



Thermal performance of squamate embryos with respect to climate, adult life history, and phylogeny

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Reptiles are important model systems for examining the effect of temperature during development on the phenotype of individuals after hatching or birth. To assess whether squamate embryos exhibit adaptive variation in thermal biology, we derived three parameters: an index of developmental rate (DRI), the upper thermal limit for successful incubation, and the lower thermal limit for development for 28 species of lizard and 12 species of snake. The associations between developmental parameters and climatic and life-history variables were examined using both conventional statistics and phylogenetically controlled analyses. Residual DRI (i.e. DRI corrected for stage at oviposition and hatchling mass) was strongly associated with phylogenetic relationship. By contrast, the upper limit for development was negatively related to the amount of precipitation during the warmest quarter of the year, and the lower thermal limit for development was positively related to temperature during the warmest quarter of the year and the activity body temperatures of adults. These latter observations indicate that embryonic thermal physiology is adapted to large-scale environmental patterns, and that global climate change will impact embryonic development directly through impacts on nest temperature *per se*, as well as indirectly through impacts on the ability of gravid females to select suitable nest sites. © 2012 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2012, **106**, 851–864.

ADDITIONAL KEYWORDS: adaptation – climate change – development – oviparity – reptile.

INTRODUCTION

Temperatures experienced by embryos during development are critically important to individual fitness. Phenotypic attributes affected by incubation temperature include survival to hatching or birth and the morphology, locomotory performance, thermal preference, growth rate, behavior, and sex of neonates (Janzen & Paukstis, 1991; Downes & Shine, 1995; Shine, Elphick & Harlow, 1997; Booth, 2006; DuRant *et al.*, 2010; Hopkins *et al.*, 2011). Temperature sensitivity is especially important for the ectothermic vertebrates that are oviparous because their embryos are exposed to environmental temperatures as they develop (Packard & Packard, 1988). Reptiles have thus become important model systems for examining the effect of incubation temperature on phenotype. Surprisingly, thermal adaptations of reptile embryos have yet to be examined in a broadly comparative

context, especially given the impressive range of characterizations of thermal adaptations of adults (Huey, 1982; van Berkum, 1986; Clusella-Trullas, Blackburn & Chown, 2011).

Thermal adaptations of adult reptiles, for example, have been examined using parameters derived from thermal performance curves (Huey & Stevenson, 1979). A performance curve is the relationship between some measure of individual fitness (e.g. survival, digestive efficiency, sprint speed) and body temperature. The shape of this curve is described by parameters such as the optimal body temperature (where performance is maximal), the optimal temperature range (OTR; where performance is above some threshold level, e.g. 90% of maximum), the minimum temperature at which performance can occur, and the maximum temperature at which performance can occur. The activity body temperature of adults in nature (T_b) or the body temperature selected by adults in a thermal gradient (T_{sel}) in the laboratory is the body temperature at which many

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performance characteristics are optimized (Huey, 1982). Exposure of embryos to environmental temperatures, and their inability to thermoregulate behaviourally, suggests that they should be adapted to their thermal environments (i.e. exhibit variation in thermal parameters on geographical gradients, as do adults).

Using methodological approaches pioneered by Huey & Stevenson (1979), we defined developmental parameters (Fig. 1) from performance curves that relate hatching success (e.g. survival to hatching

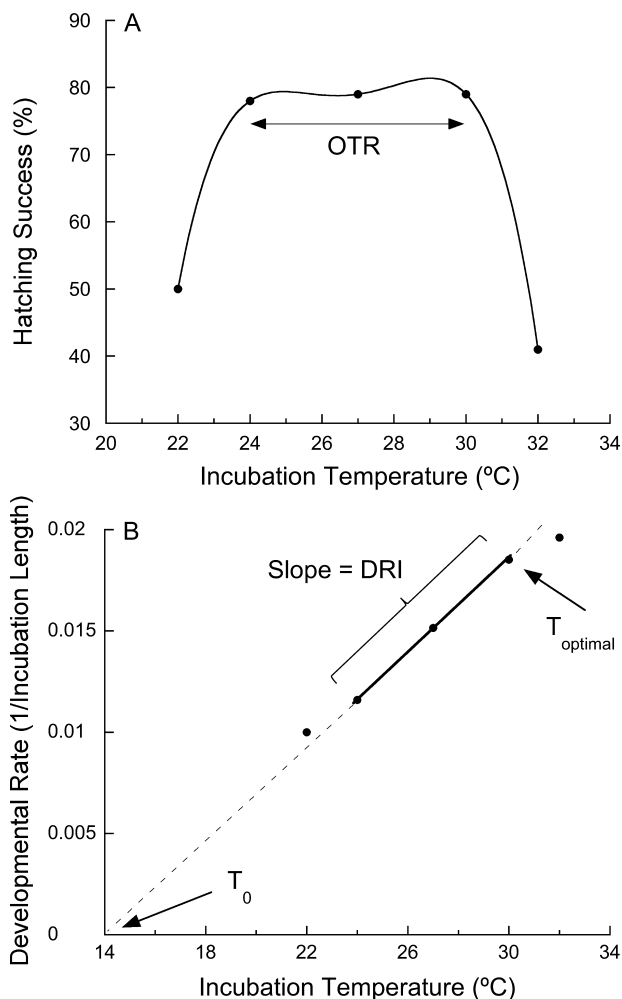


Figure 1. Developmental performance of *Elaphe taeniura* embryos (*sensu* Du & Ji, 2008). A, performance curve relating hatching success to incubation temperature. The optimal temperature range (OTR) is indicated. Note the low hatching success at incubation temperatures above and below the OTR. B, the relationship between developmental rate ($DR = 1/\text{incubation length}$) and incubation temperature. The slope of the regression for observations within the OTR is the developmental rate index (DRI). The highest incubation temperature in the OTR is T_{opt} . The intercept (T_0) is the temperature when the rate of development is 0.

