Heritability and developmental plasticity of growth in an oviparous lizard

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Abstract

Selective processes act on phenotypic variation yet the evolutionary potential of any given trait relies on underlying heritable variation. Developmental plasticity is an important source of phenotypic variation, but it can also promote changes in heritability by modifying environmental sources of variability. Here, we quantified the influence of developmental temperature on an important fitness trait, growth, in delicate skinks (*Lampropholis delicata*). We partitioned the total phenotypic variance using an animal model fitted with a genomic relatedness matrix. We measured mass growth for 262 individuals (n_{hot} = 126, n_{cold} = 136) over 16 months ($n_{observations}$ = 3,002); estimating heritability and maternal effects over time from animals experiencing two thermal developmental environments. Our results show that lizards reared in cold developmental temperatures had a higher initial mass compared to lizards that were reared in hot developmental temperatures. However, developmental temperature did not impact the rate of growth. On average, additive genetic variance, maternal effects and heritability were higher in the 'hot' developmental temperature treatment. Interestingly, heritability increased with age, whereas maternal effects decreased upon hatching but increased again at a later age. Our work suggests that evolutionary potential of growth is complex, age dependent and not overtly affected by extremes in natural nest temperatures.

Keywords

Body mass, growth rate, additive genetic variance, incubation temperature, maternal effects, temperature-size rule, cryptic genetic variation

Introduction

Developmental plasticity plays a key role in generating phenotypic variation (Noble et al 2018; Ghalambor et al., 2007; West-Eberhard, 2003). The complex interplay between an individual's genotype, and the developmental environment in which that genotype finds itself, means that a range of different phenotypes can arise (Monaghan, 2008; West-Eberhard, 2003). Phenotypic changes resulting from distinct early life experiences can have persistent effects on individual fitness (Monaghan, 2008; Noble et al., 2018). Changes induced by developmental environments may result in a better match between the adult phenotype and the subsequent selective environment. However in some cases, maladaptive phenotypes can arise if there is a mismatch between later-life environments and those experienced early in development (Beaman et al., 2016; Ghalambor et al., 2007). Regardless, phenotypic plasticity represents a promising immediate solution for threatened populations by allowing them to better track adaptive optima and persist (Beldade et al., 2011; Noble et al., 2019; West-Eberhard, 2003). Understanding the consequences of developmental environments on phenotypes and fitness is therefore critical to predict how populations will survive in stressful conditions (Botero et al., 2015; Reed et al., 2010).

A population's capacity to evolve depends not only on the strength of selection but also on the underlying standing genetic variation (Lynch & Walsh, 1998). It has long been recognised that selection and genetic variation change across environments (Falconer & Mackay, 1996). As such, a great deal of effort has been put towards understanding the circumstances under which genetic variation may change with the environment and the magnitude of those changes (Charmantier & Garant, 2005; Fischer et al., 2020; Hoffmann & Merilä, 1999; Noble et al., 2019; Rowiński & Rogell, 2017; Wood & Brodie, 2015). Genetic variance in novel environments may decrease as a result of stronger selection that erodes genetic variation (Hoffman & Parsons, 1991; Hoffmann & Merilä, 1999). In contrast, novel environments might also increase genetic variance when mutation rates are higher or buffering mechanisms breakdown triggering a release of 'cryptic genetic variation' in stressful conditions (Paaby & Rockman, 2014). Low cross-environment genetic correlations or condition-dependence of gene expression can also affect the amount of genetic variance in different environments (Charmantier & Garant, 2005; Coltman et al., 2001). Environmental dependence of genetic variance implies that under the same selection pressure, the speed of evolutionary change will be expected to change making it difficult to predict genetic adaptation.

Comparative studies have shown that the influence of environmental stress on genetic variance during development is not straightforward (Charmantier & Garant, 2005; Hoffmann & Merilä, 1999; Rowiński & Rogell, 2017). In lab studies, elevated developmental stress has been shown to increase the heritability of morphological traits (Hoffmann & Merilä, 1999), whereas wild, non-domestic populations tend to have higher heritability in favourable environments (Charmantier & Garant, 2005). Lack of consensus may be related to increased environmental heterogeneity in wild populations, making them more difficult to compare with lab studies. It has been suggested that responses to different developmental stressors (e.g. heat shock vs. starvation) may be associated with disparate patterns of gene expression making broad comparisons more variable (Charmantier & Garant, 2005; Dahlgaard & Hoffmann, 2000). Importantly, environmental comparisons of heritability have been criticised as the ratio nature of its calculations can mask changes in the relative contributions of non-genetic and genetic variance (Rowiński & Rogell, 2017). For example, a meta-analysis found that heritability of life history traits which has been argued to be more important to fitness, did not change between control and stressful conditions (Rowiński &

Rogell, 2017). The same pattern was observed for morphological traits (Fischer et al., 2020). Upon closer inspection, both additive genetic and environmental variance of life history traits increased under stressful conditions whereas the opposite was true for morphological traits (Rowiński & Rogell, 2017). The dynamics of both genetic and non-genetic sources of variation under different developmental environments can thus influence the evolutionary potential of fitness related traits.

Body size is fundamental to fitness and is both heritable and environmentally responsive (Noordwijk et al., 1988; Stillwell & Fox, 2009). Developmental environments, such as temperature and nutritional stress can drive substantial variation in body size, largely through shifts in how organisms grow (Eyck et al., 2019; Noble et al., 2018). Maternal investment in offspring are also important sources of body size variation (Noble et al., 2014; Wilson & Réale, 2006). Variation among mothers in egg investment, nest site selection or timing of birth (Mitchell et al., 2018; Shine & Harlow, 1996; Uller & Olsson, 2010) are expected to contribute the most to offspring body size early in development (Mousseau & Fox, 1998). However, these effects have shown to decline with age as maternal investment subside (Krist, 2010; Wilson, Kruuk, et al., 2005). Additionally, environmental factors such as shared habitats or long-term seasonal effects can also account for a substantial proportion of variability in body size (Kruuk, 2004). For example, permanent environmental effects that varied across years explained 26% – 35% of body size variation in bighorn sheep (Réale et al., 1999). Similarly, 56% of variation in body mass was attributed to nest boxes shared among siblings in blue tit chicks (Charmantier et al., 2004). As such, the various sources that influence body size variation (genetic, environmental, maternal) are predicted to vary across ontogeny and temporal approach is therefore needed in order to evaluate when evolutionary potential of body size is greatest.

