# Title

# Abstract

# Keywords

# Introduction

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Developmental plasticity plays a key role in generating phenotypic variation. Environmentally-induced changes may result in a better match between adult phenotype and the selective environment if developmental cues predict later-life conditions (Beldade et al., 2011), however in some cases maladaptive phenotypes can arise (Ghalambor et al., 2007). As such, developmental plasticity is considered as a promising immediate solution for populations to adjust to environmental fluctuations (West-Eberhard, 2003). Over longer terms, populations can either migrate to more favourable habitats or evolve genetic adaptations in response to environmental change (Sgrò & Hoffmann, 2004). However, the capacity to evolve may be dependent on developmental environments because environmental cues experienced during development can also affect the underlying standing genetic variation which select acts on. Understanding how phenotypic and genotypic variation respond to developmental environments is necessary in order to predict population persistence.

Environmental dependence of genetic variation implies that evolutionary change may speed up or slow down under certain contexts. Such implications has sparked researchers to describe the conditions under which genetic variation may change (Charmantier & Garant, 2005; Hoffmann & Merilä, 1999; Rowiński & Rogell, 2017). Several hypotheses have been proposed to explain environmentally-induced changes in genetic variance (Charmantier & Garant, 2005; Hoffmann & Merilä, 1999; Rowiński & Rogell, 2017). Notably, higher mutation rates (Hoffman & Parsons, 1991); reduced selection pressures which allows for the accumulation of genetic variation (Hoffmann & Merilä, 1999) and the breakdown of buffering mechanisms that releases of cryptic genetic variation are expected to increase genetic variation under novel, stressful environments (Paaby & Rockman, 2014). On the other hand, genetic variation can also decrease under stressful environments if gene expression is condition-dependent (Coltman et al., 2001) or under strong selection (Hunt et al., 2007).

Comparative studies have shown that the influence of developmental stress on genetic variance is not straightforward (Charmantier & Garant, 2005; Hoffmann & Merilä, 1999; Rowiński & Rogell, 2017). In lab studies, high developmental stress have 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 lab and wild conditions, with the latter being more variable and therefore more difficult to detect environmental patterns. The nature of environmental stressor under investigation (e.g. heat shock vs. habitat quality) could also yield variable patterns of gene expression if they are not strongly genetically correlated , making comparisons more heterogenous (Charmantier & Garant, 2005; Dahlgaard & Hoffmann, 2000). Environmental comparisons of heritability have been criticised as estimates may show no changes, but the relative contributions of non-genetic variance actually differ. Indeed, additive genetic and environmental variance of life history traits which is supposedly more important to fitness components were found to increase under high stress conditions. The opposite holds true for morphological traits, where the same variance components decreased in high stress environments. Interestingly, h2 showed no differences among environments which suggests that the capacity to evolve is contingent on non-genetic sources of variance as well as the trait of interest (Rowiński & Rogell, 2017).

Body size is a heritable, fitness related trait and is strongly affected by a number of factors. Developmental environments such as temperature and nutrition play pivotal roles on variation in body size, largely through shifts in how organisms grow and develop (Eyck et al., 2019; Noble et al., 2017). Naturally, maternal contributions on offspring development environments such as in egg size, nest site selection or timing of birth can also promote variation in body size and growth. Selection on body size is therefore likely to vary across ontogeny. For example, high juvenile mortality has favoured high maternal investment to larger birth weights in Soay sheep (Wilson, Coltman, et al., 2005). Accordingly, maternal contributions are expected to dissipate with age presumably because mothers cannot continue to influence her offspring’s phenotype post-weaning (Krist, 2010; Wilson, Kruuk, et al., 2005). Moreover, body size at sexual maturity is likely a major target of selection as it strongly predicts reproductive success and survival (Bartheld et al., 2015; Calsbeek & Sinervo, 2002) and may have favoured the evolution of compensatory growth strategies which offsets body size variance at later stages (Hector & Nakagawa, 2012). Given that selection operates on body size at certain life stages, it is reasonable to expect that its genetic and non-genetic variance to change across ontogeny. A more temporal approach is needed in order to evaluate when evolutionary potential of body size is great.

Here we investigated the impact of developmental temperature on growth and mass in an oviparous skink (*Lampropholis delicata*) and how developmental environments affect evolutionary potential in these traits. Using 8,433 SNP markers, we derived a genomic relatedness matrix to estimate quantitative genetics parameters to address the following key questions: 1) How does developmental temperature affect the shape of growth trajectories (initial mass, growth rate and curvature of growth trajectory)?; 2) Do differences in growth result in variation in age at maximum mass?; 3) How does developmental temperature affect genetic and non-genetic variance components as well as heritability overall and across age? Based on the temperature-size rule, we expect lizards that experienced cold developmental temperatures to have larger initial masses, slower growth rates and would reach maximum size at a later age compared to lizards that experienced hot developmental temperatures (Angilletta Jr et al., 2017). We expect genetic and non-genetic to differ among treatments, and predict that additive genetic.

