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Mitochondrial divergence between slow- and fast-aging garter snakes

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ABSTRACT

Mitochondrial function has long been hypothesized to be intimately involved in aging processes — either directly through declining efficiency of mitochondrial respiration and ATP production with advancing age, or indirectly, e.g., through increased mitochondrial production of damaging free radicals with age. Yet we lack a comprehensive understanding of the evolution of mitochondrial genotypes and phenotypes across diverse animal models, particularly in species that have extremely labile physiology. Here, we measure mitochondrial genome-types and transcription in ecotypes of garter snakes (*Thamnophis elegans*) that are adapted to disparate habitats and have diverged in aging rates and lifespans despite residing in close proximity. Using two RNA-seq datasets, we (1) reconstruct the garter snake mitochondrial genome sequence and bioinformatically identify regulatory elements, (2) test for divergence of mitochondrial gene expression between the ecotypes and in response to heat stress, and (3) test for sequence divergence in mitochondrial protein-coding regions in these slow-aging (SA) and fast-aging (FA) naturally occurring ecotypes. At the nucleotide sequence level, we confirmed two (duplicated) mitochondrial control regions one of which contains a glucocorticoid response element (GRE). Gene expression of protein-coding genes was higher in FA snakes relative to SA snakes for most genes, but was neither affected by heat stress nor an interaction between heat stress and ecotype. SA and FA ecotypes had unique mitochondrial haplotypes with amino acid substitutions in both CYTB and ND5. The CYTB amino acid change (Isoleucine → Threonine) was highly segregated between ecotypes. This divergence of mitochondrial haplotypes between SA and FA snakes contrasts with nuclear gene-flow estimates, but correlates with previously reported divergence in mitochondrial function (mitochondrial oxygen consumption, ATP production, and reactive oxygen species consequences).

1. Introduction

How mitochondrial function changes with advancing age and relates to senescent phenotypes are questions at the forefront of aging research (Allison et al., 2014; Trifunovic and Ventura, 2014). As the primary provider of cellular energy (Attardi and Schatz, 1988), mitochondria must respond to the energetic demands and environmental stresses imposed upon the organism (Blier and Guderley, 1993; Chamberlin, 2004; Pichaud et al., 2011). For example, mitochondria may modulate the production rate of ATP and/or reactive oxygen species (ROS) in response to environmental stimuli (Fangue et al., 2009; Seebacher et al., 2010), or with advancing chronological age (reviewed in Muller et al., 2007; Pinto and Moraes, 2015), or in association with lifespan (Brand, 2010; Lambert et al., 2010). Such plasticity in production rates of ATP and/or ROS is accomplished partially through variation in transcription, RNA processing, and translation within the mitochondria (Blomain and McMahon, 2012; Koc and Koc, 2012). Much of the focus of mitochondrial function viz. aging has been on the role of ROS and accumulating damage to mtDNA, lipids, and proteins. Additional mechanistic hypotheses for senescent deterioration involve an overall decrease in the ability of mitochondria to respond appropriately to environmental and physiological stresses.

An understanding in diverse animal models of how mitochondria function under benign and physiological stress conditions, and how such responses to stress vary across genotypes, aging rates, and environments (i.e., types of stress) may provide insights into the aging process and how aging evolves. Mutations in the mitochondrial genome and in nuclear-encoded mitochondrial genes that affect mitochondrial and organismal function can regulate life-history traits that impact survival and reproduction (Ballard and Melvin, 2010; Clancy, 2008; Kayser et al., 2004). As such, mitochondrial function and plasticity of function are predicted to be targets of natural selection (Ballard and Melvin, 2010; Blier et al., 2001) in the sense that natural selection may target genetic variation at loci underlying variation in function. However, studies attempting to link variation in mitochondrial function (e.g. respiration rates, ROS production, temperature adaptation, etc.) to variation in

Abbreviations: CRs, mitochondrial control regions; CytB, Cytochrome B gene; ND5, NADH dehydrogenase 5 gene; SA, slow-aging ecotype of garter snake; FA, fast-aging ecotype of garter snake; SNP, single nucleotide polymorphism.

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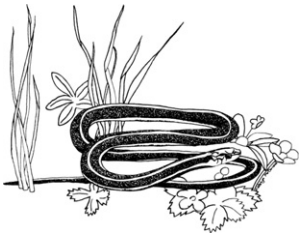
mitochondrial DNA (mtDNA) nucleotide sequences and/or variation in the environment have had mixed success (Amo and Brand, 2007; Flight et al., 2011; Fontanillas et al., 2005; Glanville and Seebacher, 2006; Hicks et al., 2012). Arguably the most direct evidence has come from studies on populations of *Drosophila simulans* with divergent mitochondrial haplotypes that correlate with divergence in life-history (Ballard et al., 2007). In *D. simulans*, detailed studies have demonstrated that variation in mtDNA sequences directly impacts mitochondrial metabolism (Pichaud et al., 2012), the thermosensitivity of mitochondrial function (Pichaud et al., 2011) and, ultimately, the fitness of individual fruit flies (James and Ballard, 2003).

