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Merging "Morphology/Performance/Fitness" and Life-History Theories: An Empirical Assessment in the Eagle Lake Garter Snake Research Project.

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RRH: Ecomorphology and life history of garter snakes

Synopsis

The morphology-performance-fitness method for estimating selection on morphological traits has seen decades of successful application. At the same time, life-history approaches using matrix methods and perturbation studies have also allowed the direct estimate of selection acting on vital rates and the traits that comprise them. Both methodologies have been successfully applied to the garter snakes of the long-term Eagle Lake research project to reveal selection on morphology, such as color pattern, number of vertebrae, gape size; and life-history traits such, such as birth size, growth rates, and juvenile survival. Here we conduct a reciprocal transplant study in a common laboratory environment to partition genetic and environmental sources of variation in morphology and life-history. To place our results in the ecomorphology paradigm, we measure performance outcomes (feeding, growth, insulin growth factor 1 titres) of morphological variation (body size, condition) and their fitness consequences for juvenile survival – a trait that has large fitness sensitivities in these garter snake populations and therefore to be subject to strong selection. To better merge these two complementary theories, we end by discussing our findings in a continuum of morphology – performance – fitness – life-history to highlight what these approaches, when combined, can reveal about selection in the wild.

Introduction

The "morphology-performance-fitness" paradigm was put forth by Stevan Arnold in these pages (Arnold 1983) from a SICB (formerly American Society of Zoologists) 1981 symposium on snake feeding mechanisms. In that paper, Arnold presented a statistical method to test for the fitness consequences, and thereby adaptive significance, of changes to morphology that impact fitness through organismal performance traits, such as behavior or physiology. This ecomorphology theory included regression methods for tracing the effects of morphological variation on organismal performance, and in turn, on an individual's fitness. When framed in microevolutionary selection theory, the strength of natural selection on these phenotypes could be quantitatively assessed (Arnold and Wade 1984b; Lande and Arnold 1983) and the adaptive significance of this variation tested. Ensuing studies of morphology/performance/fitness – i.e., the fitness consequences of morphological variation as mediated through behavior and physiology – helped to change the field of micro-evolution from adaptive story-telling (Gould and Lewontin 1979) to a comprehensive and robust assessment of adaptation in diverse taxa (Dudley 1996; Losos 1990; Schluter 1995).

At the same time, the field of life-history theory (Stearns 1992), and the advent of matrix methods to test for the adaptive significance of variation in life-history traits (Caswell 2001) – complex traits that would not yield easily to the ecomorphology paradigm - rapidly enhanced our ability to ask how and when variation in life-history traits (themselves mediated by morphology and performance) could reveal adaptation. Specifically, matrix models of population demography allowed for the quantification of the strength of natural selection on individual vital rates and life-history traits, as well as comprehensive tests for adaptive significance of such variation – i.e., parallel questions could be addressed both in the realms of morphology/behavior

and life-history/demography. Because variation in vital rates (such as birth rates, juvenile survival, etc.) are underlain by variation in morphology and performance, these two theoretical constructs have a straight-forward complementarity in their use to assess selection gradients (Lande 1982b).

Arnold's long-term Eagle Lake garter snake study system (Lassen County, California) has figured prominently in empirical tests of ecomorphology theory and, more recently, in empirical tests of life-history theory. On the ecomorphology side, Arnold's pioneering work on the adaptive significance of morphological variation – feeding mechanisms, scale counts, and vertebral variation - produced textbook examples of the utility of using this framework to draw strong inferences on the adaptive significance of morphological variation e.g., (Arnold 1992; Arnold and Wade 1984a; Ayres and Arnold 1983; Kelley and others 1997; Manier and others 2007). see also (Arnold and Bennett 1984) for *T. radix* example. Furthermore, this paradigm was extended to link behavioral variation to subsequent variation in performance and fitness (cf. (Arnold 1988). At the same time, discovered through our decades of mark/recapture efforts, these same populations of Eagle Lake garter snakes harbor two distinct life-history phenotypes whose study have provided examples of evolution of life histories within closely located populations (Bronikowski 2000; Bronikowski and Arnold 1999; Miller and others 2011; Miller and others 2014).

Conventionally, this ecomorphological theory has been concerned with measuring traditional morphology (e.g., coloration, shape, number of vertebrae) and mapping these traits in a statistical model with traditional performance traits (e.g., prey capture, slither speed) and fitness. We contend that the possibilities for considering performance are much broader and include traits that represent rates or endpoints of rates. For example, in our previous work on the

Eagle Lake garter snake system, we have measured non-traditional estimates of performance such as innate and acquired immune function (measures of natural antibodies production, bactericidal capability, and mitogen-stimulated lymphocyte proliferation (Palacios and others 2013; Palacios and others 2011)); hormonal titers of glucocorticoids ((Palacios and others 2012) and IGF-1 (Reding and others 2016; Sparkman and others 2009) because they are the main mediators of stress and growth hormone signaling, respectively; and metabolic function (Gangloff and others 2015). Measurement of these traits are in addition to more standard measures of performance such as growth efficiency and feeding behavior (Gangloff and others 2015). Of these, we focus on the endocrine system in this study, proposing that it mediates relationships among morphology, fitness, and life-history traits similar to its role in mediating trade-offs among life-history traits (Ketterson and Nolan 1999; Lailvaux and Husak 2014; Ricklefs and Wikelski 2002; Zera and Bottsford 2001). Specifically, insulin-like growth factor-1 (IGF1) facilitates variation in life-histories (e.g., (Dantzer and Swanson 2012; Lewin and others 2017; Reding and others 2016; Schwartz and Bronikowski 2016). IGF1, the primary hormone of the insulin/insulin-like signaling (IIS) system, is a peptide hormone that is a paralog of insulin and has been highly conserved across amniotes (Annunziata and others 2011; Denley and others 2005; Duan 1998; McGaugh and others 2015; Sparkman and others 2012; Zhu and others 2017). On the cellular level, IGF1 stimulates cell proliferation, differentiation, and migration, while on the organismal scale it triggers overall growth and maturation.

Lailvaux and Husak (Lailvaux and Husak 2014) argued convincingly to make the linkages between ecomorphology and life-history direct and explicit. Following their lead, to better merge ecomorphology and life-history theory, and to make clear the connections between them, we undertake here an explicit test of the relationships among morphology, performance,

fitness, life-history, and demography in the Eagle Lake populations of garter snakes. Because of the clear function that IGF1 has for organismal growth, and because of the association of IGF1 with life-history phenotypes in the Eagle Lake garter snake system, we focus our performance measures on IGF1, feeding, and growth. Specifically, we address: 1) How does morphological variation at birth translate into variation in performance - measured as feeding, growth, and circulating IGF1? 2) How do variation in these morphological and performance traits impact fitness – measured as survival until sexual maturation? And 3) how do phenotypes defined along specific morphology, performance, fitness trajectories map to variation in population demographic estimates of fitness – measured as population growth rates?

Methods

Study system

Populations of western terrestrial garter snakes (*Thamnophis elegans*) in the vicinity of Eagle Lake (Lassen County), California are characterized by either slow or fast-paced life history ecotypes. Lower elevation lakeshore snakes exhibit fast growth, large adult body size, early reproductive maturation with large reproductive effort, and low annual survival. Higher elevation meadow snakes exhibit slow growth, smaller adult body size, later maturation, low annual reproduction, high annual survival, and measurable demographic senescence with advancing adult age ("L-fast" versus "M-slow" ecotypes; (Bronikowski and Arnold 1999; Schwartz and others 2015). Selection gradients vary between these fast and slow ecotypes for survival and growth in the pre-adult stages (Miller and others 2011). As well, genetic differentiation persists among populations of contrasting ecotypes with significant F_{ST} for both nuclear markers (Manier and others 2007), and mitochondrial genomes (Schwartz and others 2015). Many ecological factors differ between the two ecotypes: predation rates are higher in the lakeshore habitat

(Sparkman and others 2013) as is prey abundance and average temperature (Bronikowski and Arnold, 1999). In wild-caught animals, variation in total plasma IGF-1 is driven by interactions among body size (snout-to-vent length), ecotype, and life-history stage. IGF-1 is also higher in wild-caught pregnant snakes Sparkman and others (2009). At the same time, IGF-1 is sensitive to ecological variation: levels vary across year and covary with precipitation – likely due to the relationship between precipitation and prey availability (Miller and others 2011; Sparkman and others 2009).