Here we investigated the impact of developmental temperature on body size (mass) and growth in an oviparous skink (*Lampropholis delicata*) – two traits that are critically important to fitness. We also test how developmental environments affect evolutionary potential in these traits. Growth trajectories ($n_{observations}$ = 3,002) for lizards that hatched from two incubation treatments (n_{hot} = 126, n_{cold} = 136), were measured over the first 16 months of life (nearly half their life). Using 8,433 single nucleotide polymorphic (SNP) markers, we derived a genomic relatedness matrix to estimate quantitative genetic parameters. Using these data, we address two key questions: 1) How does developmental temperature affect the rate and shape of growth trajectories (initial mass, growth rate and curvature of growth trajectory)? and 2) How does developmental temperature affect genetic and non-genetic variance across age? According to the 'temperature-size rule', we expect lizards experiencing cold developmental temperatures to have larger initial masses and slower growth rates – possibly resulting in lizards reaching sexual maturity at a later age compared to lizards experiencing hot developmental temperatures (Angilletta Jr et al., 2017). In addition, we predicted greater amount of genetic variance under higher developmental temperatures, after controlling for non-genetic sources of variance. We expected maternal effects and permanent environment effects to manifest early in development and dissipate over time.

Materials and Methods

Lizard collection and husbandry

From 2015 – 2017, we established a breeding colony of adult *L. delicata* ($n_{females}$ = 144, n_{males} = 50) using wild individuals collected across five sites throughout the Sydney region between August and September 2015. Using a half-sib breeding design, we paired three females with a single male in opaque plastic enclosures measuring 35cm × 25cm

× 15cm (L × W × H). Enclosures were kept under UV lights (12L:12D) in a temperature-controlled room set to 24ºC. Lizards were given access to a heat lamp that elevated temperatures to between 28-32 ºC. Each enclosure was lined with newspaper and lizards had constant access to water. Tree bark was used as refuge. Adult lizards were fed medium sized crickets *ad libitum* (*Acheta domestica*) dusted with calcium powder and multi-vitamin every two days. From the beginning of the egg laying season (October of each year), we replaced newspaper lining with garden potting mix and placed an opaque plastic box (12 cm × 17.5 cm × 4.3 cm) containing moistened vermiculite in each enclosure for females to oviposit their eggs. During this time, enclosures were sprayed with water every second day to maintain a relatively humid environment. From October to November, egg boxes were checked every day. Tail tissue samples (~1 mm) were taken from adults that were from enclosures producing eggs for DNA extraction (see below). All tissues were stored in 70% ethanol. Animal collection was approved by the New South Wales National Parks and Wildlife Service (SL101549) and all procedures were approved by the Macquarie University Ethics committee (ARA 2015/015) and University of New South Wales Animal Care and Ethics committee (ACEC 15/51A).

Developmental Temperature Manipulations

Eggs were collected between October to March, over two reproductive seasons from 2016 and 2017. As soon as eggs were found, they were weighed using a digital scale to the nearest 0.01g (Ohaus Scout SKX123). We also measured egg length (distance between the furthest points along the longest axis of the egg) and egg width (distance between the widest points along the axis perpendicular to the longest axis of the egg) using digital callipers to the nearest 0.01mm. Following measurements, each egg was placed in a plastic cup (80ml) containing three grams of vermiculite and four grams of water. Each cup was then covered using cling wrap and secured using an elastic band. We used a split-clutch design where eggs from single clutch were pseudo-randomly assigned to one of two developmental temperature treatments. We used two incubators to precisely control the temperature of eggs (LabWit, ZXSD-R1090). The 'hot' treatment was exposed to a mean temperature of 29ºC whereas the 'cold' treatment was exposed to a mean temperature of 23ºC. Both incubators fluctuated +/- 3ºC over a 24-hour period around these mean temperatures to simulate natural nest site temperature variability. These treatments represent the temperature extremes of natural nest sites for *L. delicata* (Cheetham et al., 2011). Egg cups were rotated within each incubator weekly to avoid uneven heat circulation within incubators. Incubators were also checked daily for hatchlings.

Quantifying Growth Rate

Newly emerged hatchlings were weighed to the nearest 0.01g and a small tail tip clipping (~2mm) was taken for genetic analyses. Ventral photographs were taken for digital measurement (Nikon Coolpix A900). For the first two months, photographs of hatchlings were taken approximately every 14 days. After which, hatchlings were photographed at approximately a 35-day interval. From six months onwards, we manually measured hatchling SVL using a clear ruler to the nearest ~0.5mm. We also recorded the mass of the individual each time photographs or SVL measurements were taken. Growth measurements continued until we had approximately 16 measures per individual (mean = 11.5, SD = 4.71). By the end of the study, the mean age for hot incubated lizards was 335.82 (range: 0 – 711) and for cold incubated lizards it was 384.8 (range: 0 – 707) which is approximately 40 – 50% of their total lifespan (Chapple et al., 2014). From the photographs, we extracted snout-vent-length (SVL; from tip of snout to the beginning of the cloaca opening) using ImageJ software (Rueden et al., 2017). For the first initial nine months, hatchlings were housed individually in opaque plastic

enclosures (32.3cm x 18.5cm x 6cm) lined with newspaper. Hatchlings were fed the same number of crickets every second day and had constant access to a tree bark refuge and water. Hatchling enclosures were placed in a temperature control room under the same conditions as described above for the adult colony. For logistical reasons, at approximately nine months, hatchlings were housed in groups of five in opaque bins with the same measurements as the adult enclosures. We pseudo-randomised individuals to each shared enclosure while maintaining a similar number of individuals from each treatment.

