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Research

Among-individual heterogeneity in maternal behaviour and physiology affects reproductive allocation and offspring life-history traits in the garter snake *Thamnophis elegans*

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Oikos
127: 705–718, 2018
doi: 10.1111/oik.04204

Subject Editor: Nigel G. Yoccoz
Editor-in-Chief: Dries Bonte
Accpeted 7 September 2017

Accumulating evidence suggests that within-individual plasticity of behavioural and physiological traits is limited, resulting in stable among-individual differences in these aspects of the phenotype. Furthermore, these traits often covary within individuals, resulting in a continuum of correlated phenotypic variation among individuals within populations and species. This heterogeneity, in turn, affects individual fitness and can have cross-generational effects. Patterns of trait covariation, among-individual differences, and subsequent fitness consequences have long been recognized in reptiles. Here, we provide a test of patterns of among-individual heterogeneity in behaviour and physiology and subsequent effects on reproduction and offspring fitness in the garter snake *Thamnophis elegans*. We find that measures of activity levels vary among individuals and are consistent within individuals in reproductive female snakes, indicating stable behavioural phenotypes. Blood hormone and glucose concentrations are not as stable within individuals, indicating that these traits do not describe consistent physiological phenotypes. Nonetheless, the major axes of variation in maternal traits describe behavioural and physiological phenotypes that interact to predict offspring body condition and mass at birth. This differential allocation of energy to offspring, in turn, strongly influences subsequent offspring growth and survival. This pattern suggests the potential for strong selection on phenotypes defined by behaviour–physiology interactions.

Introduction

Patterns of covariance within individuals suggest that trait combinations are not random and that natural selection may operate to maintain trait combinations that optimize fitness outcomes (Dall et al. 2004, Careau et al. 2008, Réale et al. 2010b). Thus the adaptive significance of within-individual stability and among-individual variation has become the focus of studies in numerous areas of

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organismal biology, including physiology (Bennett 1987, Williams 2008, Cockrem 2013, Roche et al. 2016, Taff and Vitousek 2016), behavior (Sih et al. 2004, Réale et al. 2010a), life history (Wilson and Nussey 2010, Vindenes and Langangen 2015, Hamel et al. 2016) and population ecology (Bolnick et al. 2011, Dochtermann and Gienger 2012). Such phenotypic patterns can come about through several genetic mechanisms, including linkage disequilibrium, pleiotropy, and the non-random adaptive association of alleles maintained by selection (Falconer and Mackay 1996, Lynch and Walsh 1998). Specifically, the relationship between behavioural and physiological traits has received much attention, with patterns of covariation of these potentially labile traits evident in a wide range of taxa (Niemelä et al. 2012, Careau et al. 2014, Dubuc-Messier et al. 2016, Kern et al. 2016, Metcalfe et al. 2016, Monestier et al. 2016, Rádai et al. 2017). Early reviews of the animal personality literature have provided important insights into the fitness consequences of among-individual heterogeneity (Réale et al. 2007, Smith and Blumstein 2008, Biro and Stamps 2010, Carere et al. 2010) and recent work has added studies of ectothermic (non-avian) reptiles to this paradigm (Careau and Garland 2012). Reptiles exhibit great flexibility in physiology (e.g. infrequent feeding in snakes, extreme hypoxia tolerance in turtles) and variation in life histories (e.g. indeterminate growth and fecundity). Thus studies in these taxa testing the impact of among-individual heterogeneity on life-history traits, as well as covariation in life-history traits with physiological and behavioural phenotypes, will provide important insights into the universality of these patterns across vertebrate taxa. With the current study, we test how maternal physiological and behavioural phenotypes interact to influence fitness (offspring growth and survivorship) and bear cross-generational consequences for life-history traits in a model reptile system, the western terrestrial garter snake *Thamnophis elegans*.

Behavioural, physiological, and life-history traits are predicted to covary in predictable directions within individuals (Careau et al. 2008, Réale et al. 2010b, Careau and Garland 2012, Roche et al. 2016). However, the mechanistic relationships between these traits and components of fitness, and how this covariation may change across environmental or social conditions, are not well understood. One potential outcome is the emergence of a continuum of 'energetic phenotypes' in which physiological traits related to energy allocation and mobilization are coupled with behavioural traits related to food acquisition and energy expenditure to maintain energy balance (Careau et al. 2008, Biro and Stamps 2010). For example, within-individual measures of energy intake, energy expenditure, and growth were strongly correlated in a field study of the common garter snake *Thamnophis sirtalis*, supporting the expectation that behaviour and physiology covary within individuals to match foraging effectiveness and influence individual growth rates (Peterson et al. 1998). More recently, studies in the common lizard *Zootoca vivipara* have found limited support for coadaptation among behavioural, metabolic, and locomotor traits. However, captive-born offspring did experience correlated selection such that individuals with reduced exploration behaviour and high metabolic rates, as well as the opposite combination, survived better in outdoor mesocosms (Le Galliard et al. 2013). In adults of the same species, there is no evidence of correlated selection among measures of performance (sprint speed), thermoregulatory behaviour and energy metabolism (Artacho et al. 2015). Interestingly, however, large lizards with high metabolic rates enjoyed higher survival rates in captive mesocosms but had reduced reproductive output. This suggests a classic survival–reproduction tradeoff mediated by energetic phenotype.

Thermal preference can also drive variation in behaviour, as in mountain log skinks *Pseudemoia entrecasteaux* in which thermal preference covaries with activity and aggressiveness (Stapley 2006). Furthermore, recent work in the delicate skink *Lampropholis delicata* provides evidence of a syndrome whereby traits related to energy flux, mediated through thermal preferences, covary with behavioural traits including activity, exploration, boldness and social behavior (Goulet et al. 2017a, b). The shape of behavior–physiology relationships across ontogeny remain largely untested, though a recent study suggests that such relationships are stable. In the snake *Boa imperator*, heart and breath rates were positively correlated with agonistic behaviors in response to potential stressors across early life-history stages (Šimková et al. 2017). Given this emerging support for physiology–behaviour syndromes and the few studies conducted to test links between among-individual variation and fitness in non-avian reptiles, the conditions under which different trait correlations are advantageous (or disadvantageous) are still largely unknown (Réale et al. 2007).

