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Evolution of Senescence in Nature: Physiological Evolution in Populations of Garter Snake with Divergent Life Histories

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abstract: Evolutionary theories of aging are linked to life-history theory in that age-specific schedules of reproduction and survival determine the trajectory of age-specific mutation/selection balances across the life span and thus the rate of senescence. This is predicted to manifest at the organismal level in the evolution of energy allocation strategies of investing in somatic maintenance and robust stress responses in less hazardous envirnments in exchange for energy spent on growth and reproduction. Here we report experiments from long-studied populations of western terrestrial garter snakes (*Thamnophis elegans*) that reside in low and high extrinsic mortality environments, with evolved long and short life spans, respectively. Laboratory common-environment colonies of these two ecotypes were tested for a suite of physiological traits after control and stressed gestations. In offspring derived from control and corticosterone-treated dams, we measured resting metabolism; mitochondrial oxygen consumption, ATP and free radical production rates; and erythrocyte DNA damage and repair ability. We evaluated whether these aging biomarkers mirrored the evolution of life span and whether they were sensitive to stress. Neonates from the long-lived ecotype (1) were smaller, (2) consumed equal amounts of oxygen when corrected for body mass, (3) had DNA that damaged more readily but repaired more efficiently, and (4) had more efficient mitochondria and more efficient cellular antioxidant defenses than short-lived snakes. Many ecotype differences were enhanced in offspring derived from stress-treated dams, which supports the conclusion that nongenetic maternal effects may further impact the cellular stress defenses of offspring. Our findings reveal that physiological evolution underpins reptilian life histories and sheds light on the connectedness between stress response and aging pathways in wild-dwelling organisms.

Keywords: DNA damage, free radical, oxidative stress, reptile, senescence, stress response.

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Introduction

Aging (senescence) is a complex biological process of progressive functional decline in a range of physiological functions that culminates in death and leaves its population-level signature as increasing mortality probability with age (Bronikowski and Promislow 2005). Pleiotropic evolutionary theories of aging, framed in a life-history perspective, posit a trade-off between investing in growth and reproduction versus investing in survival (Kirkwood and Rose 1991). Williams (1957; formalized by Hamilton [1966]) proposed that organisms in more hazardous, high-mortality environments are expected to evolve both rapid development and early and high reproductive effort, with associated costs of decreased survival and shortened life span. Conversely, those in less hazardous, low-mortality environments should evolve delayed maturation and reproduction in favor of growth and enhanced somatic maintenance and life span (see also Austad 1997; Kirkwood 1977). The extent to which these predictions hold true, however, depends on not only the sources and magnitude of mortality but also the degree to which the mortality source interacts with individual condition (Williams and Day 2003; Roach and Gampe 2004; Roach et al. 2009), density (Abrams 1993), and other traits not normally considered in the traditional life-history realm (e.g., immune function: Sparkman and Palacios 2009; reviewed in Promislow and Bronikowski 2006). Identifying and understanding variation in life span within and among species remains a central area of biological research. However, understanding the processes responsible for variation in life span is limited by the paucity of research into the evolution of senescence in natural settings. For example, field data show that wild mammals senesce (e.g., Promislow 1991), often at rates and ages different from their lab counterparts (baboon: Bronikowski et al. 2002; mouse: Miller et al. 1999). An additional and striking example of this difference between wild and captive senescence rates has been reported in a neriid fly (Kawasaki et al. 2008). However, for vertebrates, few nonendothermic tractable systems for studying aging exist for long-lived vertebrates, yet they are nec-

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essary for testing assumptions of evolutionary theories for aging (e.g., regarding ectothermy and indeterminate growth/fecundity). The snake populations in this article afford an excellent opportunity for understanding life span and life-history evolution in nature.

Studies among endothermic species that quantify how life span mirrors physiology have a long tradition in comparative biology (e.g., Rubner 1908; Pearl 1928; Kleiber 1975; Austad and Fisher 1991), with recent emphasis on phylogenetic-based analyses (Promislow 1993, 1994; Speakman 2005). Few reports exist that address this same relationship across ectotherm species (but see Robert et al. 2007). Another approach is to take advantage of the tremendous individual variation within a species' energy expenditure, physiology, and life history to ask how senescence evolves, that is, study the physiological axis, which by definition is related to trade-offs between competing energetic demands (Austad 1996; Speakman et al. 2004). How energy metabolism and longevity relate to each other within a species has been studied using various methods (transgenic or mutant lines, strain comparisons, and environmental manipulation such as caloric restriction), and these have produced very different patterns of association, ranging from positive to negative to those of no significance (reviewed in Speakman 2005). Furthermore, testing these physiological differences among naturally occurring populations that differ in their longevity has many advantages. Perhaps the most compelling is the ability to overlay an ecological context on measured physiological differentiation (or lack thereof) and, ultimately, on the evolution of senescence.

One of the best-supported mechanisms of aging is the free radical or oxidative-stress hypothesis of aging. First put forth by Harman (1956) as the "rate-of-living" hypothesis, the current form of this theory posits that senescent deterioration occurs due to accumulating damage to DNA, proteins, and lipids over the lifetime, which causes cellular, tissue, and somatic deterioration and ultimately death (Finkel and Holbrook 2000). This oxidative-stress mechanism of aging enjoys much empirical support (e.g., Robert et al. 2007). The processes by which oxidative and other stressors cause damage to accumulate, and the relative contribution of damage to aging, still constitute a very active area of research (e.g., Ungvari et al. 2008; Monaghan et al. 2009). Here, we test key predictions of the oxidative-stress mechanistic hypothesis of aging in a study system that has yielded many insights into the evolution of life-history trade-offs and the ecological drivers of this evolution.

The western terrestrial garter snake (*Thamnophis elegans*) has been a study species for more than 30 years of evolutionary and ecological research, and it presents a unique opportunity to examine within-species differences in the physiology of aging. The specific populations under study originated from one ancestral source population (Manier and Arnold 2005) that became differentiated into two distinct and genetically diverged ecotypes that evolved separate life-history strategies. Numbering more than 35 populations, this system can be divided into the lakeshore (L)-fast ecotype, which grows fast, matures young, annually devotes enormous energy to reproduction, and dies young, and the meadow (M)-slow ecotype, which grows slowly, matures late, has small litters every third year (at most), and is long lived (table 1). Earlier work has demonstrated that these life-history strategies are evolutionarily divergent, with significant population differentiation (F_{st}) between L-fast and M-slow populations despite low levels of gene flow between the two ecotypes (Bronikowski 2000; Manier et al. 2007). The putative source of mortality differences, and one of the main evolutionary drivers of this system, is avian predators. In addition, resource availability (prey and water) is predictably limited in M-slow habitats, and this may also be a potent selective pressure for slow life histories. Within years, ephemeral ponds dry before the end of the growth season. Among years, when low winter precipitation results in no standing water—and no anuran breeding—there is no available food (or water) for the snakes. Overall, abundant phenotypic plasticity persists for growth and indeed all of the life-history characteristics, despite their natural placement on a slow-to-fast pace-of-life continuum (Bronikowski and Arnold 1999; Sparkman et al. 2007).