Genomic Relatedness Matrix

We derived a genomic relatedness matrix (GRM) using single nucleotide polymorphism (SNP) genotypes for all 262 offspring with growth data (132 putative parents; $n_{females} = 69$, $n_{males} = 63$). While our half-sib breeding design allowed us to assign parentage to derive a pedigree, high levels of sperm storage and low levels of multiple paternity (94% of females had been sired by a single male) meant our pedigree had low resolution to effectively estimate additive genetic variation. Recent studies have shown that GRM derived from SNPs have low error rates (<0.3%) and are able to reconstruct pedigree relationships in much finer detail when at least 200 SNP loci are used (Bérénos et al., 2014; Huisman, 2017). Moreover, both relatedness and heritability values estimated from a GRM have been shown to be very similar to those inferred using a pedigree (Bérénos et al., 2014; Huisman, 2017). Single nucleotide polymorphism libraries were designed and animals genotyped using DArTseq™ (Diversity Arrays Technology) methods. For more details on DNA extraction and SNP genotyping see ESM.

Prior to deriving our GRM, we filtered our SNPs using the R package *dartR* (Gruber et al., 2018). We filtered loci based on various metrics in the following order: 1) read depth (8 – 40); reproducibility (> 0.996); call rate by loci (> 0.97) and then by individual (> 0.80); monomorphic loci; minor allele frequencies (> 0.02); Hamming Distance among loci (> 0.25) and Hardy Weinberg Equilibrium. This clean-up process resulted in a dataset of 8,438 loci with an average call rate of 98.5% (see ESM and provided code). Using these 8,438 loci we derived a GRM, which describes the proportion of the genome that is identical by descent (VanRaden, 2008). We calculated a GRM for all hatchlings using the *snpReady* R package (Granato et al., 2018) following methods described by VanRaden, 2008:

$$GRM = \frac{ZZ'}{2\sum p_i(1-p_i)}$$

where Z is the centered squared matrix of SNP genotypes of all individuals. This is calculated from a matrix of where heterozygote SNP genotypes (AT) were coded as 0, homozygote genotypes for the SNP allele (AA) were coded as 1 and homozygotes for the original allele (TT) were coded as -1. p_i is the frequency of the second locus at locus position *i.* The denominator scales the GRM matrix so that the values approximate a relatedness matrix derived from a pedigree. The GRM was then inverted for modelling fitting (see ESM and provided code).

Statistical Analyses

All analyses were performed using *R* (Core Team, 2013). We checked the data for potential input errors using histograms, scatterplots and Cleveland plots. We fitted Bayesian linear mixed effects models (LMM) in *brms* with interfaces with Stan (Bürkner, 2017; Gelman et al., 2015). Mass was log-transformed, and age was z-transformed. For all models we used noninformative priors with 4000 iterations with a burn in of 1500, sampling from the

posterior distribution every fifth iteration. We ensured proper mixing by inspecting trace plots and checked that scale reduction factors were less than 1.01. We report posterior means and 95% credible intervals for all parameters throughout.

Impact of Developmental Temperature on Additive Genetic Variance and Maternal Effects Across Age

First, we tested whether developmental temperature influenced the overall heritability of mass and the relative contributions of variance irrespective of age. For each treatment group, we fitted intercepts only in the fixed effects with random intercepts for additive genetic variance (*G*), maternal effects (*M*) and permanent environmental effects (*PE*) as we had repeated measures of the same individuals (Wilson et al., 2010). The model also estimated residual variance (*R*). We included our GRM to estimate additive genetic variation. Overall. Heritability (h^2) of mass using this intercept (*I*) model was calculated as:

$$h^2 = \frac{G_I}{(G_I + M_I + PE_I + R_I)}$$

To then test how *G*, *M* and h^2 change across age, we used model selection to determine the most appropriate random effects structure for our data as we had no *a priori* knowledge of what (or how) variance components change with age (Wilson & Réale, 2006). We fitted seven models with varying complexity in their random effects and compared their Watanabe–Akaike Information Criterion (WAIC) values (Table S1). We fitted random intercepts and random slopes by including either a linear age term or both linear and quadratic age terms to partition variance across age. Two models were equally supported, the first included a random linear and quadratic slope for *G* and *M* and *PE*. (Model 3 - Table S1) and the second included a random linear and quadratic slope for *G* and *M,* respectively, and a random intercept for *PE* (Model 7 – Table S1). To avoid overfitting, we selected the more parsimonious model and used this random effect structure for the remaining analyses unless stated otherwise.

Residual variance may be conflated with estimates of other variance components if it changes over time (heterogenous variance) and is not properly accounted for. We therefore explicitly modelled residual variance to verify if this was the case and compared homogenous and heterogenous residual variance models using WAIC. We fitted two models, both of which had the same fixed and random effects structure as Model 7 described above. The first model had homogenous residual variance whereas in the second model we modelled residual variance with a linear slope thereby allowing it to vary with age. The model with heterogenous variance was best supported (Table S2), we therefore modelled heterogenous variance in all subsequent models unless stated otherwise.

