptile lineage have already brought new insights into the biology of aging and are promising avenues of future research.

Comparative study of reptile aging requires knowledge of the demography, ecology, physiology, and genomics of more reptile species. We recommend four actions to remediate these deficiencies: (1) extend and expand long-term population monitoring of reptiles, (2) develop reptile cell lines to aid cellular biology (3) conduct more comparative studies of reptile morphology and physiology sampled along relevant life-history axes and (4) sequence the genomes of more reptile species to help solve the sequencing disparity (David, Wilson, & Halanych, 2019). Given the untapped potential of reptile biodiversity, and its particular alignment with relevant dimensions of aging biology, acting on these research initiatives promises fruitful rewards for our understanding of how and why animals age.

Author Contributions

All authors conducted literature review, drafted sections, and contributed to the final manuscript.

Data Archiving

This manuscript does not use data.

References

Abrams, P.A. (1993) Does increased mortality favor the evolution of more rapid senescence? *Evolution,* **47,** 877-887.

Allison D.B., Antoine L.H., Ballinger S.W. et al. (2014) Aging and energetics' 'Top 40' future research opportunities 2010-2013. *F1000Research*, **3**, 219.

Alper, S.J., Bronikowski, A.M. & Harper, J.M. (2015) Comparative cellular biogerontology: Where do we stand? *Experimental Gerontology,* **71,** 109-117.

Alvarado, A.S. (2000) Regeneration in the metazoans: why does it happen? *Bioessays,* **22,** 578-590.

Andrew, A.L., Perry, B.W., Card, D.C., Schield, D.R., Ruggiero, R.P., McGaugh, S.E., Choudhary, A., Secor, S.M. & Castoe, T.A. (2017) Growth and stress response mechanisms underlying post-feeding regenerative organ growth in the Burmese python. *BMC Genomics,* **18**.

Austad, S.N. (2010) Methusaleh's zoo: how nature provides us with clues for extending human health span. *Journal of Comparative Pathology,* **141,** S10-S21.

Austad, S.N. & Fischer, K.E. (2016) Sex differences in lifespan. *Cell Metabolism,* **23,** 1022-1033.

Avens, L., Goshe, L.R., Coggins, L., Shaver, D.J., Higgins, B., Landry, A.M. & Bailey, R. (2017) Variability in age and size at maturation, reproductive longevity, and long-term growth dynamics for Kemp's ridley sea turtles in the Gulf of Mexico. *PLOS ONE,* **12**.

Avens, L., Goshe, L.R., Coggins, L., Snover, M.L., Pajuelo, M., Bjorndal, K.A. & Bolten, A.B. (2015) Age and size at maturation-and adult-stage duration for loggerhead sea turtles in the western North Atlantic. *Marine Biology*, **162,** 1749-1767.

Babich Morrow, C., Kerkhoff, A.J. & Ernest, S.K.M. (2019) Macroevolution of dimensionless life history metrics in tetrapods. *bioRxiv***,** 520361.

Barja, G. & Herrero, A. (2000) Oxidative damage to mitochondrial DNA is inversely related to maximum life span in the heart and brain of mammals. *Faseb Journal,* **14,** 312-318.

Bartke, A. (2017) Somatic growth, aging, and longevity. *Npj Aging and Mechanisms of Disease*, **3**.

Bartke, A., Sun, L.Y. & Longo, V. (2013) Somatotropic signaling: trade-offs between growth, reproductive development, and longevity. *Physiological Reviews,* **93,** 571-598.

Barzilai, N., Huffman, D.M., Muzumdar, R.H. & Bartke, A. (2012) The critical role of metabolic pathways in aging. *Diabetes*, **61,** 1315-1322.

Beverton, R. & Holt, S. (1959) A review of the lifespans and mortality rates of fish in nature, and their relation to growth and other physiological characteristics. *Ciba Foundation Symposium-The Lifespan of Animals (Colloquia on Ageing)*, pp. 142-180. Wiley Online Library.

Blackburn, E.H. (2005) Telomeres and telomerase: their mechanisms of action and the effects of altering their functions. *Febs Letters,* **579,** 859-862.

Blanco, M.A. & Sherman, P.W. (2005) Maximum longevities of chemically protected and non-protected fishes, reptiles, and amphibians support evolutionary hypotheses of aging. *Mech Ageing Dev,* **126,** 794-803.

Booth, L.N. & Brunet, A. (2016) The aging epigenome. *Molecular Cell,* **62,** 728-744.

Brand, M.D. & Nicholls, D.G. (2011) Assessing mitochondrial dysfunction in cells (vol 435, pg 297, 2011). *Biochemical Journal,* **437,** 575-575.

Bronikowski, A.M. (2008) The evolution of aging phenotypes in snakes: a review and synthesis with new data. *Age*, **30,** 169-176.

Bronikowski, A.M., Alberts, S.C., Altmann, J., Packer, C., Carey, K.D. & Tatar, M. (2002) The aging baboon: Comparative demography in a non-human primate. *Proceedings of the National Academy of Sciences of the United States of America*, **99,** 9591-9595.

Bronikowski, A.M., Altmann, J., Brockman, D.K., Cords, M., Fedigan, L.M., Pusey, A., Stoinski, T., Morris, W.F., Strier, K.B. & Alberts, S.C. (2011) Aging in the natural world: Comparative data reveal similar mortality patterns across primates. *Science*, **331,** 1325-1328.

Brown, A.L., Graham, D.E., Nissley, S.P., Hill, D.J., Strain, A.J. & Rechler, M.M. (1986) Developmental regulation of insulin-like growth factor II messenger-RNA in different rat tissues. *Journal of Biological Chemistry*, **261,** 3144-3150.

Brown, W.S. (2016) Lifetime reproduction in a northern metapopulation of timber rattlesnakes (*Crotalus horridus*). *Herpetologica*, **72,** 331-342.

Brown, W.S & Simon, M.G. 2018. Record life span in a population of timber rattlesnakes (Crotalus horridus). *Herpetological Review*, **49**, 442-445.

Campisi, J. (2018) Cellular senescence, aging, and cancer. *Innovation in Aging*, **2,** 798-798.

Caro, P., Gomez, J., Lopez-Torres, M., Sanchez, I., Naudi, A., Portero-Otin, M., Pamplona, R. & Barja, G. (2008) Effect of every other day feeding on mitochondrial free radical production and oxidative stress in mouse liver. *Rejuvenation Research*, **11,** 621-629.

Castanet, J. (1994) Age estimation and longevity in reptiles. *Gerontology*, **40,** 174-192.