In this study, we use an integrative approach to measure a range of physiological variables, including whole-animal physiology (oxygen consumption, $\dot{V}O_2$), DNA damage and repair, and cellular physiology (mitochondrial oxygen consumption and reactive oxygen species [ROS] production) in neonatal snakes from replicate populations of both L-fast and M-slow ecotypes (three populations of each ecotype). We hypothesize that physiological differentiation is present from birth, based on our previous findings of behavioral, morphological, and life-history differences present at birth. We evaluate these traits within a species with both short (lakeshore ecotype) and long life spans (meadow ecotype). Furthermore, our L-fast and M-slow litters derived from mothers that were either control (no treatment) or treated with corticosterone (CORT treated) during pregnancy to test for an interaction of maternal CORT-related activation of the hypothalamic-pituitary-adrenal (HPA; or HPI for interrenal in reptiles) stress response axis and cellular oxidative-stress indicators. Stress is known to impact the same cellular signaling pathways that regulate aging and can mimic faster (or slower) aging at the cellular level (Greer and Brunet 2008). Following from the oxidative-stress mechanistic hypothesis combined with William's evolutionary hypothesis, we expected

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Table 1: Summary of *Thamnophis elegans* differences between ecotypes (Bronikowski and Arnold 1999; Bronikowski 2000; Sparkman et al. 2007; Robert et al. 2009)

	Lakeshore (3 populations)	Meadow (3 populations)
Neonate birth size	191 mm (range 176–209 mm), 3.27 g (range 2.5–4.2 g)	177 mm (range 147–197 mm), 2.85 g (range 1.8–3.5 g)
Sexual maturation	Later of 425 mm SVL or 3 years	Earlier of 400 mm or 6 years
Adult body size (mm)	660 (range 425–876)	538 (370–598)
Fecundity	Increases with body size	No change with body size
Litter size (liveborn)	8.8 (range 1–21)	4.3 (range 1–6)
Probability (adult survival)	.48/year	.77/year
Trematode (*Alaria* spp.) infection rate	0%	86%
Prey	Fish (*Rhinichthys* spp.), leech (*Erpobdella*)	Frog (*Pseudacris regilla*), leech (*Erpobdella*)
Elevation (m)	1,555	1,630–2,055
Summer ambient temperature (C)	20°–34°	Averages 5°–10° cooler
Corticosterone field baseline (ng/mL)	7.7 ± 15.3	50.5 ± 7.9
Laboratory corticosterone level:		
Dam (ng/mL)	133.5 ± 10.5	102.6 ± 10.2
CORT neonates (ng/mL)	123.88 ± 14.9	102.7 ± 15.7
Control neonates (ng/mL)	143.12 ± 13.7	102.5 ± 12.9

Note: Size (mm) is snout-to-vent length (SVL); hormones measured in plasma.

that the longer-lived ecotype would exhibit reduced metabolic rates, reduced DNA damage and/or increased DNA repair, and efficient mitochondria that produce reduced amounts of oxidants in comparison with the short-lived ecotype. By overlaying a stress experienced as embryos during gestation, we further ask how these variables respond to this nongenetic maternal effect.

Material and Methods

Species and Study Populations

The western terrestrial garter snake (*Thamnophis elegans)* is a viviparous nonvenomous snake that is widely distributed throughout western North America. The focus of our study is on six populations in close proximity (5–10 km) to Eagle Lake, Lassen County, California. They occur in two primary habitats (lakeshore and meadow) with the associated life-history patterns (L-fast and M-slow) described above. Perhaps as part of this life-history syndrome, or distinct from it, the corticosterone stress response appears to be more reactive in the L-fast ecotype. Specifically, L-fast adult females have lower baseline levels of corticosterone but higher stress-responsive levels of corticosterone than M-slow adult females (Robert et al. 2009).

The L-fast populations are characterized by indeterminate growth and fecundity; although average life span for an L-fast snake is only 4 years, those that manage to survive to old age (15+ years) are the largest and most fecund of the L-fast populations (Sparkman et al. 2007). Conversely, individuals of the M-slow ecotype have an average life span of 8 years, and those that live beyond the mean do so with no increases to their reproductive output with age. The L-fast and M-slow snakes are characterized by high neonatal mortality (>80%) and diverge thereafter, with M-slow animals having high annual survival throughout the juvenile and adult years in contrast to juveniles and adults of the L-fast ecotype. These differences in survival undoubtedly reflect a combination of external and internal mortality sources (Bronikowski and Arnold 1999; unpublished).

Throughout June and July 2006, we collected pregnant females from three L-fast and three M-slow focal populations and returned them to the laboratory until parturition (August/September; 35 L-fast, 30 M-slow). Of these 65 pregnant females, 27 were treated with CORT (15 L-fast and 12 M-slow) during the last third of pregnancy to examine stress effects during gestation on neonate physiology. The remaining 38 dams were untreated (20 L-fast and 18 M-slow). CORT was delivered transdermally to the snakes using a mixture of the steroid hormone and sesame oil at a concentration of 0.55 $\mu g\ g^{-1}$ mass (see Robert et al. 2009 for details). Dermal application of CORT mimicked stress-response levels of CORT levels in dams (table 1); CORT levels did not differ between the offspring of CORT-treated and untreated dams (table 1). Before parturition, all dams were housed individually in 10-gal glass aquaria with paper substrate and water dishes with hollowed rims that doubled as shelter sites. Pregnant females were fed twice weekly on a diet of live goldfish (3–4 per feeding) and were provided heating and lights on a 12 : 12 LD cycle. Following birth females were returned to the field and released at their point of capture.

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On the day of birth, offspring were removed from their mothers' cages, sexed, weighed (g), measured (snout-vent length in mm), given a unique number (marked with permanent pen), and then housed in litter groups with water provided ad lib. Resting metabolic rate (RMR) was measured at 1 month of age; all other assays were performed 1 week post-RMR measurement.

Neonatal Resting Metabolic Rate

The RMR (volume of oxygen consumed at rest: $\dot{V}O_2$) was measured for each snake at 1 month of age using closed-system respirometry (Vleck 1987) at 28°C, which corresponds to preferred body temperature (Arnold and Peterson 2002). Snakes were randomly placed within metabolic chambers in replicate 28°C incubators the night before trials to become accustomed to test conditions (Hare et al. 2004). Aluminum chambers were sealed with lids that had a tube with a stopcock on the end. Each chamber was fitted with a thermocouple thermometer, and temperature was measured at 15-min intervals to ensure chamber temperatures equaled incubator temperatures. Two hours before the start of each trial, 5 cm^3 of water was injected into each chamber to ensure saturated air, and 50 cm^3 of room air was drawn with 60-cm syringes and placed within the incubators. At the start of each trial this air was injected into the chambers and mixed by plunging and withdrawing the syringe twice, and then 30 cm^3 of air was removed from the chamber (this sample was used to determine the initial oxygen concentration within the can). Stopcocks were then sealed. At the end of 1 h, 30 cm^3 of air was removed from each chamber to determine final oxygen concentration. Air pressure was also noted at the start of each trial to calculate oxygen consumption and to allow correction to standard temperature and pressure.

Air samples (both initial and final) were measured for oxygen concentration using an Ametek N-37M oxygen sensor and an Ametek S-3A/11 oxygen analyzer per established protocols (Vleck 1987; Robert et al. 2007). The rate of oxygen consumption was determined by the method of Vleck (1987). Mass-independent measures were computed by using mass as a covariate in the statistical analysis (see "Statistical Analysis" below).

One week post–RMR measurement, animals were exsanguinated, and the organs were dissected according to Iowa State University Institutional Animal Care and Use Committee protocol 3-2-5125-J. All blood- and mitochondrial-dependent variables were collected on the same day as dissection.

Mitochondrial Physiology and Free Radical Production

Extracted livers were transferred to ice-cold mitochondrial isolation buffer (250 mM sucrose, 5 mM Tris, 2 mM EGTA, pH 7.4). Livers were pooled for each litter, and occasionally over multiple litters, to obtain sufficient quantities of mitochondria. Mitochondria were isolated from liver tissue by differential centrifugation (Palloti and Lenaz 2001) after homogenizing the tissue (detailed protocol in Robert et al. 2007). A Bradford protein determination was performed on the isolate to ensure mitochondrial concentrations of >30 mg mL^{-1}.