To test for treatment differences in variance components, we subset data for each treatment group and fitted an intercept-only model with our best supported random effect structure (Model 7) and heterogenous residual variance. We estimated a genetic variance-covariance matrix for each treatment (G), where the diagonal elements represent the additive genetic variances for the intercept (G_I), slope (G_S) and the quadratic (G_C) across age. The off-diagonal elements are the additive genetic covariances between the growth curve parameters, for example, $Cov_{I,C}$ is the additive genetic variance between the intercept and the quadratic slope.

$$G = \begin{bmatrix} G_I & Cov_{I,S} & Cov_{I,C} \\ Cov_{I,S} & G_S & Cov_{S,C} \\ Cov_{I,C} & Cov_{S,C} & G_C \end{bmatrix}$$

Similarly, the variance-covariance matrix for dams (M) can be decomposed in the same manner as G.

$$M = \begin{bmatrix} M_I & Cov_{I,S} & Cov_{I,C} \\ Cov_{I,S} & M_S & Cov_{S,C} \\ Cov_{I,C} & Cov_{S,C} & M_C \end{bmatrix}$$

For each treatment group, we then calculated additive genetic variance at a given age G_x using the random slope terms and their covariances following (Schielzeth & Nakagawa, 2020):

$$G_x = G_I + (x^2.G_S) + (x^4.G_C) + (2x.Cov_{I,S}) + (2x^2.Cov_{I,C}) + (2x^3.Cov_{S,C})$$

where x is a specific age. Age-specific maternal effect M_x was calculated using the same formula but with the relevant variance components from M. Age-specific heritability, h_x^2, is thus a ratio of all variance components at a given age x. The proportion of variance explained by maternal effects (m^2) is calculated in the same manner.

$$h_x^2 = \frac{G_x}{(G_x + M_x + PE_I + R_I)}$$

As the mean body mass increases over time, the variance may also increase concurrently due to scale effects and potentially bias estimates of quantitative genetic parameters (Wilson, Kruuk, et al., 2005). We therefore calculated coefficients of variation (CV) across age for each variance component by dividing variance by the predicted mean mass at a given age. Interpretations using CV estimates did not change our overall conclusions for additive genetic variance or maternal effects, we therefore present the raw estimates of each variance component below (See ESM).

The Influence of Developmental Temperature on Growth Trajectories

To test how developmental temperatures affect average growth trajectories, we also fitted three models that varied in their fixed effect structure to determine how developmental temperatures affect: 1) initial mass (intercept of curve), 2) linear rate of growth (linear slope) and 3) curvature of the growth trajectory (quadratic term). We also wanted to test for treatment differences in age at which lizards reach their maximum mass by solving for the maxima of quadratic regression equation. We fit mass as the response accounting for the same random effects described above. The first model included the main effect of developmental temperature and the linear and quadratic term for age (Table S2). The other two models differed in their interaction terms between developmental temperature with age and age^2 (Table 2, S3). We then compared WAIC values to select the best model for our data that explained changes in mass across age between the two developmental temperature treatments (Table 1).

Results

Over two years, we collected 3,002 observations of mass data for a total of 261 individuals (n_{hot} = 125, n_{cold} = 136). On average, the incubation period for the 'hot' treatment was 29.36 days (SD = 2.17, range = 15 - 49) days and 48.48 days (SD = 4.18, range = 25 - 56) for the 'cold' treatment.

Overall, additive genetic variance, permanent environmental variance and heritability (h^2) of growth appears to be higher in the hot developmental temperature treatment (Fig. 1). However, there were no significant differences among treatment groups (Table S3).

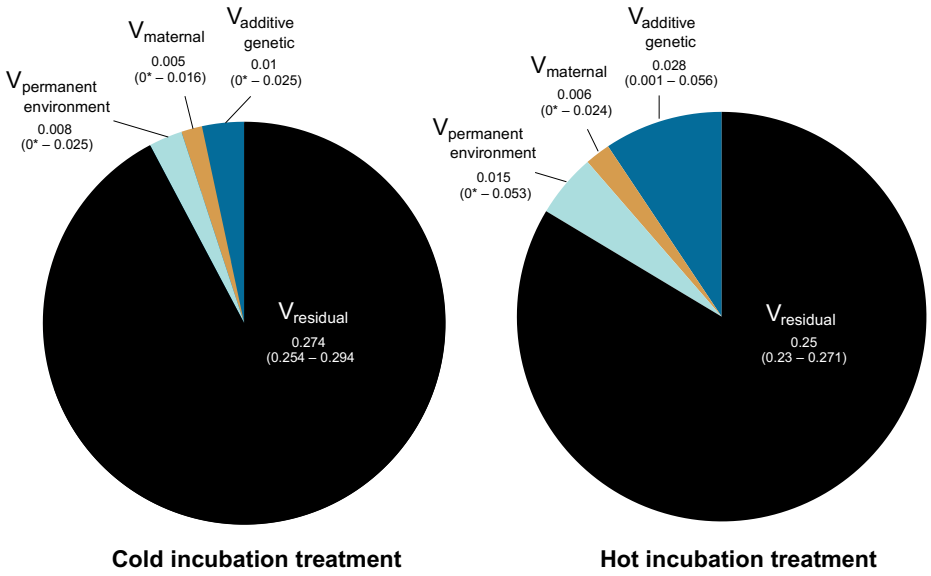
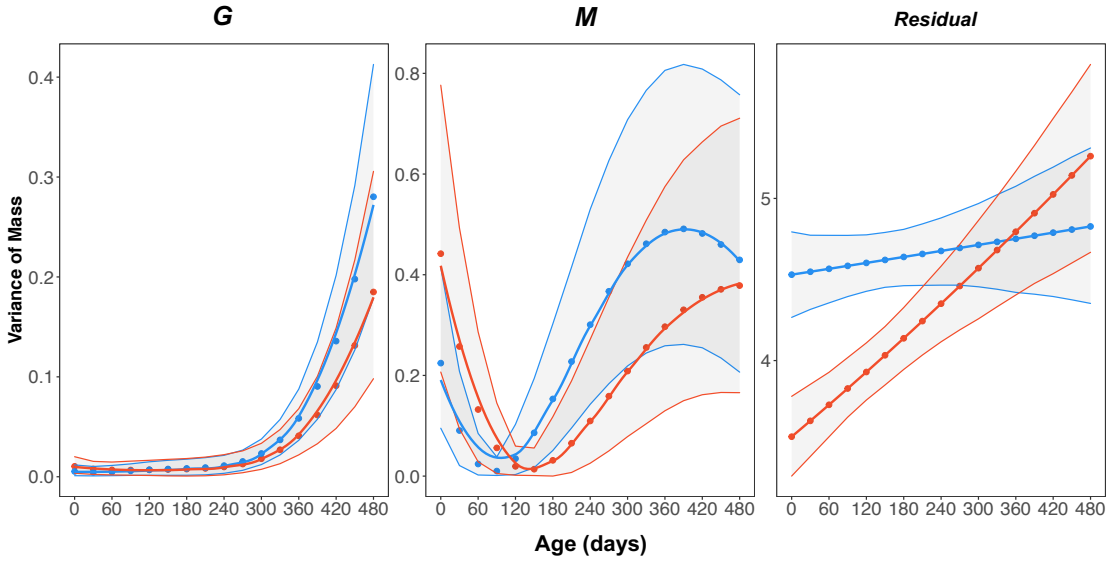