Study animals and treatment groups

Wild IGF1 and body size

To assess the long-term trends in IGF1 plasma levels versus body size in wild animals, we assayed IGF1 plasma concentrations in an additional set (N=31) of wild-caught animals (males and non-pregnant females) from 2009 and 2010 to complement our published study of IGF1 concentration in the wild from 2006 – 2008 (Sparkman and others 2009).

Cohort morphology, growth, feeding, and survival

In June 2010, we collected 40 pregnant females from six populations of *Thamnophis elegans* around Eagle Lake, California: three replicate populations of each of the lakeshore (L-fast) and meadow (M-slow) ecotypes. We transported these pregnant females to Iowa State University and housed them individually through gestation in 10-gallon glass aquaria with ground corncob substrate and a plastic bowl that served as both water dish and retreat site. We maintained them in a 20°C room with their tanks placed on a thermal gradient for 24 hours per day (range: 25-

34°C across each tank), kept on a 12:12 light:dark schedule, and offered 1-2 mice once a week until parturition.

Offspring (*n*=257) from these 40 pregnant females were born between 12 August and 19 September 2010 (Table 1). Within 24 hours of birth, we measured offspring sex, weight, and snout-vent length (SVL, mm), and placed individual neonates in plastic home cages with paper substrate and a water bowl. We divided litters randomly, with sex split nearly evenly, into two temperature-treatment groups designed to mimic the differing thermal regimes of the warmer L-fast and cooler M-slow habitats (Bronikowski 2000). Ambient room temperature was 20°C, with the warmer rearing treatment ("warm") receiving 16 hours of supplemental heating per day and the cooler rearing treatment ("cool") receiving supplemental heating for only 8 hours per day. This supplemental heating, supplied by under-tank heating elements, provided a gradient of 22°C to 32°C in each home box during heating (20°C during hours of no heat), allowing the animals to behaviorally thermoregulate. All juveniles were kept on a 12:12 light:dark schedule and offered thawed pinky mice once a week. Individuals that consumed all food were offered a greater amount in subsequent feedings. At every feeding, food consumption was recorded in grams of mouse eaten. L-fast and M-slow juveniles residing in the warm and cool treatments are referred to throughout as Lwarm, Lcool, Mwarm, and Mcool. Animals were housed accordingly for life.

Repeated measures of IGF1 and growth

A subset of the full colony, sampled from each sex × ecotype × rearing treatment group, were sampled for short-term repeated measures analysis of total plasma IGF1 concentration and growth to assess the relationship between IGF1 titer and short-term growth rate. These 130 snakes from the 2010 cohort (Table 1) were weighed and measured on 6 May 2011; 14 Sept.

2011; 1 November 2011; and 12 January 2012 – approximately 10, 14, 16, and 18 months of age – and blood-sampled on the latter three dates. Blood was collected from the caudal vein in heparin-rinsed and dried syringes. Plasma was separated from red blood cells by centrifugation and was frozen and stored at -80°C. Treatment of all experimental animals was in accordance with Iowa State University Institutional Animal Care and Use Committee protocol #3-2-5125-J.

IGF-1 Radioimmunoassay

Plasma IGF-1 concentrations were determined by radioimmunoassay (RIA) using GroPep Protocol #3002 (GroPep Ltd., Adelaide, Australia) via competitive binding with ^{125}I labeled IGF-1 (PerkinElmer #NEZ033). This assay uses an anti-human IGF-1, but was validated for the Western terrestrial garter snake by Sparkman and others (2009). IGF binding proteins were removed from IGF-1 by acid-ethanol extraction. All samples were assayed in duplicate within a single assay. Intra-assay variation was 7.5%.

Statistical analysis

All data were analyzed using SAS v9.4 (SAS Institute, Cary, NC). Significance was set at $\alpha = 0.05$. All data were first inspected for normality and homogeneity of variances and transformed to meet these assumptions as necessary (noted below). In all linear mixed model analyses, denominator degrees of freedom for *F*-tests were estimated using the Kenward-Roger Degrees of Freedom Approximation, which weights the denominator degrees of freedom according to the variance of the effect (Kenward and Roger 1997). Figures were made with the 'ggplot' package (Wickham 2009) for R (R Core Team 2014).

Wild IGF1 and body size

We analyzed blood plasma concentrations of IGF-1 in field-caught animals, excluding reproductive females, from both ecotypes across five years (2006-2010). Data from 2006 – 2008 were from Sparkman and others (2009). Data for 2009 and 2010 were from our standard blood field collecting. We utilized generalized linear models and concentrations were $\log_{10}$-transformed to meet assumptions of normality. The initial model included the main effects of size (SVL in mm), ecotype (L-fast, M-slow), year (2006 – 2010), and life-history stage (adult or juvenile, with juvenile defined as snakes with SVL < 425 mm for L-fast and < 400 mm for M-slow; Sparkman and others 2009), the four-way interaction of these effects, and all lower-order interactions. We retained statistically significant interactions and those of biological interest, resulting in a final model of:

$$log_{10}IGF1 = \mu + SVL + Ecotype + Life\ stage + Year + SVL \times Ecotype + SVL \times Life\ stage + SVL \times Year + SVL \times Ecotype \times Life\ stage + \varepsilon$$

where μ represents the grand mean and ε is the error term.

Cohort morphology, growth, feeding, and survival

Morphology

We analyzed body size across lifetime as the snout-vent length (SVL in mm) of individual snakes from birth until death. We used mixed linear models to test for the effects of ecotype, rearing treatment (warm/cool), age (in days, assuming an average birthday of 1 Sept. 2010), and

interactions thereof, on body size. We also included sex as a fixed effect in this model, though no interactions with this term because models did not converge when a large number of interactions were included and these interactions are not of primary biological concern. Population nested within ecotype was included in the model as a fixed effect to account for among-population habitat heterogeneity within ecotypes (Palacios and others 2013). We also included the random effect of litter nested within population and ecotype, which accounts for among-litter variation within populations (Robert and Bronikowski 2010). The final mixed linear models used for analysis of body size was:

$$SVL = \mu + Ecotype + Rearing\ Treatment + Age + Sex + Ecotype \times Rearing\ Treatment + Ecotype \times Age + Rearing\ Treatment \times Age + Ecotype \times Rearing\ Treatment \times Age + Population(Ecotype) + Litter(Population\ Ecotype) + \varepsilon$$

Growth

We analyzed growth as change in snout-vent length (SVL in mm) from the first uniform measurement date (29 Nov. 2010) to 10 Dec. 2014. We used mixed linear models to test for the effects of ecotype (L-fast, M-slow), rearing treatment (warm, cool), sex, and interactions thereof, on growth. To account for variation in growth due to size, we used initial size (i.e., at 29 Nov. 2010, approximately 2 months post-birth) as a covariate in the analysis. To account for elapsed time, we used number of growth days (days between 29 Nov. 2010 and an individual's final length measurement). As above, we included population nested within ecotype as a fixed effect and litter nested within population and ecotype as a random effect. The interaction of ecotype × rearing treatment was left in the model as this interaction is of biological interest; the remaining

non-significant interaction terms were removed from the model. The final mixed linear model used for our analysis of lifetime growth was:

$$\textit{Change in SVL} = \mu + \textit{Growth Days} + \textit{Initial Size} + \textit{Ecotype} + \textit{Rearing Treatment} + \textit{Sex} + \textit{Ecotype}\times\textit{Rearing Treatment} + \textit{Population(Ecotype)} + \textit{Litter(Population Ecotype)} + \varepsilon$$

Feeding

We analyzed the amount of food consumed (the number of fractional pieces of frozen/thawed pinkies consumed, converted to grams) from 29 Nov. 2010 until death. Covariates included Feeding Days, which is the number of days between 29 Nov. 2010 and death, and Initial Size, which is the 29 Nov. 2010 SVL. Using a model-reduction process identical to the above, we removed non-significant interaction terms and utilized this final model of lifetime food consumption:

$$\textit{Amount Consumed} = \mu + \textit{Feeding Days} + \textit{Initial Size} + \textit{Ecotype} + \textit{Rearing Treatment} + \textit{Sex} + \textit{Ecotype*Rearing Treatment} + \textit{Population(Ecotype)} + \textit{Litter(Population Ecotype)} + \varepsilon$$

