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Abstract

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Keywords

Eagle Lake, California, USA, garter snake, hormonal plasticity, IGF-1, insulin-like growth factor-1, life-history evolution, trade-offs, *Thamnophis elegans*

Disciplines

Other Ecology and Evolutionary Biology | Terrestrial and Aquatic Ecology | Zoology

Comments

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Evolutionary ecology of endocrine-mediated life-history variation in the garter snake *Thamnophis elegans*

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Abstract. The endocrine system plays an integral role in the regulation of key life-history traits. Insulin-like growth factor-1 (IGF-1) is a hormone that promotes growth and reproduction, and it has been implicated in the reduction of lifespan. IGF-1 is also capable of responding plastically to environmental stimuli such as resource availability and temperature. Thus pleiotropic control of life-history traits by IGF-1 could provide a mechanism for the evolution of correlated life-history traits in a new or changing environment. An ideal system in which to investigate the role of IGF-1 in life-history evolution exists in two ecotypes of the garter snake *Thamnophis elegans*, which derive from a single recent ancestral source but have evolved genetically divergent life-history characteristics. Snakes from meadow populations near Eagle Lake, California (USA) exhibit slower growth rates, lower annual reproductive output, and longer median adult lifespans relative to populations along the lakeshore. We hypothesized that the IGF-1 system has differentiated between these ecotypes and can account for increased growth and reproduction and reduced survival in lakeshore vs. meadow snakes. We tested for a difference in plasma IGF-1 levels in free-ranging snakes from replicate populations of each ecotype over three years. IGF-1 levels were significantly associated with adult body size, reproductive output, and season in a manner that reflects established differences in prey ecology and age/size-specific reproduction between the ecotypes. These findings are discussed in the context of theoretical expectations for a trade-off between reproduction and lifespan that is mediated by pleiotropic endocrine mechanisms.

Key words: Eagle Lake, California, USA; garter snake; hormonal plasticity; IGF-1; insulin-like growth factor-1; life-history evolution; trade-offs; *Thamnophis elegans*.

INTRODUCTION

How complex traits evolve, singly or in tandem with other traits, is a question central to understanding how populations diverge, and ultimately how speciation may occur. While much research examines genomic and/or morphological manifestations of divergence, few studies have sought the physiological mechanisms between the level of genes and the whole-organism phenotype that may mediate, and to some extent orchestrate, changes among populations.

The endocrine system is critically involved in the determination of fundamental life-history traits involved in evolutionary fitness, such as growth, reproduction, and even, as recent studies have indicated, aging and lifespan (e.g., Sinervo and Licht 1991, Tatar et al. 2001, 2003, Flatt et al. 2005, 2007, Bellino 2006). Life-history theory has long focused on how constraints involved in allocation of energy and other resources create trade-offs among life-history traits both within and among taxa (Kirkwood 1977, Roff 1992, Stearns 1992, Charlesworth 1994). However, the endocrinological mechanisms that may underlie many of these trade-offs and

restrict life histories to certain commonly observed patterns—such as an association between fast growth, high reproduction, and short lifespan—are only just beginning to be explored (Stearns 1989, Ketterson and Nolan 1992, 1999, Finch and Rose 1995, Zera and Botsford 2001, Ricklefs and Wikelski 2002).

Recent studies addressing the role of hormones in life-history trade-offs have given promising results. Experimental manipulation of follicle-stimulating hormone (FSH) in the side-blotched lizard, for instance, has been shown to generate the classic life-history trade-off between offspring size and number (Sinervo and Licht 1991). In birds, testosterone has been linked to the evolution of trade-offs in such traits as mate choice and reproductive success, and survival (reviewed in Ketterson and Nolan 1999, Reed et al. 2006). And in insects, juvenile hormone has proved a key actor in trade-offs between reproduction and lifespan (Zera and Denno 1997, Tatar and Yin 2001, Flatt and Kawecki 2007).

Currently much attention in model organisms has been focused on the role of insulin-like growth factor/insulin molecules in the regulation of life-history traits (reviewed in Tatar et al. 2003, Bartke 2005, 2008). While invertebrate species may exhibit several to dozens of ligands that bind to a single receptor, vertebrates have evolved three homologous but distinct ligands—insulin, IGF-1, and IGF-2—that bind to three separate recep-

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tors (though some degree of cross-reactivity among them still exists; reviewed in Nakae et al. 2001). While insulin chiefly coordinates metabolic activities, IGF-1 is heavily involved in stimulating cell survival, proliferation, migration, and differentiation. It is secreted primarily by the liver in response to pituitary growth hormone (GH), and stimulates growth, development, and reproduction in all vertebrates yet examined—a list that includes mammals, fish, reptiles, and birds (e.g., Kagawa et al. 1994, Perez-Sanchez et al. 1995, Guillette et al. 1996, Hiney et al. 1996, Beccavin et al. 2001, Beckman et al. 2003, Uchida et al. 2003). However, it appears that these constructive actions of IGF-1 do not come without a cost. IGF-1 (along with insulin) triggers a signaling pathway that is homologous to the IGF/insulin (IIS) pathway in invertebrates that has negative impacts on longevity (reviewed in Giannakou and Partridge 2007). Evidence for a role of IGF-1 in vertebrate aging is growing (reviewed in Bartke 2008), and one landmark study has shown that reduced expression of IGF-1 receptors leads to extended lifespan and reduced oxidative stress in mice (Holzenberger et al. 2003).