Chaloupka, M. & Limpus, C. (2005) Estimates of sex- and age-class-specific survival probabilities for a southern Great Barrier Reef green sea turtle population. *Marine Biology*, **146,** 1251-1261.

Chaloupka, M.Y. & Limpus, C.J. (2002) Survival probability estimates for the endangered loggerhead sea turtle resident in southern Great Barrier Reef waters. *Marine Biology*, **140,** 267-277.

Chaloupka, M.Y. & Musick, J.A. (1997) Age, growth, and population dynamics *Biology of sea turtles* (eds P.L. Lutz & J.A. Musick), pp. 233-276. CRC Press, Boca Raton, FL, USA.

Chang, J., Knowlton, A.A. & Wasser, J.S. (2000) Expression of heat shock proteins in turtle and mammal hearts: relationship to anoxia tolerance. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology*, **278,** R209-R214.

Charlesworth, B. (2000) Fisher, Medawar, Hamilton and the evolution of aging. *Genetics*, **156,** 927-931.

Christiansen, J.L., Henderson, E.R., Budke, B., Lynch, M., Lu, Q. & Johnson, J. (2001) A final report of studies of the Hayflick limit in reptiles, a test of potential immortality. *Proceedings of the Iowa Space Grant Consortium*, **10**.

Colchero, F., Jones, O.R., Conde, D.A., Hodgson, D., Zajitschek, F., Schmidt, B.R., Malo, A.E., Alberts, S.C., Becker, P.H., Bouwhuis, S., Bronikowski, A.M., De Vleeschouwer, K.M., Delahay, R.J., Dummermuth, S., Fernandez-Duque, E., Frisenvaenge, J., Hesselsoe, M., Larson, S., Lemaitre, J.F., McDonald, J., Miller, D.A.W., O'donnell, C., Packer, C., Raboy, B.E., Reading, C.J., Wapstra, E., Weimerskirch, H., While, G.M., Baudisch, A., Flatt, T., Coulson, T. & Gaillard, J.M. (2019) The diversity of population responses to environmental change. *Ecology Letters*, **22,** 342-353.

Congdon, J.D., Gibbons, J.W., Brooks, R.J., Rollinson, N. & Tsaliagos, R.N. (2013) Indeterminate growth in long-lived freshwater turtles as a component of individual fitness. *Evolutionary Ecology*, **27,** 445-459.

Congdon, J.D., Nagle, R.D., Kinney, O.M., van Loben Sels, R.C., Quinter, T. & Tinkle, D.W. (2003) Testing hypotheses of aging in long-lived painted turtles (*Chrysemys picta*). *Exp Gerontol,* **38,** 765-772.

Congdon, J.D. & van Loben Sels, R.C. (1993) Relationships of reproductive traits and body-size with attainment of sexual maturity and age in Blanding's turtles (*Emydoidea blandingi*). *Journal of Evolutionary Biology,* **6,** 547-557.

Dammhahn, M., Dingemanse, N.J., Niemela, P.T. & Reale, D. (2018) Pace-of-life syndromes: a framework for the adaptive integration of behaviour, physiology and life history. *Behavioral Ecology and Sociobiology*, **72**.

Dantzer, B. & Fletcher, Q.E. (2015) Telomeres shorten more slowly in slow-aging wild animals than in fast-aging ones. *Experimental Gerontology,* **71,** 38-47.

David, K.T., Wilson, A.E. & Halanych, K.M. (2019) Sequencing disparity in the genomic era. *Molecular Biology and Evolution*.

de Magalhaes, J.P. & Costa, J. (2009) A database of vertebrate longevity records and their relation to other life-history traits. *Journal of Evolutionary Biology*, **22,** 1770-1774.

de Paoli-Iseppi, R., Deagle, B.E., McMahon, C.R., Hindell, M.A., Dickinson, J.L. & Jarman, S.N. (2017) Measuring animal age with DNA methylation: From humans to wild animals. Frontiers in Genetics, **8**.

Derocher, A.E. & Wiig, O. (2002) Postnatal growth in body length and mass of polar bears (*Ursus maritimus*) at Svalbard. *Journal of Zoology*, **256,** 343-349.

Eckhardt, F., Kappeler, P.M. & Kraus, C. (2017) Highly variable lifespan in an annual reptile, Labord's chameleon (*Furcifer labordi*). *Scientific Reports,* **7**.

Finch, C.E. (2009) Update on slow aging and negligible senescence—a mini-review. *Gerontology,* **55,** 307-313.

Flouris, A.D. & Piantoni, C. (2015) Links between thermoregulation and aging in endotherms and ectotherms. *Temperature,* **2,** 73-85.

Fontana, L., Partridge, L. & Longo, V.D. (2010) Extending healthy life span—from yeast to humans. *Science,* **328,** 321-326.

Gangloff, E.J., Holden, K.G., Telemeco, R.S., Baumgard, L.H. & Bronikowski, A.M. (2016) Hormonal and metabolic responses to upper temperature extremes in divergent life-history ecotypes of a garter snake. *Journal of Experimental Biology,* **219,** 2944-2954.

Garner, J.A. (2012) Reproductive Endocrinology of Nesting Leatherback Sea Turtles in St. Croix, US Virgin Islands. (Doctoral dissertation) Texas A&M University.

Garner, L.A. (2016) Population Status of the American Alligator (*Alligator mississippiensis*) in North Carolina. (Masters dissertation) North Carolina State University.

Gaur, U., Tu, J., Li, D., Gao, Y., Lian, T., Sun, B., Yang, D., Fan, X. & Yang, M. (2017) Molecular evolutionary patterns of NAD+/Sirtuin aging signaling pathway across taxa. *PLOS ONE,* **12,** e0182306.

Gibbons, J.W. (1987) Why do turtles live so long? *Bioscience,* **37,** 262-269.

Gill, F.B. (2006) *Ornithology,* 3rd edn. W.H. Freeman, New York.

Gladyshev, V.N. (2013) The origin of aging: imperfectness-driven non-random damage defines the aging process and control of lifespan. *Trends in Genetics,* **29,** 506-512.

Gomes, N.M.V., Shay, J.W. & Wright, W.E. (2010) Telomere biology in Metazoa. *Febs Letters,* **584,** 3741-3751.

Gomez, J., Caro, P., Naudi, A., Portero-Otin, M., Pamplona, R. & Barja, G. (2007) Effect of 8.5% and 25% caloric restriction on mitochondrial free radical production and oxidative stress in rat liver. *Biogerontology,* **8,** 555-566.

Greer, E.L. & Brunet, A. (2008) Signaling networks in aging. *Journal of Cell Science,* **121,** 407-412.