Survival

We analyzed survivorship of captive-born snakes from ages 1 to 4 years (Sept. 2011 to Dec. 2014) with semi-parametric Cox proportional hazards using PROC PHREG in SAS. We conditioned on surviving to age 1 to correspond to the timing of the repeated measures of growth and IGF1 study, and because we were interested in the effects of morphology and performance on survival after the duration of high neonatal mortality. We included the categorical factors of

rearing treatment, ecotype, and sex, as well as all possible two- and three-way interaction terms. Because the three-way interaction was significant, we retained this term and all lower-order terms in our final model. To test for the effect of morphology on survival, we included the continuous covariates of body size at one year (SVL in mm measured in Sept. 2011), and body condition at one year (residual of the log-mass on log-SVL regression). To test for the effect of performance on survival, we included lifetime feeding rate (calculated as the amount of food (in g) consumed before death divided by the number of days alive), and lifetime growth rate (calculated as the specific growth rate, a measure of growth scaled to body size: SGR = $100 \times [\ln(SVL_2) - \ln(SVL_1)]$/days; (Killen 2014; Reid and others 2011). No direct test of IGF1 titer on survival was performed because the IGF1 titers are plastic and can vary greatly over the lifetime; instead our analyses incorporates growth, for which we separately tested for an effect of IGF1 concentration (below). Additionally, we included population nested within ecotype as a fixed effect and litter as a random effect to account for potential covariance among siblings.

Repeated measures of IGF1 and growth

To test for the main effects of ecotype and temperature on growth rates during the short term IGF-1 sampling intervals, and then to test for the effects of ecotype, temperature, and growth on IGF-1 levels, we used repeated measures mixed linear models with litter (nested within population) as a random effect.

Repeated Growth

We modeled short-term growth (change in SVL) during the three intervals of IGF-1 sampling with the main fixed effects of Sex; Interval (3 intervals: 10-14 months, 14-16 months, and 16-18

months of age); Ecotype (L-fast and M-slow); Rearing Temperature Treatment (warm, cool); and Population nested within Ecotype to reflect heterogeneity among similar ecotype populations. We also included the time-varying covariates of size at the start of each interval and food consumed in the preceding interval. Food consumption was not significant, likely due to confounding with body size and rearing treatment over this short time-course, and was therefore removed from the model. For this subsample of the full cohort, offspring from two neighboring M-slow populations were combined because only one litter was included from one M-slow population. For the main effects of Time, Treatment and Ecotype, all interactions were included in the model. Our final model for growth (repeated increases in body length) was:

$$\textit{Change in SVL} = \mu + \textit{Interval start-size} + \textit{Sex} + \textit{Interval} + \textit{Ecotype} + \textit{Rearing Treatment} + \textit{Ecotype}\times\textit{Interval} + \textit{Rearing Treatment}\times\textit{Interval} + \textit{Ecotype}\times\textit{Rearing Treatment} + \textit{Ecotype}\times\textit{Rearing Treatment}\times\textit{Interval} + \textit{Population(Ecotype)} + \textit{Litter(Population)} + \varepsilon.$$

Repeated Plasma IGF1 Concentration

For the analysis of repeated measures of plasma IGF-1 concentrations, all IGF-1 values (ng/mL) were $\log_{10}$ transformed to meet normality assumptions. Because IGF-1 is a main mediator of growth hormone, we considered three different proxies for growth in our analyses: body size (mm), growth in the interval preceding the IGF-1 measure ("prior interval growth"), and growth in the interval following the IGF-1 measure ("subsequent interval growth"). This latter variable of subsequent interval growth had far fewer observations available for the last IGF-1 measure because most animals were placed immediately into hibernation after the last blood draw. Our models using each of these explanatory growth variables were in general agreement with each

other (data not shown), but because we were interested in the effect of growth *per se* (and not body size) on IGF1 we included growth in the preceding interval as the covariate in our final model. "Prior Interval Growth" was calculated by taking the change in SVL divided by the duration of the interval. Because interactions with sex were never significant, we considered only the two and three way interactions of Time, Treatment, and Ecotype. Our initial model included food consumption as a covariate over the same period as growth, but as it was not significant, we removed it from the model:

$$log_{10}IGF1 = \mu + Prior\ Interval\ Growth + Sex + Time + Ecotype + Rearing\ Treatment + Ecotype \times Time + Rearing\ Treatment \times Time + Ecotype \times Rearing\ Treatment + Ecotype \times Rearing\ Treatment \times Time + Population(Ecotype) + Litter(Population) + \varepsilon.$$

Results

Wild IGF1 plasma concentration.

IGF-1 concentrations in field-caught snakes were dependent on the interaction of SVL, ecotype, and life-history stage such that larger adult L-fast snakes had higher concentrations of plasma IGF-1, whereas size did not affect IGF-1 concentrations in juvenile L-fast snakes or M-slow snakes (Table 2, Fig. 1). Additionally, plasma IGF-1 concentrations were heterogeneous across years.

Cohort morphology, growth, feeding, and survival

Cohort morphology, growth, feeding

For the repeated measures of body size from birth to age 4 yr, all two-way interactions of ecotype, rearing treatment, and age were significant in determining absolute body size across lifetime (Table 3, Fig. 2). The main effect of ecotype was significant as well, with L-fast snakes maintaining larger body size across lifetime than M-slow snakes. Additionally, sex was a significant factor, with males slightly larger in body size than females after correcting for age. For the single measure of growth over the first 4 years, after accounting for the number of growth days and initial size, the effects of ecotype, rearing treatment, and sex were significant in determining lifetime growth. L-fast snakes grew more than M-slow snakes while snakes in the warm rearing treatment grew more than snakes in the cool rearing treatment regardless of ecotype. Additionally, female snakes grew more than male snakes, despite males maintaining slightly greater absolute size (Table 4, Fig. 3A). For amount of food consumed (g) from birth through 4 years, after accounting for the number of feeding days and initial size, the main effects of both ecotype and rearing treatment, as well as their interaction, were significant factors in determining feeding rate (Table 5, Fig. 3B). L-warm snakes ate significantly more food than snakes in any other ecotype/treatment group combination. Additionally, female snakes ate more than male snakes.

Cohort survival

Survivorship to age 4 was dependent on the interaction of ecotype, rearing treatment, and sex (Table 6, Figure 4). Generally, L-fast snakes lived longer than M-slow snakes while snakes in the warm temperature treatment outlived snakes in the cool treatment. The effect of sex was

dependent on ecotype, with females experiencing higher survivorship in the L-fast ecotype and males in the M-slow. Additionally, snakes larger at one year of age and snakes with slower growth rates lived longer than small snakes and fast-growers.

Repeated growth and IGF1

Repeated measures of growth and IGF1 plasma concentrations were undertaken over three intervals spanning 6 May, 2011 – 12 Jan. 2012, with blood draws at each interval end date. Snakes with larger body length at the start of each interval grew faster than smaller snakes during the interval (Table 7). As well, females grew faster than males. L-fast animals grew faster than M-slow animals in the first interval, but not in the second or third intervals (Table 7, Fig. 5). All animals grew faster in the warm treatment during the first and second intervals, and in the cool treatment during third interval (Fig. 5). These short-term interval results contrast with overall growth over the entire experiment in which animals in the warm treatment both consumed more food and grew more than animals in the cool treatment (see Figs. 3 and 4) for both ecotypes.

In the analysis of IGF-1 concentration, growth in the interval preceding IGF-1 concentrations negatively affected IGF-1 concentration (Table 8, Figure 6); higher rates of growth in the interval preceding IGF1 measurements corresponded to lower levels of circulating IGF-1. This negative correlation between previous growth and current IGF1 was consistent across all three intervals, particularly intervals 1 and 3. Consistent with this negative association between IGF1 plasma concentration and growth, males grew slower than females across these three sampling intervals and males had overall higher levels of plasma IGF1. Other significant effects in the model of IGF1 variation included a marginally significant 3-way interaction among

ecotype, treatment, and time (Fig. 6). Across sampling periods, snakes in the warm treatment had higher IGF-1 levels than those in the cool treatment (Table 8).