In squamate reptiles (snakes and lizards), there is often large within-cohort variation in body size, which in turn affects subsequent life-history traits, including growth and survival (Bronikowski 2000, Kissner and Weatherhead 2005, Blouin-Demers and Weatherhead 2007, Warner and Lovern 2014). Yet the adaptive significance of both within-litter and within-cohort variation remains unclear, though this is essential for understanding the evolution of reproductive tradeoffs in squamate reptiles generally (Niewiarowski and Dunham 1994, Aubret 2012). Furthermore, studies of growth trajectories in reptiles demonstrate two important themes: 1) environmental conditions, especially variation in food availability, largely determine growth rates and 2) environmental conditions of birth and early development bear lifetime consequences for individuals because size is closely tied to important fitness traits, including reproduction and survival (reviewed by Andrews and Pough 1985; squamates: Jayne and Bennett 1990, Madsen and Shine 2000, Baron et al. 2010, Miller et al. 2014, Mack et al. 2017; crocodilians: Tucker et al. 2006; turtles: Lindeman 1997, Spencer and Janzen 2010, Eguchi et al. 2012). Variation in growth trajectories is also important for individual reproduction. For example, Lake Erie watersnakes *Nerodia sipedon insularum* exhibit significant among-individual heterogeneity in asymptotic size. In turn, the fitness benefit of larger size is dependent on annual rates of survival, which may provide a mechanism to maintain growth polymorphisms within the population (King et al. 2016).

Extreme within-species diversity in life-history traits, as well as physiological correlates thereof, is well-exemplified in the metapopulation of western terrestrial garter snakes around Eagle Lake, California, USA. Though connected by gene flow (Manier and Arnold 2005), replicate populations along the lakeshore and in nearby mountain meadows diverge in life-history strategies on the pace-of-life continuum (sensu Ricklefs and Wikelski 2002). Lakeshore populations exhibit a fast-paced strategy, characterized by more rapid growth rates to larger adult body sizes, higher annual reproductive output, and relatively higher annual mortality and therefore shorter lifespans in comparison with the slow-living meadow populations (Sparkman et al. 2007, Schwartz et al. 2015). Importantly, the mountain meadow habitats are characterized by dramatic stochastic variation in seasonal rainfall and thus prey (adult and larval anuran) availability (Bronikowski and Arnold 1999, Miller et al. 2011). Given that environmental variation can maintain trait polymorphisms and that we might expect standing genetic variation for such traits in recently-diverged populations such as these, snakes from meadow populations present an ideal system in which to test for a relationship between maternal phenotypes, reproductive output, and fitness. We test two primary hypotheses: first, we assess whether maternal behavioural and physiological phenotypes influence energetic allocation to offspring at birth; second, we test the importance of offspring mass and body condition at birth on early-life growth and survival (Fig. 1).

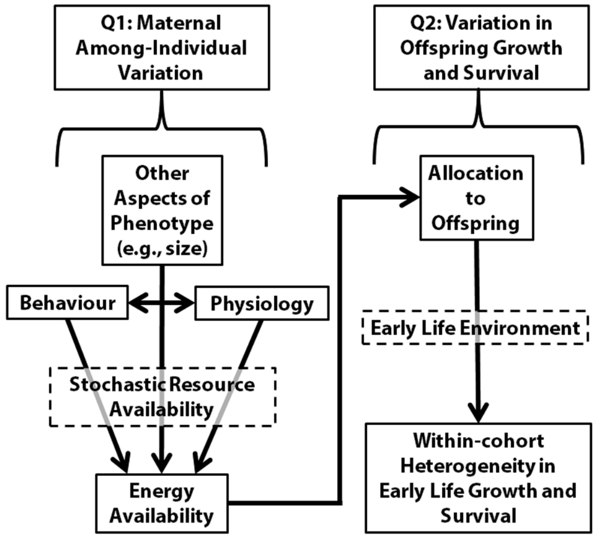

Figure 1. Theoretical schematic of how variation in maternal phenotypes is filtered through environmental conditions to affect early life growth and survivorship of offspring. Aspects of organism phenotypes in solid boxes; environmental factors in dashed boxes.

To address our first question, we quantify indices of among-individual heterogeneity in behavioural and physiological traits measured both before and after parturition in wild-caught female garter snakes from a single slow-living meadow population. The hypothalamus–pituitary–adrenal/interrenal (HPA/HPI) axis is implicated in the maintenance and coordination of energetic phenotypes because of its central role in modulating energetic allocation decisions, especially in response to perceived stressors (Sapolsky et al. 2000, Careau et al. 2008, Angelier and Wingfield 2013, Dantzer et al. 2016). In reptiles, the primary glucocorticoid is corticosterone (CORT; Norris and Jones 1987), which acts to increase glucose availability to tissues by initiating processes of glycogenolysis and gluconeogenesis (Sapolsky et al. 2000). Thus, measures of CORT and glucose concentrations provide data on both the activation of the HPI axis (CORT) and its downstream effect on energy mobilization (glucose). We predict that traits will be correlated within individuals such that snakes exhibiting more active behaviours in different contexts will also have higher indicators of energy availability (plasma glucose concentration) and mobilization (plasma corticosterone concentration). We then test whether the phenotypes of these reproductive females (hereafter, 'moms') interact to shape reproductive investment and success by quantifying the effect of these traits on the mass and body condition of offspring at birth; both of these metrics of energetic allocation have established effects on subsequent growth and survival in this system (Bronikowski 2000). To address our second question, we quantify the influence of birth body condition and mass on growth and survival to ages four months and one year. Furthermore, we test how maternal investment might be mediated through environmental conditions by raising offspring in two different thermal regimes designed to mimic potential variation in natural habitats (Bronikowski 2000, Gangloff et al. 2015). Finally, we discuss how these results may provide a potential mechanism by which phenotypic polymorphisms may be maintained in stochastic environments by fluctuating correlated selection.