Respiratory activities of liver mitochondria were measured by determining oxygen consumption in airtight chambers at 28°C, using a Clark-type oxygen electrode (Hansatech, Norfolk, UK) according to established procedures (Brand et al. 1993; Herrero and Barja 1997; Barja 1998). Details of slight modifications that we have made for optimizing reptile measurements can be found in Robert et al. (2007). We used Succinate as the electron transport chain substrate, which is utilized by complex II. Rotenone was used to inhibit complex I. Our measurements were taken using a final mitochondrial concentration of 2 mg mL^{-1}. We recorded depletion of oxygen in the presence (state III respiration) and in the absence (state IV respiration) of ADP. Mitochondrial oxygen consumption in state III and state IV is

$$\text{oxygen consumption (nmol } O_2 \text{ min}^{-1} \text{ mg}^{-1}) = \frac{(\text{nmol } O_2 \text{ mL}^{-1} \text{ min}^{-1}) \times \text{reaction volume (mL)}}{\text{mitochondria in reaction (mg)}}.$$

Respiratory control ratios (RCRs) were calculated as ratio of state III (i.e., in the presence of ADP) to state IV (resting) rates of respiration. Respiratory coefficient ratio provides an indication of the degree of uncoupling in the mitochondria (Ungvari et al. 2008). As such, values greater than 3 indicate the mitochondria have not been damaged (Brunet-Rossini and Austad 2004). Therefore, RCR is usually measured before any other assays.

The efficiency of ADP to ATP conversion is computed as the amount of ATP produced per oxygen atom consumed. This ratio (P : O) was calculated as P : O = μmol ATP formed/μmol atomic O consumed. A greater P : O ratio indicates greater mitochondrial efficiency, that is, that a smaller amount of oxygen is required to produce a given amount of ATP.

Two estimates of hydrogen peroxide were measured: rate of production by mitochondria (pmol min^{-1} mg^{-1} mitochondrial protein) and concentration (μM) in whole blood at exsanguination. Hydrogen peroxide, H_2O_2, is a

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primary reactive oxygen species produced during electron transport in mitochondria. The rate of production was measured fluorometrically using a hydrogen peroxide/peroxidase assay kit (A22188; Molecular Probes) that uses the Amplex Red reagent (10-acetyl-3, 7-dihydroxyphenoxazine) to detect hydrogen peroxide presence. The rate of H_2O_2 generation by mitochondria was measured per kit instructions as an increase in fluorescence at an excitation maximum of 530 nm and an emission maximum of 590 nm using a Synergy fluorescence spectrophotometer. Kinetic readings were taken over a 30-min period to calculate H_2O_2 production per minute for 0.5 mg mitochondria (brought up to a final volume of 100 μL). Known concentrations of H_2O_2 generated in parallel were used to construct a standard curve. For blood concentration, 5 μL of whole blood were diluted to a final volume of 100 μL, and a single reading of H_2O_2 was taken at time 1 min to quantify concentration.

DNA Damage and Repair

DNA damage and repair were assessed using single-cell gel electrophoresis on red blood cells (which are nucleated in reptiles). This assay, called the comet assay after the cometlike shape of the migrating DNA (Singh et al. 1988; Klaude et al. 1996) is a sensitive and rapid technique for quantifying and analyzing DNA damage in individual cells. Briefly, individual live cells are embedded in a thin agarose gel on a microscope slide. These cells can be exposed to mutagenic agents or other sources of DNA damage and either lysed immediately or allowed to repair before lysing. After cell lysis, the DNA is unwound and denatured under alkaline conditions. Following unwinding, the DNA is subjected to electrophoresis; the more damaged DNA in each cell is, the longer the cometlike tail of DNA migrating away from the cell body is. After SYBR green staining, each cell is quantified for the amount of fluorescence in head and tail and the length of the comet tail. The extent of DNA liberated from the head of the comet is directly proportional to the amount of DNA damage.

Details of the protocol are presented in Bronikowski (2008). For this study, blood was pooled for each litter, diluted 10,000-fold, and mixed with low-melt agarose (500 μL of diluted blood with 500 μL of 1.5% low-melt agarose). Each sample was divided into six 150-μL aliquots and immediately transferred to prewarmed slides and gently covered with a cover slip to ensure an even distribution of cells in agarose layer. Two of the six slides were assigned randomly to each of three treatment groups: baseline damage (B), inducible damage (D), and repaired damage (R). Baseline slides were immersed in lysis buffer immediately for a baseline measure of DNA damage in erythrocytes. The four remaining slides (D and R) were subjected to 312-nm ultraviolet (UV) light for 5 min. The two D slides were lysed immediately after UV exposure; the two R slides were allowed 10 min to repair DNA damage in a 28°C incubator and then lysed. Electrophoresis was performed in electrophoresis buffer (300 mM NaOH, 1 mM EDTA, pH 13) at 25 V and 300 mA for 40 min. After electrophoresis, the slides were neutralized, and the DNA was fixed by incubation in 100% ethanol for 10 min. After air drying, the slides were stained with SYBR green for image analysis. Fluorescent images were evaluated by comet analysis software to calculate the percentage of damaged DNA in the comet tail (Viscomet, Impuls Imaging, Gilching, Germany). Repair efficiency percentage was computed as $(D - R)/B \times 100$.

Statistical Analysis

General linear models were used for the analysis of all dependent variables after testing for normality and homogeneity of variances. Analyses were conducted in SAS (SAS Institute, Cary, NC) utilizing the Mixed Procedure to accommodate variance analyses with both random and fixed effects. The following models were analyzed for each of the listed dependent variables.

The general linear model (GLM) for $\dot{V}O_2$ was

$$\begin{aligned}\dot{V}O_2 = \mu + \ln(\text{mass}) + \text{sex} + \text{cort} + \text{eco} \\ + \text{eco} \times \text{CORT} + \ln(\text{mass}) \times \text{eco} \\ + \ln(\text{mass}) \times \text{CORT} + \ln(\text{mass}) \times \text{CORT} \times \text{eco} \\ + \text{pop(eco)} + \text{litter[pop(eco)]} + \varepsilon,\end{aligned}$$

where ln (mass) is the covariate natural logarithm of body mass; sex, CORT, and eco are the fixed main effects of sex (male, female), CORT (offspring born of CORT-treated dam or untreated dam), and ecotype (L-fast, M-slow); pop(eco) was the fixed effect of population nested within ecotype (three L-fast and three M-slow replicate populations); and litter[pop(eco)] was the random effect of litter nested within replicate population.

All other mitochondrial and ROS-dependent variables were collected on either pooled blood or pooled mitochondria for each litter and were analyzed with the following GLM:

$$\begin{aligned}\text{response variable} = \mu + \text{CORT} + \text{eco} \\ + \text{eco} \times \text{CORT} \\ + \text{pop(eco)} + \varepsilon,\end{aligned}$$

where the explanatory variables are as above. Because our sample sizes were smaller for the ROS (H_2O_2)-dependent

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Table 2: ANOVA for dependent variables

Source of variation	$\dot{V}O_2$	RCR	P : O	H_2O_2 mitos
	$F_{df1, df2}$			
ln (mass)	$34.2^{***}_{1, 433}$	NR	NR	NR
Ecotype	$1.0_{1, 57}$	$3.0_{1, 51}$	$4.7^{*}_{1, 51}$	$7.9^{*}_{1, 15}$
ln (mass) × ecotype	$1.6_{1, 433}$	NR	NR	NR
CORT	$1.0_{1, 433}$	$.55_{1, 51}$	$.66_{1, 51}$	$9.9^{**}_{1, 15}$
ln (mass) × CORT	$.8_{1, 433}$	NR	NR	NR
Ecotype × CORT	$.5_{1, 433}$	$.58_{1, 51}$	$.10_{1, 51}$	$8.0^{*}_{1, 15}$
ln (mass) × ecotype × CORT	$.5_{1, 433}$	NR	NR	NR
Population (ecotype)	$1.8_{4, 57}$	$1.09_{4, 51}$	$.67_{4, 51}$	NR
Sex	$1.6_{1, 433}$	NR	NR	NR

Note: $\dot{V}O_2$ = volume O_2 consumed at rest: our measure of resting metabolic rate, RCR = respiratory coefficient ratio, P : O = ATP production per atomic O, H_2O_2 mitos = H_2O_2 production min^{-1} mg^{-1} mitochondria. See text for units and measurement details. Not all effects were relevant for each model (NR); main effects include ecotype (L-fast, M-slow), CORT (CORT-treated dam, no CORT treatment of dam), and sex. One, two, and three asterisks denote significance at the .05, .001, and .0001 levels, respectively.

variables, we removed pop(eco) from the model after verifying that it had no effect.