Here we introduce naturally occurring polymorphisms in life-history phenotypes in garter snakes that have been studied in the wild for the last 40 years. We briefly review their measures of demographic aging and their underlying physiological correlates of aging, many of which point to mitochondrial function and that motivate the study of their mitochondria. We then present our new data and test hypotheses on mitochondrial genome divergence and mitochondrial transcription phenotypes. Our study system of garter snakes (*Thamnophis elegans*) inhabit a Pleistocene lake basin (Eagle Lake) in Lassen County, California, USA. They have evolved distinctive life-history strategies that correspond to micro-habitat variation in this vicinity. Termed ecotypes, snakes of the fast pace-of-life ecotype are born larger, grow faster, mature 2–3 years earlier, reproduce 3× more offspring per year, and die younger than conspecific snakes of the slow pace-of-life ecotype (Bronikowski, 2000; Bronikowski and Arnold, 1999; Miller et al., 2011; Miller et al., 2014; Sparkman et al., 2007; Sparkman et al., 2005). As a result, slow pace-of-life adults are smaller-bodied and longer-lived than fast pace-of-life snakes (median adult lifespan is 5 years versus 9 years for fast- and slow pace-of-life phenotypes, respectively see Table 1). (Slow- and

Table 1
Selected differences between *Thamnophis elegans* SA and FA ecotypes (adapted from Schwartz and Bronikowski, 2011). All reported life-history, physiology, and genetic differences are significant at $p < 0.05$). Please see cited references for more details. Original sketches by Katelyn McDonald (BPMI program, ISU).

	Characteristics	Slow-aging (SA)	Fast-aging (FA)
Habitat	Substrate	Mountain Meadow	Rocky Lakeshore
	Elevation	1630 to 2055 m	1555 m
	Average daily summer temperature	15 to 30 °C	20 to 34 °C
	Food/water availability[1,2]	Variable across years	Continuous
	Major prey types[1,3]	Anurans, Leech	Fish, Leech
Morphology	Color and stripe patterns[4]	Black with bright yellow stripe	Variable, checkered, muted grays, browns
			
Life-history	Adult body size (mean)[1]	Shorter: 538 mm (range: 370 to 598)	Longer: 660 mm (range: 425 to 876 mm)
	Growth rate (field and lab)[1,5]	Slower	Faster
	Field litter size (mean)[6]	Smaller: 5.3 liveborn (range: 1 to 6)	Larger: 8.8 liveborn (range: 1 to 21)
	Annual survival[10]	Higher	Lower
	Adult median/max lifespan[1,2]	9/18yrs	5/18yrs
Metabolic Physiology	Whole animal metabolic rate (mL O_2 h^{-1})		
	Lab born neonate (28 °C)[7]	0.52 (mean mass = 2.6 g)	Statistically equivalent
	Lab born juveniles (20 °C)[11]	Lower: 1.51 (mean mass = 7.7 g)	Higher: 1.92 (mean mass = 9.8 g)
	Field born adults (28 °C)[8]	Lower: 2.09 (mean mass = 21.6 g)	Higher: 3.12 (mean mass = 22.1 g)
	H_2O_2 production by liver mitochondria		
	Lab neonates: response to chronic in utero stress[7]	No change from control 56 nmol min^{-1} mg^{-1}	Increases from control 240 nmol min^{-1} mg^{-1}
	H_2O_2 circulating (RBC have mitochondria)		
	Lab 17-mo: response to 2-h heat stress[9]	Decreases from control	Increases from control
	Superoxide (% of cells with superoxide above threshold)		
	Lab 17-mo: response to 2-h heat stressed[9]	Decreases from control	Increases from control
Genetic	Mitochondrial diversity[a]	Haplotypes: A1 with CytB SNP (93%), A9 (7%)	Haplotypes: A2 (80%), A4 (13%), A6 (13%), B (13%)
	Mitochondrial transcription		
	Lab born 17 mo: mitochondrial mRNAs[b]	Generally lower	Generally higher[b]

[1] Bronikowski and Arnold (1999).
[2] Miller et al. (2011).
[3] Kephart and Arnold (1982).
[4] Manier et al. (2007).
[5] Bronikowski (2000).
[6] Sparkman et al. (2007).
[7] Robert and Bronikowski (2010).
[8] Bronikowski and Vleck (2010).
[9] Schwartz and Bronikowski (2013).
[10] Miller et al. (2014).
[11] Gangloff et al. (2015).
[a] This study.
[b] This study Experiment-2.