Discussion

Life-history theory postulates trade-offs among suites of life-history traits – such as along a "pace-of-life" continuum – where species are arrayed along an axis from "slow" to "fast" living (Promislow and Harvey 1990; Wikelski and others 2003). At one end are species with slow growth and delayed sexual maturation, but with extended lifespan and iteroparity. On the "fast" end of the continuum, species exhibit fast growth, rapid reproduction, semelparity or high reproductive effort in relatively fewer bouts and, a short lifespan e.g., (Lemaitre and others 2015). Extensive work has documented the reality of this continuum and the presence of underlying trade-offs among growth, reproduction, maintenance, and survival (e.g., (Ghalambor and Martin 2001; Lawson and others 2012; Lee and others 2012; Smith and others 1989); reviewed in (Nylin and Gotthard 1998; Schluter and others 1991). However, less research has focused on the genetic and evolutionary mechanisms that underlie these trade-offs (e.g., (Hendry and others 2004; Johnston and others 2013; Nussey 2009; Roff 2000) and even less research has sought to understand the physiological processes by which these trade-offs occur (Cohen and others 2012). Few studies have looked at whole organism physiology, focusing on how allocation of resources is differentially controlled (but see (Cox and others 2010; Gangloff and others 2015). Many of these animal studies of the mechanisms underlying a species' life-history placement on this continuum have been conducted in birds and mammals (Gaillard and others 1989), but work in snakes and lizards has also found that they exhibit life history characteristics congruent with a slow-to-fast pace of life continuum (Shine and Charnov 1992; Sparkman and

others 2007). This pattern is frequently documented across species; however, some studies have documented the existence of a slow-fast continuum even within a single species among populations that have experienced different selective pressures over generations (e.g. *Anolis heterodermis*, (Moreno-Arias and Urbina-Cardona 2013); *Thamnophis elegans*, (this system); *Sceloporus grammicus,* (Pérez-Mendoza and Zúñiga-Vega 2014*)*. It is here, where intra-specific variation in life-history strategies exist, that it is most fruitful to understand these polymorphisms in the ecomorphology framework. Specifically, when morphological and performance variation give rise to fitness differences among individuals, these fitness differences provide natural selection the basis to shape variation in population vital rates, including survivorship and reproduction.

On the morphological axis, we found that variation in body size can be accounted for by fixed differences between the ecotypes and interactions with thermal rearing treatment and age. Generally, warmer older snakes are larger than cooler younger snakes, particularly in the fast-pace-of-life ecotype. In turn, variation in body size affects both short-term growth – a performance measure – (with larger snakes growing more than smaller snakes) as well as survival through the juvenile stage (1 to 4 yr) – a fitness measure – (with larger snakes experiencing higher juvenile survival). Moreover, growth itself was predictive of survival with slow growth (after age 1yr) predicting higher juvenile survival. In addition to growth rate, we considered two other performance measures: feeding and circulating IGF1. Variation among individuals in feeding was not related to morphological variation directly. Generally, feeding was highly impacted by thermal rearing treatment, with snakes in the warmer treatment eating more, resulting in these animals growing more and obtaining larger body sizes. Such temperature-based plasticity of feeding behavior was further impacted by ecotype, with warm L-

fast snakes eating disproportionately more than M-slow warm animals. In the short-term repeated measures growth and IGF1 experiment, feeding (grams food consumed) and growth were essentially interchangeable – food consumption and rearing treatment were confounded; animals in the warm treatment ate more and therefore grew more. Our final performance measure, IGF1 plasma concentration (ng/mL), was considered over two time scales. First, we analyzed 5 years of field-collected blood plasma. Here we found a significant interaction among body size, ecotype, and life state (juvenile versus adult; Figure 1). These results show that for adult snakes, the manner in which body size associates with IGF1 titers is ecotype-dependent: L-fast adults have increasing IGF1 with increasing body size; M-slow adults show no dependence of circulating IGF1 on body size. Our previous work has shown that whereas M-slow adults essentially stop growing once they reach adulthood, L-fast adults continue growing over their adult lifespans with ever increasing size-associated fecundity (Bronikowski and Arnold 1999; Sparkman and others 2007).

Without additional data on individual growth rates, these field data cannot distinguish between competing functions of IGF1 among growth, reproduction, and survival in juveniles and adults. Thus we undertook here a short-term common garden growth and IGF1 study in which we document, among other effects, a short term negative association with IGF1 and growth rates (see also (Reding and others 2016). This is in contrast (discussed in more detail below) to studies in other systems (e.g., (Lodjak and others 2017).

IGF1 as a mediator of ecomorphology to life-history transition

We originally envisioned IGF1 as a measure of performance that may impact survival and which is itself potentially influenced by body size or body condition. Our results suggest it may be

more appropriate think of IGF1 as a mediator of the morphology-to-performance-to-fitness-to-life histories causative continuum. We found that variation in IGF1 is not explained by food consumption per se, and negatively relates to growth preceding IGF1 measurement. Others have found that IGF1 responds to food and/or nutrition availability (Beckman 2011; Duncan and others 2015; Hetz and others 2015; Pierce and others 2005). And indeed we have hypothesized elsewhere that lower levels of IGF1 in M-slow snakes in the wild may be related to their unpredictable food availability (Robert and Bronikowski 2010). For example, in *Sceloporus undulatus* both hepatic IGF1 mRNA levels and circulating levels of IGF1 were reduced in response to food restriction but returned to normal levels at the cessation of food restriction (Duncan amd others., 2015).

From work primarily conducted on mammals and fish, IGF1 has been shown to play an integral role in coordinating growth with internal and external cues (e.g. (Dantzer and Swanson 2012; Duan 1998; Fox and others 2006). However, as the insulin/insulin-like signaling (IIS) system is highly conserved across vertebrate taxa, it likely is involved in integrating environmental cues with growth responses in additional vertebrate species as well (e.g., Sparkman and others, 2010, Duncan and others 2015, Dantzer and Swanson 2012, McGaugh and others 2015). Most investigations of IGF1 as a mediator of life-history trade-offs have focused on mammals (e.g., above citations and see (Lewin and others 2017)). However, unlike in mammals, in reptiles environmental temperature per se can affect IGF1 levels (Avila-Mendoza and others 2016) and has a much greater impact on growth because of their ectothermic physiology. Further, most studies to date that have examined the relationship between growth and IGF1 have been correlative (but see (Duncan and others 2015; Lodjak and others 2017; Sparkman and others 2010).

Food consumption is positively correlated with growth in garter snakes – both in the present study and in the wild. However, in the short-term, prior food consumption was not correlated with IGF1 levels beyond its positive association with warm rearing treatments. Yet, prior growth was negatively correlated with titers of IGF1. These results suggest that the effects of food on growth are not directly mediated through IGF1, similar to findings in the brown house snake (Sparkman et al. 2010) and Japanese quail (Ronning et al., 2009), but contrary to the findings in other species (Pierce et al., 2001, Duncan et al. 2015). It is possible that IGF1 levels would only respond to extreme changes in food availability; since our experimental snakes were offered food *ad lib*, there was no variation in available food to affect IGF1 levels. Another possible explanation could be that food affects transcription or translation of the IGF1 gene, but we do not see those effects manifested into circulating IGF1 protein.

We found a negative correlation between prior growth and titers of circulating IGF1. This result is opposite to the pattern found in brown house snakes (Sparkman and others 2010), great tits (Lodjak and others, 2014), *Sceloporus* lizards (Duncan and others 2015), pied flycatchers (Lodjak and others 2017), and spotted hyenas (Lewin and others, 2017, but note this study examined mass not growth), but similar to that found in meadow *T. elegans* in the field (Sparkman and others 2009, but this study was looking at size not growth). Because it is unclear if IGF1 levels are indicative of previous growth, we had also looked for a correlation between IGF1 levels and growth after the measurement time point, but no relationship existed. More frequent measurements of size and IGF1 could have revealed a different relationship than what we found. For example, in salmon, the strongest relationship between IGF1 levels and growth occurred within a month prior to the hormone sampling (Beckman and others 2004).

One key component of the insulin/insulin-like signaling system axis this study did not examine was localized expression of IGF1. As we only looked at circulating levels of IGF1, we do not know how temperature and food may have affected localized production of the hormone. A companion study by Reding et al. (2016) found that mRNA of IGF1 in liver and muscle was higher in cool animals compared to those same tissues in warm animals. These results support our findings of higher levels of circulating IGF1 in cool animals at our final time point, although Reading and others (2016) found that circulating IGF1 levels were higher in warm animals. The importance of paracrine and autocrine production vs. endocrine release of IGF1 is equivocal (e.g., (Chauvigne and others 2003; Eppler and others 2007).