and/or viability of hatchlings, a direct assay of individual fitness) to incubation temperature. These parameters are the optimal temperature for development (T_{opt}) the lowest temperature at which development occurs (T_0), and an index of the rate of development (DRI) in the OTR. We expected that developmental rates and thermal limits for successful development of reptiles would exhibit adaptive variation on a global scale. This expectation is supported by observations of intraspecific variation in developmental parameters on climatic gradients (Qualls & Shine, 1998; Du *et al.*, 2010; Weber *et al.*, 2012). On the other hand, developmental parameters may be sufficiently plastic to accommodate at least some degree of climatic variation, given that embryos cannot accommodate change in the environment behaviourally or the thermal adaptations of embryos may be more strongly influenced by evolutionary relationships rather than by their immediate physical environment, or both. In either of these latter situations, large-scale patterns of adaptation would not be evident in comparative analyses.

The present study aimed: (1) to determine the extent to which variation in the thermal biology of embryonic squamates is adaptive; (2) to identify the factor or factors that act as selective agents on the thermal biology of embryos; and (3) to place variation in the temperature dependence of development in the context of global climate change. Thermal adaptations of embryos could potentially exacerbate the negative effects of climate change predicted on the basis of adult thermal biology (Kearney *et al.*, 2009; Sinervo *et al.*, 2010), or perhaps, mitigate such effects, at least in the short term. Embryonic adaptations of viviparous species are considered elsewhere (Schwarzkopf & Andrews, in press) because their thermal adaptations should parallel those of adults (Arnold & Peterson, 2002; Caley & Schwarzkopf, 2004; Lourdais *et al.*, 2004), and thus be comparably affected by climatic change.

MATERIAL AND METHODS

SPECIFIC TESTS OF CLIMATIC AND LIFE-HISTORY HYPOTHESES

For specific tests of 'climate' hypotheses, we predicted that the index of developmental rate should be more related to indices of seasonality than to annual means of climatic variables. Highly seasonal environments, for example, have restricted periods for reproduction and hence provide 'counter-gradient' selection for rapid embryonic growth. By contrast, because T_{opt} and T_0 are indices of the upper and lower thermal limits for development, we hypothesized that those thermal limits would be correlated with ambient temperatures

experienced during the period of incubation, typically during the warmest period of the year.

For specific tests of 'life-history' hypotheses, it was hypothesized that DRI would be positively related to adult body size independent of the physical environment. Species with large adult body sizes have relatively large hatchlings and commensurately long incubation periods (Andrews, 1982, 2004); rapid embryonic growth rates may be favoured by selection to compensate for long incubation periods. By contrast, we hypothesized that T_{opt} and T_0 would be correlated with the body temperatures of adults (T_b). Adult body temperature is associated with habitat (Huey & Webster, 1976; Clusella-Trullas *et al.*, 2011), and, in general, females nest in the habitats where they live (Lu *et al.*, 2006). Temperatures experienced by embryos in the nest may thus be related to the body temperature of adults.

EMBRYONIC PERFORMANCE CURVES

Data on hatching success, incubation length, and incubation temperature were obtained from a survey of the primary literature (see Appendix). We used observations at constant temperatures to allow comparisons among studies. Although nest temperatures in nature normally fluctuate on diel and seasonal cycles, hatching success and incubation length at constant as well as at fluctuating temperatures with the same mean do not differ as long as temperatures do not fluctuate to detrimental extremes (Shine & Harlow, 1996; Andrews, Mathies & Warner, 2000; Du & Ji, 2006; Lin *et al.*, 2008; Du & Shine, 2010; Oufiero & Angilletta, 2010). If observations were made at different substrate water contents, we used the observation that maximized egg survival at each incubation temperature. Important ancillary information recorded was: hatchling mass, running speed and frequency of abnormalities as a function of temperature, and embryonic stage at oviposition.

Embryonic performance curves for squamate embryos typically have flat tops and steeply descending sides, as illustrated by *Elaphe taeniura* (Fig. 1A); for a similarly-shaped curve characterizing jump distances of *Rana clamitans*, see Huey & Stevenson, (1979). Twenty-eight (85%) of the 33 species for which hatching success was reported, had such 'staple-shaped' performance curves. We therefore defined the OTR to include those temperatures at which hatching success is high (mean = 83.3%, range = 76–100%, $N = 28$). For these 28 species, temperatures outside the OTR were characterized by low hatching success, and, when the information was available, by high frequencies of developmental abnormalities, poor running performance, and low viability of hatchlings. For species for which hatchling success was not reported or

Table 1. Numbers of studies in which eggs were incubated at temperatures below the optimal temperature range (OTR), above the OTR, and within the OTR

Number of observations	0	1	2	3	4–6	Total
Below OTR	30	7	3	0	0	40
Above OTR	12	22	6	0	0	40
Within OTR	0	0	12	17	11	40

was judged unreliable ($N = 7$ and 5 , respectively), we selected the linear section of relationship between developmental rate and temperature as the OTR (see below). We did not use nonlinear curve-fitting techniques (Bulté & Blouin-Demers, 2006) to characterize the shape of the embryonic performance curves because the data sets for most species did not include sufficient observations above and below the OTR to define shape accurately (Table 1).