Figure 1 Pie charts depicting the overall relative contributions of mass variance for the hot ($n_{lizards}$ = 126) and cold ($n_{lizards}$ =136) developmental treatment group irrespective of age. Point estimates and 95% credible intervals are presented in Table S3. There were no significant differences in variance components between developmental temperature treatments. * in indicates very small values that were above 0.The influence of developmental temperature on genetic and non-genetic variance across age

Treatment groups did not differ in how the relative contributions of G and M changed with age as their 95% credible intervals overlapped (Fig. 2). Additive genetic variance remained relatively low and constant upon emergence until approximately nine months of age, after which it increased rapidly (Fig. 2). Maternal effects decreased sharply upon hatching and dropped to the minimum at approximately six months before it increased again (Fig. 2). There were some differences among developmental treatments in how residual variance



changed with age (Fig. 2C). Residual variance in cold incubated lizards had a much higher intercept compared to hot incubated lizard however their residual variance converged by eight months of age (Fig. 2).

Figure. 2 Scatterplot showing how additive genetic variance (*G*), maternal effects (*M*), residual variance changed with age for the hot developmental treatment ($n_{lizards}$ = 125, red) and the cold developmental treatment (n = 136, blue). Points represent posterior means, thin lines represent the 95% credible intervals, thick lines represent the mean for each treatment group. Note that permanent environmental effects were treated as constant across age. $V_{permanent\ environment}$ for the hot treatment group was 0.0047 [0.00017 – 0.0096], $V_{permanent\ environment}$ for the cold treatment group was 0.0047 [0.00065 – 0.0085].

We investigated whether increases in average mass over time affected variance estimates due to scaling effects between the mean and variance. However, we found that the CV of *G* and *M* followed the same pattern as the raw variance estimates suggesting that changes in variance were not the result of increasing mean body mass with age (Fig. S1).

After accounting for heterogenous residual variance, we found no treatment differences in heritability or the proportion of variance explained by maternal effects (M^2) (Fig. 3). Heritability was very low for the first year of growth in *L. delicata* and only began increasing at one year of age (Fig. 3). As predicted M^2 decreased soon after hatching, however it increased slightly again from six months of age (Fig. 3). The G and M matrices for each treatment group are presented in Table S4-S5.

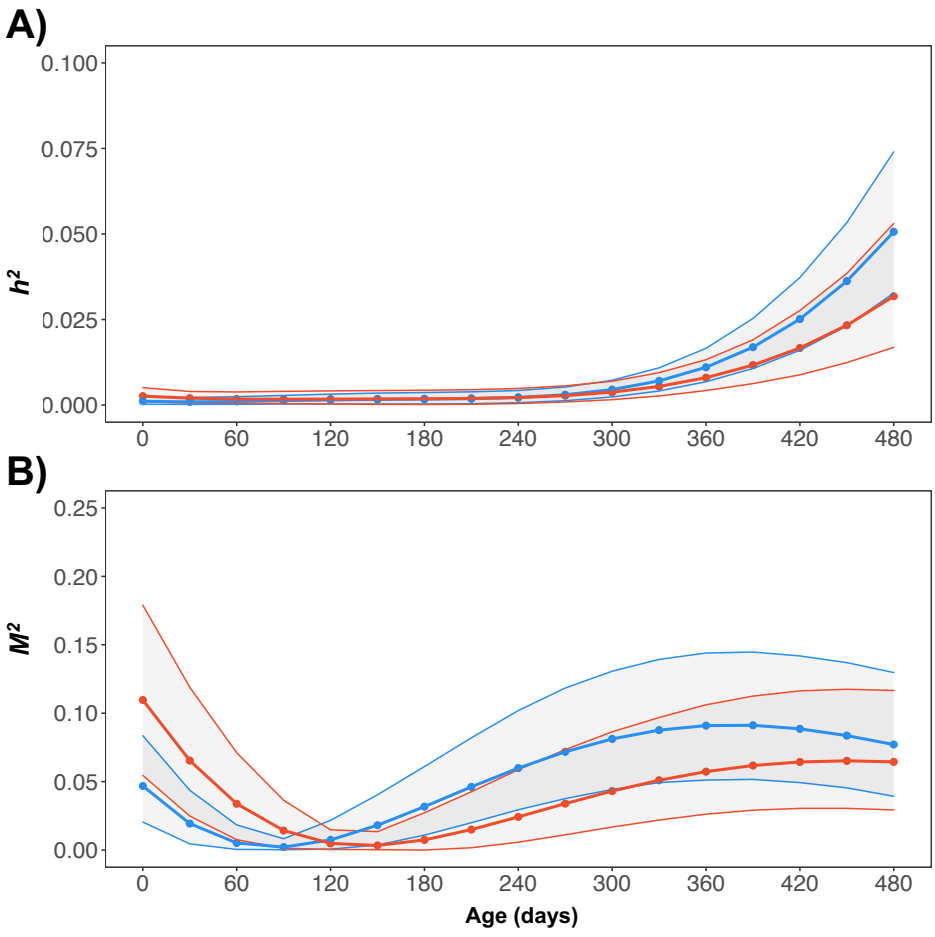


Figure 3 Heritability (h^2, **A**) and the proportion of total variance explained by maternal effect variance (M^2, **B**) across age (days) for the hot developmental treatment ($n_{lizards}$ = 125, red) and the cold developmental treatment ($n_{lizards}$ = 136, blue). Points represent estimates generated from the posterior distribution of the variance-covariance matrix, thin lines represent the 95% credible intervals, thick lines represent the mean for each treatment group.