Growth variation as a mediator of survival

As has been seen in snakes and other ectothermic vertebrates (Folkvord and Ottera, 1993; Gangloff et al. 2015; Sinervo and Adolph, 1989), initial size affects how much an individual grows. However, when considering growth over 4 years, the initial importance of birth size (or an interval's start size) disappears as animals reach sexual maturation. In the present study, this was true in both ecotypes and in both temperature treatments. Food consumption also positively predicted growth rate with more food consumed in the warm treatment and as animals increased in size (see also (Lodjak and others 2014; Sparkman and others 2010). Previous work in this system, including a different subset of individuals from this cohort, demonstrates that growth efficiency is higher in larger animals and animals in the warm rearing treatment (Gangloff and others 2015). Whole organism resting metabolic rate – an additional performance measure – may shed light on long-term versus short-term trends in our study. In both ecotypes, snakes with higher mass-independent resting metabolic rates had lower growth efficiency (a measure of

conversion of ingested food into body substance(Gangloff and others 2015). This trend of higher resting metabolism negatively associating with growth occurs in other reptiles as well (e.g., (Steyermark 2002), and is postulated to occur because animals with higher resting metabolic rates devote more energy to maintenance and have less energy available for growth.

We were not able to take into consideration the maternal condition during gestation, which can affect initial growth and size at birth. The amount of protein, for example, that the mother consumed could have affected what she deposited into the yolk. The nutritional content of the yolk could have contributed to neonatal growth. Any differences in growth between ecotypes could be influenced by maternal diet (Metcalfe and Monaghan 2001; Micke and others 2011). Robbins et al. 2012, Sikes 1996). Notwithstanding, animals that grew fastest during their 2^{nd} – 4^{th} years had lower probability of survival, despite that animals that started off larger had a higher probability of surviving their first 4 years. Although not a topic of this study, if faster growers were engaged in "catch-up" growth (for example, due to cooler rearing temperatures), then these results would suggest a downstream cost in terms of survival (see also (Marcil-Ferland and others 2013; Metcalfe and Monaghan 2003; Orizaola and others 2014) reviewed in (Lindstrom 1999).

Selection gradients: The direct link between ecomorphology and life-history

One of the elements of the morphology/performance/fitness paradigm was the calculation of selection gradients and thus the ability to infer whether and how selection was operating on specific morphological/performance traits (Arnold 1983). Accomplished through regression techniques with a proxy for fitness and multiple phenotypes ((Arnold and Wade 1984a; 1984b; Lande and Arnold 1983), the partial regression coefficients can be interpreted as the selection

differentials. On the life-history side, population demography sensitivity analyses applied to a Leslie or Lefkovitch matrix of vital rates can address how variation in vital rates affect population growth rates; how sensitive the fitness of a phenotype is to perturbations in vital rates (reviewed in(Caswell 2001)). At the same time, Russ Lande ((Lande 1982a; Lande 1982b) made explicit that these estimated sensitivities are equivalent to selection differentials. Thus, selection differentials should be a direct link between ecomorphology and life-history theory.

Selection gradients vary between these fast and slow ecotypes for survival and growth in the pre-adult stages (Miller and others 2011). Specifically, neonatal and juvenile survival are two vital rates that differ between M-slow and L-fast populations in their estimates of sensitivities (selection gradients) and elasticities (contribution to population growth rates). While much is known about the causes of neonate mortality in this system, relatively little is known about the juvenile stage. Yet, in M-slow populations, survival increases in the juvenile stage to levels equivalent to adults (ca. 0.80 probability of survival annually), whereas in L-fast populations, juvenile survival is much lower (ca. 0.50 probability of annual survival). Accordingly, the selection gradients for juvenile survival average 0.45 in M-slow populations and 0.35 in L-fast populations. Therefore we sought to better understand the sources of variation in juvenile survival. Figure 6 presents the contributions to juvenile survival through morphology and performance, but also through environmental variation and ecotype variation which we use as a proxy for genetic background. Strong positive effects of body size and negative effects of growth on juvenile survival may buffer snakes from the negative effects of low-resource years and high temperature years. The relationships among feeding behavior, hormone levels, and growth are more complex, with larger snakes eating more and growing more, yet having lower IGF1 plasma concentrations and negative association of growth with survival. We have argued elsewhere that

growing maximally rather than optimally is likely very costly in this system where food availability is unpredictable. In M-slow habitats, any given year is only 50% likely to have adequate snowfall for anuran breeding in the spring (the primary prey of meadow garter snakes). In L-fast habitats, although fish and leeches are the primary prey, fluctuations in water level and shoreline vegetation provide an annually changing gauntlet that snakes must traverse to get to their food source.

The results of the present study, combined with past work on the garter snakes in the Eagle Lake system, present a complex relationship between traits traditionally categorized as morphology, performance, and life history (Fig. 6). This web of mutually dependent traits belies simple categorization in a one-way causal path among different aspects of the phenotype. Rather, we see that traits feedback on each other through multiple pathways in which genetic background and environmental conditions interact to create distinct ecotypic syndromes at the population level. Importantly, our experimental results demonstrate that the hormone IGF-1 plays a central mediating role between body size and growth during the juvenile stage. Field observations complement this finding, showing that in the L-fast ecotype – where snakes grow faster and reach larger asymptotic sizes – plasma IGF-1 concentrations are correlated with size. Nonetheless, this relationship is not entirely straightforward and may shift across ontogeny, especially at life-history transitions when energetic allocation to growth begins to trade-off with allocation to reproduction. What is startling about the interdependent relationships among traits within individuals is that, despite this complexity, we observe rather distinct life-history ecotypes in these natural populations. Traits of individuals within each ecotype – including metabolic and energetic functions, immune capacity, hormone titres, and life-history traits – can be categorized on opposite ends of the fast-slow pace-of-life continuum. Thus, ecotype differentiation is an

emergent property of this biological system, relying not on a single causal pathway but multiple interwoven systems that feedback on each other, resulting in suites of correlated phenotypic traits adapted to local environments.

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Figure Legends

Figure 1. Scatterplots of the relationship between size (SVL in mm) and plasma IGF-1 concentration (log_{10}-transformed ng/mL) for each life-stage by ecotype combination in field-caught *Thamnophis elegans*. Snakes were sampled from 2006-2010 at populations around Eagle Lake, CA, USA. Overall levels of IGF-1 varied across years (see Table 2), so values here are normalized with respect to year (from model of log_{10}-transformed IGF-1 concentrations with year as a categorical fixed effect). The relationship between SVL and IGF-1 concentrations is significant for L-fast adults ($R^2 = 0.14$, $P < 0.001$).

Figure 2. Least-square means from the model of lifetime size (SVL in mm; see text for model details) for *T. elegans* juveniles raised in captivity, through age 4 years. Both ecotype and rearing treatment influenced lifetime growth. Error bars represent ± SE.

Figure 3. (A) Least-square means from the model of lifetime growth (SVL in mm; see text for model details) for *T. elegans* juveniles raised in captivity, from birth to age 4 years. Both ecotype and rearing treatment were significant factors in determining lifetime growth. (B) Least-square means from the model of lifetime feeding (amount consumed in g; see text for model details) for *T. elegans* juveniles raised in captivity, from birth to age 4 years. Lwarm snakes ate more than Lcool, Mwarm, or Mcool snakes (all adjusted pairwise comparisons significant with $p < 0.01$). Error bars for both panels represent ± SE.

Figure 4. Survival curves for captive-born *Thamnophis elegans* by treatment group within each sex. Plots demonstrate the significant influence of ecotype, treatment, and sex on survivorship

based on the semi-parametric Cox Proportional hazard model (see Table 6). Horizontal dotted line shows 50% survivorship. Estimates are median residual lifespans (in months) ± SE.

Figure 5. (A) Growth (change in SVL, mm) for captive-born *T. elegans* over the three intervals. Estimates are shown on the last day of an interval; the number of days in each interval varied: Interval 1 = 178 days; Interval 2 = 48 days; Interval 3 = 72 days. The significant effect of temperature × interval is seen in that during interval 1, warm snakes were growing fastest, whereas in interval 3, cooler snakes were growing fastest. The significant effect of ecotype × time can be in that during interval 1, L snakes were growing fastest, whereas in interval 3, cooler snakes were growing fastest. **(B)** Least-square means from the model of plasma IGF-1 concentration (log_{10}-transformed ng/mL). IGF-1 levels were assayed from samples collected from the final day of 3 intervals, with first measurement 267 days after birth. The significant 3-way interaction of ecotype × treatment × time can be seen in the deviation of the Mcool values from the others, Note that Mcool animals grew fastest, and faster than other categories, during interval 3. Error bars for both panels represent ± SE.