The extent of DNA damage was assessed initially with several dependent variables measured for up to 40 comets per treatment (B, D, R) per litter. Because all comet-tail-dependent variables were highly correlated and supported the same conclusion, only the percentage of DNA in tail results is reported here for ease of interpretability.

$$\begin{aligned}\%\text{DNA in tail} &= \mu + \text{CORT} + \text{eco} + \text{damage}\\ &+ \text{CORT} \times \text{eco} + \text{CORT} \times \text{damage} + \text{eco} \times \text{damage}\\ &+ \text{CORT} \times \text{eco} \times \text{damage} + \text{pop(eco)}\\ &+ \text{replicate(damage)} + \varepsilon,\end{aligned}$$

where the variables are as above with the addition of damage, which is the slide-type of B, D (UV-inducible damage), and R (repaired UV damage). Replicate is a random effect for up to 40 replicate comets per damage treatment for each pooled litter blood sample.

Results

Neonatal Resting Metabolic Rate

Body mass had a significant effect on resting metabolic rate (table 2; fig. 1). Once the effect of mass was removed, no other effects significantly explained variation in RMR.

Mitochondrial Physiology and Free-Radical Production

How mitochondria upregulate from resting to state III metabolism in vitro reflects the degree of uncoupling or damage to mitochondria induced by the isolation procedure. Respiratory coefficient ratios were not significantly affected by any of the explanatory variables (ecotype, corticosterone treatment, or source population; table 2). Moreover, the magnitude of the RCR indicates well-functioning mitochondria (mean RCR = 5.5 ± 0.46). The P : O ratio quantifies the efficiency of ADP to ATP conversion at complex V (ATP synthase) in the electron transport chain. Neither CORT treatment nor CORT × ecotype affected P : O ratio, but ecotype was a significant predictor of efficiency. The M-slow snakes were more efficient at ATP production than were the L-fast snakes (table 2; least square mean ± SE for P : O ratios: L = 0.82 ± 0.10; M = 1.18 ± 0.11).

Two assays of reactive oxygen species were conducted: H_2O_2 production by liver mitochondria (pmol min^{-1} mg^{-1} mitochondria) and H_2O_2 concentration in whole blood (μM). Mitochondrial ROS production was significantly affected by the interaction of ecotype with CORT treatment. The L-fast litters derived from CORT-treated dams had an ROS production rate that was an order of magnitude higher than that of any of the three other groups (table 2; fig. 2). Least square means ± SE (pmol min^{-1} mg^{-1}) are L-fast (CORT treated) = 240 ± 30, L-fast (untreated) = 56.2 ± 35, M-slow (CORT treated) = 66.8 ± 20, and M-slow (untreated) = 57 ± 35. Blood levels of H_2O_2 showed patterns similar to those of the mitochondrial production rate (data not shown).

DNA Damage and Repair

The M-slow (long-lived) snakes had erythrocyte DNA that was damaged more readily than that of the L-fast (short-lived) offspring when exposed to UV damage (fig. 3*A*; table 3); however, they had greater repair efficiency than lakeshore offspring (73% as opposed to 35% repair efficiency).

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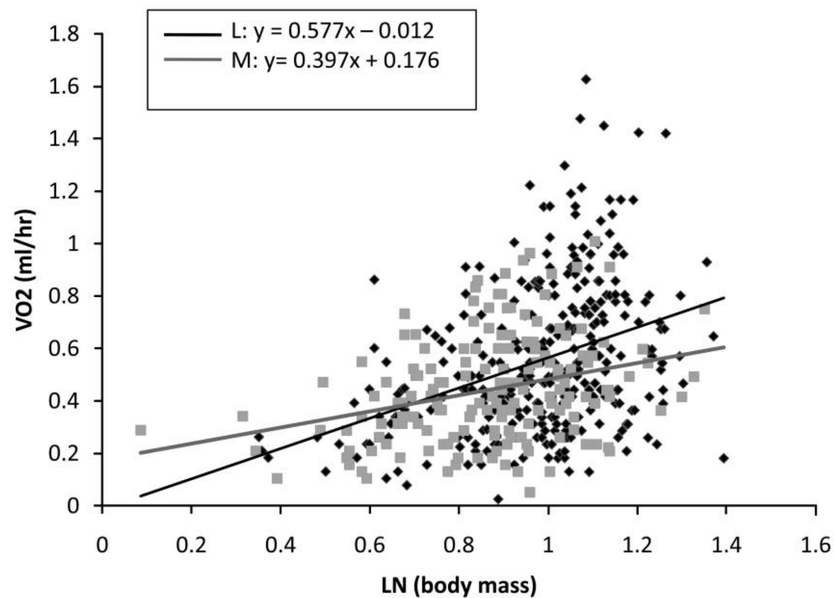

Figure 1: Resting metabolic rate as a function of the natural logarithm of body mass in juvenile garter snakes. Ecotype was not significant but is shown here (M = meadow/long-lived; L = lakeshore/short-lived). No other effects were significant predictors of metabolic rate. *Gray squares* = M-slow, and *black diamonds* = L-fast.

The amount of DNA damage was increased in L-fast (short-lived) snakes but not in M-slow (long-lived) snakes in litters derived from CORT-treated dams (fig. 3*B*). The greater repair ability of M-slow erythrocytes is maintained in these CORT-derived litters.

Discussion

Oxidative Stress, Life History, and Evolutionary Mechanism

We found that neonatal garter snakes from a natural ecotype with evolved long life span had features of their physiology that may support their longer life. Relative to an evolutionarily divergent short-lived ecotype, neonatal long-lived garter snakes produced more ATP for a given amount of consumed oxygen; that is, they had more efficient mitochondria. Simultaneously, they also had consistently low levels of reactive oxygen species production measured both as a mitochondrial production rate and as a standing concentration in the blood. Furthermore, with respect to DNA repair, erythrocytes from neonatal long-lived garter snakes had DNA that was damaged more readily but was repaired more efficiently in comparison to the short-lived ecotype. The consistency among these measures—low ROS production with high efficiency of ATP production and DNA repair—is an excellent first indicator that, as in mammals and birds, life span, aging, and physiology may be interconnected in an ectothermic vertebrate. Understanding how life span, a complex emergent life-history trait, evolves can be aided through the study of physiology and ecology. We discuss the physiological underpinnings of aging and life span and end with an ecological perspective on stress and aging in this replicated natural system of long- and short-lived snakes.

Most of the vertebrate comparative aging literature focuses on endotherms (e.g., Austad and Fisher 1991; Promislow 1991; Brunet-Rossini and Austad 2004; Lambert et al. 2007). However, some data exist on ectothermic aging biomarkers for comparison to data in this study. For example, previous work in our lab has shown that across a diverse group of colubrid snakes, neonates from species with varying life spans have oxidative-stress responses that may underlie these life-span differences (sensu Perez-Campo et al. 1998; e.g., Robert et al. 2007; Bronikowski 2008). Two results stand out that are in agreement with the current intraspecific study. First, similar to the long-lived ecotype, neonates from species of snake with long life span repaired a significantly larger fraction of DNA damage. Second, long-life-span neonates produced significantly less H_2O_2 in their mitochondria than did neonates from short-lived species, a result seen in this study in the CORT-derived litters of the L-fast ecotype and overall in blood levels of H_2O_2. DNA repair and ROS production are widely supported as important indicators of biological age and aging rate (e.g., Lambert et al. 2007;