Guraya, S.S. (1989) Germinal beds, oogenesis and folliculogenesis. *Ovarian Follicles in Reptiles and Birds*, pp. 6-21. Springer.
Hamilton, W.D. (1966) The moulding of senescence by natural selection. *Journal of theoretical biology,* **12,** 12-45.
Hariharan, I.K., Wake, D.B. & Wake, M.H. (2016) Indeterminate growth: Could it represent the ancestral condition? *Cold Spring Harbor Perspectives in Biology,* **8**.
Harrison, P.M., Khachane, A. & Kumar, M. (2010) Genomic assessment of the evolution of the prion protein gene family in vertebrates. *Genomics,* **95,** 268-277.
Hatase, H., Sudo, R., Watanabe, K.K., Kasugai, T., Saito, T., Okamoto, H., Uchida, I. & Tsukamoto, K. (2008) Shorter telomere length with age in the loggerhead turtle: a new hope for live sea turtle age estimation. *Genes & Genetic Systems,* **83,** 423-426.
Hayflick, L. & Moorhead, P.S. (1961) The serial cultivation of human diploid cell strains. *Experimental cell research,* **25,** 585-621.
Head, J.A., Mittal, K. & Basu, N. (2014) Application of the LUminometric methylation assay to ecological species: tissue quality requirements and a survey of DNA methylation levels in animals. *Molecular Ecology Resources,* **14,** 943-952.
Heikkila, J.J. (2017) The expression and function of hsp30-like small heat shock protein genes in amphibians, birds, fish, and reptiles. *Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology,* **203,** 179-192.
Hoekstra, L.A., Weber, R.C., Bronikowski, A.M. & Janzen, F.J. (2018) Sex-specific growth, shape, and their impacts on life history of a long-lived vertebrate. *Evolutionary Ecology Research,* **19**, 639-657.
Horvath, S. & Raj, K. (2018) DNA methylation-based biomarkers and the epigenetic clock theory of ageing. *Nature Reviews Genetics,* **19,** 371-384.
Hossie, T.J., Hassall, C., Knee, W. & Sherratt, T.N. (2013) Species with a chemical defence, but not chemical offence, live longer. *Journal of Evolutionary Biology,* **26,** 1598-1602.
Hulbert, A.J. (2010) Metabolism and longevity: Is there a role for membrane fatty acids? *Integrative and Comparative Biology,* **50,** 808-817.
Hulbert, A.J., Pamplona, R., Buffenstein, R. & Buttemer, W.A. (2007) Life and death: metabolic rate, membrane composition, and life span of animals. *Physiological Reviews,* **87,** 1175-1213.
Isaksson, C., While, G.M., Olsson, M., Komdeur, J. & Wapstra, E. (2011) Oxidative stress physiology in relation to life history traits of a free-living vertebrate: the spotted snow skink, *Niveoscincus ocellatus*. *Integrative Zoology,* **6,** 140-149.
Jacyniak, K., McDonald, R.P. & Vickaryous, M.K. (2017) Tail regeneration and other phenomena of wound healing and tissue restoration in lizards. *Journal of Experimental Biology,* **220,** 2858-2869.
Jena, B.S., Das, S. & Patnaik, B.K. (1995) Effect of age on lipid-peroxidation in a short-lived species of reptile, *Calotes versicolor*. *Archives of Gerontology and Geriatrics,* **20,** 263-272.
Jena, B.S., Das, S. & Patnaik, B.K. (1996) Age related modifications of soluble proteins in various organs of male garden lizard, *Calotes versicolor*. *Archives of Gerontology and Geriatrics,* **22,** 1-10.
Jones, O.R., Scheuerlein, A., Salguero-Gomez, R., Camarda, C.G., Schaible, R., Casper, B.B., Dahlgren, J.P., Ehrlen, J., Garcia, M.B., Menges, E.S., Quintana-Ascencio, P.F., Caswell, H., Baudisch, A. & Vaupel, J.W. (2014) Diversity of ageing across the tree of life. *Nature,* **505,** 169.
Kennedy, B.K., Berger, S.L., Brunet, A., Campisi, J., Cuervo, A.M., Epel, E.S., Franceschi, C., Lithgow, G.J., Morimoto, R.I., Pessin, J.E., Rando, T.A., Richardson, A., Schadt,

E.E., Wyss-Coray, T. & Sierra, F. (2014) Geroscience: linking aging to chronic disease. *Cell,* **159,** 708-712.
Klaips, C.L., Jayaraj, G.G. & Hartl, F.U. (2018) Pathways of cellular proteostasis in aging and disease. *Journal of Cell Biology,* **217,** 51-63.
Koons, D.N., Birkhead, R.D., Boback, S.M., Williams, M.I. & Greene, M.P. (2009) The effect of body size on cottonmouth (*Agkistrodon piscivorus*) survival, recapture probability, and behavior in an Alabama swamp. *Herpetological Conservation and Biology,* **4,** 221-235.
Krishnamurthty, J., Torrice, C., Ramsey, M.R., Kovalev, G.I., Al-Regaiey, K., Su, L.S. & Sharpless, N.E. (2004) Ink4a/Arf expression is a biomarker of aging. *Journal of Clinical Investigation,* **114,** 1299-1307.
Krivoruchko, A., Storey, K.B. (2010) Regulation of the heat shock response under anoxia in the turtle Trachemys scripta elegans. J*ournal of Comparative Physiology B,* **180**, 403-414.
Ku, H.H., Brunk, U.T. & Sohal, R.S. (1993) Relationship between mitochondrial superoxide and hydrogen-peroxide production and longevity of mammalian-species. *Free Radical Biology and Medicine,* **15,** 621-627.
Labbadia, J. & Morimoto, R.I. (2015) The biology of proteostasis in aging and disease. *Annual Review of Biochemistry,* **84,** 435-464.
Lahdenperä, M., Mar, K.U., Courtiol, A., & Lummaa, V. (2018) Differences in age-specific mortality between wild-caught and captive-born Asian elephants. *Nature communications*, **9**, 3023.
Lambert, A.J., Boysen, H.M., Buckingham, J.A., Yang, T., Podlutsky, A., Austad, S.N., Kunz, T.H., Buffenstein, R. & Brand, M.D. (2007) Low rates of hydrogen peroxide production by isolated heart mitochondria associate with long maximum lifespan in vertebrate homeotherms. *Aging Cell,* **6,** 607-618.
Liu, Y., Zhou, Q., Wang, Y.J., Luo, L.H., Yang, J., Yang, L.F., Liu, M., Li, Y.R., Qian, T.M., Zheng, Y., Li, M.Y., Li, J., Gu, Y., Han, Z.J., Xu, M., Wang, Y.J., Zhu, C.L., Yu, B., Yang, Y.M., Ding, F., Jiang, J.P., Yang, H.M. & Gu, X.S. (2015) *Gekko japonicus* genome reveals evolution of adhesive toe pads and tail regeneration. *Nature Communications,* **6**.
Lopez-Otin, C., Blasco, M.A., Partridge, L., Serrano, M. & Kroemer, G. (2013) The hallmarks of aging. *Cell,* **153,** 1194-1217.
Lozito, T.P. & Tuan, R.S. (2017) Lizard tail regeneration as an instructive model of enhanced healing capabilities in an adult amniote. *Connective Tissue Research,* **58,** 145-154.
Lyson, T.R., Rubidge, B.S., Scheyer, T.M., de Queiroz, K., Schachner, E.R., Smith, R.M.H., Botha-Brink, J. & Bever, G.S. (2016) Fossorial origin of the turtle shell. *Current Biology,* **26,** 1887-1894.
MacRae, S.L., Croken, M.M., Calder, R., Aliper, A., Milholland, B., White, R.R., Zhavoronkov, A., Gladyshev, V.N., Seluanov, A. & Gorbunova, V. (2015) DNA repair in species with extreme lifespan differences. *Aging (Albany NY),* **7,** 1171.
Manibabu, P.V. & Patnaik, B.K. (1991) Age-related changes in brain and spinal-cord lipids of the male garden lizard. *Archives of Gerontology and Geriatrics,* **13,** 43-50.
Massot, M., Clobert, J., Montes-Poloni, L., Haussy, C., Cubo, J. & Meylan, S. (2011) An integrative study of ageing in a wild population of common lizards. *Functional Ecology,* **25,** 848-858.
Maue, A.C. & Haynes, L. (2009) CD4+T Cells and Immunosenescence - A Mini-Review. *Gerontology,* **55,** 491-495.