MEASURES OF EMBRYONIC DEVELOPMENTAL PARAMETERS: DRI, T_{opt} , AND T_0

The OTR (see above) established from developmental performance curves was used to establish three developmental parameters for each species. The developmental rate index (DRI) is the slope of the regression of developmental rate on incubation temperature for observations in the OTR (Fig. 1B). Developmental rate is the reciprocal of incubation length (Shine & Harlow, 1996; Jarosik *et al.*, 2004), a transformation that linearizes the relationship between incubation length and temperature within the OTR. DRI is an overall index of developmental rate; species with high values of DRI also have high absolute developmental rates at temperatures in the OTR. DRI is positively correlated with developmental rate at 23 °C, which is at the low end of the OTR for most species ($F_{1,38} = 41.8$, $P < 0.0001$, $R^2 = 0.51$). The association is stronger at 30 °C ($F_{1,38} = 224.9$, $P < 0.0001$, $R^2 = 0.85$), the modal value of the highest incubation temperature in the OTR. DRI is related to the stage at oviposition (Stage) and hatchling mass (H_{mass}) (Birchard & Marcellini, 1996; Andrews & Mathies, 2000) as:

$$\text{DRI} = -0.00207 + 0.000144 \text{ Stage} - 0.0010 \text{ Log}_{10} H_{\text{mass}} \\ (F_{2,30} = 9.8, P < 0.001, R^2 = 0.40),$$

and both Stage and $\text{Log}_{10} H_{\text{mass}}$ contribute significantly to the overall model ($P = 0.0024$ and 0.0003 , respectively). Therefore, Studentized residuals of this relationship (ResidDRI) were used in all subsequent analyses to control for when in development eggs are laid and the size of hatchlings.

The second performance parameter is T_{opt} , the highest incubation temperature in the OTR (Fig. 1B). This temperature is 'optimal' because, on the one hand, incubation length decreases with temperature, eggs incubated at T_{opt} have relatively short incubation periods. Short incubation periods may reduce the risk of egg mortality as a result of predation and/or provide enhanced opportunities for growth of neonates in seasonal environments because hatching occurs relatively early (Shine, 1985; Olsson & Shine, 1997; Brown & Shine, 2006; Warner & Shine, 2007). On the other hand, incubation temperatures higher than T_{opt} are physiologically stressful as indicated by reduced rates of development, decreased hatching size, increased abnormalities, and increased mortality. Because experimental temperatures are systematically biased (i.e. they are often widely spaced and/or selected to avoid potentially stressful temperatures); however, our estimates of T_{opt} are likely lower than their 'true' values. We assume that this bias is randomly distributed in the data set and will not affect the conclusions of comparative analyses.

The third performance parameter is the lower limit of temperature at which development occurs (T_0 ; Fig. 1B). T_0 was estimated as the temperature at which DRI was zero as extrapolated from individual regressions of developmental rate on incubation temperature (Shine & Harlow, 1996) for observations in the OTR. Although hatching success at constant temperatures approaching T_0 is low, T_0 has biological significance because some development presumably occurs during short-term exposure when nest temperature approaches T_0 during normal diel or seasonal fluctuations (Du & Shine, 2010).

INDICES OF PHYSICAL ENVIRONMENTS OF EMBRYOS

Standard climatic data were used to characterize the environment of each species at the latitude and longitude of the study site. For species whose initial source was unknown (*Chamaeleo calytratus*, *Paroedura pictus*, *Eublepharis macularius*), we arbitrarily selected a latitude and longitude at the center of their geographical range. Climatic data were accessed from WorldClim (Hijmans *et al.*, 2005) for all species except *Zootoca vivipara* for which data were from González Taboada & Anadón Álvarez (2011). To minimize problems associated with 'data dredging' and 'overfitting', we limited the number of climatic variables used in the statistical models (Anderson, 2008; Knappe & de Valpine, 2011). Climatic variables were selected to test the specific a priori hypotheses: indices of seasonality (T_{seas} , SD of monthly values of mean annual temperature, and P_{seas} , the coefficient of variation for mean monthly precipitation) and temperature and rainfall during the time of year when

squamate reproduction typically occurs (T_{warm} , mean temperature of the warmest quarter of the year, and P_{warm} , precipitation during the warmest quarter of the year). Mean annual temperature (T_{ann}) and mean annual precipitation (P_{ann}) were used, in part, as control variables with the rationale that, as general descriptors of climate, they should perform more poorly than specific climatic variables associated with the time of year when development occurs. Observations of T_{warm} were transformed as $(T_{\text{warm}}/10)^{3.75}$ to meet normality criteria.

LIFE-HISTORY VARIABLES

Choice of predictor variables for the life-history hypotheses was limited to observations widely available in the literature because studies on embryonic development seldom included information on adults. The first was mean activity temperature of adults (T_b). Field measures of active body temperatures (daytime T_b for diurnal species and night-time T_b for nocturnal species) were used to characterize the thermal niche of adults. Selected body temperatures from laboratory studies were used when T_b was not available. When T_b was available for different seasons, we used the highest mean value provided.