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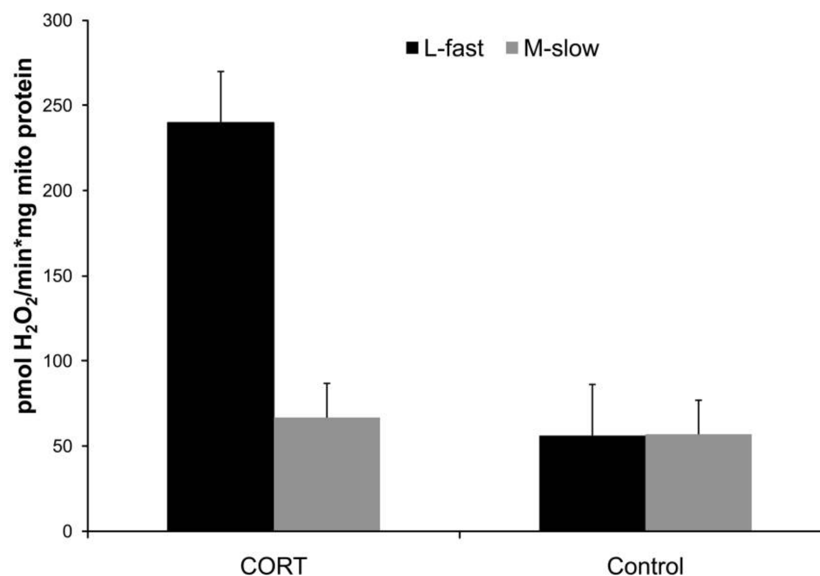

Figure 2: Mitochondrial reactive oxygen species production (pmol H_2O_2 min^{-1} mg^{-1} mitochondrial protein) in offspring produced from mothers treated with corticosterone (*CORT*) and in offspring produced from untreated (*Control*) mothers. Values are least square means from full-model analyses $\pm$ 1 SE (see table 2 for models).

Buffenstein et al. 2008; Campisi and Yaswen 2009; Pérez et al. 2009), and while the exact mechanism of action has yet to be fully elucidated, lower production of damaging molecules or heightened repair mechanisms are consistently supported as important in life-history traits predictive of long life (reviewed for reptiles by Schwartz and Bronikowski [2010]). One of our dependent variables, mitochondrial efficiency, is open to debate as far as its interpretation. For example, some evidence suggests that decreases in mitochondrial efficiency due to the action of uncoupling proteins may actually enhance life span by decreasing ROS production (Brand 2000). In contrast, recent studies on lizard ROS (e.g., Olsson et al. 2008*a*, 2009*b*) found that administering an uncoupling reagent in lizards resulted in increased, not decreased, ROS production. Without additional assays on the expression and function of uncoupling proteins in this species, mitochondrial efficiency differences alone could not unequivocally address the oxidative stress hypothesis. Notwithstanding, the consistency between mitochondrial efficiency and the physiological aging indices used in this study suggests a link between components of oxidative stress and life span.

Researchers of aging recognize the value of wild-derived models of aging (Harper et al. 2006; Harper 2008; Ungvari et al. 2008) and studies of senescence in nature (for vertebrates, primarily on mammals and birds; Bergeron et al. 2008; Nussey et al. 2008; Bouwhuis et al. 2009; reviewed in Ricklefs 2008). Whereas the former category has revealed genetic variation for life span that apparently has been lost in laboratory stocks, the latter has confirmed that senescence is common in the wild, and in many cases, causative variables can be identified, such as differential-mortality environments envisioned by Williams (1957) and Hamilton (1966). Our findings support Williams's (1957) evolutionary hypothesis that high-mortality environments put a selective premium on individuals that can mature fast and reproduce young, which can result in shortened life with potentially rapid senescence. Specifically, populations that evolved in hazardous high-mortality habitats, those along the lakeshore in our system, have individuals with shorter expected life span than those that evolved in more salubrious natural settings. One famous study (Austad 1993) that tested this hypothesis contrasted the vital rates and anatomical features of aging in opossums that were protected from predation with those that were not. As in our study, the opossums with low predation not only lived longer but had longer reproductive life spans and "younger" collagen profiles. Perhaps the most widely cited and digested test of the Williams's high-risk environment hypothesis is that of Trinidadian guppies (e.g., Bronikowski and Promislow 2005; Williams et al. 2006). Reznick and colleagues (Bryant and Reznick 2004; Reznick et al. 2004) carefully reared second-generation fish deriving from high- and low-mortality habitats. Contrary to expectations (but see Baudisch 2005), the longest-lived and most fecund individuals were from high-mortality envi-

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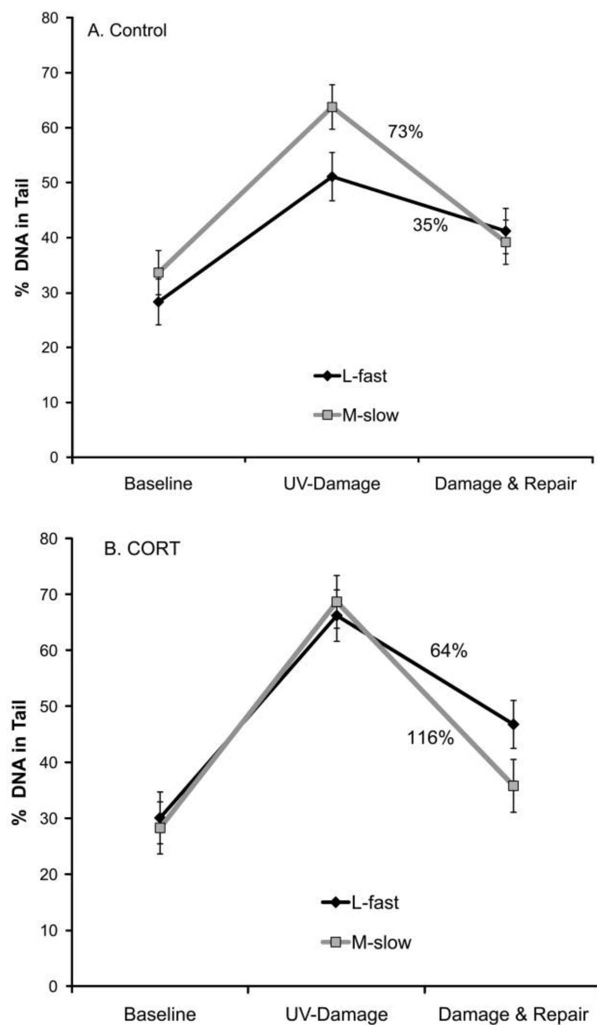

Figure 3: Least square means (±1 SE) of percent DNA in comet tails of erythrocytes in offspring from mothers originating from lakeshore-short-lived (*black diamonds*) or meadow-long-lived (*gray squares*) ecotype (*A*) and percent DNA in comet tails of erythrocytes in offspring from mothers treated with stress hormone during pregnancy originating from lakeshore-short-lived (*black diamonds*) or meadow-long-lived (*gray squares*) ecotypes (*B*). Efficiency of repair expressed as percent baseline damage ([UV damage − damage and repair]/ baseline) computed with least square means from ANOVA (see table 3 for model).

ronments. Although at least one additional measurement did show signs of senescence (swimming behavior), these findings highlighted the need to consider condition (Williams and Day 2003) and density (Abrams 1993) effects within natural populations that may be made irrelevant in laboratory settings (Reznick et al. 2004).

In our system, over 30 years of mark/recapture, scan sampling, and opportunistic predation observations have revealed that lakeshore habitat is frequented by a large diversity and frequency of known avian predators of these snakes (A. Sparkman, J. Billings, A. Bronikowski, and S. J. Arnold, unpublished data). Although indirect, these data support that avian predation differences between lakeshore and meadow habitat underlie the nearly twofold differences in annual survival of juveniles and adults. One likely result of constant high-mortality conditions in lakeshore habitats is to place a selective advantage on rapid growth and early maturation, coupled with high reproductive effort (Charlesworth 1994). These growth and reproduction traits trade off with survival and life span and apparently with the oxidative-stress response. At the same time, these same long-term data have revealed that M-slow snakes experience years with no prey or water, on average every other year (Bronikowski and Arnold 1999). This results in a permanent, albeit fluctuating, difference between the two ecotypes in food and water availability (constant for L-fast, fluctuating among years from abundant to absent for M-slow). It is likely that high predation at lakeshore sites and low food availability at meadow sites are both important selective pressures for the placement of their resident snake phenotypes on the fast-to-slow pace-of-life continuum.

