# Title

# Abstract

# Keywords

# Introduction

For decades, early life experiences have been recognised as an important evolutionary process as they can have a lasting effect on an organism’s phenotype. Theoretical and experimental work suggests that developmental plasticity is adaptive when early life cues predict later life environments. The ability for embryos to pre-emptively match themselves to the environment will be important for ectotherms as mean temperatures as well as temperature variability continue to increase. Oviparous reptiles typically deposit their eggs in an untended nest, leaving embryos to develop in the microhabitat chosen by the mother. The incubation conditions will go on and shape the hatchling’s phenotype and impact its fitness and survival.

There is a wealth of studies that illustrate that incubation temperatures can have a profound influence on the hatchling’s phenotype.

Growth rate is a key life history trait that can influence an individual’s fitness and survival. Fast growth rates enable individuals to reach sexual maturity earlier but may also compromise their lifespan compared to those with a slow growth rate (ref). The temperature-size rule describes the widespread phenomenon that individuals or species that experience cold environments tend to grow more slowly and mature at the larger size compare to those exposure to hot environments. In order to attain a large body size in cool environments, individual must prolong growth and. Thermal plasticity in body size has been documented in

[What are the effects of incubation temperature on growth rate? Temperature-size rule, examples, are they all mean sizes? Need to know how trajectories are altered because responses are not linear, comparisons/differences may be time/age specific which has implications on…?]

Evolutionary changes in growth can occur if it is heritable and there is sufficient additive genetic variation for selection to act on. [Examples]

Despite there being some evidence that suggests that growth can evolve, we do not currently know how heritability and additive genetic variance of growth varies with environment. [What are the theoretical expectations? How does temperature/environment changeAdditive genetic variance?]. Moreover, our understanding on how heritability and genetic variance changes with age is limited.

Here we investigated how incubation temperatures affects growth trajectories and the additive genetic variance of growth using an oviparous skink (*Lampropholis delicata*). Using 8,433 SNP markers, we derived a genomic relatedness matrix to [novel stuff] address the following questions 1) How does developmental temperature affect growth trajectories? (initial mass, linear growth rate and curvature of trajectory) 2) Does developmental temperature affect the relative contributions of growth variance? 3) How does developmental temperature change the relative contributions of growth variance as individuals age?

# Materials and Methods

## Lizard husbandry, Breeding Design and Egg Collection

From 2015 – 2017, we established a breeding colony of adult *L. delicata* (nfemales = 144, nmales = 50) using wild individuals collected across five sites throughout the Sydney region between 28 August and 8 September 2015. We used a half-sib breeding design where up to three females were housed with a single male in an opaque plastic enclosure measuring 35cm 25cm 15cm (L W H). Enclosures were kept under UV lights (12L:12D) in a temperature control room set at 24ºC and they 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 and tree bark 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 egg laying seasons (October of each year), we replaced newpaper 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 gently with water every second day to maintain a relatively humid environment. From October to November, egg boxes were checked every day. We took tail tip tissue samples from adults that were from enclosures that produced 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 Treatments

Eggs were collected over two years from 2016 -2017 and were typically laid between October –March. 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 by an elastic band. We assumed that eggs that were found together in the substrate box were from the same clutch as eggs from a single female are often stuck together. Each clutch was 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. However, both incubators fluctuated +/- 3ºC over 24 hours. These treatments represent the temperature extremes of natural nest sites of *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 dead eggs and emergent hatchlings. 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.

## 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 (see below). 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 approximately six months onwards, we manually measured hatchling SVL using a clear ruler. Growth measurements continued until we had approximately 16 measures per individual (mean = 11.5 , range = 1 - 18). 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.

Pedigree and Genomic Relatedness

Tail tissue samples from adults and hatchlings were used to determine parentage of offspring. DNA extraction and single nucleotide polymorphism (SNP) genotyping was performed by Diversity Arrays Technology, a commercial company which utilises a technique called DArTseq™. For more details on DNA extraction and SNP genotyping see ESM.

DNA was extracted from tissue samples using a Qiagen DNeasy Blood and Tissue Kits following the manufacturer’s instructions. Diversity Arrays Technology (DArT) combines next generation sequencing platforms and genome complexity reduction methods (Kilian et al., 2012) to select the most appropriate method for *L.delicata.*

[Insert section from DArT here on complexity reduction methods, DArT tested four methods in *Gambusia* so it’s unclear what method was used for us].