Figure. 6. The complex interplay of phenotypic traits involved in the morphology–performance–life-history web. Black arrows represent relationships between aspects of the phenotype, with solid lines indicating positive relationships and dashed lines indicating negative. Solid gray arrows represent the direct impact of environmental characteristics on traits. Zig-zag lines show traits in which genetic canalization has resulted in differences between distinct life-history ecotypes. Traits and factors shown in bold black type are included in the present study; traits and factors in gray type have been established in previous studies (Bronikowski & Arnold 1999, Sparkman and others 2007, Miller and others 2011, Sparkman and others 2013).

Table 1. Sample Sizes and measuring dates for full colony of *T. elegans* neonates raised in captivity.

Study	N neonates	Ecotype (litters, neonates)	Female-warm	Female-cool	Male-warm	Male-cool
Cohort morphology, growth, feeding, survival study						
[1]Birth	257 (65 released)					
[2]Assigned a treatment	192	M-slow (19, 72)	18	20	18	16
		L-fast (21, 120)	31	29	30	30
29 Nov. 2010	189					
[3]1 Nov. 2011	153					
15 Nov. 2012	66					
19 Aug. 2013	56					
10 Dec. 2014	28					
[4]IGF1-growth study	130	M-slow (18, 47)	10	10	15	12
		L-fast (21, 83)	22	20	21	20

[1] Birth occurred 12 Aug – 19 Sept 2010.
[2] 65 neonates were released shortly after birth into their natal populations from very large litters.
[3] Survival analysis was conditioned on survival to one-year (i.e., survival through the neonate stage) and used these 153 1-year olds.
[4] IGF1-growth study initiated 14 Sept 2011.

Table 2. Generalized linear model results of log_{10}-transformed field IGF-1 plasma concentration measures collected from male and non-gravid female *T. elegans* from 2006-2010.

Source of Variation	Estimate	df_1, df_2	*F*	Pr > F	Direction of significant factors
SVL	-0.00059	1, 539	1.40	0.24	
Ecotype	-0.33	1, 539	8.16	**0.0044****	L-fast > M-slow
Life stage	-0.25	1, 539	3.78	0.052	
Year	--	4, 539	8.52	**< 0.0001****	2006 > 2007 > 2009 > 2010 > 2008
SVL × Ecotype	0.00097	1, 539	7.63	**0.0059****	See Figure 1
SVL × Life stage	0.00081	1, 539	3.83	0.051	See Figure 1
SVL × Year	--	4, 539	1.21	0.31	
SVL × Ecotype × Life Stage	-0.00035	1, 539	9.66	**0.0020****	See Figure 1

Table 3: Mixed linear model analysis of *T. elegans* body size from Birth—Dec. 2014 (SVL in mm).

Source of Variation	Estimate	df_n, df_d	*F*	Pr > F	Direction of significant factors
Ecotype	10.35	1, 31.6	14.33	**0.0006****	L-fast > M-slow
Rearing treatment	-4.16	1, 137	1.36	0.25	--
Age	0.086	1, 140	1047.5	**< 0.0001****	Older > Younger
Sex	-4.39	1, 140	9.70	**0.0022***	Males > Females
Ecotype×Rearing treatment	5.24	1, 137	3.93	**0.050***	See Figure 2
Ecotype×Age	0.04	1, 140	15.70	**0.0001****	See Figure 2
Rearing treatment×Age	0.038	1, 140	12.35	**0.0006****	See Figure 2
Ecotype×Treatment×Age	-0.023	1, 140	2.50	0.12	--
Population(Ecotype)	--	5, 31.4	1.53	0.21	--

Table 4: Mixed linear model analysis of *T. elegans* growth from Nov. 2010—Dec. 2014 (change in SVL in mm).

Source of Variation	Estimate	df_n, df_d	F	Pr > F	Direction of significant factors
Growth Days	0.13	1, 137	535.63	**< 0.0001****	More days > Less days
Initial size	-0.025	1, 84.4	0.01	0.91	--
Ecotype	5.29	1, 37.8	9.98	**0.0031****	L-fast > M-slow, See Fig. 3A
Rearing treatment	33.82	1, 126	46.06	**< 0.0001****	Warm > Cool, See Fig. 3A
Sex	12.18	1, 138	6.97	**0.0092****	Females > Males
Ecotype×Rearing treatment	-6.19	1, 126	0.46	0.50	--
Population(Ecotype)	--	5, 27.1	1.48	0.23	--

Table 5: Mixed linear model analysis of *T. elegans* feeding from Nov. 2010—Dec. 2014 (weight of frozen/thawed pinky mice, in g).

Source of Variation	Estimate	df_n, df_d	F	Pr > F	Direction of significant factors
Feeding Days	0.15	1, 173	1632.79	**< 0.0001****	More days > Less days
Initial size	0.18	1, 112	1.29	0.26	--
Ecotype	18.58	1, 41.6	5.87	**0.020***	L-fast > M-slow
Rearing treatment	6.64	1, 150	25.15	**< 0.0001****	Warm > Cool
Sex	7.75	1, 170	5.65	**0.019***	Females > Males
Ecotype×Rearing treatment	17.86	1, 149	8.25	**0.0047****	See Fig. 3B
Population(Ecotype)	--	5, 31.1	1.20	0.33	--

Table 6. Results of semi-parametric Cox proportional hazards model of survivorship from age 1 to age 4 years in captive-born *T. elegans*.

Source	χ^2	Adj. df	*p*	Direction of significant factors
Size at 1 year	5.23	0.88	**0.018***	Larger snakes > Smaller snakes
Body Condition at 1 year	0.34	0.85	0.49	
Lifetime feeding Rate	0.29	0.89	0.54	
Lifetime growth rate	20.23	0.88	**< 0.0001****	Slow growers > Fast growers
Sex	2.63	0.84	0.083	
Ecotype	0.63	0.41	0.18	
Rearing treatment	1.28	0.90	0.23	
Sex × Ecotype	11.67	0.88	**0.0005****	Female L-fast > Male L-fast Male M-slow > Female M-slow
Sex × Rearing treatment	1.52	0.93	0.20	
Sex × Ecotype × Rearing treatment	3.81	0.93	**0.046***	See Figure 4
Population(Ecotype)	3.19	2.38	0.26	

Table 7. Repeated measures mixed model analysis of *T. elegans* growth over three intervals measured as change-in-SVL (in mm).

Source of Variation	Estimate	df_n, df_d	F	Pr > F	Direction of significant factors
Size at start of interval	-0.071	1, 92.2	10.95	**0.0013****	Larger > Smaller
Sex	3.26	1, 50.7	9.30	**0.0036****	Females > Males
Interval	--	2, 109	257	**< 0.0001****	Interval 1 > 3 > 2
Ecotype	-2.15	1, 50.6	8.33	**0.0057****	L-fast > M-slow
Rearing treatment	-6.22	1, 41	25.6	**< 0.0001****	Warm > Cool
Ecotype×Interval	--	2, 80.9	3.99	**0.0222***	See Figure 4
Rearing Treatment×Interval	--	2, 82.9	21.4	**< 0.0001****	See Figure 4
Ecotype×Rearing Treatment	6.11	1, 38.3	0.46	0.50	--
Ecotype×Rearing*Interval	--	2, 81.7	0.62	0.54	--
Population(Ecotype)	--	3, 20.8	0.13	0.94	--

Table 8. Repeated measures mixed model analysis for *T. elegans* plasma log_{10}IGF-1 with prior growth (growth over the preceding interval) as a covariate across three time points.