Methods

Animal collection and care

On 15 June 2012, we hand-captured 21 pregnant western terrestrial garter snakes from a single meadow population (elevation 1645 m) near Eagle Lake, California, USA (population 'M1' from Sparkman et al. 2007, and population 'PAP' from Manier and Arnold 2005). Snakes were bled (approximately 150 μl) from the caudal vein using heparin-rinsed syringes. Baseline blood samples were taken within 10 min of capture for 11 pregnant females; the remaining 10 females were caught in association with several other snakes, and were consequently not bled within 10 min (before plasma CORT concentration is elevated in this species; Palacios et al. 2012). We kept whole blood on ice for a short time (< 3 h), aliquoted and snap-froze plasma in liquid nitrogen, and stored samples at –80°C until measurements. All snakes were weighed (mass in g; range: 33–90, SD = 15.8), measured (snout–vent length [SVL] in mm; range: 407–549, SD = 41.6), gently palpated for the presence of embryos, and transferred to Iowa State University. There we kept snakes under common-garden conditions (see husbandry details in Gangloff et al. 2015). We collected mom blood samples and weights/measures at two subsequent time points: after moms had been in captivity for about one month (hereafter 'pre parturition'; range 27–28 days after capture) and one month after giving birth (hereafter 'post parturition'; range 31–35 days after birth, 80–106 days post capture).

Offspring were live-born between 6–28 August (total n = 116; median litter size = 5, range: 2–10) and were weighed, measured, sexed, and moved to individual housing within 24 h of birth (mass range: 1.31–3.94 g, SD = 0.45; SVL range: 145–212 mm, SD = 11.7). We excluded from analysis four stillborn snakes and one snake born with a severely kinked back that died at age four days. Offspring were randomly assigned, with the restriction of being evenly divided among litters and between sexes, to either a 'warm' or 'cool' rearing environment mimicking the range of environmental conditions experienced in natural habitats (details in Gangloff et al. 2015). We recorded the amount of food consumed with each weekly feeding and measured offspring mass and SVL again at approximately 4 and 12 months of age. Snakes were checked a minimum of three times per week, so dates of death are accurate within 48 h. We calculated body condition at each time point as the residual of the $\log_{10}$-mass on $\log_{10}$-SVL regression including all live animals (Weatherhead and Brown 1996). We calculated specific growth rate ($SGR = 100 \times (\ln(SVL_2) - \ln(SVL_1))$ / days), which is numerically equivalent to the change in percentage body size per day and has been used to describe growth in snakes from this system (Addis et al. 2017).

Corticosterone and glucose assays

We measured CORT and glucose concentrations in mom plasma collected at three time points: time of capture, pre-parturition and post-parturition. CORT concentration was measured with double-antibody radioimmunoassay kits (kit no. 07120102, MP Biomedical) following the protocol described in Robert et al. (2009) with samples diluted 1:80. We measured CORT concentration using two kits and assessed inter-assay variability with kit-provided controls, yielding coefficients of variation (CV%) of 8.2% and 3.8% for high and low controls, respectively. All samples were run in duplicate and re-run if CV% between the duplications was $> 10\%$. We measured glucose from 1.5 µl of the thawed plasma using a glucometer. Samples measuring below the lower threshold of the glucometer (20 mg dl^{-1}; n = 3 samples) were assigned a value of 20 mg dl^{-1}. We were unable to collect baseline blood samples in the wild for 10 individuals and plasma limitations precluded measures in five and four samples for captive measures of CORT and glucose, respectively. We analyzed a total of n = 48 measures for CORT and n = 49 measures for glucose.

Behaviour assays

We quantified behaviour of moms in repeated open-field tests to measure two axes of personality: exploratory escape behaviour and boldness toward a predator (Réale et al. 2007, Carter et al. 2013, Gangloff et al. 2017a). We conducted two assays, 72 h apart, both pre- and post-parturition, for a total of four assays for each individual. The order of the trials was randomized within each testing day. Following identical protocols for each individual, we placed moms in a neutral test arena (circular plastic container with blocked escape portals) where we left the snake undisturbed for 15 s. Snakes were then subjected to a simulated predator attack consisting of five movements of a foam-tipped rod directed toward the snake's head but not contacting the snake. Such simulated predator attacks have been demonstrated to elicit an antipredator response in snakes similar to behaviours observed in nature (Herzog et al. 1989). Attacks were repeated three times at 20 s intervals. Following the final set of simulated attacks (after 60 s), we lifted the gate to reveal the escape portals. We continued to subject the snakes to simulated attacks at 20 s intervals until they escaped the arena or until the total trial time reached 180 s. As a metric of exploratory escape behaviour, we quantified the number of tongue flicks exhibited in the first 15 s in the test arena, which indicates information-gathering via olfaction and is an established index of both exploratory and antipredatory behaviour in squamate reptiles (Chiszar et al. 1976, Gove and Burghardt 1983, Šimková et al. 2017). We quantified boldness as the latency to escape from the test arena after the gate was opened (Debecker et al. 2016, Mell et al. 2016). Trials were video recorded and behaviours scored using JWatcher (Blumstein et al. 2006) by one author, with intra-rater reliability validated by rescoring 10% of the trials (Kendall's coefficient of concordance for tongue flicks: $W = 0.91$, $p = 0.0037$; escape latency: $W = 0.94$, $p = 0.0008$; Burghardt et al. 2012).

Statistical methods

To estimate the repeatability of physiological traits (CORT and glucose concentrations) we calculated consistency repeatability, which provides a measure of the relative amount of variation explained by differences among individuals after accounting for changes in measures across time (Biro and Stamps 2015). We created models with the dependent variables CORT concentration and glucose concentration with the fixed effect of time point and a random effect of individual, modeled with a compound symmetric covariance structure. CORT and glucose concentrations were $\log_{10}$-transformed before analysis to meet assumptions of normality (Sokal and Rohlf 2011). Our repeatability estimates are the ratio of between-individual variance to total variance, with significance assessed by a likelihood ratio test using

χ^2-distribution with one and zero degrees of freedom ($\chi^2_{0:1}$ in results; Snijders and Bosker 2012, Wolak et al. 2012).

To estimate the repeatability of behavioural traits (tongue flicks and latency to escape), we estimated Kendall's coefficient of concordance (*W*) using the 'magree' macro in SAS (Gwet 2002). The distribution of the escape latency times were largely bimodal, with snakes either escaping within the first few seconds or not leaving the test arena at all. Furthermore, the count data of tongue-flicks did not meet assumptions of normality. As a non-parametric measure of repeatability, Kendall's *W* compares changes the rank-order of individuals across trials and is therefore insensitive to changes in mean trait values over time, providing a statistic of rank agreement on the same scale (0–1) as repeatability (Sokal and Rohlf 2011).