Sequences from all lanes were then processed using DArT specific pipelines. The main pipeline filters our ‘poor’ quality sequences [Details needed from DArT]. For our samples, this filtering process by DArT resulted in a total of 185,963 SNPs. ]

In order to estimate quantitative genetic parameters, we derived a genomic relatedness matrix (GRM) using our SNP dataset for 261 offspring growth data (132 putative parents; nfemales = 69, nmales = 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 when at least 200 SNP loci are used (Bérénos et al., 2014; Huisman, 2017). Moreover, both relatedness and heritability values estimated from a GRM are strongly correlated to those inferred using a pedigree (Bérénos et al., 2014; Huisman, 2017).

Prior to deriving our GRM, we filtered our SNPs using the R package *dartR* (Gruber et al., 2018). Using the in-built functions of *dartR*, 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:

where *Z* is the centered matrix of SNP genotypes of all individuals. This is calculated from a matrix of SNP genotypes coded as -1, 0, 1 for homozygote for the reference allele, heterozygote and homozygote for the SNP allele. *pi*is the frequency of the second locus at position locus *i.* The denominator scales the GRM matrix so that the values approximates to 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 *MCMCglmm* (Hadfield, 2010). Mass was log-transformed, and age was z-transformed. Due to natural mortality, missing measurement occasions and equipment malfunctions, we were not able to get all 16 complete measurements across age for each lizard. In total, N = 165/261 lizards had missing data. As such, missing measurement occasions were assumed as NA and we used data augmentation with *MCMCglmm* to deal with missing data (REFS). Data augmentation deals with missing data extremely well and can counter the loss of statistical power when using complete case approaches (REFS). For all models we used noninformative priors with 253,000 MCMC iterations with a burn in of 3000 and sampling the posterior every100 iterations. We ensured proper mixing by inspecting trace plots and checked samples were not strongly auto-correlated using the *autocorr* function from *coda* (Plummer et al., 2006). 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. To do this, we fitted intercept only models for each treatment group 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*). Overall. Heritability (*h2*) of mass using this intercept (*I*) model was calculated as:

To then test how *G*, *M* and *h2* 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 how the variance components change with age. We fitted six intercept only models with varying complexity in their random effects and compared their DIC 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. The best supported model included a random linear and quadratic slope for *G* and *M* and a random intercept for *PE*. (Table S1).

We therefore fitted an intercept only model using this random effect structure for each treatment group. We estimated a genetic variance-covariance matrix for each treatment (), where the diagonal elements represent the additive genetic variances for the intercept (), slope () and the quadratic () across age. The off-diagonal elements are the additive genetic covariances between the growth curve parameters, for example, is the additive genetic variance between the intercept and the quadratic slope.

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

For each treatment group, we then calculated additive genetic variance at a given age using the random slope terms and their covariances as follows,

where is a specific age. Age-specific maternal effect was calculated using the same formula structure but with the relevant variance components from . Age-specific heritability is thus a ratio of all variance components at a given age ,

The Influence of Developmental Temperature on Growth Trajectories

We 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 tracjetory (quadratic term). We also wanted to test for treatment differences in age at which lizards reach their maximum mass (maxima of quadratic curve). We fitted mass as the response and the most supported random effect structure we identified 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 age2 (Table 2, S3). We then compared DIC 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 3002 observations of mass data for a total of 261 individuals ( = 125, = 136). The average age for ‘hot’ incubated lizards was 335.82 days (range: 0 – 711) and for ‘cold’ incubated lizards it was 384.8 days (range: 0 – 707). On average, a lizard had 11.5 measurements.

Developmental plasticity in growth trajectories in response to temperature

While a model containing an interaction between treatment and age (linear and quadratic) was best supported (Table 1; see also Table S2–3) the improvement was marginal, given DIC has a tendency to favour slightly more complex models (REFS). Linear growth rate and curvature of the growth trajectory did not differ significantly between the two developmental temperature treatments (Table 2, Table S3). Irrespective of treatment, a lizard mass increased by 1.65g for every SD units increase in age. Developmental temperature did however impact initial mass (Table 2, Fig. 1). 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 (378.53 days, 95% CI: 353.4 – 407.16) compared to lizards from ‘hot’ treatment (408.1 days, 95% CI: 374.73 – 448.26).