This study tests for a role of IGF-1 in the evolutionary divergence of life-history traits in replicate wild populations of the garter snake *Thamnophis elegans*. These populations are derived from the same ancestral source lineage but have differentiated into two distinct ecotypes that differ in scalation, coloration, and life history (Bronikowski and Arnold 1999, Bronikowski 2000, Manier and Arnold 2005, Manier et al. 2007). Individuals in one ecotype, which occupies lakeshore habitats, exhibit fast growth, early maturation, large adult body size, high annual reproduction, and low annual survival. In contrast, individuals in the second ecotype, which occupies mountain meadow habitats, exhibit slow growth, late maturation, small adult body size, low annual reproduction, and high annual survival (Bronikowski and Arnold 1999). Long-term mark-recapture and reproduction data have shown distinct differences in age-specific reproduction between ecotypes (Sparkman et al. 2007). The respective lifespans of the two ecotypes reflect these fast/slow life-history strategies, with fast-growth lakeshore snakes maintaining a shorter median adult lifespan (4 years) than slow-growth meadow snakes (8 years) (A. M. Bronikowski and S. J. Arnold, unpublished data). Furthermore, a genetic basis for differences in growth rates between the two ecotypes has been established with neonates raised in a common environment (Bronikowski 2000).

All populations of fast-growth snakes along the lakeshore were derived from an ancestral meadow lineage (Manier and Arnold 2005). There are a number of ecological factors that are likely to have influenced the evolution of life-history traits upon dispersal to lakeshore habitats, including higher mean temperatures, year-to-year stability and abundance of prey, and increased susceptibility to predation (Bronikowski and

Arnold 1999; S. J. Arnold, unpublished data). Such conditions are conducive to faster growth and the attainment of larger body sizes, as well as a correlated increase in reproductive output. IGF-1, which strongly influences the expression of all of these traits, responds plastically to experimental manipulations of nutrition and temperature (Duan and Plisetskaya 1993, Gabillard et al. 2003). Thus the life-history evolution which has occurred in lakeshore snakes may have involved evolution of the GH-IGF-1 axis in response to novel environmental cues encountered in the lakeshore habitat. If this were the case, it is also possible that increased IGF-1 receptor signaling incurred a trade-off in lifespan.

The goals of the study were twofold. First, it was necessary to establish basic biological patterns of IGF-1 in free-ranging snakes, as this constitutes the first study of IGF-1 in squamate reptiles, and one of only a handful of studies conducted in the wild (e.g., Crain et al. 1995a,b, Guillette et al. 1996, Webster et al. 1996, Schmidt and Kelley 2001). In particular, the activity of IGF-1 in life histories characterized by indeterminate growth and reproduction is poorly understood, and while IGF-1 secretion is positively associated with food intake and varies seasonally in some fish and mammals (Duan et al. 1995, Webster et al. 1996, Schmidt and Kelley 2001, Uchida et al. 2003), how levels might vary from year to year according to food availability is unknown. To understand the influence of these factors, both reproductive and nonreproductive snakes of a wide range of sizes were sampled during three years that differed dramatically with respect to climate-driven food availability. The second, and most pertinent, goal was to test for differences in plasma IGF-1 between ecotypes that would account for differences in growth and reproductive rates and support the possibility of its involvement in a trade-off with lifespan.

METHODS

Study populations and sample collection

Free-ranging western terrestrial garter snakes (*Thamnophis elegans*) living in the vicinity of Eagle Lake in Lassen County, California, USA, were collected during 2006, 2007, and 2008. They were sampled from four distinct lakeshore populations scattered intermittently along the Eagle Lake shoreline (L1–L4; population descriptors in accordance with those described in Bronikowski and Arnold [1999]) and three montane meadow populations located at increasing elevations just southeast of the lake (M1–M3). Only two southern lakeshore populations, L1 and L2, were sampled in 2006, but sampling was extended to two northern populations, L3 and L4, in 2007. All three meadow populations were sampled in 2006, but only M1 and M3 meadows were sampled in 2007, as M2 was dry and virtually devoid of snakes in that year. All seven populations were sampled in 2008. A total of 229 snakes were sampled in 2006, 342 in 2007, and 194 in 2008.