While repeatability statistics as described above provide a measure of the relative stability of individual traits over time, we also sought to identify the major axes of variation in both behavioural and physiological traits. A principal components analysis (PCA) permits us to identify the primary axes of among-individual variation in all measures to illustrate an individual's behavioural or physiological phenotype while simultaneously retaining variation in the original data. For physiological traits, we $\log_{10}$-transformed measures of plasma CORT and glucose concentrations from the three time points and used a correlation matrix to identify the major axes of variation and the corresponding loadings. We utilized a rank-order correlation matrix for the PCA of behavioural traits (Helsel 2012). We also inverted the escape latency times so that the behavioural traits would exhibit the same directionality (higher values corresponded both to more tongue flicks and a quicker escape time). PCA analyses were conducted with the prcomp function and missing values imputed with the imputePCA function from the 'missMDA' package (Josse and Husson 2016) for R (<www.r-project.org>). To test for within-individual correlations, we both regressed the individual scores of the first PC axes for behavioural and physiological traits using an ordinary least-squares regression and calculated Kendall's *W*.

To test for the influence of behavioural and physiological phenotypes on body condition and mass of offspring at birth, we utilized mixed-effects models with mom size (SVL from first measure in captivity), behaviour PC1, physiology PC1, and their interaction as fixed effects and the random effect of litter, modeled with a compound symmetric covariance structure. We normalized SVL to a mean of zero and standard variance so that parameters for other fixed effects would be estimated at the mean mom size.

Last, we tested the impact of body condition and mass on subsequent specific growth rate across two time intervals (birth to four months and birth to one year) with mixed-effects linear models and probability of survival (to ages four months and one year) with mixed-effects logistic regression models. Because offspring birth body condition and mass can be considered distinct measures of maternal allocation to offspring, we conducted the analyses of condition and mass separately (see discussion of size versus condition in Weatherhead et al. 1999). Initial models included the fixed effect of offspring sex and all possible two- and three-way interactions with treatment and body condition/mass, but these effects were removed as they did not significantly explain variance in these traits (models of growth: all $p > 0.29$; models of survival: all $p > 0.20$). Initial models of survival probability included the main effects of sex, treatment and body condition/mass, as well as the two-way interaction of rearing treatment and body condition/mass (logistic regression models did not converge when all possible two-way interactions were included). We removed the non-significant effects of sex to simplify models. For all models, we included litter as a random effect modeled with a compound symmetric covariance structure to account for non-random covariance among littermates.

We utilized proc mixed and proc glimmix in SAS ver. 9.4 (SAS Inst.) for mixed linear models and logistic regression models, respectively. We estimated the denominator degrees of freedom for *F*-tests tests for all mixed models using the Kenward–Roger degrees of freedom approximation (Kenward and Roger 1997). To display the relationships among behaviour, physiology, and offspring phenotype, we present the surface defined by a restricted cubic spline function produced with the 'rms' package in the R programming language (<www.r-project.org>). Splines were created using three knots for each axis, with linear tails and interactions restricted to not be doubly nonlinear (Harrel 2015). Surface is presented within the range of the original data using the 'rgl' package. Remaining plots were created with the 'ggplot2' package (Wickham 2009). For visualization of survival probabilities (Fig. 3), we present natural cubic splines fitted with the 'geom_smooth' function in 'ggplot2'.

Field and laboratory work with live animals was approved by the Iowa State University Institutional Animal Care and Use Committee (protocols no. 3-2-5125-J and no. 1-12-7285-J) and the California Department of Fish and Game (SC-11973). Wild-caught animals were released at point of capture after data collection and captive-born snakes were maintained in the lab colony.

Data deposition

Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.fs57v> (Gangloff et al. 2017b).

Results

Repeatability of physiological and behavioural traits

To estimate repeatability of physiological biomarkers (plasma CORT and glucose concentrations), we utilized consistency repeatable (R_c), which accounts for changes in group means across measures made in the field, before parturition, and after parturition. Our estimates of R_c were at the high end of the range generally reported for hormonal measures in other taxa (e.g. in birds Holtmann et al. 2017). Estimates of R_c for

Table 1. Repeatability estimates of physiological measures and behaviours in adult female *Thamnophis elegans* (n = 21). CORT and glucose concentrations were measured at time of capture, after one month in captivity, and one month after parturition. Behaviours were measured in two trials 72 h apart after one month in captivity and two trials one month after giving birth (four trials total). Significant results ($p < 0.001$) shown in bold with two asterisks. R_c = consistency repeatability. See text for statistical details.

Measure	Statistic	Estimate	Test statistic	Significance
CORT concentration	R_c	0.19	$\chi^2_{0:1} = 1.1$	$p = 0.15$
Glucose concentration	R_c	0.27	$\chi^2_{0:1} = 2.36$	$p = 0.062$
Tongue-flicks	Kendall's *W*	**0.48****	$F_{18.5,55.5} = 2.73$	**p = 0.0020****
Escape latency	Kendall's *W*	**0.50****	$F_{18.5,55.5} = 3.00$	**p = 0.0008****

glucose measures approached significance while measures of CORT were not significantly repeatable (Table 1).

Both measures of behaviour (tongue-flicks and escape latency) were significantly repeatable and in accord with repeatability estimates from other behaviour studies, including those with snakes (Brodie and Russell 1999, Bell et al. 2009, Mayer et al. 2016, Gangloff et al. 2017a). This indicates that individuals retained their rank-order position in these measures across the four behavioural trials (two pre-parturition; two post-parturition). Additionally, we calculated concordance statistics for pairwise comparisons both within and across pre- and post-parturition trials to confirm no pattern of upward bias in overall calculations resulting from high estimates in the temporally-clustered pairs.