The second life-history variable was adult body size. We characterized adult body size as the mass of the largest adult individual recorded for each study population or species. Maximum snout-vent length (SVL) was converted to mass using the mass-SVL relationships for *Sceloporus occidentalis* for lizards (Andrews, 1982) and *Waglerophis merremii* for snakes (Vitt, 1983). The use of maximum SVL to estimate asymptotic species size is appropriate for some squamates at least (Stamps & Andrews, 1992), and for the present study in particular, given that adult mass spanned three orders of magnitude (7–4655 g); imprecision in estimated size for any one species is small compared to the variance in size among species.

PHYLOGENETIC RELATIONSHIPS

To determine whether relationships between developmental parameters and predictor variables were confounded by phylogenetic relationships, we compared results of conventional phylogenetically uncontrolled (PU) and phylogenetically controlled (PC) analyses. Conventional statistical procedures were conducted using JMP, version 8.0.1 (SAS Institute). Phylogenetically controlled analyses were conducted using the PDAP: PDTREE module in MESQUITE (Midford, Garland & Maddison, 2005; Maddison & Maddison, 2009). Analyses were based on a composite phylogenetic tree constructed from independent molecular phylogenies. Relationships of squamate families were

conducted based on those reported by Wiens (2008). For families with three or more species, relationships were based those reported by Gamble *et al.* (2008) and A. Bauer (unpublished data) (gekkotans); Brandley, Schmitz & Reeder (2005), Honda *et al.* (2000), Skinner (2007), and Smith *et al.* (2007) (Scincidae); Ota *et al.* (2002) and Hipsley *et al.* (2009) (Lacertidae); Wang *et al.* (1999), Utiger *et al.* (2002) and Wiens *et al.* (2008) (snakes); Schulte, Valladares & Larson (2003) (Iguanidae *sensu lato*); and Hugall *et al.* (2008) (Agamidae). Phylogenies were pruned to include only the subset of species for which data were available for all pertinent variables. Branch lengths were set to 1.0 or transformed so that they met diagnostic criteria for independent contrasts analyses. If variables were significantly correlated in both the PU and PC analyses, we judged that their relationship was independent of phylogeny (Midford *et al.*, 2005).

STATISTICAL ANALYSIS

To evaluate relationships between embryonic performance measures and climatic and life-history predictor variables, we built independent sets of models for DRI, T_{opt} , and T_0 . Climate variables that were correlated ($P < 0.10$) were not included in the same model (e.g. T_{ann} and T_{warm} , P_{ann} and P_{warm}). Information theoretic approaches based on Akaike's information criterion (AIC_c) were used for model selection (Burnham & Anderson, 2002; Anderson, 2008). This approach provides an objective process by which to rank and compare models and thus obviates biases associated with stepwise regression and similar approaches (Whittingham *et al.*, 2006). AIC_c and log likelihood values for each model were obtained using general linearized models (JMP, version 8.0.1). Because of the small number ($N = 5-8$) of models, we report AIC_c values for all of them. Relative model weights (w_i) were used to rank models; w_i is a relative estimate of the weight of evidence in favour of i being the best model (Burnham & Anderson, 2002).

Data collected on development, adult life history, and climate used in analyses are available upon request.

RESULTS

We found sufficient information on 40 species of squamate, representing two families of snakes and seven families of lizards, that enabled characterization of the performance curves and/or developmental parameters (Table 1). The number of observations within the OTR was in the range 2–6 (mode = 3). Approximately two-thirds of the species ($N = 28$) had one or more observations above the OTR. By contrast, only ten of

40 species had observations (1–2) below the OTR. Because of missing data for stage at oviposition for seven species, ResidDRI was determined for 33 species. Missing data for T_b for 12 species limited the sample size for analyses in which T_{opt} and T_0 were dependent variables to 28.

The most highly ranked model for predicting ResidDRI involved only T_{seas} (Table 2). This model was three-fold better than the second ranked model and more than five-fold better than the remaining models. ResidDRI was related to T_{seas} as ResidDRI = $-0.970 + 0.000168 T_{\text{seas}}$ ($F_{1,31} = 4.9$, $P = 0.035$, $R^2 = 0.136$, least squares analysis). ResidDRI and T_{seas} , however, were not correlated in the phylogenetically corrected analyses (Table 3). This result indicates that the association between these variables was removed by accounting for phylogeny.

The most highly ranked model for T_{opt} involved only P_{warm} (Fig. 2A, Table 2). This model was 2.0- and 2.2-fold more likely than the second and third ranked models that included P_{warm} and T_{warm} and that included T_b and P_{warm} , respectively, as explanatory variables. P_{warm} was thus included in all three of the most highly ranked models. P_{warm} by itself explained 16% of the variation in T_{opt} ($T_{\text{opt}} = 31.3 - 0.00453 P_{\text{warm}}$, $F_{1,26} = 5.11$, $P = 0.032$, $R^2 = 0.16$).

The most highly ranked model for T_0 had two predictor variables, T_b and T_{warm} . This model was almost six-fold better than the second ranked model and more than nine-fold better than the third ranked model (Table 2). The first ranked model was $T_0 = 2.51 + 0.360 T_b + 0.065 T_{\text{warm}}$ ($F_{2,25} = 10.52$, $P < 0.0001$, $R^2 = 0.46$). T_b ($F_{1,25} = 10.55$, $P = 0.0033$) explained more variance in T_0 than T_{warm} ($F_{1,25} = 6.17$, $P = 0.02$) (Fig. 2B, C). The best models for both T_{opt} and T_0 , were also supported by phylogenetically controlled analyses (Table 3). T_{opt} was correlated with P_{warm} in both PU and PC analyses and T_0 was correlated with T_b , T_{warm} and T_{ann} , in both PU and PC analyses.