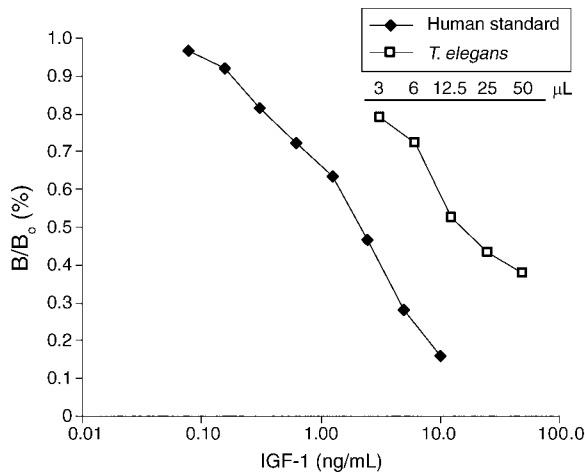


FIG. 1. Validation of a human radioimmunoassay (RIA) for IGF-1 in *Thamnophis elegans*. The figure shows percentage I-125 IGF-1 binding (B/B_0) against concentration of IGF-1 (ng/mL) for human standards of known concentration and against volume (μL) of pooled snake plasma.

In 2006 and 2007, sampling occurred from mid-May, within a few weeks of emergence from hibernation, through peak foraging season in June and July. In 2008, sampling occurred just as emergence and initial dispersal from hibernacula were beginning during two weeks from early to late May in 2008. Snakes were captured by hand from under rocks and vegetation or while basking or actively foraging. Previous work shows negligible diel variation in plasma IGF-1, so sampling was performed throughout the day (Lee and Rosenfeld 1987). Each snake was bled caudally within 10 minutes of capture. Blood samples were spun in a field centrifuge and plasma was frozen in liquid nitrogen for later analysis. All snakes were weighed, measured (snout-to-vent length [SVL]), sexed, and checked for ecdysis (i.e., blue eyes and/or shedding skin) on site, and females were palpated for embryos to determine reproductive status. A subset of the gravid females were returned to the laboratory in 2006 and 2007, and reproductive output was estimated by total litter mass upon parturition, using the combined masses of undeveloped embryos and both live and stillborn young (Robert and Bronikowski, *in press*).

Radioimmunoassay

Field plasma samples were assayed for IGF-1 using a standard radioimmunoassay (RIA) protocol (GroPep Ltd. Protocol #3002; Zovozyms GroPep, Adelaide, Australia). The assay was validated for *T. elegans* via serial plasma dilution. Briefly, a human standard curve was generated with recombinant human IGF-1 standards ranging from 0.078–10 ng/mL. A pool of acid-ethanol extracted plasma from *T. elegans* was aliquoted in 50, 25, 12.5, 6.25, and 3.125 μL volumes and assayed in duplicate along with the human standards using human IGF-1 antibody, and I-125 labeled human IGF-

1. Percentage I-125 IGF-1 binding (B/B_0) for all samples is shown in Fig. 1. The serial dilutions of snake plasma yielded a curve parallel to the human standard curve.

All field samples were extracted and assayed in duplicate following each field season. Randomly assigned groups of samples were assayed back-to-back within a 48-hour time period using the same reagents to reduce inter-assay variability. A pooled positive control was run with each successive assay. For 2006 assays, the intra-assay coefficient of variation (CV) averaged 2.2% and the inter-assay CV was 9.3%; for 2007 the intra-assay CV averaged 4.2% and the inter-assay CV was 6.7%; for 2008 the intra-assay CV averaged 2.9% and the inter-assay CV was 4.3%.

Climate

To assess the climatic variation among years, temperature and snow depth data were obtained from the National Climate Data Center and the California Data Exchange Center respectively, for all three years of study (data available online).^{2,3} Snow-depth measures were taken from a nearby weather station at Feather River Meadow, and monthly temperature means were taken from Susanville, a nearby city. It was clear from these data, and from that of multiple nearby locations, that snow depth was much reduced during the spring of 2007 vs. 2006 (April, 161.8 vs. 52.1 cm; May, 168.9 vs. 16.0 cm). Furthermore, spring temperatures reached an early high of 8.1°C in March of 2007, while in 2006 the March mean temperature was 2.2°C and did not reach into temperatures $>4^\circ\text{C}$ until April. 2008 was an intermediate year with respect to these variables, with snow depth high in April (143.0 cm), but low in May (48.3 cm), with a March mean temperature of 4.7°C. Negligible spring rainfall suggests that 2008, like 2007, will be a drought year. However, standing water from recent snow melt was still present in the meadows during the two-week sampling period.