Principal component analysis

Maternal physiology

We present results of important of PCA axes determined by the Kaiser–Guttman rule (eigenvalues > 1; Niemelä et al. 2013, Goulet et al. 2017b). The first two axes explained a total of 67.2% of the variation in measures of maternal physiological biomarkers (plasma CORT and glucose concentrations) made in the field, before parturition, and after parturition (Table 2). PC1 loadings distinguished between individuals with high concentrations of plasma CORT and glucose across all measurements and individuals with low concentrations across all measures, with measures of field glucose and pre-parturition CORT exhibiting the highest loadings. This demonstrates that the primary axis of physiological variation is among individuals displaying consistently high levels of these markers and those exhibiting consistently low levels of these markers. PC2 describes variation among individuals which decreased glucose concentration between field and lab measures while increasing CORT concentration between these measures, or the opposite pattern (increasing glucose while decreasing CORT between field and lab measures).

Maternal behaviour

For measures of maternal behaviour, the first three axes of the PCA (eigenvalues > 1) explained a total of 66.5% of variation in the rank-transformed data (Table 2). PC1 loadings distinguished 'high reactive' individuals – with high numbers of tongue flicks and rapid escape in all trials – from 'low reactive' individuals with low numbers of tongue flicks and slow escape times. PC2 describes differences between the two behavioural measures within individuals: either exhibiting high numbers of tongue flicks and slow escape behaviours or vice versa. PC3 describes changes in reactivity in both measures before and after parturition (Table 2). Despite the fact that the first axes of both PCA analyses described variation among individuals with 'high reactive' and 'low reactive' behavioural and physiological phenotypes, there was no significant among-individual correlation between scores on behaviour PC1 and physiology PC1 (linear regression slope estimate: 0.29 ± 0.23 SE, $t_{19} = 1.25$, $p = 0.23$; Kendall's *W*: 0.66, $F_{19,19} = 1.95$, $p = 0.078$). In practice, this means that individuals with high reactive physiology could be either high or low reactive behaviourally and vice versa.

Table 2. Results of principal component analyses of behaviour and physiology measures in reproductive female *Thamnophis elegans* (n = 21). Higher loading values for escape latency describe a more rapid escape from the test arena. See text for statistical details.

	Behaviour				Physiology	
	PC1	PC2	PC3		PC1	PC2
Proportion of variance	35.80%	17.30%	13.40%	Proportion of variance	42.30%	24.90%
Loadings				Loadings		
Tongue flicks trial 1	0.277	–0.502	0.347	Field glucose	0.569	–0.300
Tongue flicks trial 2	0.224	–0.542	0.171	Pre-parturition glucose	0.282	0.545
Tongue flicks trial 3	0.389	–0.027	–0.255	Post-parturition glucose	0.372	0.192
Tongue flicks trial 4	0.436	–0.237	–0.082	Field CORT	0.342	0.415
Escape latency trial 1	0.125	0.4	0.788	Pre-parturition CORT	0.536	–0.096
Escape latency trial 2	0.477	0.285	0.187	Post-parturition CORT	0.231	–0.628
Escape latency trial 3	0.278	0.336	–0.207			
Escape latency trial 4	0.46	0.207	–0.281			

Table 3. Results of mixed linear model analysis of the effects of maternal phenotypes on offspring body condition and mass at birth ($\log_{10}$-transformed in g) in *Thamnophis elegans* (n = 111 offspring from n = 21 mothers). Models also include litter as a random effect (see text for statistical details). Significant effects designated with a single ($p < 0.05$) or double ($p < 0.01$) asterisk.

	Birth body condition			Birth mass		
Source of variation	Estimate (SE)	F (df_n, df_d)	Pr > F	Estimate (SE)	F (df_n, df_d)	Pr > F
Mom SVL	0.0063 (0.0070)	0.79 (1,12.7)	0.39	0.034 (0.0080)	17.51 (1,11.8)	**0.0013****
Behaviour PC1 (linear)	–0.0019 (0.0051)	0.13 (1,14.3)	0.72	–0.0065 (0.0058)	1.25 (1,13.2)	0.28
Physiology PC1 (linear)	–0.0037 (0.0052)	0.50 (1,14.7)	0.49	0.011 (0.0059)	3.21 (1,13.5)	0.096
Behaviour PC1 (quadratic)	–0.0016 (0.0020)	0.58 (1,13.2)	0.46	–0.0088 (0.0023)	14.56 (1,12.3)	**0.0024****
Physiology PC1 (quadratic)	–0.00093 (0.0024)	0.15 (1,14.5)	0.71	0.0062 (0.0028)	5.07 (1,13.3)	**0.042***
Behaviour PC1 × Physiology PC1	0.0087 (0.0040)	4.79 (1,14.2)	**0.046***	0.013 (0.0045)	8.19 (1,13.1)	**0.013***

Effects of maternal phenotype on reproductive allocation

The first principal component axes of behaviour and physiology interacted to influence offspring body condition and mass at birth with an effect size greater than any of the linear or quadratic terms. The effect of the interaction on both body condition and mass at birth describes a saddle-shaped function with highest values of both condition and body mass at birth at the extremes of maternal trait combinations (high–high or low–low) and especially low values for individuals with high behaviour and low physiology scores. Additionally, the first axes of behaviour and physiology exhibited non-linear effects on offspring mass at birth (Table 3, Fig. 2). We recognize the limited sample size of this dataset in such a multivariate approach and so conducted a second analysis utilizing multivariate clustering (Ward's minimum variance method). This analysis provided support for dividing the snakes into two groups, with one group exhibiting lower behaviour and physiology values across measures and the second group with high values. Comparing the litter qualities (condition and mass at birth) provides the same qualitative result as the PCA analysis presented here (results not shown). We found no evidence in mothers of a tradeoff between litter size and either offspring mass or offspring condition (ordinary least-squares regression $p = 0.88$ and $p = 0.77$, respectively).

Effects of body condition at birth, mass at birth, and rearing environment on growth and survival

Body condition at birth (a measure of size-corrected mass) and birth mass were collinear (ordinary least-squares regression, $p < 0.001$), with heavier snakes also being in better condition. Given that these measures provide different interpretations of potential energetic stores (relative versus absolute), we present analyses of both the effects of body condition and mass at birth to subsequent growth and survival. Growth rate for the first four months was determined by an interaction of birth condition and rearing treatment, such that condition had a more pronounced and positive effect on offspring in the warm rearing treatment. Neither body condition nor mass at birth influenced growth rates through one year. Survival to four months of age was determined by birth mass and rearing treatment such that snakes born heavier and snakes in the cool rearing treatment had a higher probability of survival. For survival to one year of age (i.e. including a snake's first hibernation), snakes born in better body condition and snakes born heavier were more likely to survive (Table 4, Fig. 3).