**Table 1** Comparisons of DIC of three models with different combinations of treatment interactions with age parameters. = 3982

|  |  |  |
| --- | --- | --- |
| Formula | DIC | DIC |
| Treatment \* Age + Treatment\*Age2 | -3332.981 | 0 |
| Treatment \* Age + Age2 | -3329.495 | -3.486 |
| Treatment + Age + Age2 | -3329.845 | -3.136 |

**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. = 3982

|  |  |  |  |
| --- | --- | --- | --- |
| Variable | Estimate | Lower | Upper |
| Intercept | **-0.987** | **-1.008** | **-0.965** |
| Treatment | **-0.083** | **-0.115** | **-0.051** |
| Age | **0.501** | **0.476** | **0.526** |
| Age2 | **-0.2** | **-0.221** | **-0.18** |
| Treatment\*Age | 0.008 | -0.023 | 0.039 |
| Treatment\*Age2 | 0.023 | -0.009 | 0.056 |

**Table 3.** Matrices for G and M depicting the variance-covariance and correlations between growth trajectory parameters (intercept, linear slope and quadratic slope) for the overall population (n = 261, nobs = 3982). PE variance is also presented. Variances are represented along the diagonal, covariances are represented in the upper triangle and correlations are represented in the lower triangle. Bolded estimates are significantly different from zero. Values in the brackets represent the 95% credible intervals. See main text for further details

|  |  |  |  |
| --- | --- | --- | --- |
| *G* | | | |
|  | Intercept | Linear Slope | Quadratic Slope |
| Intercept | **0.01  (0.004 - 0.017)** | 0  (-0.004 - 0.003) | **-0.007  (-0.011 - -0.004)** |
| Linear Slope | -0.018  (-0.156 - 0.116) | **0.015  (0.011 - 0.02)** | **0.011  (0.007 - 0.015)** |
| Quadratic Slope | **-0.289  (-0.392 - -0.178)** | **0.366  (0.297 - 0.423)** | **0.015  (0.011 - 0.02)** |
| *M* | | | |
|  | Intercept | Linear Slope | Quadratic Slope |
| Intercept | **0.002  (0 - 0.006)** | -0.001  (-0.004 - 0.002) | -0.001  (-0.004 - 0) |
| Linear Slope | -0.107  (-0.442 - 0.305) | **0.005  (0.001 - 0.01)** | 0  (-0.002 - 0.003) |
| Quadratic Slope | -0.211  (-0.47 - 0.299) | 0.024  (-0.381 - 0.356) | **0.001  (0 - 0.003)** |
| *PE* | | *Residuals* | |
| **0.004  (0.001 - 0.008 )** | | **0.019  (0.017 - 0.02)** | |

A screenshot of a cell phone

Description automatically generated

**Figure 1** Model predictions of log-transformed mass over age for a random subset of 40 lizards from the two developmental temperatures. Red points represent mean estimates for each lizard from the hot developmental treatment, blue points represent mean estimates for each lizard from the cold developmental treatment (blue). Thick lines represent average growth curve for each treatment. for each treatment. Faint grey lines are each individual’s growth curve.

The influence of developmental temperature on additive genetic variance and maternal effects across age

Overall, additive genetic variance, permanent environmental variance and heritability of growth appears to be higher in the hot developmental temperature treatment (Fig. 2, Table S4). However, there were no significant differences among treatment groups (Table S4). Both treatment groups showed similar patterns in how the relative contributions of *G* and *M* changed with age (Fig. 3, Table 4-5). Initially, additive genetic variance was very low and remained constant until approximately nine months of age, after which it increased steadily (Fig. S1). Maternal effects decreased sharply upon hatching and dropped to the minimum at approximately six months (Fig. S1). From six months onwards, *M* gradually increased until lizards were approximately one year of age. Consequently, *h2* peaked at approximately six months and slowly decreased until nine months where it showed a gradual increase again (Fig. 3A). In contrast, maternal effects explained a majority of the total phenotypic variance upon emergence (*M2*) and at approximately six months of age (Fig. 3B).

**Figure 2** Pie charts depicting the overall relative contributions of mass variance for the hot (n = 126) and cold (n =136) developmental treatment group irrespective of age. Point estimates and 95% credible intervals are presented in Table S4. There were no significant differences between developmental temperature treatments.