Statistical analyses

Full model: ecotype, population, time, group, and ecdysis.—All statistical analyses were conducted using SAS software (SAS 9.1.3; SAS Institute, Cary, North Carolina, USA) using log-transformed values for IGF-1. IGF-1 was analyzed separately for each year as the dependent variable in an analysis of covariance (ANCOVA) using the following linear model, $Y = \mu + \text{day} + \text{ecotype} + \text{population}(\text{ecotype}) + \text{gravidity} + \text{ecdysis} + \text{day} \times \text{ecotype} + \text{day} \times \text{gravidity} + \text{gravidity} \times \text{ecotype} + \varepsilon$, where μ is the population mean, day (representing Julian day, the number of days elapsed between January 1 and the date of capture) is the covariate, and ε is the error term. Ecotype is the fixed effect of lakeshore vs. meadow habitat, and population nested within ecotype represents the effect of the seven different lakeshore and

² (www.ncdc.noaa.gov)

³ (www.cdec.water.ca.gov)

meadow populations. Population was treated as a fixed rather than a random effect to reflect complex micro-habitat variation among populations even within ecotypes. Gravidity is the effect of reproductive status and is divided in two categories: (1) male and non-gravid females and (2) gravid females. Ecdysis is the effect of the presence or absence of ecdytic characteristics (i.e., blue eyes and/or shedding skin). Originally, all two- and three-way interactions between effects were included in the model, but all nonsignificant interactions were removed to produce the model presented above.

Adult body size model.—SVL was excluded from the full model as it exhibited no significant relationship to IGF-1 when all body sizes were included in the analysis. However, when the data set was restricted only to non-reproductive adult snakes, a relationship between IGF-1 and SVL become evident. This relationship was assessed by year using a separate model, $Y = \mu + \text{ecotype} + \text{population}(\text{ecotype}) + \text{SVL} + \text{ecotype} \times \text{SVL} + \varepsilon$, where SVL is the covariate consisting of a range of body sizes in mature snakes (maturity occurs at ~400 mm in meadow and 425 mm in lakeshore snakes; see Bronikowski and Arnold [1999], Sparkman et al. [2007]). Sex and day showed no significant effects and thus were not included in the model.

Reproductive output model.—Gravid females were excluded from the above body size analysis since reproductive output is highly correlated to body size, making it difficult to dissociate the effect of reproductive activity from any independent effect of body size (Sparkman et al. 2007). The relationship between field levels of IGF-1 and final reproductive output was originally assessed using a full model analysis including ecotype, population nested within ecotype, and day, but as none of these effects showed any significance, the analysis was reduced to a simple linear regression between field plasma IGF-1 and total litter mass by year.

RESULTS

Full model: ecotype, population, day, gravidity, and ecdysis

In 2006, ecotype differences in IGF-1 emerged as a significant gravidity-by-ecotype interaction (Table 1). Comparison of least square means revealed that fast-growth lakeshore gravid females had significantly higher IGF-1 levels than slow-growth meadow gravid females ($P = 0.009$), while meadow males and non-reproductive females exhibited higher IGF-1 levels than their lakeshore counterparts ($P = 0.033$). There was also a nearly significant interaction ($P = 0.06$) between gravidity and days into the growing season, where IGF-1 remained constant in males and non-gravid females, but was low in May and rose to fairly constant levels in June and July in gravid females (Fig. 2A). Finally, ecdytic (shedding) snakes had significantly higher values of IGF-1 than non-shedding individuals (Table 1).

TABLE 1. Full model: analysis of covariance (ANCOVA) with log IGF-1 as the response variable.

Sources of variation	df	F	P
2006			
Ecotype	1, 217	0.09	0.7672
Population (ecotype)	3, 217	1.48	0.2211
Gravidity	1, 217	3.29	0.0710
Julian days	1, 217	12.00	0.0006*
Julian days \times ecotype	1, 217	0.67	0.6690
Julian days \times gravidity	1, 217	3.53	0.0617
Gravidity \times ecotype	1, 217	6.63	0.0003*
Ecdysis	1, 217	9.03	0.0030*
2007			
Ecotype	1, 330	22.79	<0.0001*
Population (ecotype)	4, 330	10.79	<0.0001*
Gravidity	1, 330	8.84	0.0032*
Julian days	1, 330	39.94	<0.0001*
Julian days \times ecotype	1, 330	9.69	<0.0001*
Julian days \times gravidity	1, 330	25.84	0.0002*
Gravidity \times ecotype	1, 330	0.40	0.5274
Ecdysis	1, 330	17.18	<0.0001*
2008			
Ecotype	1, 181	2.84	0.0937
Population (ecotype)	5, 181	11.05	0.2114
Gravidity	1, 181	4.40	0.2535
Gravidity \times ecotype	1, 181	0.55	0.0318*

Note: Asterisks denote significant effects ($P < 0.05$).

In 2007, a strong ecotypic effect was revealed in the ecotype-by-days interaction, where fast-growth lakeshore snakes retained constant levels of IGF-1 throughout the season (Fig. 2B), but slow-growth meadow snakes showed a gradual but highly significant decline as the season progressed (Fig. 2C). This proved a strongly assorting ecotypic effect, as all meadow populations showed significant decline throughout the season, while all of the lakeshore populations remained constant.