DISCUSSION

The thermal biology of squamate embryos varied as a function of climate, preferred body temperature of adults, and phylogenetic relationship. Our observations thus document, for embryos, adaptive and historical parallels with the thermal biology of adults. For example, in a study involving almost 400 species of squamate, Clusella-Trullas *et al.* (2011) report that, although thermal parameters of adult squamates are related to climate and life history, models that included phylogenetic information had greater explanatory power than those that did not; thus, phylogenetic effects enhanced conclusions otherwise based on only climate and life-history variables. Below, we first discuss the specific results of our

Table 2. Best model determinations for the developmental parameters, ResidDRI, T_{opt} , and T_0 , using Akaike's information criterion (AIC_c) ($N = 33, 28,$ and $28,$ respectively)

Variable	Model	K	$-2 \log(L)$	AIC_c	Δ	Likelihood	w_i
ResidDRI	T_{seas}	3	89.28	96.11	0	1.000	0.547
	$T_{\text{seas}}, P_{\text{seas}}$	4	88.82	98.26	2.15	0.341	0.187
	T_{ann}	3	92.55	99.39	3.28	0.194	0.106
	P_{ann}	3	93.75	100.58	4.47	0.107	0.059
	$\text{Log}_{10}\text{AdMass}$	3	93.90	100.73	4.81	0.099	0.054
	P_{seas}	3	94.01	100.84	4.73	0.094	0.051
T_{opt}	P_{warm}	3	121.06	128.06	0	1.000	0.388
	$T_{\text{warm}}, P_{\text{warm}}$	4	119.76	129.50	1.44	0.487	0.189
	$T_{\text{b}}, P_{\text{warm}}$	4	119.90	129.65	1.59	0.452	0.175
	T_{b}	3	123.40	130.40	2.34	0.310	0.120
	P_{ann}	3	123.90	130.90	2.84	0.242	0.094
	T_{warm}	3	125.90	132.90	4.84	0.089	0.035
	T_{ann}	3	126.02	133.03	4.97	0.083	0.032
	$T_{\text{b}}, T_{\text{warm}}$	4	123.40	133.13	5.07	0.079	0.031
T_0	$T_{\text{b}}, T_{\text{warm}}$	4	121.92	131.67	0	1.000	0.746
	T_{b}	3	128.10	135.11	3.44	0.179	0.133
	$T_{\text{b}}, P_{\text{warm}}$	4	126.38	136.12	4.45	0.108	0.080
	T_{warm}	3	131.79	138.79	7.12	0.028	0.021
	T_{ann}	3	131.90	138.96	7.29	0.026	0.019
	$T_{\text{warm}}, P_{\text{warm}}$	4	131.28	141.02	9.35	0.009	0.007
	P_{ann}	3	138.96	145.96	14.29	0.001	0.001
	P_{warm}	3	139.03	146.03	14.36	0.001	0.001

DRI, index of developmental rate; w_i , relative model weight.

analyses with regards to phylogeny, climate, and life history, and then consider our results with regards to climate change. Because our sample of 40 species is a relatively small subset of squamates, results from a wider survey might differ in details of the association between developmental parameters and climate. Nonetheless, the present study provides the first documentation of the widescale adaptation of reptile embryos to their environment.

DEVELOPMENTAL PARAMETERS AND PHYLOGENETIC RELATIONSHIPS

The developmental parameters T_{opt} and T_0 were related to climate and/or life history variables independent of phylogeny (Tables 2, 3). By contrast, the apparently strong relationship between ResidDRI and T_{seas} in phylogenetically uncontrolled analyses disappeared when analyses were controlled for phylogeny. The reason is that lineages that have high values of ResidDRI occur in more seasonal environments than lineages that have low values of ResidDRI but ResidDRI and T_{seas} are not correlated within lineages. For example, mean ResidDRI for families (lacertids, scincids, elaphids, colubrids, gekkotans, iguanids, agamids, and chamaeleonids, listed from highest to

lowest values) is positively correlated with mean T_{seas} ($R = 0.85, P = 0.008, N = 8$). Our hypothesis that selection would favour rapid developmental rates in highly seasonal environments was therefore rejected. Rather, the rate of development is a life-history attribute at the family level. The explanation for this trend is not obvious. For example, ResidDRI is not correlated with T_{b} as might be expected if lineages with a high T_{b} also had high developmental rates. Nonetheless, many life-history attributes are conserved across taxonomic groups, and therefore not strongly influenced by adaptation at the species level. ResidDRI may be linked to such conserved attributes. Possible linkages are that: (1) the rate of anabolic processes is taxon specific, such that growth rates of individuals post-hatching and during development are correlated, and (2) developmental rate is related to adult life span, at least in birds and mammals (Ricklefs, 2010); hence, if life span is taxon-specific in squamates, then ResidDRI would be linked to phylogenetic relationships.