Source of Variation	Estimate	df_n, df_d	F	Pr > F	Direction of significant factors
Prior interval growth	-0.0014	1, 271	4.88	**0.0280***	Slower > Faster
Sex	-0.053	1, 93.9	7.17	**0.0087****	Males > Females
Time	--	2, 217	10.12	**< 0.0001****	
Ecotype	0.14	1, 106	3.58	0.06	
Rearing Treatment	0.15	1, 112	6.15	**0.0146***	Warmer > Cooler
Ecotype×Time	--	2, 178	1.26	0.28	--
Rearing Treatment×Time	--	2, 193	1.73	0.18	--
Ecotype×Rearing Treatment	-0.13	1, 102	2.18	0.14	--
Ecotype×Rearing*Time	--	2, 183	2.49	0.08	See Figure 6
Population(Ecotype)	--	3, 100	0.51	0.67	--

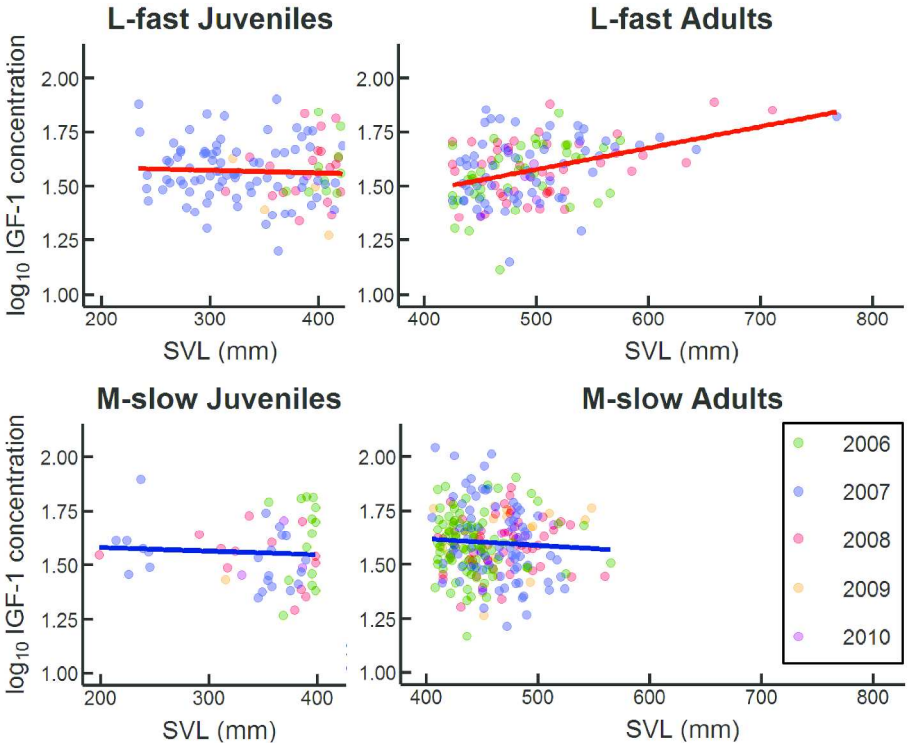

Figure 1. Scatterplots of the relationship between size (SVL in mm) and plasma IGF-1 concentration (log10-transformed ng/mL) for each life-stage by ecotype combination in field-caught Thamnophis elegans. Snakes were sampled from 2006-2010 at populations around Eagle Lake, CA, USA. Overall levels of IGF-1 varied across years (see Table 2), so values here are normalized with respect to year (from model of log10-transformed IGF-1 concentrations with year as a categorical fixed effect). The relationship between SVL and IGF-1 concentrations is significant for L-fast adults (R2 = 0.14, P < 0.001).

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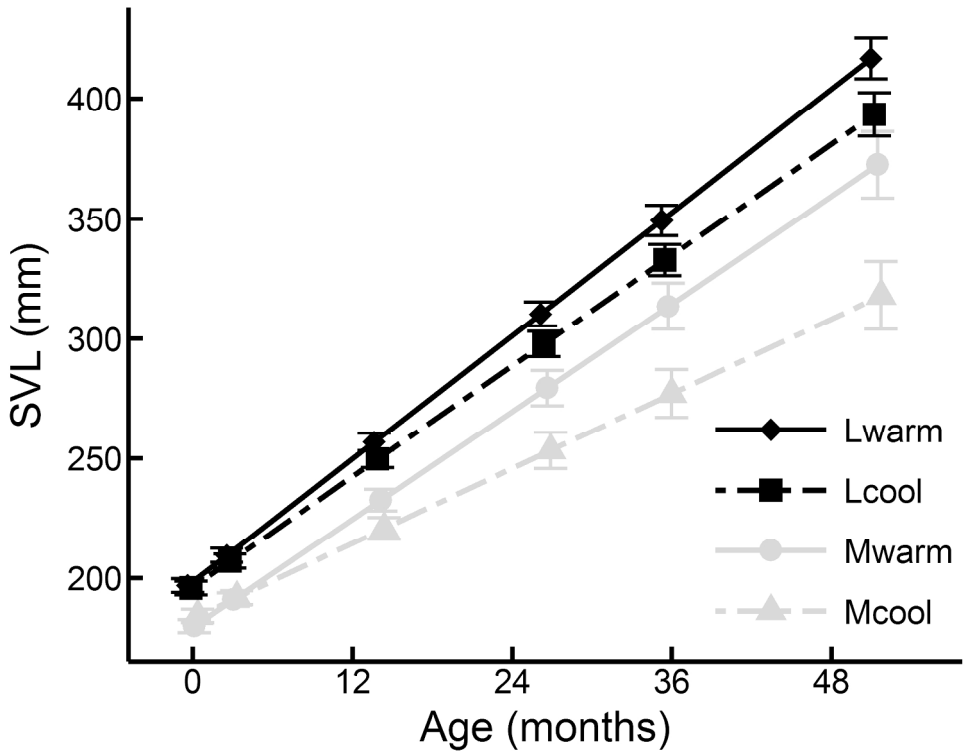

Figure 2 . Least-square means from the model of lifetime size (SVL in mm; see text for model details) for T. elegans juveniles raised in captivity, through age 4 years. Both ecotype and rearing treatment influenced lifetime growth. Error bars represent ± SE.

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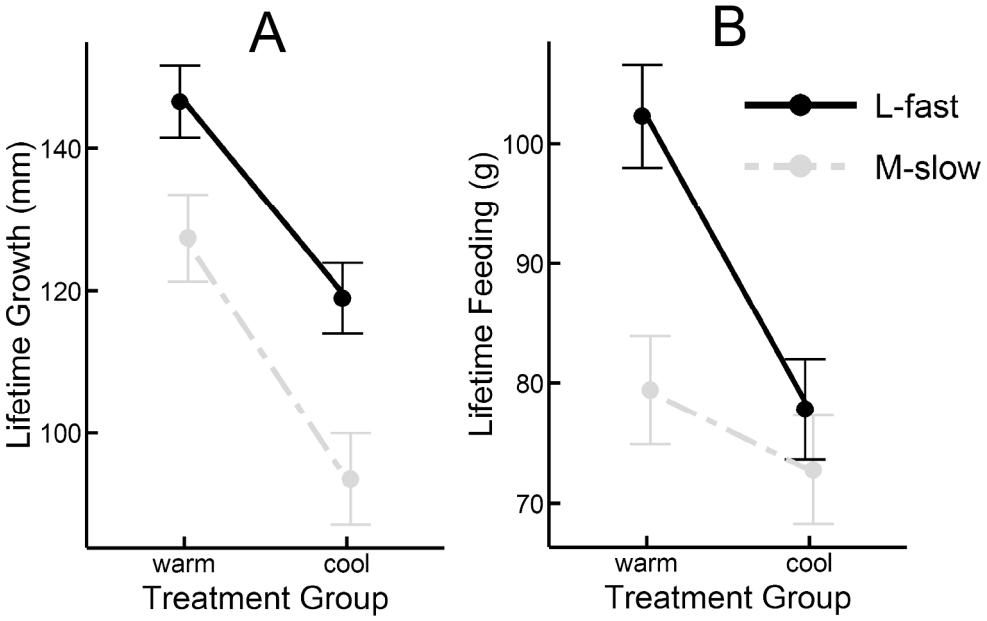

Figure 3. (A) Least-square means from the model of lifetime growth (SVL in mm; see text for model details) for T. elegans juveniles raised in captivity, from birth to age 4 years. Both ecotype and rearing treatment were significant factors in determining lifetime growth. (B) Least-square means from the model of lifetime feeding (amount consumed in g; see text for model details) for T. elegans juveniles raised in captivity, from birth to age 4 years. Lwarm snakes ate more than Lcool, Mwarm, or Mcool snakes (all adjusted pairwise comparisons significant with $p < 0.01$). Error bars for both panels represent ± SE.