Gravidity also emerged as a significant effect in 2007, with males and non-gravid females having higher levels of IGF-1 than gravid females. There was no significant gravidity-by-ecotype interaction, as fast-growth lakeshore snakes from each group had higher levels of IGF-1 than slow-growth meadow snakes from corresponding groups. As in 2006, ecdysis also emerged as a significant effect in 2007, with non-shedding snakes having lower values than shedding snakes (Table 1).

In 2008, a gravidity-by-ecotype interaction was again evident, with lakeshore gravid females having significantly higher levels of IGF-1 than all other groups (lakeshore gravid vs. lakeshore non-gravid, $P = 0.0197$; vs. meadow non-gravid, $P = 0.0234$; vs. meadow gravid, $P = 0.0103$).

Adult body-size model

In both 2006 and 2007 there was a significant interaction between ecotype and SVL, where plasma IGF-1 levels increased with adult body size in fast-growth lakeshore snakes (2006, $P = 0.013$, $n = 40$, Fig. 3A; 2007, $P = 0.011$, $n = 62$, Fig. 3C), but either remained constant (2006, $P = 0.823$, $n = 85$; Fig. 3B) or

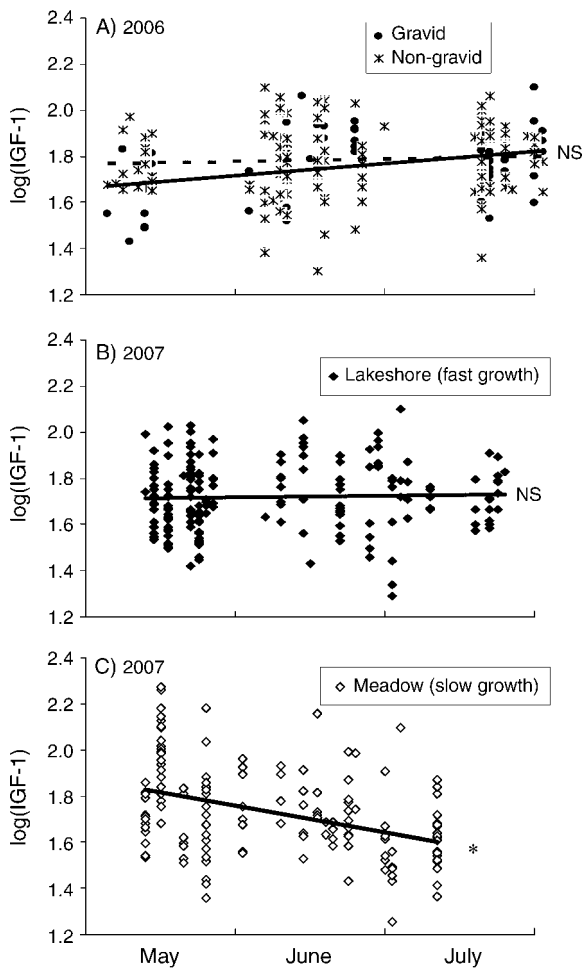


FIG. 2. Full model. Curve of log IGF-1 against Julian days (A) in 2006 for gravid and non-gravid snakes in both ecotypes, and (B, C) in 2007 for both fast-growth lakeshore and slow-growth meadow snakes individually. Months are denoted on the x-axis for the sake of clarity. The asterisk (*) denotes a significant slope ($P < 0.05$); NS = nonsignificant.

actually declined (2007, $P = 0.003$, $n = 69$; Fig. 3D) with body size in slow-growth meadow snakes (Table 2). In 2008, the year with a reduced sampling period, IGF-1 was positively correlated to SVL in both ecotypes ($P = 0.0182$, $n = 82$).

Reproductive output model

Regression of IGF-1 on total litter mass revealed the same pattern in both 2006 and 2007: increasing IGF-1 levels were associated with increasing reproductive output (2006, $P = 0.003$, $R^2 = 0.25$, $n = 34$; 2007, $P = 0.046$, $R^2 = 0.12$, $n = 34$; Fig. 4).

DISCUSSION

IGF-1 is well known for its close involvement in growth, reproduction, and survivorship in a variety of species throughout the vertebrate lineage (e.g., Kagawa

et al. 1984, Buonomo et al. 1987, Daughaday and Rotwein 1989, Scanes et al. 1989, Holzenberger et al. 2003). The plasticity of IGF-1 in response to a variety of environmental stimuli, including nutrition, temperature, and stress, has been particularly well documented in laboratory fish, birds, and mammals (e.g., Duan and Plisetskaya 1993, Schmidt and Kelley 2001, Gabillard et al. 2003), suggesting that studies situated in a natural ecological context are of great interest. However, IGF-1 activity in wild populations has been largely unexplored (but see Crain et al. 1995a, Guillette et al. 1996, Webster et al. 1996). This study presents the first data set on plasma IGF-1 in squamate reptiles, and explores its relationship to both environmental and phenotypic variation. In so doing, it becomes the first to elucidate a relationship between IGF-1 and ecotypic differences in life history in any wild species.