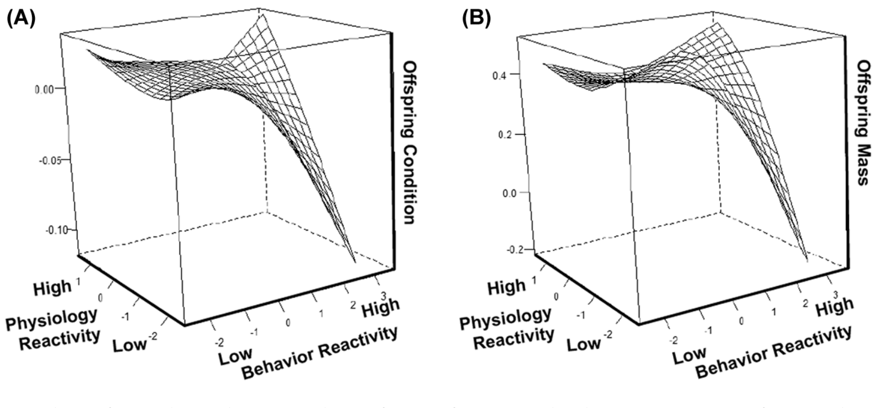

Figure 2. Three-dimensional surface plots showing the effects of maternal phenotypes on offspring body condition and mass ($\log_{10}$-transformed g) at birth in *Thamnophis elegans* (n = 111 offspring from n = 21 mothers). First principal component axis for maternal behavioural and physiological traits are shown on the x-axis and y-axis, respectively, for both plots. Body condition at birth (A) and mass at birth (B) are on the z-axis. Surface is defined by spline function (see text for details) and is constrained to the range of data used in analysis.

Table 4. Results of analysis of the effects of body condition at birth and mass at birth ($\log_{10}$-transformed in g) on growth (mixed linear model) and on probability of survival (mixed logistic regression model) in *Thamnophis elegans* (n = 111 offspring from n = 21 mothers). Models include litter as a random effect (see text for statistical details). Significant effects designated with a single ($p < 0.05$) or double ($p < 0.01$) asterisk.

Source of variation	Growth through four months (n = 94)	Probability of survival to four months	Growth through first year (n=24)	Probability of survival to first year
Birth condition				
Estimate (SE)	0.17 (0.057)	6.43 (7.59)	0.17 (0.13)	17.16 (6.93)
F (df_n, df_d)	41.33 (1,61.8)	0.72 (1,105.5)	2.59 (1,16.4)	6.13 (1,75.7)
Pr $>$ F	**$<$ 0.0001****	0.4	0.13	**0.016***
Rearing treatment				
Estimate (SE)	–0.0053 (0.0026)	–1.72 (0.75)	0.0065 (0.0065)	–0.26 (0.42)
F (df_n, df_d)	4.07 (1,81.6)	5.22 (1,99.7)	1.03 (1,18.4)	0.32 (1,95.4)
Pr $>$ F	**0.047***	**0.024***	0.32	0.57
Birth condition × Rearing treatment				
Estimate (SE)	0.15 (0.067)	–	–0.080 (0.15)	–
F (df_n, df_d)	4.82 (1,83.3)		0.29 (1,19.7)	
Pr $>$ F	**0.031***		0.6	
Birth mass				
Estimate (SE)	0.022 (0.041)	16.49 (4.91)	0.15 (0.11)	20.63 (7.14)
F (df_n, df_d)	2.96(1,43.9)	11.27 (1,31.4)	1.13 (1,17.9)	8.35 (1,63.2)
Pr $>$ F	0.093	**0.0021****	0.3	**0.0053****
Rearing treatment				
Estimate (SE)	–0.029 (0.022)	–2.19 (0.73)	0.078 (0.055)	–0.033 (0.47)
F (df_n, df_d)	1.64 (1,85.5)	9.06 (1,107.9)	1.98 (1,19.7)	0.01 (1,83.4)
Pr $>$ F	0.2	**0.0033****	0.18	0.94
Birth condition × Rearing treatment				
Estimate (SE)	0.061 (0.054)	–	–0.16 (0.12)	–
F (*df_n*, *df_d*)	1.30 (1,84.8)		1.80 (1,19.6)	
Pr $>$ F	0.26		0.19	

Discussion

These results demonstrate that major axes of variation in maternal traits describe a continuum of reactivity, with a strong pattern for behavioural traits and a weak pattern for physiological traits. Furthermore, these among-individual differences in maternal phenotypes then interact to determine the body condition and mass of offspring at birth, suggesting a tradeoff between maternal behavioural and physiological decisions and energetic allocation to offspring. Moms with matched phenotypes ('high–high' or 'low–low' for physiological and behavioural traits) gave birth to offspring with greater energetic stores. In turn, these higher-quality offspring grew faster in the first months of life and were more likely to survive to one year of age. These results demonstrate that among-individual differences in physiological and behavioural traits have trans-generational effects, influencing important early life-history traits of offspring, including growth rate and survival probability. Additionally, growth and survival of neonates was influenced by immediate environmental conditions, indicating that environmental variation may impact the relative influence of maternal traits on fitness. These results from a single population at a single point in time provide a foundation for future studies to elucidate the mechanisms maintaining stable maternal phenotypes and driving energetic tradeoffs between maternal energy use and allocation to offspring across populations and over time.