Maynard, S., Fang, E.F., Scheibye-Knudsen, M., Croteau, D.L. & Bohr, V.A. (2015) DNA damage, DNA repair, aging, and neurodegeneration. *Cold Spring Harbor Perspectives in Medicine*, **5**.
McDiarmid, R.W., Foster, M.S., Guyer, C., Chernoff, N. & Gibbons, J.W. (2012) *Reptile biodiversity: standard methods for inventory and monitoring*. Univ of California Press.
McGaugh, S. & Schwartz, T.S. (2017) Here and there, but not everywhere: repeated loss of uncoupling protein 1 in amniotes. *Biology Letters*, **13**.
McGaugh, S.E., Bronikowski, A.M., Kuo, C.H., Reding, D.M., Addis, E.A., Flagel, L.E., Janzen, F.J. & Schwartz, T.S. (2015) Rapid molecular evolution across amniotes of the IIS/TOR network. *Proc Natl Acad Sci U S A*, **112,** 7055-7060.
McKenzie, J., Page, B., Goldsworthy, S.D. & Hindell, M.A. (2007) Growth strategies of New Zealand fur seals in southern Australia. *Journal of Zoology*, **272,** 377-389.
Medawar, P.B. (1952) *An unsolved problem of biology*. Lewis, London.
Miller, D.A., Clark, W.R., Arnold, S.J. & Bronikowski, A.M. (2011) Stochastic population dynamics in populations of western terrestrial garter snakes with divergent life histories. *Ecology*, **92,** 1658-1671.
Miller, D.A., Janzen, F.J., Fellers, G.M., Kleeman, P.M. & Bronikowski, A.M. (2014) Biodemography of ectothermic tetrapods provides insights into the evolution and plasticity of mortality patterns. *Sociality, Hierarchy, Health: Comparative Biodemography, National Academies Press, Washington*.
Miller, J.K. (2001) Escaping senescence: demographic data from the three-toed box turtle (*Terrapene carolina triunguis*). *Exp Gerontol*, **36,** 829-832.
Moorad, J., Promislow, D. & Silvertown, J. (2019) Evolutionary ecology of senescence and a reassessment of Williams' "extrinsic mortality" hypothesis. *Trends in Ecology & Evolution*.
Mumby, H.S., Chapman, S.N., Crawley, J.A.H., Mar, K.U., Htut, W., Soe, A.T., Aung, H.H. & Lummaa, V. (2015) Distinguishing between determinate and indeterminate growth in a long-lived mammal. *Bmc Evolutionary Biology*, **15**.
Nilsen, F.M., Parrott, B.B., Bowden, J.A., Kassim, B.L., Somerville, S.E., Bryan, T.A., Bryan, C.E., Lange, T.R., Delaney, J.P., Brunell, A.M., Long, S.E. & Guillette, L.J. (2016) Global DNA methylation loss associated with mercury contamination and aging in the American alligator (*Alligator mississippiensis*). *Science of the Total Environment*, **545,** 389-397.
Olsson, M., Pauliny, A., Wapstra, E., Uller, T., Schwartz, T. & Blomqvist, D. (2011a) Sex differences in sand lizard telomere inheritance: paternal epigenetic effects increases telomere heritability and offspring survival. *PLOS ONE*, **6**.
Olsson, M., Pauliny, A., Wapstra, E., Uller, T., Schwartz, T., Miller, E. & Blomqvist, D. (2011b) Sexual differences in telomere selection in the wild. *Molecular Ecology*, **20,** 2085-2099.
Olsson, M., Wapstra, E. & Friesen, C. (2018) Ectothermic telomeres: it's time they came in from the cold. *Philosophical Transactions of the Royal Society B-Biological Sciences*, **373**.
Olsson, M., Wilson, M., Uller, T. & Isaksson, C. (2009) Free radicals run in lizard families without (and perhaps with) mitochondrial uncoupling. *Biology Letters*, **5,** 345-346.
Olsson, M., Wilson, M., Uller, T., Mott, B., Isaksson, C., Healey, M. & Wanger, T. (2008) Free radicals run in lizard families. *Biology Letters*, **4,** 186-188.
Omeyer, L.C.M., Fuller, W.J., Godley, B.J., Snape, R.T.E. & Broderick, A.C. (2018) Determinate or indeterminate growth? Revisiting the growth strategy of sea turtles. *Marine Ecology Progress Series*, **596,** 199-211.