Here we report a complex and novel picture of how plasma IGF-1 differs within the two ecotypes of *Thamnophis elegans* in question. A basic understanding of the growth-stimulating effects of IGF-1 suggests that if lakeshore snakes are growing faster than meadow snakes, they should exhibit higher circulating IGF-1 levels on the whole. Surprisingly, a simple differentiation in plasma IGF-1 levels between slow-growth meadow and fast-growth lakeshore ecotype snakes was not evident. Instead, the way in which ecotype influenced IGF-1 levels in the complete sample of individuals was through interactions with three key variables: Julian day, body size, and reproductive status ("gravidity").

IGF-1 and Julian day

Interactions between Julian day and both gravidity and ecotype illuminate various aspects of how IGF-1 changes in relation to seasonal and interannual climate variability. In 2006, gravid females from both ecotypes showed an increase in IGF-1 levels from May to June (Fig. 2A). This timeline coincides with the onset of embryogenesis in females that have recently emerged from hibernation and mated, and matches seasonal elevations of IGF-1 seen in reproductive sea turtles (Crain et al. 1995a). IGF-1 levels in gravid females do not show a similar change from May to June in 2007, but this can easily be explained by an earlier emergence from hibernacula, owing to dramatically reduced snow depth and earlier highs in spring temperatures in 2007 than in 2006 (see *Methods: Climate*). Presumably adult females mated and commenced embryogenesis a few weeks before blood sampling was initiated in that year.

From June through July in 2006 for both ecotypes, and throughout 2007 in lakeshore snakes, IGF-1 did not change with Julian day (Fig. 2B). In 2007, however, meadow snakes showed declining IGF-1 levels from May to July (Fig. 2C). According to predictions from long-term records, amphibians, the primary prey source for *T. elegans* in meadow habitats, were scarce in 2007 due to the low levels of snow and rainfall (Bronikowski and Arnold 1999). As IGF-1 is known to decline with

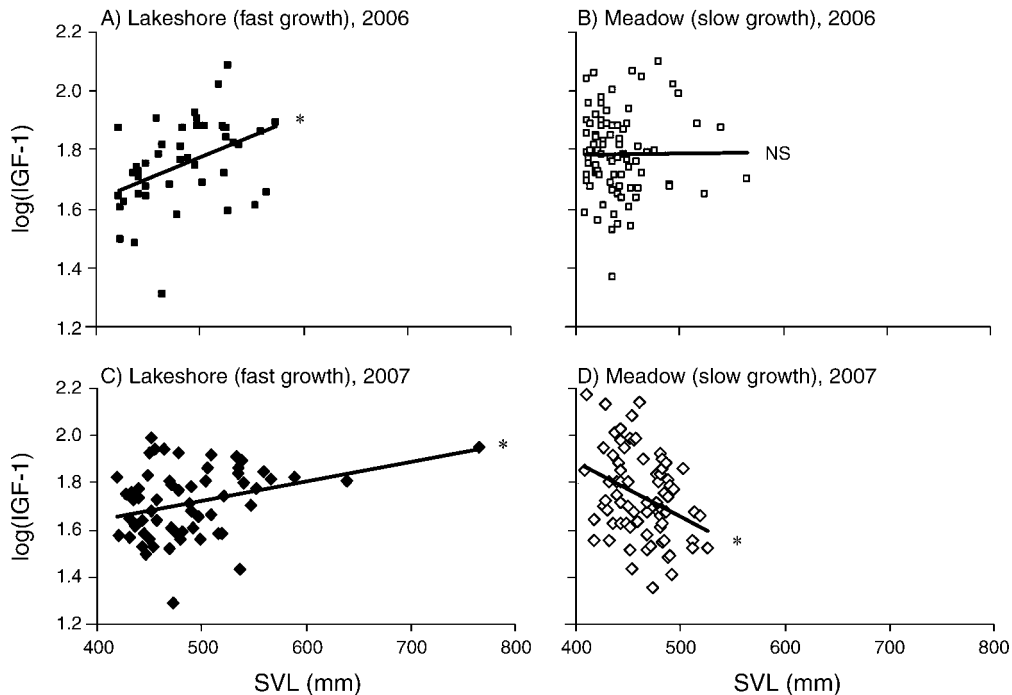


FIG. 3. Adult body size model. Curves of log IGF-1 against snout-vent length (SNV) for two years: (A) 2006 fast-growth lakeshore, (B) 2006 slow-growth meadow, (C) 2007 lakeshore, and (D) 2007 meadow snakes. Asterisks denote significant effects ($P < 0.05$). Note that significant effect remains even if large snakes are excluded from panel C.

low nutrition (e.g., Webster et al. 1996, Uchida et al. 2003), the seasonal decline in IGF-1 levels in meadow snakes during 2007 is thus readily interpretable as a response to declining availability of amphibian prey as the meadows continued to dry over the summer months. Given that scarcity of prey frequently occurs in meadow habitats due to highly variable annual precipitation from year to year (Bronikowski and Arnold 1999), this has important ramifications for differences in circulating IGF-1 between the two ecotypes. If meadow snakes regularly experience years of diminished IGF-1 levels, it follows that lakeshore snakes—which feed primarily on fish that are consistently available in the lake—may experience cumulatively higher levels of IGF-1 across years than meadow snakes.