We found that exploratory and antipredator behaviours within individuals where highly consistent (Kendall's $W = 0.48$ and 0.50, respectively) and the primary axis of behavioural variation represents a continuum of overall activity levels in both behavioural traits across all measures. Given this pattern, these two behaviour metrics likely represent different aspects of a single behavioural axis, that of general activity (cf. Réale et al. 2007). This finding correlates with a study of behaviours in field-caught adults from this garter snake system, in which both tongue-flick rate and activity level were correlated and repeatable within individuals (Gangloff et al. 2017a). The physiological indicators we measured are reliable biomarkers of potential tradeoffs in energetic allocation decisions within individuals: increases in circulating CORT promote the mobilization of energy stores, while glucose concentration serves as a direct measure of energy availability (Sapolsky et al. 2000). After accounting for differences in group means across measured time points, consistency repeatability of plasma glucose concentration was in the high range for physiological traits generally ($R_c = 0.27$) and approached significance. Repeatability of plasma CORT concentration was lower ($R_c = 0.19$) and not statistically significant, consistent with hormonal repeatabilities in this and other taxa (Sparkman et al. 2014, Pavitt et al. 2016, Holtmann et al. 2017). Such a pattern of stable glucose concentrations within

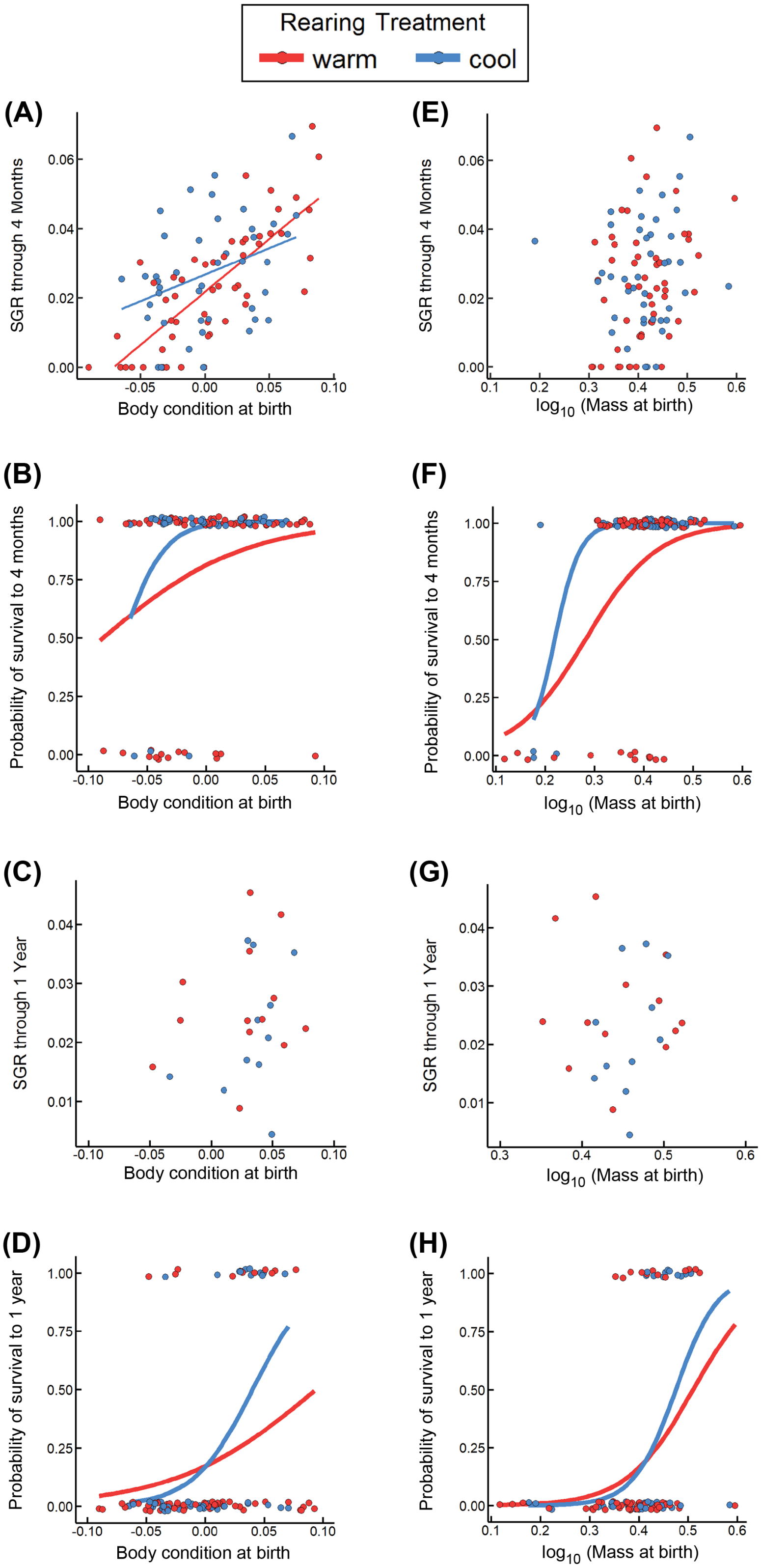

Figure 3. Scatterplots of the effect of rearing treatment and body condition at birth (A–D) and mass at birth (E–H) on growth through four months of age (n = 94), probability of survival to 4 months of age (n = 111), growth rate through one year of age (n = 24), and probably of survival to one year of age (n = 111) for captive-born *Thamnophis elegans* (from n = 21 litters). Linear regression lines are shown for significant effect of body condition at birth on growth through 4 months of age and cubic splines are shown for survival probability figures (see text for details). Separate curves are shown for offspring raised in warm rearing treatment (red line) and cool rearing treatment (blue).

individuals but less consistency in CORT may be expected if the HPI axis operates to maintain glucose concentrations within a narrow homeostatic range through the modulating effects of CORT. That is, we expect that circulating CORT concentrations respond to both external and internal conditions (such as food consumption, temperature variation, shedding cycles, etc.) and act to maintain stable energetic availability in the form of glucose. Thus, it is likely that measures of plasma glucose concentration describe differences in energetic phenotypes more accurately than measures of CORT. For example, previous work in this system demonstrates that across a range of temperatures, adult females from the fast-living and slow-living ecotypes differ in glucose concentrations, but not CORT (Gangloff et al. 2016).

As with our measures of behaviour, the first axis of variation from our physiological PCA reveals that snakes primarily vary on a reactivity continuum, though physiological measures were not consistent within individuals. Given that baseline CORT concentrations mediate metabolic tradeoffs and influence general activity levels (Landys et al. 2006), we predicted that CORT and glucose concentrations would be correlated with the behavioural activity levels within individuals. Higher levels of the physiological biomarkers promote higher activity levels, thus requiring more energy but also increasing the possibility of food acquisition. We were not able to identify such a correlation between proactive behaviours and proactive physiologies (by comparing the first PCA axes in both traits). Though we found maternal phenotypes across the range of possible combinations, individuals with matched behavioural and physiological phenotypes (either ‘high–high’ or ‘low–low’) gave birth to heavier offspring in better condition. This suggests that an energetic tradeoff between maternal energetic phenotype and reproductive allocation acts to promote variation in birth mass and condition within populations, consistent with other studies in squamates that have identified maternal energetic constraints as driving variation in offspring size and condition (Weatherhead et al. 1999, Warner and Andrews 2002, Waters and Burghardt 2005, Oufiero et al. 2007).