Oppliger, A. & Clobert, J. (1997) Reduced tail regeneration in the Common Lizard, *Lacerta vivipara*, parasitized by blood parasites. *Functional Ecology*, **11,** 652-655.
Page, M.M. & Stuart, J.A. (2012) Activities of DNA base excision repair enzymes in liver and brain correlate with body mass, but not lifespan. *Age*, **34,** 1195-1209.
Pal, S. & Tyler, J.K. (2016) Epigenetics and aging. *Science Advances*, **2**.
Palacios, M.G. & Bronikowski, A.M. (2017) Immune variation during pregnancy suggests immune component-specific costs of reproduction in a viviparous snake with disparate life-history strategies. *Journal of Experimental Zoology Part a-Ecological and Integrative Physiology*, **327,** 513-522.
Palacios, M.G., Cunnick, J.E. & Bronikowski, A.M. (2013) Complex interplay of body condition, life history, and prevailing environment shapes immune defenses of garter snakes in the wild. *Physiological and Biochemical Zoology*, **86,** 547-558.
Palacios, M.G., Sparkman, A.M. & Bronikowski, A.M. (2011) Developmental plasticity of immune defence in two life-history ecotypes of the garter snake, Thamnophis elegans–a common-environment experiment. *Journal of Animal Ecology*, **80,** 431-437.
Palackdharry, S., Sadd, B.M., Vogel, L.A. & Bowden, R.M. (2017) The effect of environmental temperature on reptilian peripheral blood B cell functions. *Hormones and Behavior*, **88,** 87-94.
Parrott, B.B. & Bertucci, E.M. (2019) Epigenetic aging clocks in ecology and evolution. *Trends in Ecology & Evolution*, **In Press**.
Passow, C.N., Bronikowski, A.M., Blackmon, H., Parsai, S., Schwartz, T.S. & McGaugh, S.E. (2019) Contrasting patterns of rapid molecular evolution within the p53 network across mammal and sauropsid lineages. *Genome Biol Evol*.
Perez, V.I., Buffenstein, R., Masamsetti, V., Leonard, S., Salmon, A.B., Mele, J., Andziak, B., Yang, T., Edrey, Y., Friguet, B., Ward, W., Richardson, A. & Chaudhuri, A. (2009) Protein stability and resistance to oxidative stress are determinants of longevity in the longest-living rodent, the naked mole-rat. *Proceedings of the National Academy of Sciences of the United States of America*, **106,** 3059-3064.
Promislow, D.E.L. (1994) DNA-repair and the evolution of longevity—a critical analysis. *Journal of theoretical biology*, **170,** 291-300.
Promislow, D.E.L. & Bronikowski, A. (2006) Evolutionary genetics of senescence. *Evolutionary Genetics: Concepts and Case Studies* (eds C.W. Fox & J.B. Wolf), pp. 464–481. Oxford University Press, Oxford, UK.
Quesada, V., Freitas-Rodriguez, S., Miller, J., Perez-Silva, J.G., Jiang, Z.F., Tapia, W., Santiago-Fernandez, O., Campos-Iglesias, D., Kuderna, L.F.K., Quinzin, M., Alvarez, M.G., Carrero, D., Beheregaray, L.B., Gibbs, J.P., Chiari, Y., Glaberman, S., Ciofi, C., Araujo-Voces, M., Mayoral, P., Arango, J.R., Tamargo-Gomez, I., Roiz-Valle, D., Pascual-Torner, M., Evans, B.R., Edwards, D.L., Garrick, R.C., Russello, M.A., Poulakakis, N., Gaughran, S.J., Rueda, D.O., Bretones, G., Marques-Bonet, T., White, K.P., Caccone, A. & Lopez-Otin, C. (2019) Giant tortoise genomes provide insights into longevity and age-related disease. *Nature Ecology & Evolution*, **3,** 87-95.
Rando, T.A. & Chang, H.Y. (2012) Aging, rejuvenation, and epigenetic reprogramming: resetting the aging Clock. *Cell*, **148,** 46-57.
Rayl, J.M., Wellehan, J.F.X., Bunick, D. & Allender, M.C. (2019) Development of reverse-transcriptase quantitative PCR assays for detection of the cytokines IL-1β, TNF-α, and IL-10 in chelonians. *Cytokine*, **119,** 16-23.
Reding, D.M., Addis, E.A., Palacios, M.G., Schwartz, T.S. & Bronikowski, A.M. (2016) Insulin-like signaling (IIS) responses to temperature, genetic background, and growth

variation in garter snakes with divergent life histories. *Gen Comp Endocrinol,* **233,** 88-99.

Refsnider, J.M., Palacios, M.G., Reding, D.M. & Bronikowski, A.M. (2015) Effects of a novel climate on stress response and immune function in painted turtles (*Chrysemys picta*). *Journal of Experimental Zoology Part a-Ecological Genetics and Physiology,* **323,** 160-168.

Regan, J.D., Carrier, W., Samet, C. & Olla, B.L. (1982) Photoreactivation in two closely related marine fishes having different longevities. *Mechanisms of ageing and development,* **18,** 59-66.

Reinke, B.A., Hoekstra, L.A., Bronikowski, A.M., Janzen, F.J. & Miller, D.A. (2019) Joint estimation of growth and survival from mark-recapture data to improve estimates of senescence in wild populations. *Ecology,* **In review**.

Ressler, S., Bartkova, J., Niederegger, H., Bartek, J., Scharffetter-Kochanek, K., Jansen-Durr, P. & Wlaschek, M. (2006) p16(INK4A) is a robust in vivo biomarker of cellular aging in human skin. *Aging Cell,* **5,** 379-389.

Rhodin, A.G.J., Stanford, C.B., van Dijk, P.P., Eisemberg, C., Luiselli, L., Mittermeier, R.A., Hudson, R., Horne, B.D., Goode, E.V., Kuchling, G., Walde, A., Baard, E.H.W., Berry, K.H., Bertolero, A., Blanck, T.E.G., Bour, R., Buhlmann, K.A., Cayot, L.J., Collett, S., Currylow, A., Das, I., Diagne, T., Ennen, J.R., Forero-Medina, G., Frankel, M.G., Fritz, U., Garcia, G., Gibbons, J.W., Gibbons, P.M., Gong, S.P., Guntoro, J., Hofmeyr, M.D., Iverson, J.B., Kiester, A.R., Lau, M., Lawson, D.P., Lovich, J.E., Moll, E.O., Paez, V.P., Palomo-Ramos, R., Platt, K., Platt, S.G., Pritchard, P.C.H., Quinn, H.R., Rahman, S.C., Randrianjafizanaka, S.T., Schaffer, J., Selman, W., Shaffer, H.B., Sharma, D.S.K., Shi, H.T., Singh, S., Spencer, R., Stannard, K., Sutcliffe, S., Thomson, S. & Vogt, R.C. (2018) Global conservation status of turtles and tortoises (Order Testudines). *Chelonian Conservation and Biology,* **17,** 135-161.