Developmental plasticity in growth trajectories in response to temperature

While the model containing a full interaction between treatment and linear and quadratic age was best supported, the improvement in WAIC value was marginal (Table 1). Moreover, the linear growth rate (Age) and curvature of the growth trajectory (Age^2) did not differ significantly between the two developmental temperature treatments in any of the models containing interactions (Table S7 - S9). Irrespective of treatment, lizard mass increased by 1.65 g for every 1 SD unit increase in age.

Table 1 Comparisons of WAIC values of four models (n_{obs} = 2926) with different combinations of treatment interactions with age parameters. $\Delta ELPD$ represents the difference in expected log predicted density. Age measured in days was z-transformed (mean = 361.34, SD = 185.16)

Formula of Fixed Effects	WAIC	$\Delta ELPD$	Std. Error $\Delta ELPD$
Treatment + Age + Age^2 + Treatment × Age + Treatment × Age^2	-3301	0	0
Treatment + Age + Age^2 + Treatment × Age	-3295	-0.62	1.182
Treatment + Age + Age^2 + Treatment × Age^2	-3300	-2.798	1.375
Treatment + Age + Age^2	-3292	-4.452	1.563

Developmental temperature did, however, influenced hatching mass (Table 1, Fig. 3). Lizards from the 'cold' treatment were on average 0.030 g (0.018g – 0.041g) heavier compared to lizards from the 'hot' treatment (Table. 2). Larger initial masses meant that lizards from the 'cold' treatment reached their maximum mass slightly earlier (382.97 days, 95% CI: 358.84 – 409.78) compared to lizards from 'hot' treatment (413.04 days, 95% CI: 379.70 – 452.34). *G* and *M* matrices from this model, along with other variance components, are presented in Table S6.

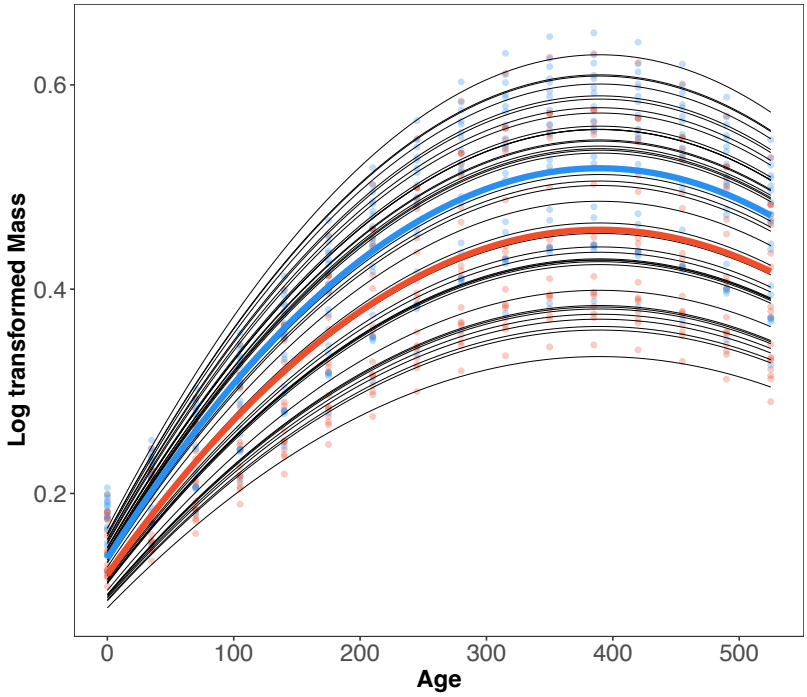


Figure 3 Model predictions of log-transformed mass over age from the two developmental temperatures. We randomly subset 40 lizards (20 from each treatment) to plot their individual growth curves. Points represent mean estimates for each lizard from the hot developmental treatment (hot) and the cold developmental treatment (blue). Thick lines represent average growth curve for each treatment. Faint grey lines are each individual's growth curve. Model predictions were generated from the full model where interaction terms between treatment and both the linear component and quadratic component were included

Table 2 Coefficient estimates from full model testing the effects of developmental treatment on mass and how mass changes with age. Bolded estimates are significantly different from zero. * indicates that value is above zero prior to rounding. n_{obs} = 2926. Age measured in days was z-transformed (mean = 361.34, SD = 185.16). *G* and M matrices for this model is presented in Table S6.

Parameter	Estimate	Lower	Upper
Intercept	**-0.991**	**-1.01**	**-0.971**
Treatment	**-0.083**	**-0.114**	**-0.05**
Age	**0.5**	**0.476**	**0.526**
Age^2	**-0.196**	**-0.216**	**-0.178**
Treatment × Age	0.008	-0.021	0.037
Treatment × Age^2	0.022	-0.007	0.052

Discussion

Early development at hot temperatures resulted in smaller body sizes compared to development at cold temperatures. Growth trajectories, however, were not significantly impacted by early thermal environments – lizards from both temperatures grew at the same rate despite cold animals remaining larger throughout life. Marginalising over age, we found

that developmental temperature did not impact the relative contributions of additive genetic, maternal, permanent environment or residual variance. The environmental component of the phenotype (residual variance) explained most of the variability in body mass. Congruently, heritability of mass was generally low across ontogeny, increasing at one year of age. As we predicted, maternal effects on offspring mass declined in the first few months, presumably because maternal non-genetic contributions were less influential on mass over time. Unexpectedly, maternal effects increased again at approximately six months possibly from maternal genetic factors affecting mass. Upon hatching, the residual variance component of body mass was much higher in lizards that were reared at cold incubation temperatures, suggesting that aspects of development environment played a bigger role in determining their hatching mass.