Environmental Stress, Organismal Stress Response, and Cellular Oxidative Stress

Two of our physiological response variables, H_2O_2 mitochondrial production rate and inducible DNA damage, were increased in L-fast neonates whose mothers had been treated with CORT during the final third of gestation. This effect of maternal CORT was absent in M-slow neonates. However, DNA repair efficiency was enhanced in both ecotypes in neonates from CORT-treated dams. Little is known regarding how external stresses, such as predation or prey availability, influence the oxidative-stress responses of wild-dwelling organisms. Notwithstanding, a stress-related nongenetic maternal effect impacted the oxidative-

Table 3: ANOVA for percent DNA in tail of comets

Source of variation	$F_{\mathrm{df1,\ df2}}$	P
Ecotype	$.72_{1,\ 804}$	.8717
CORT	$5.98_{1,\ 804}$	.0147
Damage	$182.3_{2,\ 110}$	<.0001
Ecotype × CORT	$11.5_{1,\ 804}$	.0007
Ecotype × damage	$11.2_{2,\ 804}$	<.0001
CORT × damage	$7.57_{2,\ 804}$	.0006
Ecotype × CORT × damage	$.13_{2,\ 804}$	.8834
Population (ecotype)	$19.2_{4,\ 804}$	<.0001

Note: Data show main effects of ecotype (L-fast, M-slow), CORT (CORT-treated dam, no CORT treatment of dam), and damage (baseline, damage, repaired). Replicate within damage type is a random effect in this model.

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stress responses of offspring. It is likely that the impacts of ecological stresses are modulated by glucocorticoids through the responsiveness of the organismal HPA (HPI in reptiles) stress axis. The dissimilar response of the L-fast ecotype to CORT manipulation—heightened ROS and DNA damage—demonstrates that sensitivity to CORT differs between the two ecotypes; the similar response of the L-fast ecotype and M-slow ecotype—heightened DNA repair—demonstrates shared sensitivity to CORT in at least some downstream effects. How these differential cellular reactions to stress impact life span may be informed by considering the context of the ecological stresses encountered and the reactivity of the HPI stress response.

Baseline field levels of corticosterone were an order of magnitude lower in the short-lived L-fast ecotypic dams of this study (7.7 vs. 50.5 ng/mL; see table 1). Laboratory measures of maximal stress response CORT were slightly but significantly higher in these L-fast dams (133.5 vs. 102.6 ng/mL; see table 1). This pattern of higher stress response CORT values in L-fast gravid females is similar to nongravid adult field patterns of CORT response (A. M. Sparkman and M. G. Palacios, unpublished data). Elsewhere, we have argued that the L-fast ecotype has a more reactive HPI stress response system (Robert et al. 2009), but whether this is due to increased hazards or other factors is not known. Increased production of corticosterone can either prime the fight-or-flight escape response or mobilize necessary activities such as foraging and reproduction (Love and Williams 2008*a*). Thus, HPI induction does not necessarily indicate a negative stress (reviewed in Landys et al. 2006). In fact, the literature has many empirical examples of the beneficial physiological and life-history responses to moderate stresses that are mediated through the stress response. Modest environmental stressors have frequently been shown to enhance the average life span in a population (modeled in Mangel 2008; reviewed in Mattson 2008; see LeBourg and Rattan 2008; Love and Williams 2008*b*). Termed “hormesis” (Calabrese 2008), longevity has been enhanced by a range of stressors in, for example, mice given daily electric shocks (Ordy et al. 1967), rats subjected to cold water immersion (Holloszy and Smith 1986), roundworm and fruit flies subjected to heat shock (Butov et al. 2001; Cypser and Johnson 2002; Scannapieco et al. 2007), and dietary or caloric restriction in a diversity of species (MacKay et al. 1935; Weindruch and Walford 1988; Masoro et al. 1991; Mair et al. 2003). Our results may add to these reports in that mild stress exposure may contribute to increased life span in M-slow snakes. Although mortality is unequivocally higher in L-fast than in M-slow populations across the life span, the M-slow (longer-lived) snakes have at least two forms of nonlethal stresses unique to their meadow habitats.

One of the ecological stresses that meadow animals face is the unpredictable resource availability within and between years for both food and water mentioned above. If we consider the variable and low prey availability in meadow habitats as a form of natural caloric restriction, then the resultant evolved slow-growth phenotype can be thought of as derived from this natural selective breeding experiment. Simply stated, long-term (across-generation) evolution in a diet-restricted habitat would place a selective premium on slow growth, small size, and conservation of resources for reproduction over longer interbirth intervals (after Charlesworth 1994), with the correlated evolutionary response of lengthened life span. Even in years with bountiful prey, channeling energy to reproductive stores would still be advantageous over growing to large adult body sizes that are simply not sustainable in bad resource years. Short-term (within-generation) caloric restriction would be expected to manifest as downregulated glucocorticoid and insulin signaling, the latter of which may be an important mechanistic pathway to life-span extension under caloric restriction (reviewed in Masoro 2005). Evidence beyond the data reported herein for this putative relationship among food availability, life-history evolution, and cellular processes is found in a key pleiotropic hormone in this system. Insulin-like growth factor 1 (IGF1), a signaling molecule in a major vertebrate aging pathway, tracks this changing food availability in M-slow snakes while remaining constant in L-fast snakes (Sparkman et al. 2009). The net result is that L-fast snakes have a larger lifetime exposure to IGF1, an effect consistent with reports that increased IGF1-related signaling causes rapid aging and shortened life (reviewed in Greer and Brunet 2008). The interplay among ecological stress, the (glucocorticoid) stress response, and IGF1 signaling in these populations of snakes is beyond the scope of this study. It is interesting to note, however, that the higher baseline plasma CORT levels in the wild M-slow snakes (Robert et al. 2009) occurred in a good condition year when food and water were plentiful, which may enhance foraging behavior in such bountiful years (e.g., Landys et al. 2006).

The second, and less understood, potential moderate stress in meadow habitats is the high occurrence of trematode infection in wild-living M-slow snakes. For more than 30 years, we have noted the incidence of *Alaria* (see table 1) infection in meadow snakes (and its complete absence in lakeshore snakes). Snakes are an intermediate host in the complex life cycle of this invertebrate. The extent of trematode-induced damage to the tails of snakes can be severe, and indirect effects of infection, such as blood loss and impaired locomotion, have been documented (A. M. Bronikowski and S. J. Arnold, unpublished data). Direct costs of infection to reproductive success and survival are not apparent. Notwithstanding, the upregulation of either the innate and/or acquired immune components are the-

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oretically expected to have associated costs along a life-history axis (Lee 2006; Martin et al. 2006; Sparkman and Palacios 2009). To date, our first tests of trade-offs between life-history and innate immunity confirm that L-fast snakes invest more heavily in innate immunity than M-slow snakes (Sparkman and Palacios 2009). Interactions between innate and/or acquired immunity and life history in the lab and in the field are active areas of research in this model natural system.

Our findings contribute to the growing literature that utilizes the natural genetic variability within wild populations for life history to test hypothesized mechanisms of aging rate and life span. We have explored the ecological circumstances and physiological responses in a test of the microevolution of aging determinants in high- and low-mortality populations. Our findings suggest a considerable need for a multilevel approach to answering how external stress interacts with the glucocorticoid stress response and cellular stress responses to impact the evolution of life span and other life-history traits.

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Literature Cited

Abrams, P. 1993. Does increased mortality favor the evolution of more rapid senescence? Evolution 47:877–887.

Arnold, S. J., and C. R. Peterson. 2002. A model for reaction norms: the case of the pregnant garter snake and her temperature-sensitive embryos. American Naturalist 160:306–316.

Austad, S. N. 1993. Retarded senescence in an insular population of Virginia opossums (*Didelphis virginiana*). Journal of Zoology 229: 695–708.

———. 1996. The uses of intraspecific variation in aging research. Experimental Gerontology 31:453–463.

———. 1997. Comparative aging and life histories in mammals. Experimental Gerontology 32:23–38.