Rice, M.E., Forman, R.E., Chen, B.T., Avshalumov, M.V., Cragg, S.J. & Drew, K.L. (2002) Brain antioxidant regulation in mammals and anoxia-tolerant reptiles: balanced for neuroprotection and neuromodulation. *Comparative Biochemistry and Physiology C-Toxicology & Pharmacology,* **133,** 515-525.

Richard, M., Massot, M., Clobert, J. & Meylan, S. (2012) Litter quality and inflammatory response are dependent on mating strategy in a reptile. *Oecologia,* **170,** 39-46.

Ricklefs, R.E. (2010) Insights from comparative analyses of aging in birds and mammals. *Aging Cell,* **9,** 273-284.

Riera, C.E., Merkwirth, C., De Magalhaes, C.D. & Dillin, A. (2016) Signaling networks determining life span. *Annual Review of Biochemistry, Vol 85,* **85,** 35-64.

Robert, K.A. & Bronikowski, A.M. (2010) Evolution of senescence in nature: physiological evolution in populations of garter snake with divergent life histories. *Am Nat,* **175,** 147-159.

Robert, K.A., Brunet-Rossinni, A. & Bronikowski, A.M. (2007) Testing the 'free radical theory of aging' hypothesis: physiological differences in long-lived and short-lived colubrid snakes. *Aging Cell,* **6,** 395-404.

Rollings, N., Uhrig, E.J., Krohmer, R.W., Waye, H.L., Mason, R.T., Olsson, M., Whittington, C.M. & Friesen, C.R. (2017) Age-related sex differences in body condition and telomere dynamics of red-sided garter snakes. *Proceedings of the Royal Society B-Biological Sciences,* **284**.

Ross, C.A. & Poirier, M.A. (2004) Protein aggregation and neurodegenerative disease. *Nature Medicine,* **10,** S10-S17.

Ruby, J.G., Smith, M. & Buffenstein, R. (2018) Naked mole-rat mortality rates defy Gompertzian laws by not increasing with age. *elife*, **7,** e31157.

Ruf, T., Bieber, C. & Turbill, C. (2012) Survival, Aging, and Life-History Tactics in Mammalian Hibernators. *Living in a seasonal world: thermoregulatory and metabolic adaptations* (eds T. Ruf, C. Bieber, W. Arnold & E. Millesi), pp. xiv, 563 p. Springer, Berlin, Heidelberg.

Salmon, A.B., Richardson, A. & Perez, V.I. (2010) Update on the oxidative stress theory of aging: Does oxidative stress play a role in aging or healthy aging? *Free Radical Biology and Medicine*, **48,** 642-655.

Sanchez, E. & Refsnider, J.M. (2017) Immune activity, but not physiological stress, differs between the sexes during the nesting season in painted turtles. *Journal of Herpetology*, **51,** 449-453.

Sanchez-Roman, I., Gomez, A., Perez, I., Sanchez, C., Suarez, H., Naudi, A., Jove, M., Lopez-Torres, M., Pamplona, R. & Barja, G. (2012) Effects of aging and methionine restriction applied at old age on ROS generation and oxidative damage in rat liver mitochondria. *Biogerontology*, **13,** 399-411.

Sanders, J.L., Guo, W., O'Meara, E.S., Kaplan, R.C., Pollak, M.N., Bartz, T.M., Newman, A.B., Fried, L.P. & Cappola, A.R. (2017) Trajectories of IGF-I predict mortality in older adults: the Cardiovascular Health Study. *The Journals of Gerontology: Series A*, **73,** 953-959.

Sauer, J.R. & Slade, N.A. (1987) Size-based demography of vertebrates. *Annual Review of Ecology and Systematics*, **18,** 71-90.

Schield, D.R., Card, D.C., Hales, N.R., Perry, B.W., Pasquesi, G.M., Blackmon, H., Adams, R.H., Corbin, A.B., Smith, C.F. & Ramesh, B. (2019) The origins and evolution of chromosomes, dosage compensation, and mechanisms underlying venom regulation in snakes. *Genome Research*.

Schwanz, L., Warner, D.A., McGaugh, S., Di Terlizzi, R. & Bronikowski, A. (2011) State-dependent physiological maintenance in a long-lived ectotherm, the painted turtle (*Chrysemys picta*). *J Exp Biol*, **214,** 88-97.

Schwartz, T.S., Arendsee, Z.W. & Bronikowski, A.M. (2015) Mitochondrial divergence between slow- and fast-aging garter snakes. *Exp Gerontol*, **71,** 135-146.

Schwartz, T.S. & Bronikowski, A.M. (2011) Molecular stress pathways and the evolution of life histories in reptiles. *Molecular Mechanisms of Life History Evolution* (eds T. Flatt & A. Heyland). Oxford University Press, Oxford, UK.

Schwartz, T.S. & Bronikowski, A.M. (2013) Dissecting molecular stress networks: identifying nodes of divergence between life-history phenotypes. *Mol Ecol*, **22,** 739-756.

Scott, N.M., Haussmann, M.F., Elsey, R.M., Trosclair, P.L. & Vleck, C.M. (2006) Telomere length shortens with body length in *Alligator mississippiensis*. *Southeastern Naturalist*, **5,** 685-692.

Secor, S.M. & Diamond, J. (1998) A vertebrate model of extreme physiological regulation. *Nature*, **395,** 659-662.

Sena, L.A. & Chandel, N.S. (2012) Physiological roles of mitochondrial reactive oxygen species. *Molecular Cell*, **48,** 158-167.

Shaffer, H.B., Minx, P., Warren, D.E., Shedlock, A.M., Thomson, R.C., Valenzuela, N., Abramyan, J., Amemiya, C.T., Badenhorst, D., Biggar, K.K., Borchert, G.M., Botka, C.W., Bowden, R.M., Braun, E.L., Bronikowski, A.M., Bruneau, B.G., Buck, L.T., Capel, B., Castoe, T.A., Czerwinski, M., Delehaunty, K.D., Edwards, S.V., Fronick, C.C., Fujita, M.K., Fulton, L., Graves, T.A., Green, R.E., Haerty, W., Hariharan, R., Hernandez, O., Hillier, L.W., Holloway, A.K., Janes, D., Janzen, F.J., Kandoth, C.,

Kong, L., de Koning, A.P., Li, Y., Literman, R., McGaugh, S.E., Mork, L., O'Laughlin, M., Paitz, R.T., Pollock, D.D., Ponting, C.P., Radhakrishnan, S., Raney, B.J., Richman, J.M., St John, J., Schwartz, T., Sethuraman, A., Spinks, P.Q., Storey, K.B., Thane, N., Vinar, T., Zimmerman, L.M., Warren, W.C., Mardis, E.R. & Wilson, R.K. (2013) The western painted turtle genome, a model for the evolution of extreme physiological adaptations in a slowly evolving lineage. *Genome Biol,* **14,** R28.