DEVELOPMENTAL PARAMETERS, CLIMATE, AND LIFE HISTORY

Our prediction that developmental parameters would be related to climate and to life history was supported

Table 3. Correlation between developmental parameters, ResidDRI, T_{opt} , and T_0 , and climatic and life-history variables in conventional (phylogenetically uncontrolled; PU) and phylogenetically controlled (PC) analyses

Pairwise correlations	R , PU	P	R , PC	P
ResidDRI: T_{seas}	0.37	0.035**	0.20	0.26
ResidDRI: P_{seas}	-0.05	0.79	0.07	0.71
ResidDRI: T_{ann}	-0.21	0.23	0.11	0.52
ResidDRI: P_{ann}	0.10	0.58	-0.15	0.40
ResidDRI: Log_{10} AdMass	-0.08	0.68	0.03	0.88
T_{opt} : T_b	0.30	0.12	0.14	0.46
T_{opt} : T_{warm}	0.08	0.68	0.05	0.78
T_{opt} : P_{warm}	-0.40	0.032**	-0.36	0.057*
T_{opt} : T_{ann}	-0.05	0.82	-0.11	0.57
T_{opt} : P_{ann}	-0.27	0.16	-0.19	0.34
T_0 : adult T_b	0.57	0.002**	0.45	0.017**
T_0 : T_{warm}	0.48	0.010**	0.57	0.0014**
T_0 : P_{warm}	0.01	0.95	0.31	0.10
T_0 : T_{ann}	0.47	0.011**	0.64	0.0002**
T_0 : P_{ann}	0.05	0.80	0.16	0.41

R , correlation coefficients (PU, REML method; PC, Pearson product-moment correlations). DRI, index of developmental rate. * $P < 0.06$, ** $P < 0.05$ (in bold font). The number of observations is the same as that reported in Table 2.

for T_{opt} and T_0 . Importantly, the best climatic predictors of T_{opt} and T_0 were variables associated with the incubation period *per se*; none of the first ranked models included the ‘control’ variables (annual means of temperature or precipitation), and models that included these variables were poorly supported overall (Table 2). Second, correlations between T_{opt} and T_0 and climate and life-history variables were independent of phylogeny, implying that these developmental parameters have evolved in response to the physical environment of embryos.

We hypothesized that the upper thermal limit for incubation (T_{opt}) and the lower thermal limit for development (T_0) would be related to ambient temperature experienced during the warmest quarter of the year and/or to body temperatures of adults. This expectation was met, although, for T_{opt} , not quite as expected. Precipitation during the warmest quarter of the year (P_{warm}) (rather than temperature, T_{warm} , as expected) was not only the best predictor of T_{opt} but was included in the top three models. T_{opt} was related to P_{warm} such that the lower the precipitation during the warmest quarter of the year, the higher T_{opt} . T_{opt} is the embryonic parallel of adult T_b , the temperatures that maximize the performance of embryos and adults, respectively. We therefore examined the rela-

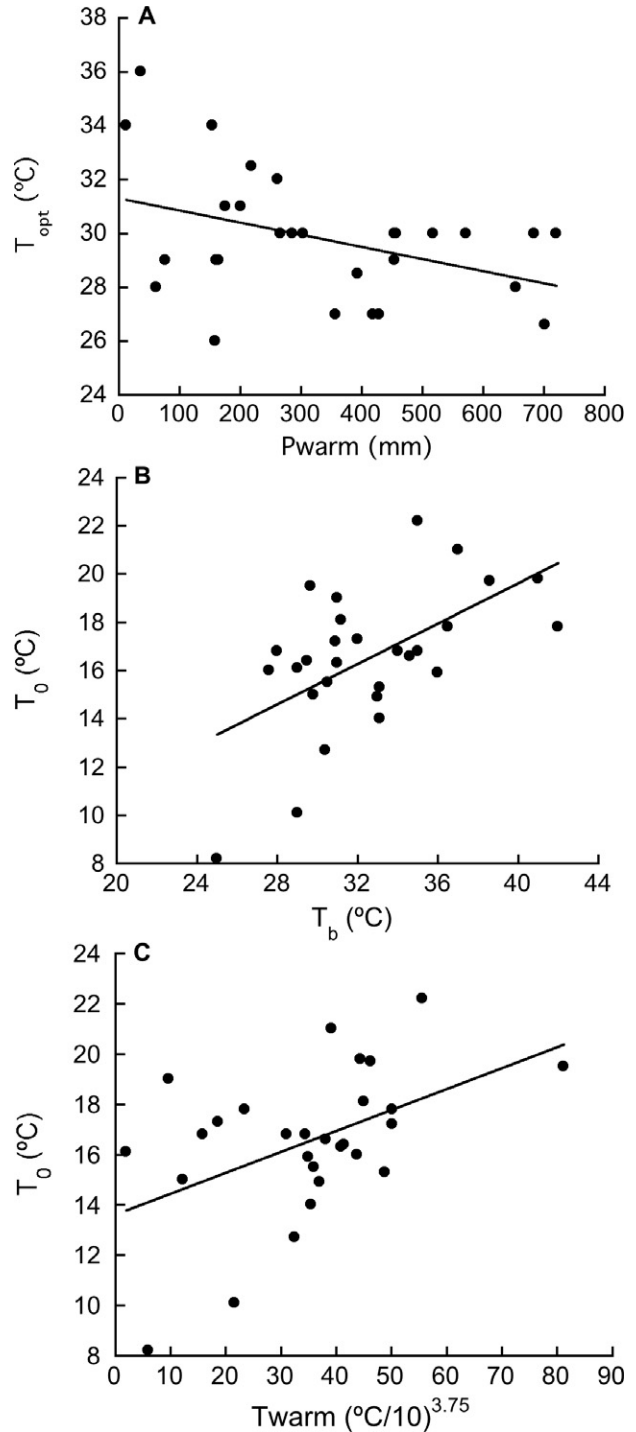


Figure 2. Response plots of developmental performance variables. A, T_{opt} as a function of P_{warm} . B, T_0 as a function of T_b . C, T_0 as a function of T_{warm} .

tionship between T_b and P_{warm} in our data set and also found an inverse correlation ($R = -0.32$, $P = 0.10$, $N = 28$). Similarly, in the study by Clusella-Trullas *et al.* (2011), the best predictor of adult T_b was

precipitation during the driest month of the year. The negative relationships between T_{opt} and T_{b} and precipitation have a functional interpretation; precipitation is associated with cloud cover (Aguilar *et al.*, 2005), and hence regions with low cloud cover during the warmest or driest months of the year would provide enhanced thermoregulatory opportunities for adult squamates and warmer nest (soil) temperatures as well.