Austad, S. N., and K. E. Fisher. 1991. Mammalian aging, metabolism, and ecology: evidence from the bats and marsupials. Journal of Gerontology B 46:47–53.

Barja, G. 1998. Kinetic measurement of free radical production. Pages 533–548 *in* B. P. Yu, ed. Methods in aging research. CRC, Boca Raton, FL.

Baudisch, A. 2005. Hamilton's indicators of the force of selection. Proceedings of the National Academy of Sciences of the USA 23: 8263–8268.

Bergeron, P., M. Festa-Bianchet, A. von Hardenberg, and B. Bassano. 2008. Heterogeneity in male horn growth and longevity in a highly sexually dimorphic ungulate. Oikos 117:77–82.

Bouwhuis, S., B. C. Sheldon, S. Verhulst, and A. Charmantier. 2009. Great tits growing old: selective disappearance and the partitioning of senescence to stages within the breeding cycle. Proceedings of the Royal Society B: Biological Sciences 276:2769–2777.

Brand, M. D. 2000. Uncoupling to survive? the role of mitochondrial inefficiency in aging. Experimental Gerontology 35:811–820.

Brand, M. D., M. E. Harper, and H. C. Taylor. 1993. Control of the effective P : O ratio of oxidative phosphorylation in liver mitochondria and hepatocytes. Biochemical Journal 291:739–748.

Bronikowski, A. M. 2000. Experimental evidence for the adaptive evolution of growth rate in the garter snake *Thamnophis elegans*. Evolution 54:1760–1767.

———. 2008. The evolution of aging phenotypes in snakes: a review and synthesis with new data. Age: Journal of the American Aging Society 30:169–176.

Bronikowski, A. M., and S. J. Arnold. 1999. The evolutionary ecology of life history variation in the garter snake *Thamnophis elegans*. Ecology 80:2314–2325.

Bronikowski, A. M., and D. E. L. Promislow. 2005. Testing evolutionary theories of aging in wild populations. Trends in Ecology & Evolution 20:271–273.

Bronikowski, A. M., S. C. Alberts, J. Altmann, C. Packer, K. D. Carey, and M. Tatar. 2002. The aging baboon: comparative demographic senescence in a model non-human primate. Proceedings of the National Academy of Sciences of the USA 99:9591–9595.

Brunet-Rossini, A. K., and S. N. Austad. 2004. Ageing studies on bats: a review. Biogerontology 5:211–222.

Bryant, M. J., and D. N. Reznick. 2004. Comparative studies of senescence in natural populations of guppies. American Naturalist 163:55–68.

Buffenstein, R., Y. H. Edrey, T. Yang, and J. Mele. 2008. The oxidative stress theory of aging: embattled or invincible? insights from non-traditional model organisms. Age: Journal of the American Aging Society 30:99–110.

Butov, A., T. Johnson, J. Cypser, I. Sannikov, M. Volkov, M. Sehl, and A. Yashin. A. 2001. Hormesis and debilitation effects in stress experiments using the nematode worm *Caenorhabditis elegans*: the model of balance between cell damage and HSP levels. Experimental Gerontology 37:57–66.

Calabrese, E. J. 2008. Converging concepts: adaptive response, preconditioning, and the Yerkes-Dodson law are manifestations of hormesis. Ageing Research Reviews 7:8–20.

Campisi, J., and P. Yaswen. 2009. Aging and cancer cell biology. Aging Cell 8:221–225.

Charlesworth, B. 1994. Evolution in age-structured populations. 2nd ed. Cambridge University Press, New York.

Cypser, J., and T. Johnson. 2002. Multiple stressors in *Caenorhabditis elegans* induce stress hormesis and extended longevity. Journal of Gerontology 57:B109–B114.

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Finkel, T., and N. J. Holbrook. 2000. Oxidants, oxidative stress and the biology of ageing. Nature 408:239–247.

Greer, E. L., and A. Brunet. 2008. Signaling networks in aging. Journal of Cell Science 121:407–412.

Hamilton, W. D. 1966. The moulding of senescence by natural selection. Journal of Theoretical Biology 12:12–45.

Hare, K. M., S. Pledger, M. B. Thompson, J. H. Miller, and C. H. Daugherty. 2004. Conditioning reduces metabolic rate and time to steady-state in the lizard *Naultinus manukanus* (Reptilia: Gekkonidae). Comparative Biochemistry and Physiology A 139:245–250.

Harman, D. 1956. Aging: a theory based on free radical and radiation therapy. Journal of Gerontology 11:298–300.

Harper, J. M. 2008. Wild-derived mouse stocks: an underappreciated tool for aging research. Age: Journal of the American Aging Society 30:135–145.

Harper, J. M., S. J. Durkee, R. C. Dysko, S. N. Austad, and R. A. Miller. 2006. Genetic modulation of hormone levels and life span in hybrids between laboratory and wild-derived mice. Journal of Gerontology 61A:1019–1029.

Herrero, A., and G. Barja. 1997. Sites and mechanisms responsible for the low rate of free radical production of heart mitochondria in the long-lived pigeon. Mechanisms of Ageing and Development 98:95–111.

Holloszy, J. O., and E. K. Smith. 1986. Longevity of cold-exposed rats: a reevaluation of the "rate of living theory." Journal of Applied Physiology 61:1656–1660.

Kawasaki, N., C. E. Brassil, R. C. Brooks, and R. Bonduriansky. 2008. Environmental effects on the expression of life span and aging: an extreme contrast between wild and captive cohorts of *Telostylinus angusticollis* (Diptera: Neriidae). American Naturalist 172:346–357.

Kirkwood, T. B. L. 1977. Evolution of ageing. Nature 270:301–304.

Kirkwood, T. B. L., and M. R. Rose. 1991. Evolution of senescence: late survival sacrificed for reproduction. Philosophical Transactions of the Royal Society B: Biological Sciences 332:15–24.

Klaude, M., S. Eriksson, J. Nygren, and G. Ahnstrom. 1996. The comet assay: mechanisms and technical considerations. Mutation Research/DNA Repair 363:89–96.

Kleiber, M. 1975. Metabolic turnover rate: a physiological meaning of the metabolic rate per unit body weight. Journal of Theoretical Biology 53:199–204.

Lambert, A. J., H. M. Boysen, J. A. Buckingham, T. Yang, A. Podlutsky, S. N. Austad, T. H. Kunz, R. Buffenstein, and M. D. Brand. 2007. Low rates of hydrogen peroxide production by isolated heart mitochondria associate with long maximum life span in vertebrate homeotherms. Aging Cell 6:607–618.

Landys, M. M., M. Ramenofsky, and J. C. Wingfield. 2006. Actions of glucocorticoids at a seasonal baseline as compared to stress-related levels in the regulation of periodic life processes. General and Comparative Endocrinology 148:132–149.

Lee, K. A. 2006. Linking immune defenses and life history at the levels of the individual and the species. Integrative and Comparative Biology 46:1000–1015.

LeBourg, E., and S. I. S. Rattan. 2008. Mild stress and healthy aging: applying hormesis in aging research and interventions. Springer, Dordrecht.

Love, O. P., and T. D. Williams. 2008*a*. The adaptive value of stress-induced phenotypes: effects of maternally derived corticosterone on sex-biased investment, cost of reproduction, and maternal fitness. American Naturalist 172:E135–E149.

———. 2008*b*. Plasticity in the adrenocortical response of a free-living vertebrate: the role of pre- and post-natal developmental stress. Hormones and Behavior 54:496–505.

Mair, W., P. Goymer, S. D. Pletcher, and L. Partridge. 2003. Demography of dietary restriction and death in *Drosophila*. Science 19:1731–1733.

Mangel, M. 2008. Environment, damage and senescence: modeling the life-history consequences of variable stress and caloric intake. Functional Ecology 22:422–430.

Manier, M. K., and S. J. Arnold. 2005. Population genetic analysis identifies source-sink dynamics for two sympatric garter snake species (*Thamnophis elegans* and *Thamnophis sirtalis*). Molecular Ecology 14:3965–3976.