Shine, R. & Charnov, E.L. (1992) Patterns of survival, growth, and maturation in snakes and lizards. *American Naturalist,* **139,** 1257-1269.

Shine, R. & Iverson, J.B. (1995) Patterns of survival, growth and maturation in turtles. *Oikos,* **72,** 343-348.

Sohal, R.S., Ku, H.H., Agarwal, S., Forster, M.J. & Lal, H. (1994) Oxidative damage, mitochondrial oxidant generation and antioxidant defenses during aging and in response to food restriction in the mouse. *Mechanisms of ageing and development,* **74,** 121-133.

Sparkman, A.M., Arnold, S.J. & Bronikowski, A.M. (2007) An empirical test of evolutionary theories for reproductive senescence and reproductive effort in the garter snake Thamnophis elegans. *Proc Biol Sci,* **274,** 943-950.

Sparkman, A.M., Schwartz, T.S., Madden, J.A., Boyken, S.E., Ford, N.B., Serb, J.M. & Bronikowski, A.M. (2012) Rates of molecular evolution vary in vertebrates for insulin-like growth factor-1 (IGF-1), a pleiotropic locus that regulates life history traits. *Gen Comp Endocrinol,* **178,** 164-173.

Speakman, J.R., Blount, J.D., Bronikowski, A.M., Buffenstein, R., Isaksson, C., Kirkwood, T.B.L., Monaghan, P., Ozanne, S.E., Beaulieu, M., Briga, M., Carr, S.K., Christensen, L.L., Cocheme, H.M., Cram, D.L., Dantzer, B., Harper, J.M., Jurk, D., King, A., Noguera, J.C., Salin, K., Sild, E., Simons, M.J.P., Smith, S., Stier, A., Tobler, M., Vitikainen, E., Peaker, M. & Selman, C. (2015) Oxidative stress and life histories: unresolved issues and current needs. *Ecology and Evolution,* **5,** S745-S757.

Spencer, R.J. & Janzen, F.J. (2010) Demographic consequences of adaptive growth and the ramifications for conservation of long-lived organisms. *Biological Conservation,* **143,** 1951-1959.

Spencer, R.J. & Thompson, M.B. (2005) Experimental analysis of the impact of foxes on freshwater turtle populations. *Conservation Biology,* **19,** 845-854.

Stark, G., Tamar, K., Itescu, Y., Feldman, A. & Meiri, S. (2018) Cold and isolated ectotherms: drivers of reptilian longevity. *Biological Journal of the Linnean Society,* **125,** 730-740.

Stier, A., Dupoue, A., Picard, D., Angelier, F., Brischoux, F. & Lourdais, O. (2017) Oxidative stress in a capital breeder (*Vipera aspis*) facing pregnancy and water constraints. *Journal of Experimental Biology,* **220,** 1792-1796.

Sun, A.X., Londono, R., Hudnall, M.L., Tuan, R.S. & Lozito, T.P. (2018) Differences in neural stem cell identity and differentiation capacity drive divergent regenerative outcomes in lizards and salamanders. *Proceedings of the National Academy of Sciences of the United States of America,* **115,** Eb256-Eb265.

Takii, R., Fujimoto, M., Matsuura, Y., Wu, F.X., Oshibe, N., Takaki, E., Katiyar, A., Akashi, H., Makino, T., Kawata, M. & Nakai, A. (2017) HSF1 and HSF3 cooperatively regulate the heat shock response in lizards. *PLOS ONE,* **12**.

Tanaka, H., Horikawa, L., Barrett, J.C. & Oshimura, M. (2005) Evidence for inactivation of distinct telomerase repressor genes in different types of human cancers. *International Journal of Cancer,* **115,** 653-657.

Thorbjarnarson, J.B. (1996) Reproductive characteristics of the order Crocodylia. *Herpetologica,* **52,** 8-24.

Ujvari, B., Biro, P.A., Charters, J.E., Brown, G., Heasman, K., Beckmann, C. & Madsen, T. (2017) Curvilinear telomere length dynamics in a squamate reptile. *Functional Ecology,* **31,** 753-759.
Ujvari, B. & Madsen, T. (2009) Short telomeres in hatchling snakes: erythrocyte telomere dynamics and longevity in tropical pythons. *PLOS ONE,* **4**.
Uphoff, S., Reyes-Lamothe, R., de Leon, F.G., Sherratt, D.J. & Kapanidis, A.N. (2013) Single-molecule DNA repair in live bacteria. *Proceedings of the National Academy of Sciences of the United States of America,* **110,** 8063-8068.
Varriale, A. & Bernardi, G. (2006) DNA methylation in reptiles. *Gene,* **385,** 122-127.
Vaupel, J.W., Baudisch, A., Dolling, M., Roach, D.A. & Gampe, J. (2004) The case for negative senescence. *Theor Popul Biol,* **65,** 339-351.
Vitt, L.J. & Cooper, W.E. (1986) Tail loss, tail color, and predator escape in *Eumeces* (Lacertilia, Scincidae)—age-specific differences in costs and benefits. *Canadian Journal of Zoology-Revue Canadienne De Zoologie,* **64,** 583-592.
Waits, D., Simpson, D., Sparkman, A.M., Bronikowski, A.M. & Schwartz, T.S. (2019) The utility of reptile blood transcriptomes. *Molecular Ecology,* **In review**.
Wallace, D.C. (2005) A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: A dawn for evolutionary medicine. *Annual Review of Genetics,* **39,** 359-407.
Warner, D.A., Miller, D.A., Bronikowski, A.M. & Janzen, F.J. (2016) Decades of field data reveal that turtles senesce in the wild. *Proc Natl Acad Sci U S A,* **113,** 6502-6507.
Weigl, R. (2014) Longevity of crocodiles in captivity. *Intern. Zool. News,* **61,** 363-374.
Wilkinson, P.M., Rainwater, T.R., Woodward, A.R., Leone, E.H. & Carter, C. (2016) Determinate growth and reproductive lifespan in the American alligator (*Alligator mississippiensis*): evidence from long-term recaptures. *Copeia,* **104,** 843-852.
Williams, G.C. (1957) Pleiotropy, natural selection, and the evolution of senescence. *Evolution,* **11,** 398-411.
Williams, P.D. & Day, T. (2003) Antagonistic pleiotropy, mortality source interactions, and the evolutionary theory of senescence. *Evolution,* **57,** 1478-1488.
Woodhead, A.D., Setlow, R.B. & Grist, E. (1980) DNA repair and longevity in three species of cold-blooded vertebrates. *Experimental Gerontology*, **15**, 301-304.
Xu, M., Wu, X.B., Yan, P. & Zhu, H.T. (2009) Telomere length shortens with age in Chinese alligators (*Alligator sinensis*). *Journal of Applied Animal Research,* **36,** 109-112.
Zhang, Z.B., Chen, B.J., Yuan, L. & Niu, C.J. (2015) Acute cold stress improved the transcription of pro-inflammatory cytokines of Chinese soft-shelled turtle against *Aeromonas hydrophila*. *Developmental and Comparative Immunology,* **49,** 127-137.
Zhao, Y., Ren, J.L., Wang, M.Y., Zhang, S.T., Liu, Y., Li, M., Cao, Y.B., Zu, H.Y., Chen, X.C., Wu, C.I., Nevo, E., Chen, X.Q. & Du, J.Z. (2013) Codon 104 variation of p53 gene provides adaptive apoptotic responses to extreme environments in mammals of the Tibet plateau. *Proceedings of the National Academy of Sciences of the United States of America,* **110,** 20639-20644.
Zhou, X.X., Guo, Q.L. & Dai, H.P. (2009) Molecular characterization and expression profiles in response to bacterial infection of Chinese soft-shelled turtle interleukin-8 (IL-8), the first reptilian chemokine gene. *Developmental and Comparative Immunology,* **33,** 838-847.
Zimmerman, L.M., Bowden, R.M. & Vogel, L.A. (2014) A vertebrate cytokine primer for eco-immunologists. *Functional Ecology,* **28,** 1061-1073.
Zimmerman, L.M., Carter, A.W., Bowden, R.M. & Vogel, L.A. (2017) Immunocompetence in a long-lived ectothermic vertebrate is temperature dependent but shows no decline in older adults. *Functional Ecology,* **31,** 1383-1389.