Our hypothesis that the lower thermal limit for development (T_0) would be related to T_{warm} and/or to T_{b} was supported; the top-ranked model included both these variables. The association between T_0 and T_{warm} is likely direct, reflecting the environmental temperatures to which embryos are exposed during development. The association between T_0 and T_{b} is likely indirect and related to nest site selection by gravid females. T_{b} is related both to habitat and to phylogeny (Andrews, 1998, 2008a; Clusella-Trullas *et al.*, 2011), and females choose nest sites according to their thermal preferences in the habitats where they themselves live (Lin *et al.*, 2008). As a consequence, embryos will be exposed to nest temperatures that correspond to adult preferences, as well as to environmental temperatures.

Our hypothesis that species with large body sizes would exhibit faster embryonic growth (higher ResidDRI) than species with small body size was rejected; the model with adult body size as the predictor variable was the second lowest ranked of all models for ResidDRI (Table 2). Adult size thus does not affect developmental rate independently of hatchling size *per se* (ResidDRI controlled for hatchling size in our analyses).

THERMAL BIOLOGY OF EMBRYOS AND CLIMATE CHANGE

Observations on the thermal biology of adult ectothermic tetrapods (amphibians and reptiles) have been used to predict how populations will respond to global climatic change. This research agenda includes studies that describe the relationship between geographical variation in thermal biology and global climatic patterns (Clusella-Trullas *et al.*, 2011), studies that document local reduction in population density and extinctions associated with climate change (Huey *et al.*, 2009; Sinervo *et al.*, 2010), and studies that predict future changes in species ranges and persistence (Kearney, Shine & Porter, 2009; Sinervo *et al.*, 2010). To date, however, observations on the impact of climatic change have focused entirely on the thermal biology of adult individuals.

Climate change could have both positive and negative effects on squamate embryos, and hence potentially mitigate or exacerbate impacts on squamate

populations. Some predicted climatic changes will affect embryos more than adults. For example, global temperature increases are asymmetrically distributed over annual (Schwartz, Ahas & Aasa, 2006) and diurnal timeframes (Karl *et al.*, 1995; DeGaetano & Allen, 2002); mean night-time temperatures are increasing at twice the rate of corresponding daytime averages (Easterling *et al.*, 1997, 2000). This means that, at least initially, in some places or for some species, developmental rates will be enhanced, whereas the ability of diurnally active adults to thermoregulate to preferred levels will be less affected (Chamailé-Jammes *et al.*, 2006; Clarke & Zani, 2012). Because soil temperatures are lower than, and fluctuate less than air temperature (Packard & Packard, 1988), daytime nest temperatures will increase less than night-time nest temperature. Mean nest temperature will thus increase without a proportional increase in maximum nest temperature. As a result of asymmetrical warming, embryos will develop faster overall and incubation length will be shortened (Clarke & Zani, 2012). Shortened incubation periods could reduce egg mortality and increase neonate survival. These effects are, of course, dependent on the extent to which (1) egg mortality is proportional to the length of incubation and (2) early hatching conveys benefits such as enhanced growth or survival (Shine, 1985; Warner & Shine, 2007). Where successful development is currently limited by low ambient temperature, for example, higher mean incubation temperature will increase reproductive success because embryos will develop at more favourable temperatures (Lourdais *et al.*, 2004; Hare, Pledger & Daugherty, 2008). Moreover, increased length of breeding seasons in the Northern Hemisphere (Schwartz *et al.*, 2006) will mean that early and late clutches of multiple-clutching species will be exposed to more favourable thermal conditions for development and the number of clutches that can be produced per year may increase as well (Clarke & Zani, 2012).

On the other hand, because the optimal temperature for development (T_{opt}) and the lower limit for development (T_0) are positively correlated with environmental temperature and with the activity temperatures of adults, the effects of climate warming on embryos will likely exacerbate the thermal challenges faced by adults. For example, climate changes that force gravid females to be active at a relatively high T_{b} (Huey *et al.*, 2009; Sinervo *et al.*, 2010), or to select nest sites with temperatures that are outside the OTR for development, would reduce hatching success and produce hatchlings with low fitness. For many species, even the modest changes in thermoregulation and nest site selection that might ameliorate impacts of climate change on development are not possible.

For example, eggs of the tuatara incubate successfully at very low nest temperatures; natural tuatara habitat is limited to small islands off the coast of New Zealand. The thermal refugia that would allow tuatara to nest successfully will simply no longer exist if temperature continues to rise (Nelson *et al.*, 2004; Huey & Janzen, 2008). This dire outcome has already been realized for some populations of cool-adapted lizards on isolated mountaintops (Sinervo *et al.*, 2010). The other side of this scenario is that climate warming will facilitate the invasion of high elevation habitats by lowland species and rainforest interior habitats by heliothermic species (Huey *et al.*, 2009). In this situation, the correlated adaptations of embryos and adults to warm environments will enhance their shift in geographical range at the expense of species with cool adapted embryos and adults.