**Figure 3** Scatterplot showing the relationship of the how heritability (*h2*) and the proportion of total variance explained by maternal effects (*M2*) changed with age for the hot developmental treatment (n = 125, red) and the cold developmental treatment (n = 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.

**Table 4.** Matrices for *G* and *M* depicting the variance-covariance and correlations between growth trajectory parameters (intercept, linear slope and quadratic slope) for lizards from the hot developmental temperature treatment group (n = 125, nobs = 1892). PE variance is also presented. Variances are represented along the diagonal, covariances are represented in the upper triangle and correlations are represented in the lower triangle. Bolded estimates are significantly different from zero. Values in the brackets represent the 95% credible intervals. See main text for further details



|  |  |  |  |
| --- | --- | --- | --- |
| *G* | | | |
|  | Intercept | Linear Slope | Quadratic Slope |
| Intercept | **0.009  (0.002 - 0.02)** | -0.001  (-0.007 - 0.005) | **-0.007  (-0.014 - -0.002)** |
| Linear Slope | **-0.063  (-0.335 - 0.2)** | **0.015  (0.008 - 0.026)** | **0.008  (0.003 - 0.015)** |
| Quadratic Slope | **-0.371  (-0.476 - -0.186)** | **0.305  (0.136 - 0.43)** | **0.011  (0.005 - 0.018)** |
| *M* | | | |
|  | Intercept | Linear Slope | Quadratic Slope |
| Intercept | **0.089  (0.011 - 0.242)** | **0.151  (0.051 - 0.293)** | **-0.048  (-0.094 - -0.016)** |
| Linear Slope | **0.384  (0.175 - 0.491)** | **0.295  (0.201 - 0.426)** | **-0.093  (-0.137 - -0.063)** |
| Quadratic Slope | **-0.422  (-0.495 - -0.299)** | **-0.287  (-0.329 - -0.246)** | **0.031  (0.019 - 0.047)** |
| *PE* | | *Residuals* | |
| **0.006  (0.001 - 0.01 )** | | **0.019 (0.017 - 0.02)** | |

**Table 5.** Matrices for G and M depicting the variance-covariance and correlations between growth trajectory parameters (intercept, linear slope and quadratic slope) for lizards from the cold developmental temperature treatment group (n = 136, nobs = 2036). PE variance is also presented. Variances are represented along the diagonal, covariances are represented in the upper triangle and correlations are represented in the lower triangle. Bolded estimates are significantly different from zero. Values in the brackets represent the 95% credible intervals. See main text for further details

|  |  |  |  |
| --- | --- | --- | --- |
| *G* | | | |
|  | Intercept | Linear Slope | Quadratic Slope |
| Intercept | **0.008  (0.002 - 0.017)** | 0  (-0.004 - 0.006) | **-0.006  (-0.012 - -0.002)** |
| Linear Slope | 0.012  (-0.202 - 0.208) | **0.015  (0.009 - 0.022)** | **0.01  (0.005 - 0.015)** |
| Quadratic Slope | **-0.292  (-0.418 - -0.128)** | **0.349  (0.233 - 0.431)** | **0.013  (0.009 - 0.019)** |
| *M* | | | |
|  | Intercept | Linear Slope | Quadratic Slope |
| Intercept | **0.2  (0.077 - 0.361)** | **0.23  (0.131 - 0.358)** | **-0.096  (-0.15 - -0.055)** |
| Linear Slope | **0.481  (0.419 - 0.499)** | **0.275  (0.191 - 0.393)** | **-0.114  (-0.163 - -0.079)** |
| Quadratic Slope | **-0.398  (-0.478 - -0.325)** | **-0.352  (-0.384 - -0.319)** | **0.049  (0.033 - 0.072)** |
| *PE* | | *Residuals* | |
| **0.006  (0.002 - 0.009 )** | | **0.014 (0.013 - 0.015)** | |

# Discussion

Key results:

Initial mass differs between treatment groups

Growth rate and curvature does not

There appeared to more additive genetic variance and permanent environmental variance in the hot treatment however treatment differences in heritability and variance components were not significant.

The relative contributions of mass variance were not constant over age. Notably geneti

# Conclusion

# Author contributions

# Data accessibility

Datasets and code used to generate results of this study is accessible via Open Science Framework (DOI: XXXXXXXXXXX)

# Acknowledgements