# Heritability and developmental plasticity of growth in an oviparous lizard

Fonti Kar1, Shinichi Nakagawa1,2, Daniel W.A. Noble1,3

1 *School of Biological Earth and Environmental Sciences, Ecology and Evolution Research Centre, University of New South Wales, Sydney, NSW, Australia*

2 *Diabetes and Metabolism Division, Garvan Institute of Medical Research, 384 Victoria Street, Darlinghurst, Sydney, NSW 2010, Australia*

3 *Division of* *Ecology and Evolution, Research School of Biology, The Australian National University, Canberra, ACT, Australia*

Corresponding author: Daniel Noble

Correspondence email: [daniel.noble@anu.edu.au](mailto:fonti.kar@gmail.com)

# Abstract

Selective processes act on phenotypic variation however the evolutionary potential of any given trait relies on the underlying heritable variation. Developmental plasticity is an important source of phenotypic variation, but it can also promote changes in genetic variation, yet we have a limited understanding on how they are both impacted. Here, we quantified the influence of developmental temperature on the growth in delicate skinks (*Lampropholis delicata*) and partitioned the total variance using an animal model fitted with a genomic relatedness matrix. We measured mass for 262 individuals (nhot = 126, ncold = 136) over 16 months (nobservations = 3,002) and estimated heritability and maternal effects over time. Our results show that lizards reared in cold developmental temperatures had consistently higher mass across development 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 hot developmental temperature treatment, however these differences were not statistically significant. Heritability increased with age, whereas maternal effects decreased upon hatching but increased again at a later age which could be driven by social competition or intrinsic changes in the expression of variation as individual’s growth. 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 ([Ghalambor *et al*, 2007](#_ENREF_35); [Noble *et al*, 2018](#_ENREF_59); [West-Eberhard, 2003](#_ENREF_89)). 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](#_ENREF_55); [West-Eberhard, 2003](#_ENREF_89)). Phenotypic changes resulting from distinct early life experiences can have persistent effects on individual fitness ([Monaghan, 2008](#_ENREF_55); [Noble *et al*, 2018](#_ENREF_59)). 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](#_ENREF_3); [Ghalambor *et al*, 2007](#_ENREF_35)). 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](#_ENREF_4); [Chevin, 2010](#_ENREF_17); [Noble *et al*, 2019](#_ENREF_58); [West-Eberhard, 2003](#_ENREF_89)). 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](#_ENREF_8); [Reed *et al*, 2010](#_ENREF_66)).

A population’s capacity to evolve depends not only on the strength of selection but also on the underlying standing genetic variation ([Lynch and Walsh, 1998](#_ENREF_51)). It has long been recognised that both selection and genetic variation change across environments ([Falconer and Mackay, 1996](#_ENREF_28)). 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 and Garant, 2005](#_ENREF_13); [Fischer *et al*, 2020b](#_ENREF_30); [Hoffmann and Merilä, 1999](#_ENREF_43); [Noble *et al*, 2019](#_ENREF_58); Rowiński and Rogell, 2017; [Wood and Brodie, 2015](#_ENREF_97)). Genetic variance in novel environments may increase due to relaxation of selection pressures combined with higher mutation rates ([Hoffman and Parsons, 1991](#_ENREF_42); [Hoffmann and Merilä, 1999](#_ENREF_43)). An increase in genetic variance is also expected when buffering mechanisms breakdown triggering a release of ‘cryptic genetic variation’ ([Paaby and Rockman, 2014](#_ENREF_62)). However, other mechanisms, such as low cross-environment genetic correlations or condition-dependence of gene expression can also affect the amount of genetic variance in different environments ([Charmantier and Garant, 2005](#_ENREF_13); [Coltman *et al*, 2001](#_ENREF_18)). Under the same selection pressure, the speed of evolutionary responses can change depending on the environment making it potentially difficult to predict genetic adaptation.