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APPENDIX

Table A1. Species and sources

Family	Species	Source
Agamidae	<i>Agama impalearis</i>	El Mouden <i>et al.</i> (2001); Znari & El Mouden (1998)
Agamidae	<i>Calotes versicolor</i>	Ji <i>et al.</i> (2002); Qui <i>et al.</i> (2009); Radder <i>et al.</i> (2002)
Agamidae	<i>Calotes versicolor2</i>	Vani <i>et al.</i> (2010)
Agamidae	<i>Chlamydosaurus kingii</i>	Christian <i>et al.</i> (1996); Harlow & Shine (1999)
Agamidae	<i>Ctenophorus decresii</i>	Harlow (2000); Greer (1989)
Agamidae	<i>Leiolepis reevesii</i>	Lin <i>et al.</i> (2007); Ji, pers. comm.
Chamaeleon	<i>Chamaeleon calypttratus</i>	Andrews (2008a, b)
Colubridae	<i>Elaphe carinata</i>	Ji & Du (2001b); Ji, pers. comm.
Colubridae	<i>Pituophis melanoleucus</i>	Burger & Zappalorti (1988); Gutzke & Packard (1987); Diller & Wallace (1996)
Colubridae	<i>Elaphe taeniura</i>	Du & Ji (2008); Ji, pers. comm.
Colubridae	<i>Dinodon rufozonatum</i>	Ji <i>et al.</i> (1999); Ji, pers. comm.
Colubridae	<i>Natrix natrix</i>	Löwenborg <i>et al.</i> (2010); Isaac & Gregory (2004)
Colubridae	<i>Ptyas korros</i>	Du & Ji (2002); Ji, pers. comm.
Colubridae	<i>Rhabdophis tigrinus</i>	Cai <i>et al.</i> (2007); Chen & Ji (2002)
Colubridae	<i>Tropidonophis mairii</i>	Bell (2010), pers. comm.
Colubridae	<i>Xenochrophis piscator</i>	Ji <i>et al.</i> (2001)
Colubridae	<i>Zaocys dhumnades</i>	Lin <i>et al.</i> (2010)
Elaphidae	<i>Naja atra</i>	Ji & Du (2001a); Lin <i>et al.</i> (2008); Lin <i>et al.</i> (2005); Ji, pers. comm.
Elaphidae	<i>Bungarus multicinctus</i>	Ji <i>et al.</i> (2007); Ji, pers. comm.
Gekkonidae	<i>Gekko japonicus</i>	Hu & Du (2007); Ji (1992); Tokunaga (1985); Ji, pers. comm.
Gekkonidae	<i>Eublepharis macularius</i>	Autumn & De Nardo (1995); Viets <i>et al.</i> (1993); Wise <i>et al.</i> (2009)
Gekkonidae	<i>Hemidactylus bowringii</i>	Xu & Ji (2007); Xu <i>et al.</i> (2007); Ji, pers. comm.
Gekkonidae	<i>Oedura lesueurii</i>	Doughty (1997)
Gekkonidae	<i>Paroedura pictus</i>	Blumberg <i>et al.</i> (2002); Kratochvil <i>et al.</i> (2006); Noro <i>et al.</i> (2009)
Iguanidae	<i>Dipsosaurus dorsalis</i>	DeWitt (1967); Muth (1977, 1980)
Iguanidae	<i>Anolis carolinensis</i>	Goodman (2007); Goodman & Walguarnery (2007); Sanger <i>et al.</i> (2008)
Iguanidae	<i>Cyclura nubila</i>	Alberts <i>et al.</i> (1997); Christian <i>et al.</i> (1986)
Iguanidae	<i>Sceloporus undulatus</i>	Andrews (1998); Andrews <i>et al.</i> (2000)
Lacertidae	<i>Eremias argus</i>	Hao <i>et al.</i> (2006); Zhao <i>et al.</i> (2008)
Lacertidae	<i>Podarcis muralis</i>	Amo <i>et al.</i> (2004); Brana & Ji (2000); Ji & Brana (1999); Van Damme <i>et al.</i> (1992)
Lacertidae	<i>Takydromus septentrionalis</i>	Du, Lu & Shen (2005a); Ji (1992); Ji <i>et al.</i> (1996); Du & Ji (2006); Yang <i>et al.</i> (2008)
Lacertidae	<i>Takydromus stejnegeri</i>	Chen <i>et al.</i> (2010)
Lacertidae	<i>Takydromus wolteri</i>	Pan & Ji (2001)
Lacertidae	<i>Zootoca vivipara</i>	Rodríguez-Díaz <i>et al.</i> (2010)
Scincidae	<i>Bassiana duperryii</i>	Shine & Harlow (1996); Shine (1995); Telemeco <i>et al.</i> (2010)
Scincidae	<i>Ctenotus robustus</i>	Greg Brown, pers. comm.; Greer (1989)
Scincidae	<i>Eumeces chinensis</i>	Du <i>et al.</i> (2005b); Ji & Zhang (2001); Ji <i>et al.</i> (1995)
Scincidae	<i>Eumeces elegans</i>	Du <i>et al.</i> (2000); Du <i>et al.</i> (2003)
Scincidae	<i>Oligosoma suteri</i>	Hare & Daugherty (2002); Hare <i>et al.</i> (2004); Hare <i>et al.</i> (2008)
Varanidae	<i>Varanus albigularis</i>	Phillips & Packard (1994); Greer (1989)