Thermal developmental plasticity in growth

In ectotherms, temperature plays a pervasive role in phenotypic development (Eyck et al., 2019; Noble et al., 2018; O'Dea et al., 2019; While et al., 2018). Contrary to other reptile studies, we did not show that growth rate differed between developmental temperatures. Some researchers reported increases in growth at higher incubation temperatures (Elphick & Shine, 1999; Hare et al., 2004; Verdú-Ricoy et al., 2014), while have others found either the opposite result or no differences at all (Andrews et al., 2000; Goodman, 2008). The directionality of change is highly variable, even among studies of the same species (e.g. Bassiana dupreyi, Elphick & Shine, 1998, 1999; Flatt et al., 2001; Telemeco et al., 2010). Lack of generality may be related to how growth is statistically modelled. Very few studies account for individual variation in hatching mass or growth trajectories. Indeed, if we did not account for among individual variance in our models, significant treatment differences in growth can be detected (Table S10). We emphasise the importance of partitioning confounding sources of variance such as individual or clutch effects as they can misconstrue conclusions about developmental impacts on late life phenotypes. Moreover, future studies should make use of all repeated measures of mass instead of averaging across individuals as the former approach not only increases statistical power but also provide more accurate estimates of growth.

Consistent with other squamates, we found that the cold incubation treatment group attained higher hatching mass compared to their hot counterparts (Dayananda et al., 2016; Downes & Shine, 1999; Flatt et al., 2001; Goodman et al., 2013). These results support the temperature-size-rule whereby organisms reared in cold temperatures tend to have larger body sizes (Angilletta Jr et al., 2017). Larger hatching size can be achieved through prolonged development at cooler temperatures during embryonic stages (Forster & Hirst, 2012). It is well known that cold developmental temperatures result in longer incubation periods in many reptiles (Booth, 2006; Dayananda et al., 2016; Downes & Shine, 1999; Elphick & Shine, 1998; R. M. Goodman, 2008). Longer developmental time may allow embryos to assimilate yolk nutrients more efficiently thus increasing mass at hatching (Storm & Angilletta, 2007). Indeed, turtle embryos exposed to high temperatures have enhanced mitochondrial metabolism and metabolic enzymic activity which constrains developmental time and reduced overall hatching size (Ji et al., 2003; Sun et al., 2015). Thermal plasticity in embryonic development may be adaptive for lizards born late in the season when nest temperatures are generally colder (Warner & Shine, 2008; While et al., 2015). Indeed, female *L. delicata* have an extended oviposition period (September to February in our population) and nest temperatures during this time can be highly variable in the wild (Cheetham et al., 2011). Heavier weight at emergence could mean that hatchlings are in better condition to

compete with lizards that hatched earlier or have sufficient body reserves to survive harsher condtions in more seasonal environments (Downes & Shine, 1999; Gifford et al., 2017; Qualls & Shine, 2000). Understanding how body mass affects survival will be necessary to elucidate the adaptative potential of developmentally plastic responses in the wild.

Thermal developmental environments and the evolutionary potential of body mass

Adaptative evolutionary responses depend not only on the amount of selection operating on a trait but on also its underlying additive genetic variance (Falconer, 1952; Ghalambor et al., 2007; Hoffmann & Merilä, 1999). Stressful developmental environments are hypothesized to lead to the release of 'cryptic' genetic variation (Fischer et al., 2020; Noble et al., 2019; Rowiński & Rogell, 2017; Wood & Brodie, 2015), possibly increasing the evolutionary potential of a given trait. Higher genetic variation, combined with stronger selection may facilitate rapid evolutionary responses that may allow populations to adapt to novel environments (Hoffmann & Merilä, 1999; Falconer and Mackay 1996). Contrary to these hypotheses, we found no statistical differences in additive genetic variance for mass between our developmental temperature treatments. In fact, heritability for mass was overall quite low echoing heritability values for mass in various animal systems [e.g., bighorn sheep – 0.03 to 0.31 (Réale et al., 1999), macaques – 0.39 (Kimock et al., 2019) lizards – 0 to 0.54 – (Martins et al., 2019; Noble et al., 2014)]. It should be noted that decoupling additive genetic variances from other non-genetic variance such as maternal effects requires considerable paternal links in the study design and pedigree (Kruuk, 2004). Indeed, when this variance partitioning is done accordingly, heritability estimates are often low (e.g., Noble et al. 2014). In the case of our study, we found relatively low levels of multiple paternity (<1% of clutches were sired by multiple fathers), as such the number of half-sibs were generally low which may have affected our genomic relatedness matrix and estimates of quantitative genetic parameters.