# Materials and Methods

## Lizard collection and husbandry

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. 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 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. 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 over two years from 2016–2017 and were typically laid between October to 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 using an elastic band. 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 a 24 hour period. 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 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.

Genomic Relatedness Matrix

We derived a genomic relatedness matrix (GRM) using single nucleotide polymorphism (SNP) genotypes for all 261 offspring with 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). 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:

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 (Hadfield, 2010). Data augmentation deals with missing data extremely well and can counter the loss of statistical power when using complete case approaches (Noble & Nakagawa). For all models we used noninformative priors with 253,000 MCMC iterations with a burn in of 3000, sampling from the posterior distribution every100 iterations. We ensured proper mixing by inspecting trace plots and checked that samples were not 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*). We included our GRM to estimate additive genetic variation. 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 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 proportion of variance explained by maternal effects (*m2*) is calculated in the same manner.

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 trajectory (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 1 SD unit 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). The population *G* and *M* matrices and other variance components are presented in Table S4.

**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 |



**Figure 1** Model predictions of log-transformed mass over age for a random subset of 40 lizards from the two developmental temperatures. 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.

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 S5). Treatment groups did not differ in how the relative contributions of *G* and *M* changed with age as the 95% credible intervals overlapped (Fig. 3). 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). The *G* and *M* matrices and other variance components for each treatment group are presented in Table S6-S7.



**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 in variance components 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.

# Discussion

Lizards from hot early developmental environments weighed less than lizards from cold temperatures. However, growth trajectories were not significantly impacted by thermal environment. In addition, developmental temperature did not impact the expression of additive genetic and maternal effect variance. Heritability of mass varied over ontogeny, peaking at six months before gradually increasing again due to a rise in additive genetic variance around nine months of age. As we predicted, maternal effects on offspring mass declined in the first month presumably because maternal non-genetic contributions were less influential on mass over time. Unexpectedly, maternal effect variance increased again at approximately six months and continued to remain high, possibly resulting from maternal genetic factors impacting mass. Our study suggests that variation in mass is largely driven by environmental sources and both genetic and non-genetic sources of maternal effects could influence the evolutionary potential of body mass over time.

1. Developmental temp did not affect growth
   1. Hot smaller than cold but not evidence of compensatory growth
      1. What did other studies find on hatching mass?
      2. What is the mechanism? Fast incubation period, faster development, and used egg resources faster and less efficiently?
      3. Hatching mass 🡪 higher survival, maternal effect
   2. Some evidence that developmental temperature influenced when you reach sexual maturity earlier through larger hatching mass.
      1. Variation in developmental temperature is related to temporal/seasonal shifts in temperature, cold born late reaching earlier may have been favoured prior to end of autumn. Selective benefit EXAMPLE?
      2. Life history is strongly linked with growth and age at maturity could be influenced by developmental temperatures. (Cite Chapter 1 - POL). Variation in age at maturity could lead to changes in population dynamics under warming, more variable climate regimes

Temperature has a pervasive influence on ectotherm phenotypes.

1. Overall there was very little additive genetic variation across both treatments, environmental sources contributed most to variance in body size.
   1. Genetic variation may only increase outside of constant/ad lib lab conditions
      1. Hypothesis in Hoffman/Rowinski, Uller and Radar – gene variation only increase in certain environments “If the genotypes that are capable of producing a maximal trait value can do so only under optimal conditions, the poor growth conditions experienced under stress may constrain the expression of genetic variation, and hence decrease genetic vari- ation under stress (Hebhardt-Hendrich and Van Noordwijk 1991; Hoffman and Merila ̈ 1999)”, “co-seggreation of genes and environment”
   2. Need long term studies in semi natural conditions
2. Maternal effects did indeed decline upon hatching but increased later in life
   1. Heritability peaked due to a decrease in maternal effects at 6 months
   2. Acknowledge that we don’t know why maternal variance increases again
   3. Genetic vs non-genetic contributions of maternal effects
      1. Which first? What are some examples for genetic and non genetic effects and how do they affect offspring body size/growth/sexual maturity
   4. From 9 months onwards individuals were in shared enclosures and may have to compete for resources and coincided with an increase of genetic m2?
      1. Suggests that social “stress” increases maternal contributions
   5. Need more studies that manipulate maternal effect cross factorial stress no stress pre and post hatch maybe
      1. Thought: developmental temp is considered a maternal effect irrespective of what temperature mothers lay eggs, you still see the same pattern…..nest site selection, is non genetic, clutch mass…clutch timing etc
      2. Remember the genetic effects are associated with MOTHERS GENES.

# Conclusion

# Author contributions

FK, DN, 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 (DOI: XXXXXXXXXXX)

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