Zimmerman, L.M., Vogel, L.A. & Bowden, R.M. (2010) Understanding the vertebrate immune system: insights from the reptilian perspective. *Journal of Experimental Biology*, **213,** 661-671.

Table 1. A selection of maximum observed lifespan estimates for long-lived reptile species, defined here as >40 years, (AnAge database; de Magalhães & Costa 2009; Accessed March 13, 2019)

species	max lifespan (years)
Galapagos tortoise (*Chelonoidis nigra*)	177[1]
Aldabra tortoise (*Aldabrachelys gigantea*)	152[1]
eastern box turtle (*Terrapene carolina carolina*)	138
Greek tortoise (*Testudo graeca*)	127[1]
European pond turtle (*Emys orbicularis*)	120[1]
other turtles (7 species)	40-77
other turtles and tortoises (11 species)	40-70.3[1]
tuatara (*Sphenodon punctatus*)	90[1]
West African dwarf crocodile (*Osteolaemus tetraspis*)	70[1]
other crocodiles (10 species)	41-56[1]
Komodo dragon (*Varanus komodoensis*)	62
other squamates (6 species)	40.4-54[1]

[1] estimated from a captive individual

Table 2. Summary of evidence for mechanistic pillars of aging in reptiles (*sensu* Kennedy et al., 2014). Within each "pillar of aging" category, we summarize published evidence supporting the effect of "specific mechanism(s)" on the aging process of reptiles. In the absence of functional studies on mechanisms of aging in reptiles, we highlight work on molecular sequence conservation and evolution within canonical aging pathways.

pillar of aging	specific mechanism	effect on reptiles (# of citations)
macromolecular damage	DNA damage	+ correlation with age/lifespan (3^{A})
		no association with age (3^{B})
	lipid & protein damage	accumulates with age (2^{C})
epigenetics	methylation	decreases with age (1^{D})
inflammation	adaptive immune system	no change with age (2^{E})
		+ association with aging (1^{F})
	innate immune system	no change with age (3^{G})
		expansion of gene families (1^{H})
adaptation to stress	reduced oxidative stress	+ association with longevity (4^{I})
		mixed results (2^{J})
proteostasis	homology	sequence conservation (3^{K})
		expansion of gene families (2^{L})
stem cells & regeneration	limits on cell division	correlated with lifespan (1^{M})
	telomere dynamics	declines with age (3^{N})
		sex-specific pattern (3^{O})
		no change with age (1^{P})
	regeneration	unknown
	p53 pathway	molecular evolution (1^{Q})
metabolism	nutrient sensing	molecular evolution (1^{R})

[A] Bronikowski, 2008; Robert & Bronikowski, 2010; Schwartz & Bronikowski, 2015

[B] Woodhead, Setlow & Grist, 1980; Regan et al., 1982; Schwanz et al., 2011

[C] Manibabu & Patnaik, 1991; Jena, Das, & Patnaik, 1995

[D] Nilsen et al., 2016

[E] Zimmerman et al., 2017; Palacios & Bronikowski, 2017

[F] Richard et al., 2012

[G] Sparkman & Palacios, 2009 ; Palacios, Sparkman, & Bronikowski, 2011; Schwanz et al., 2011

[H] Quesada et al., 2019

[I] Robert et al. 2007; Olsson et al. 2008; Olsson et al. 2009; Robert & Bronikowski, 2010

[J] Schwartz & Bronikowski, 2013; Iskasson et al. 2011

[K] Shaffer et al., 2013; Quesada et al., 2018, Schield et al., 2019

[L] Harrison, Khachane, & Kumar, 2010; Shaffer et al., 2013

[M] Christiansen et al., 2001

[N] Scott et al., 2006; Xu et al., 2009; Ujvari et al., 2017

[O] Bronikowski, 2008; Olsson et al., 2011b; Rollings et al., 2017

[P] Ujvari & Madsen, 2009

[Q] Passow et al., 2009

[R] Sparkman et al., 2012; McGaugh et al., 2015

Figure legends

Figure 1. Unique features of reptile life history may alter selection on the rate of senescence and affect the evolution of underlying aging mechanisms. A. Indeterminant growth in reptiles, contrasted with mostly determinant growth in mammals and birds, in combination with age-dependent fecundity, increases selection against senescence. B. Basic phylogenetic relationships among reptiles and mammals with examples of reptiles with unprotected phenotypes. C. Senescence rates are influenced by variation in seven underlying pillars of aging as described in Kennedy et al., 2014. The seven pillars are color coded by the theory of aging they relate to: Red, oxidative stress theory; Purple, pace-of-life theory; Green, thermal theories. Photo Credits: Eirini Pajak (western diamondback rattlesnake and desert tortoise), John Finger (alligator)