Lack of differences in genetic variation between developmental temperature environments support findings from recent meta-analyses. Fisher et al. (2020) assessed the degree to which stressful thermal environments result in the release of genetic variation. They found that these effects manifested in only a third of the studied cases – in mainly clonal organisms (Fischer et al., 2020). Furthermore, of the 25 cases where genetic variance changed across thermal environments there was no consistent direction (i.e., 11 increased and 14 decreased under thermal stress). Noble et al. (2019) also showed that the release of 'cryptic' genetic variation depends on the study design – studies not able to partition out non-genetic sources of variation supported a release of genetic variation whereas studies that did showed the opposite pattern. As a caveat, defining an environment as stressful or novel is a difficult task which requires detailed knowledge of a given species' past environmental exposure – information that is often unknown (Roelofs et al., 2010). While our incubation temperatures were selected based on temperature extremes of naturally occurring *L. delicata* nests (Cheetham et al., 2011), it is nonetheless possible they were not 'stressful' from an evolutionary perspective. Indeed, egg mortality did not differ across incubation treatments which suggests that lizards from both treatments experienced a similar level of thermal stress as embryos (the estimate of treatment difference: 0.80 [-0.04 -1.73]). Furthermore, treatment differences may be harder to detect under realistic fluctuating temperature regimes. As such, lizards were not exposed to extreme temperatures over extended periods which might be more important in orchestrating changes in genetic variation (Bonamour et al., 2019). Overall, our results suggest that the thermal extremes experienced by natural nest sites do not modify the evolutionary potential of mass. However this should be interpreted with caution

as estimates of quantitative parameters from laboratory studies can differ from wild populations (Sgrò & Hoffmann, 2004; Weigensberg & Roff, 1996).

Ontogenetic changes in genetic and non-genetic contributions to body mass

Genetic contributions to body size are expected to vary throughout ontogeny (Lynch & Walsh, 1998). Selection pressures on body size are likely to increase at critical life stages, such as at birth or at sexual maturation, thereby reducing genetic variance at certain ages (Rollinson & Rowe, 2015). On the contrary, we found that additive genetic variance of mass was very low upon hatching but slowly increased by the end of the first year. This result parallels those seen in big horn sheep (Réale et al., 1999), soay sheep (Wilson et al., 2007) and ladybird beetles (Dmitriew et al., 2010). While the underlying cause of this pattern is not well established, it coincided with changes in the social environment (shared housing). This suggests that perhaps competition for resources (basking sites or food) may orchestrate changes in genetic variation (Dmitriew et al., 2010; Hoffmann & Merilä, 1999). Alternatively, the gradual increase in additive genetic variance may be related to initial genotypic changes underpinning sexual maturation (~14 months) as *L.delicata* are sexually dimorphic in various morphological traits including body size (Chapple et al., 2014). Nonetheless, ontogenetic variation in genetic variance implies that potential rates of evolution varies with age (Houle, 1998), however this depends on non-genetic sources of variance as well.

Maternal non-genetic contributions to offspring body size are expected to be highest during early life stages and decline as offspring mature, particularly in precocial species (Cheverud, 1984; Wilson, Kruuk, et al., 2005). In accordance with other studies, maternal effects did in fact decline after hatching (Dmitriew et al., 2010; Lindholm et al., 2006; Pick et al., 2016; Wilson, Coltman, et al., 2005; Wilson, Kruuk, et al., 2005). Maternal investment, such as investment in clutch number or egg quality, has been shown to influence hatching size in lizards (Brown & Shine, 2009; Noble et al., 2014; Warner & Lovern, 2014), however, as predicted, these effects dissipated post-hatching (Pick et al., 2016; Réale et al., 1999). Interestingly, maternal contributions increased at a later age and remained moderately low for the remainder of the study. The cause of resurgence in maternal effect variance is unclear, however this pattern may indicate other maternally inherited components such as maternal genetic effects (e.g., mitochondrial genetic variation) that promote variation in body size (Pick et al., 2016). Indeed, variation in mitochondrial function has been linked to an individual's metabolic rate and growth – explaining as much as ~50% of the variation in food intake and growth (Salin et al., 2016, 2019). Therefore, it is likely an important driver of body size variability. Similar to additive genetic variance, resurgence of maternal effects also cooccurred with changes in the shared environment (housing conditions), suggesting that maternal effects on offspring body size is likely to be environmentally driven.

Traits under strong selection are expected to show low evolutionary potential as selection acts to remove genetic variation. While low evolutionary potential is at least in part due to reduced levels of additive genetic variance, it is also a result of larger proportions of environmental variance. In our study, the environmental component of the phenotype accounted for over 80% of variation in body mass which is in line with values reported in great tits (53 –74%) and soay sheep (70 – 96%) (Noordwijk et al., 1988; Wilson et al., 2007). Interestingly, cool developmental temperatures increased the amount of environmental variance attributed to body mass at an early age. What mechanisms are comprised in this

environmental component? Variation in developmental period between developmental temperatures may explain these differences. In many ectotherms, developmental time exhibits a nonlinear reaction norm with temperature (Marshall et al., 2020; Noble et al., 2018).This means that developmental time decelerates with temperature following an negative exponential function. As a result, hot incubated lizards are more constrained in their development time compared to lizards that were reared a cooler temperature. In actual fact, the cold developmental temperature treatment had much greater variance in incubation duration. With a longer incubation period, embryos can maximise the yolk resources left by their mothers which can vary considerably within clutch (Wallace et al., 2007). Our results suggest that thermodynamic effects of development time can give rise greater environmental heterogeneity in hatching mass and may affect potential for evolution at early life stages.

Conclusion

Our work illustrates the pervasive role of developmental temperature on phenotypic variation. The impact of developmental temperature on body mass manifested early and persisted through life (Monaghan, 2008). This has profound implications as developmentally induced variation in body mass may drive life history differences within populations and alter their vulnerability to environmental change (Botero et al., 2015; Marshall et al., 2020; Reed et al., 2010). In contrast, genetic variance of body mass was robust to thermal extremes experienced by natural nests and suggests that the potential to genetically adapt to warming climate may be limited. However, more stressful incubation temperatures are needed to elucidate the capacity for this species to reveal new genetic material for selection to act on. Non-genetic sources of variance were responsible for most of the variability in body mass and their dynamics with age means that effectiveness of evolution is everchanging. Understanding the complexities of adaptive evolution in response to climate change may require intensive long-term studies in wild populations.

Author contributions

FK, DN and SN conceived the study, FK and DN collected and analysed the data, FK wrote the first draft, FK, DN and SN edited the manuscript.

Data accessibility

Datasets and code used to generate results of this study is accessible via Open Science Framework (https://bit.ly/2Uy72id)

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