The first year represents a critical period in snake life-history, with neonates experiencing low survivorship in these and other natural snake populations (Brodie 1992, Bronikowski and Arnold 1999, Baron et al. 2010, Miller et al. 2014). Furthermore, studies in both natural populations and experimental mesocosms demonstrate that snakes born large and/or in better condition grow more and experience higher survivorship, especially while overwintering (Brown and Shine 2005, Kissner and Weatherhead 2005, Manjarrez and San-Roman-Apolonio 2015, Mack et al. 2017). Our data corroborate these findings by demonstrating that maternal energetic allocation to offspring, as measured by body condition and mass at birth, affected the early-life growth and survival of neonates in captivity. As expected from previous work in this and other snake systems, longer moms produced heavier offspring (Bronikowski and Arnold 1999, Brown and Shine 2005), though not necessarily offspring in better condition (Table 4, Fig. 3). The importance of body mass and condition at birth on subsequent growth and survival is well-established in snakes (Bronikowski 2000, Mack et al. 2017) and in animals generally (reviewed by Roff 1992). However, we cannot assume that low rates of growth are necessarily unfavorable in this system. For example, reduced growth rates may be optimal under specific environmental conditions (Bronikowski 2000, Addis et al. 2017) and/or may represent a tradeoff between allocation options, such as between metabolic rate and growth (Gangloff et al. 2015). While captivity provides artificial conditions lacking important environmental variables (e.g. predation), such experimental designs nevertheless provide important data on factors such as growth, survival, and underlying tradeoffs between traits, that would not otherwise be possible to obtain from wild populations (Bronikowski 2000, Addis et al. 2017).

Placing these early results in the broader ecological context points to exciting avenues for future research. In temperate regions, garter snakes mate immediately upon emergence from hibernation and utilize energy stores from the previous year during vitellogensis (Gregory and Skebo 1998, Gregory 2006, Tuttle and Gregory 2014, Feriche et al. 2016). In the Eagle Lake system, precipitation serves as a proxy for food (anuran prey) availability, which in turn influences reproduction the following spring. ‘Good’ years follow a year of > 500 mm total precipitation and ‘bad’ years follow a year of < 500 mm (Bronikowski and Arnold 1999, Miller et al. 2011, 2014). The total precipitation from the three weather stations nearest to Eagle Lake averaged 354 mm for 2011, classifying 2012 (the year of our experiment) as a ‘bad’ year (California Climate Data Archive <www.calclim.dri.edu/>). We found a tradeoff between maternal use and allocation to offspring, an expected life-history tradeoff when resources are limited in a ‘bad’ year such as this (sensu van Noordwijk and de Jong 1986): moms of the matched ‘high reactive’ phenotypes are presumably able to acquire more of the limited resources, while moms of the matched ‘low reactive’ phenotypes use less energy. During a poor year, these tradeoffs could lead to a fitness advantage for matched extremes of behavioural and physiological phenotypes (‘high–high’ and ‘low–low’). Future work can be directed to identify whether such tradeoffs are evident when food is plentiful, as in a ‘good’ year.

Future studies would also benefit from additional measures of energy processing, including standard metabolic rates, maximal metabolic rates, energy assimilation rates, and/or field measures of daily energy expenditure, which are known to vary among individuals within garter snake populations (Peterson et al. 1998, Gangloff et al. 2015) and are all important in understanding allocation versus acquisition tradeoffs (Careau et al. 2008, Mathot and Dingemanse 2015). Additionally, to quantify how selection might act on phenotypic trait combinations, measuring both the heritability and the underlying genetic correlations of these characters is necessary (Lande and Arnold 1983, Arnold 1987). Numerous studies have demonstrated that both behavioural and physiological traits in garter snakes are both repeatable and heritable (Arnold and Bennett 1984, Garland 1988, Brodie 1993, King et al. 2004), though generally physiological markers seem to be less consistent within individuals (Careau and Garland 2012, Sparkman et al. 2014, this study). Furthermore, common garden experiments reveal that growth, metabolic rates and endocrine function are partially determined by genetic background (Bronikowski 2000, Gangloff et al. 2015, Reding et al. 2016, Addis et al. 2017). More precise estimates of heritability will be needed to assess the potential for correlated selection to shape integrated phenotypes.

These results demonstrate that the interaction of different axes of maternal phenotypes shapes maternal–offspring energetic tradeoffs, which in turn differentially affect the life-history trajectories of individual offspring. This tradeoff provides a mechanism describing why, in squamate reptiles, there is no straightforward adaptive explanation for observations of within-cohort variation in offspring size and condition. In this long-lived species, individual heterogeneity in maternal 'energetic phenotypes' will interact with environmental conditions over several years to determine the reproductive success of an individual snake over her lifespan, the life-history trajectories of her offspring, and ultimately population demography and dynamics.

Acknowledgements – We thank K. Martin, T. Rickman, and S. Zylstra for support in field work; P. Dixon and K. Goode for statistical advice and M. Barazowski, C. Corwin, C. Hinsley and A. Wendt for assistance with behaviour assays and animal care. G. Burghardt, B. Danielson, F. Janzen and members of the Bronikowski and Janzen labs provided valuable manuscript feedback. We are grateful to S. Hamel, N. Yoccoz and J.M. Gaillard for insightful discussions and organization of the themed issue.

Funding – Funding was provided by the Society for Integrative and Comparative Biology Grants-in-Aid-of-Research, the Iowa Dept of Natural Resources, the Iowa Science Foundation (15-11), and the US National Science Foundation (IOS-0922528 to AMB). We also recognize the support of a fellowship from the ISU Office of Biotechnology to EJG.

Permits – Field and laboratory work with live animals was approved by the Iowa State University Institutional Animal Care and Use Committee (protocols no. 3-2-5125-J and no. 1-12-7285-J) and the California Department of Fish and Game (SC-11973).

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