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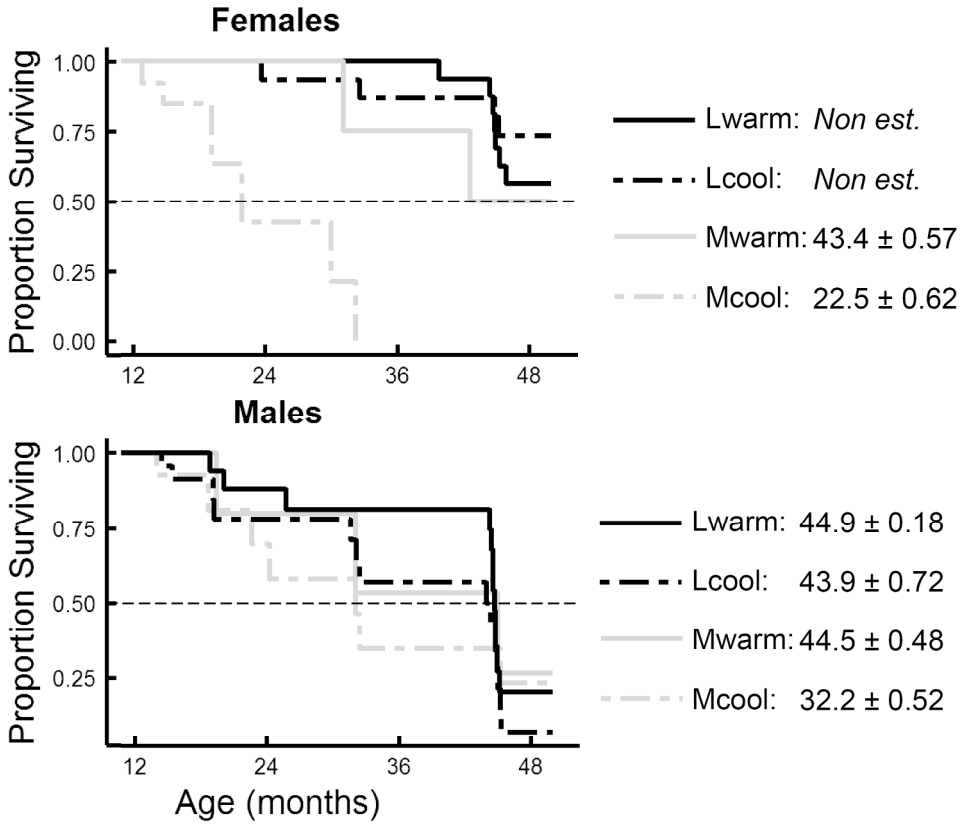

Figure 4. Survival curves for captive-born Thamnophis elegans by treatment group within each sex. Plots demonstrate the significant influence of ecotype, treatment, and sex on survivorship based on the semi-parametric Cox Proportional hazard model (see Table 6). Horizontal dotted line shows 50% survivorship. Estimates are median residual lifespans (in months) ± SE.

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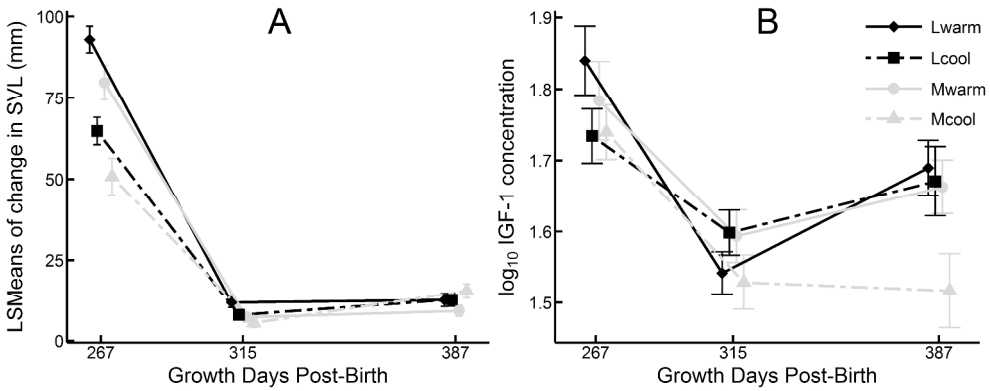

Figure 5 . (A) Growth (change in SVL, mm) for captive-born T. elegans over the three intervals. Estimates are shown on the last day of an interval; the number of days in each interval varied: Interval 1 = 178 days; Interval 2 = 48 days; Interval 3 = 72 days. The significant effect of temperature × interval is seen in that during interval 1, warm snakes were growing fastest, whereas in interval 3, cooler snakes were growing fastest. The significant effect of ecotype × time can be in that during interval 1, L snakes were growing fastest, whereas in interval 3, cooler snakes were growing fastest. (B) Least-square means from the model of plasma IGF-1 concentration (log10-transformed ng/mL). IGF-1 levels were assayed from samples collected from the final day of 3 intervals, with first measurement 267 days after birth. The significant 3-way interaction of ecotype × treatment × time can be seen in the deviation of the Mcool values from the others, Note that Mcool animals grew fastest, and faster than other categories, during interval 3. Error bars for both panels represent ± SE.

400x158mm (300 x 300 DPI)

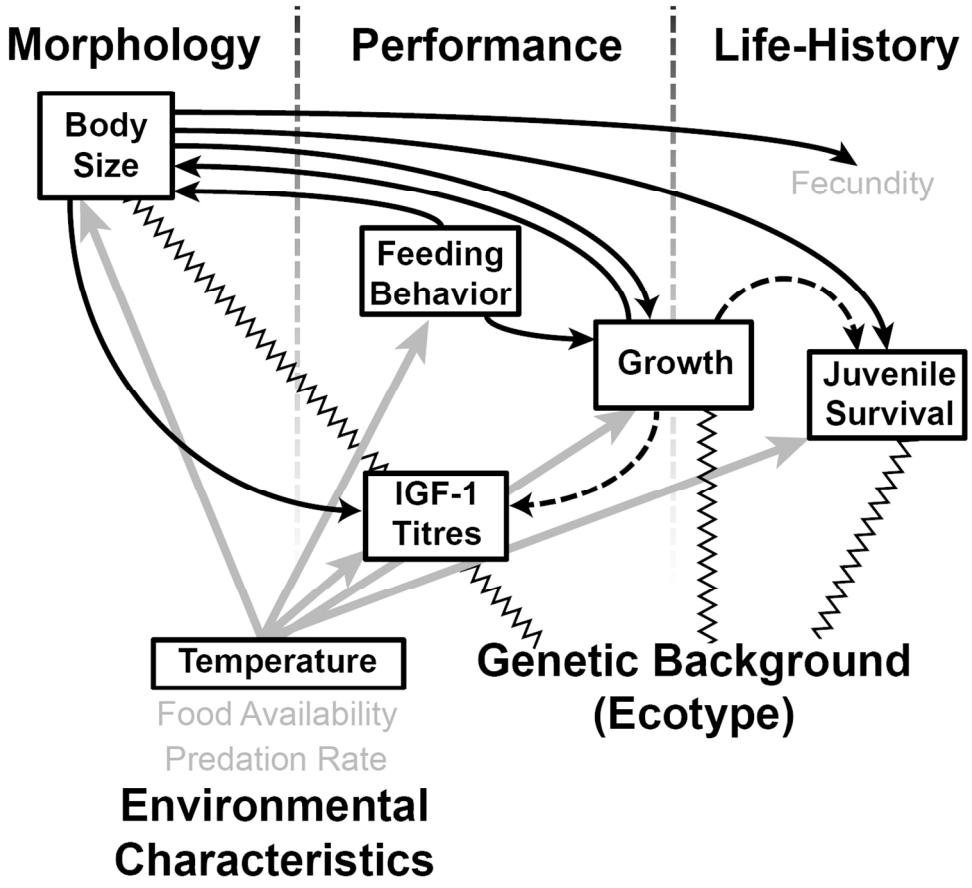

Fig. 6. The complex interplay of phenotypic traits involved in the morphology–performance–life-history web. Black arrows represent relationships between aspects of the phenotype, with solid lines indicating positive relationships and dashed lines indicating negative. Solid gray arrows represent the direct impact of environmental characteristics on traits. Zig-zag lines show traits in which genetic canalization has resulted in differences between distinct life-history ecotypes. Traits and factors shown in bold black type are included in the present study; traits and factors in gray type have been established in previous studies (Bronikowski & Arnold 1999, Sparkman and others 2007, Miller and others 2011, Sparkman and others 2013).

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