IGF-1 and reproduction

In both 2006 and 2007 there was a strong correlation between circulating IGF-1 in gravid females and reproductive output: females with larger litters had higher IGF-1 levels (Fig. 4). As fast-growth lakeshore females have higher age/size-specific reproductive output than slow-growth meadow females (Bronikowski and Arnold 1999, Sparkman et al. 2007), this relationship between IGF-1 and reproductive output presumably resulted in lakeshore females having significantly higher IGF-1 levels in both years than meadow females. While it is clear that other species have elevated IGF-1 during gravidity (Daughaday and Rotwein 1989, Crain et al. 1995a, Guillette et al. 1996), this is the first time plasma

IGF-1 levels have been shown to rise with increasing reproductive output in an indeterminately growing species. Furthermore, it supports a role for IGF-1 in the divergence of reproductive life-history traits between the two ecotypes, with higher levels of IGF-1 in the lakeshore facilitating higher reproductive output.

IGF-1 and body size

This study presents three disparate relationships between IGF-1 and body size in *T. elegans* that have

TABLE 2. SVL model: analysis of covariance (ANCOVA) with log IGF-1 as the response variable.

Source of variation	df	F	P
2006			
Ecotype	1, 118	5.61	0.0195*
Population (ecotype)	3, 118	1.62	0.1889
SVL	1, 118	2.68	0.1040
SVL × ecotype	1, 118	4.17	0.0433*
2007			
Ecotype	1, 123	1.72	0.1922
Population (ecotype)	4, 123	3.20	0.0155*
SVL	1, 123	5.66	0.0189*
SVL × ecotype	1, 123	9.41	0.0027*
2008			
Ecotype	1, 74	0.02	0.8814
Population (ecotype)	4, 74	0.53	0.7108
SVL	1, 74	4.13	0.0456*
SVL × ecotype	1, 74	0.08	0.7719

Notes: Analysis includes mature, non-gravid snakes. Asterisks denote significant effects ($P < 0.05$).

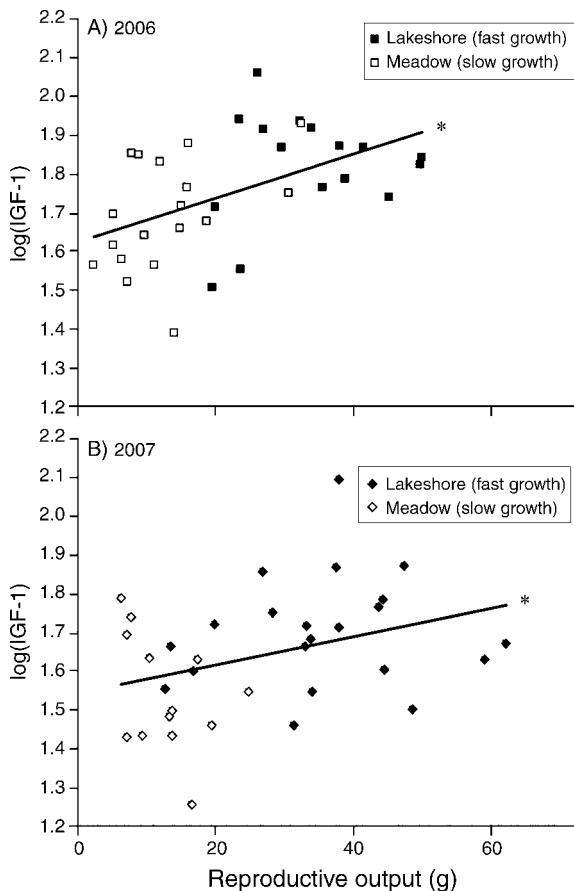


FIG. 4. Reproductive output model. Simple linear regression of log field IGF-1 against reproductive output (defined as total litter mass in grams) of fast-growth lakeshore and slow-growth meadow snakes combined for 2006 and 2007. Asterisks denote significant slopes ($P < 0.05$).

intriguing ecological and evolutionary implications (Fig. 3). In the lakeshore ecotype, where prey is continuously available, IGF-1 consistently increased with adult body size in all three years. In slow-growth meadow snakes, however, the relationship between IGF-1 levels and body size was plastic and varied annually with resource availability. During the two-week period sampled immediately post-hibernation in 2008, when tadpoles were still available (despite the promise of a dry year to come), meadow snakes, like lakeshore snakes, showed increasing IGF-1 with size. However, during the two years of extended sampling over a two month period, meadow snakes showed markedly different patterns from lakeshore snakes. In 2006, when conditions for amphibian breeding were favorable, IGF-1 levels in nonreproductive adult meadow snakes showed no relationship to size. In 2007, when conditions for amphibian breeding were unfavorable, larger mature snakes had lower plasma IGF-1 than smaller snakes.