Manier, M. K., C. M. Seyler, and S. J. Arnold. 2007. Adaptive divergence within and between ecotypes of the terrestrial garter snake, *Thamnophis elegans*, assessed with F_{ST}-Q_{ST} comparisons. Journal of Evolutionary Biology 20:1705–1719.

Martin, L. B., Z. M. Weil, and R. J. Nelson. 2006. Refining approaches and diversifying directions in ecoimmunology. Integrative and Comparative Biology 46:1030–1039.

Masoro, E. J. 2005. Overview of caloric restriction and ageing. Mechanisms of Ageing and Development 126:913–922.

Masoro, E. J., I. Shimokawa, and B. P. Yu. 1991. Retardation of the aging processes in rats by food restriction. Annals of the New York Academy of Sciences 621:337–352.

MacKay, C. M., M. F. Crowell, and L. A. Maynar. 1935. The effect of retarded growth upon the length of the life-span and ultimate body size. Journal of Nutrition 10:63–79.

Mattson, M. P. 2008. Hormesis defined. Ageing Research Reviews 7: 1–7.

Miller, R. A., S. Austad, D. Burke, C. Chrisp, R. Dysko, A. Galecki, A. Jackson, and V. Monnier. 1999. Exotic mice as models for aging research: polemic and prospectus. Neurobiology of Aging 20:217–231.

Monaghan, P., N. B. Metcalfe, and R. Torres 2009. Oxidative stress as a mediator of life history trade-offs: mechanisms, measurements and interpretation. Ecology Letters 12:75–92.

Nussey, D. H., T. Coulson, M. Festa-Bianchet, and J.-M. Gaillard. 2008. Measuring senescence in wild animal populations: towards a longitudinal approach. Functional Ecology 22:393–406.

Olsson, M., M. Wilson, T. Uller, B. Mott, C. Isaksson, M. Healey, and T. Wanger. 2008*a*. Free radicals run in lizard families. Biology Letters 4:186–188.

Olsson, M., M. Wilson, T. Uller, and C. Isaksson. 2008*b*. Free radicals run in lizard families without (and perhaps with) mitochondrial uncoupling. Biology Letters 5:345–346.

Ordy, J. M., T. Samorajski, W. Zeman, and H. J. Curtis. 1967. Interaction effects of environmental stress and deuteron irradiation of the brain on mortality and longevity of C57BL/10 mice. Proceedings of the Society for Experimental Biology and Medicine 126:184–190.

Palloti, F., and G. Lenaz. 2001. Isolation and subfractionation of mitochondria from animal cells and tissue culture lines. Pages 1–35 *in* L. A. Pon and E. A. Schon, eds. Mitochondria. Academic Press, San Diego, CA.

Pearl, R. 1928. The rate of living. University of London Press, London.

Pérez, P., R. Buffenstein, V. Masamsetti, S. Leonard, A. Salmon, J. Mele, B. Andziak, et al. 2009. Protein stability and resistance to oxidative stress are determinants of longevity in the longest-living

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rodent, the naked mole-rat. Proceedings of the National Academy of Sciences of the USA 106:3059–3064.

Perez-Campo, R., M. Lopez-Torres, S. Cadenas, C. Rojas, and G. Barja. 1998. The rate of free radical production as a determinant of the rate of aging: evidence from the comparative approach. Journal of Comparative Physiology B 168:149–158.

Promislow, D. E. L. 1991. Senescence in natural populations of mammals: a comparative study. Evolution 45:1869–1887.

———. 1993. On size and survival: progress and pitfalls in the allometry of life span. Journal of Gerontology 48:B115–B123.

———. 1994. DNA repair and the evolution of longevity: a critical analysis. Journal of Theoretical Biology 170:291–300.

Promislow, D. E. L., and A. M. Bronikowski. 2006. The evolutionary genetics of senescence. Pages 464–481 *in* J. Wolf and C. Fox, eds. Evolutionary genetics: concepts and case studies. Oxford University Press, Oxford.

Reznick, D. N., M. J. Bryant, D. Roff, C. K. Ghalambor, and D. E. Ghalambor. 2004. Effect of extrinsic mortality on the evolution of senescence in guppies. Nature 431:1095–1099.

Ricklefs, R. E. 2008. The evolution of senescence from a comparative perspective. Functional Ecology 22:379–392.

Roach, D. A., and J. Gampe. 2004. Age-specific demography in *Plantago*: uncovering age-dependent mortality in a natural population. American Naturalist 164:60–69.

Roach, D. A., C. E. Ridley, and J. L. Dudycha. 2009. Longitudinal analysis of *Plantago*: age-by-environment interactions reveal aging. Ecology 90:1427–1433.

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

Robert, K. A., C. Vleck, and A. M. Bronikowski. 2009. The effects of maternal corticosterone levels on offspring behavior in fast and slow growth garter snakes (*Thamnophis elegans*). Hormones and Behavior 55:24–32.

Rubner, M. 1908. Das Problem det Lebensdaur und seiner beziehunger zum Wachstum und Ernarnhung. Munich, Oldenbourg.

Scannapieco, A. C., J. G. Sorensen, V. Loeschcke, and F. M. Norry. 2007. Heat-induced hormesis in longevity of two sibling *Drosophila* species. Biogerontology 8:315–325.

Schwartz, T. S., and A. M. Bronikowski. 2010. Molecular stress pathways and the evolution of life histories in reptiles. *In* T. Flatt and A. Heyland, eds. Mechanisms of life history evolution. Oxford University Press, Oxford (forthcoming).

Singh, N. P., M. T. McCoy, R. R. Tice, and E. L. Schneider. 1988. A simple technique for quantitation of low levels of DNA damage in individual cells. Experimental Cell Research 175:184–191.

Sparkman, A. M., and M. G. Palacios. 2009. A test of life-history theories of immune defense in two ecotypes of the garter snake, *Thamnophis elegans*. Ecology 90:720–728.

Sparkman, A. M., S. J. Arnold, and A. M. Bronikowski. 2007. An empirical test of evolutionary theories for reproductive senescence and reproductive effort in the garter snake *Thamnophis elegans*. Proceedings of the Royal Society B: Biological Sciences 274:943–950.

Sparkman, A. M., C. Vleck, and A. M. Bronikowski. 2009. Evolutionary ecology of endocrine-mediated life history variation in the garter snake, *Thamnophis elegans*. Journal of Animal Ecology 78:1242–1248.

Speakman, J. R. 2005. Correlations between physiology and life span: two widely ignored problems with comparative studies. Aging Cell 4:167–175.

Speakman, J. R., D. A. Talbot, C. Selman, S. Snart, J. S. McLaren, P. Redman, E. Krol, D. M. Jackson, M. S. Johnson, and M. D. Brand. 2004. Uncoupled and surviving: individual mice with high metabolism have greater mitochondrial uncoupling and live longer. Aging Cell 3:87–95.

Ungvari, Z., B. F. Krasnikov, A. Csiszar, N. Labinsky, P. Mukhopadhyay, P. Pacher, A. Cooper, N. Podlutskaya, S. N. Austad, and A. Podlutsky. 2008. Testing hypotheses of aging in long-lived mice of the genus *Peromyscus*: association between longevity and mitochondrial stress resistance, ROS detoxification pathways, and DNA repair efficiency. Age: Journal of the American Aging Association 30:121–133.

Vleck, D. 1987. Measurement of O_2 consumption, CO_2 production, and water vapor production in a closed system. Journal of Applied Physiology 62:2103–2106.

Weindruch, R., and R. L. Walford. 1988. The retardation of aging and disease by dietary restriction. Thomas, Springfield, IL.

Williams, G. C. 1957. Pleiotropy, natural selection, and the evolution of senescence. Evolution 11:398–411.

Williams, P. D., and T. Day. 2003. Antagonistic pleiotropy, mortality source interactions, and the evolutionary theory of senescence. Evolution 57:1478–1488.

Williams, P. D., T. Day, Q. Fletcher, and L. Rowe. 2006. The shaping of senescence in the wild. Trends in Ecology & Evolution 21:458–463.

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