This negative relationship between IGF-1 and size may reflect differential ability to obtain prey where

smaller snakes have the advantage in times of scarcity, or else may reflect ontogenetic changes that involve a juggling of energy investment priorities. There is some support for the former hypothesis in other snake species. Feeding rate in water pythons, for instance, has been shown to be lower in larger species during drought, as they have more difficulty obtaining prey from small openings (Shine and Madsen 1997). However, the latter hypothesis also merits investigation, as we do know that meadow snakes experience age/size-specific changes in energy investment: while reproductive effort (defined as grams reproductive output per gram female) remains constant in lakeshore snakes, it actually declines with age/size in meadow snakes (Sparkman et al. 2007). This may have important ramifications for IGF-1 activity during scarcity.

Plasticity between years aside, the fact that even in a wet ("good") year, fast-growth lakeshore and slow-growth meadow snakes sampled over a two-month period exhibit fundamentally different relationships between body size and IGF-1 levels suggests the possibility of evolution in the GH-IGF-1 axis. Growth begins to slow dramatically at maturity in *T. elegans*, so the increase in IGF-1 in mature lakeshore snakes is unlikely to be tied to increasing growth rate (Bronikowski and Arnold 1999). However we do know that reproductive output increases throughout life in lakeshore females, and shows minimal to no increase in meadow females (Sparkman et al. 2007). Thus we can hypothesize that mature lakeshore snakes are exhibiting increases in IGF-1 levels that are associated with size-related increases in reproductive output, while IGF-1 levels in mature meadow snakes reflect the relative lack of change in output with age/size.

As previously discussed, higher reproductive output is associated with higher IGF-1 levels in reproductive females, which is consistent with reproductive lakeshore females having higher IGF-1 levels than meadow females. Increasing IGF-1 as age/body size increases in nonreproductive adult lakeshore females may reflect increasing devotion of resources towards future reproduction even in a nonreproductive year. Whether most males reproduce every year and how their reproductive effort varies with age/body size is unknown. We do know, however, that IGF-1 stimulates spermatogenesis, and that testis size tends to increase with body size in many ectotherms (Baker et al. 1996, Shine et al. 1998, Nader et al. 1999, Skinner and Watt 2007). Such an increase in IGF-1 with size suggests that males may also exhibit size-specific changes in reproductive investment.

Concluding remarks

This study presents three lines of evidence in support of a role for IGF-1 in mediating the evolution of life-history traits in *T. elegans*. First, IGF-1 was higher in high-reproduction lakeshore snakes than in low-reproduction meadow snakes in all three years of study. Second, in two years of two-month sampling, IGF-1

increased with body size in male and nonreproductive female adult lakeshore snakes but remained constant or declined in adult meadow snakes. Finally, IGF-1 remained high and constant across the season with constant prey availability in the lakeshore habitats, whereas it declined in meadow snakes in a year with declining prey availability (a common occurrence in meadow habitats; see Bronikowski and Arnold 1999). Whether or not these ecotype differences in IGF-1 levels owe more to plasticity in different environments than to evolutionary divergence has yet to be established, but at the very least they suggest that fast-growth snakes living in the lakeshore habitat may experience cumulatively higher IGF-1 levels over their lifespan due to its association with larger body sizes, higher reproductive output, and greater prey availability. Clearly, any assessment of whether free-ranging lakeshore snakes experience cumulatively higher IGF-1 levels over their lifespan would require many more years of close monitoring, preferably involving repeated measures of individuals. However, if lakeshore snakes do indeed experience a higher IGF-1 "load," this may directly contribute to differences in survivorship between the two ecotypes by increasing the phenotypic rate of aging via increased IGF-1 receptor signaling.

Since this study focuses on levels of IGF-1 in free-ranging snakes, its findings are necessarily correlational and cannot definitively establish causal links between IGF-1 and the evolution of growth, reproduction, and aging. To approach this system from a more experimental angle, neonates are currently being raised in a common environment to test for a genetic divergence in IGF-1 between the two ecotypes. Further investigation of key components of the IGF-1 system, such as binding proteins, receptors, and aging-related signaling pathways downstream, is also in order. Thus, the complex involvement of IGF-1 in *T. elegans* ecology, body size, and reproduction presented here provides a solid and promising foundation for further work testing for a role of the endocrine system in generating trade-offs between reproduction and survival in an ecological context.

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