Comparative studies have shown that the environmental impacts on genetic variance is not straightforward ([Charmantier and Garant, 2005](#_ENREF_13); [Hoffmann and Merilä, 1999](#_ENREF_43); Rowiński and Rogell, 2017). In lab studies, elevated developmental stress has been shown to increase the heritability of morphological traits ([Hoffmann and Merilä, 1999](#_ENREF_43)), whereas wild, non-domestic populations tend to have higher heritability in favourable environments ([Charmantier and Garant, 2005](#_ENREF_13)). 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 and Garant, 2005](#_ENREF_13); [Dahlgaard and Hoffmann, 2000](#_ENREF_19)). Importantly, environmental comparisons of heritability have been criticised because they mask changes in the relative contributions of non-genetic and genetic variance ([Hansen *et al*, 2011](#_ENREF_40); Rowiński and Rogell, 2017). For example, a meta-analysis found that heritabilityof life history traits, which have been argued to be more important to fitness, did not change between control and stressful conditions (Rowiński and Rogell, 2017). The same pattern was observed for morphological traits ([Fischer *et al*, 2020b](#_ENREF_30)). 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 and Rogell, 2017). The expression of genetic 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](#_ENREF_60); [Stillwell and Fox, 2009](#_ENREF_77)). 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](#_ENREF_26); [Noble *et al*, 2018](#_ENREF_59)). Maternal investment in offspring are also important sources of body size variation([Noble *et al*, 2014](#_ENREF_57); [Wilson and Réale, 2006](#_ENREF_95)). Variation among mothers in egg investment, nest site selection or timing of birth([Mitchell *et al*, 2018](#_ENREF_54); [Shine and Harlow, 1996](#_ENREF_75); [Uller and Olsson, 2010](#_ENREF_82)) are expected to contribute the most to offspring body size early in development([Mousseau and Fox, 1998](#_ENREF_56)). However, these effects have shown to decline with age as maternal investment subside ([Krist, 2010](#_ENREF_48); [Wilson *et al*, 2005b](#_ENREF_93)). 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](#_ENREF_49)). For example, permanent environmental effects that varied across years explained 26% – 35% of body size variation in bighorn sheep ([Réale *et al*, 1999](#_ENREF_65)). Similarly, 56% of variation in body mass was attributed to nest boxes shared among siblings in blue tit chicks ([Charmantier *et al*, 2004](#_ENREF_14)). As such, the various sources that influence body size variation (genetic, environmental, maternal) are predicted to vary across ontogeny and temporal approach is needed in order to evaluate age-specific evolutionary potential of body size – higher genetic variation at a given age would imply that, if selection were to operate, it would be more likely to lead to an evolutionary response.

Here we investigated the impact of developmental temperature on growth and mass 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 (nobservations = 3,002) for lizards that hatched from two incubation treatments (nhot = 126, ncold = 136), were measured over the first 16 months of 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](#_ENREF_2)). In addition, we predicted greater amount of genetic variance under higher developmental temperatures, after controlling for non-genetic sources of variance, as higher temperatures may release ‘cryptic genetic variation’ (Rowiński and Rogell, 2017). We expected maternal effects and permanent environment effects to manifest early in development and dissipate over time.

# Materials and Methods

## Lizard collection and husbandry

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 August and September 2015. While we collected from five different sites in Sydney, biogeographic data suggests high gene-flow across the Sydney region ([Chapple](#_ENREF_12" \o "Chapple, 2013 #84) *[et al](#_ENREF_12" \o "Chapple, 2013 #84)*[, 2013](#_ENREF_12" \o "Chapple, 2013 #84)). 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). We choose a paternal half-sib design because maternal half-sibs are difficult to generate given that females in our colony only produced a single clutch in a year (see below). 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 (~ 2 standard deviations above and below the mean - ~27 ºC) sites for *L. delicata* ([Cheetham *et al*, 2011](#_ENREF_15)). We chose these temperatures because were expect thermal environments to become more extreme and variable in the future making it of interest in knowing how the expression of genetic variation is likely to manifest in abnormal thermal conditions. While it is challenging to determine if an environment is ‘stressful’ or not without data on egg mortality ([Roelofs *et al*, 2010](#_ENREF_67)), we viewed this as atypical of what is commonly encountered in nature. 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 25 – 50% of their total lifespan ([Chapple *et al*, 2014](#_ENREF_11)). 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](#_ENREF_70)). 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. Social housing conditions may result in additive genetic and maternal effects becoming more apparent because of competition and social stress that may drive greater variation among individuals. Our modelling approaches estimate changes in variance components across age and should be able to detect any changes brought about by the release of variation (see below).

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; 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 offspring 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](#_ENREF_5); [Huisman, 2017](#_ENREF_45)). Moreover, both relatedness and heritability values estimated from a GRM can be very similar to those inferred using a pedigree ([Bérénos *et al*, 2014](#_ENREF_5); [Huisman, 2017](#_ENREF_45)). 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](#_ENREF_83)). We calculated a GRM for all hatchlings using the *snpReady* R package ([Granato *et al*, 2018](#_ENREF_39)) following methods described by ([VanRaden, 2008](#_ENREF_83)):

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. *pi*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.

# Statistical Analyses

All analyses were performed using *R* ([Team, 2023](#_ENREF_80)). 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](#_ENREF_10); [Gelman *et al*, 2015](#_ENREF_34))*.*  Mass was log-transformed, and age was z-transformed. For all models we ran 6000 iterations with a burn in of 1000, sampling from the posterior distribution every 10 iterations. 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](#_ENREF_96)). 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 *PE*, *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 what (or how) variance components change with age ([Wilson and Réale, 2006](#_ENREF_95)). We fitted models with varying complexity in their random effects and used Leave-One Out (LOO) cross validation to compare model fit and select the model with best predictive performance. Using LOO, the expected log pointwise predictive density for a model can be calculated, and these can be used to compare model performance – by calculating the difference between expected log pointwise predictive density of various models. Differences of less than 4 mean that models are comparable ([Sivula *et al*, 2020](#_ENREF_76)). For differences greater than 4, then the standard error (SE) of the differences in expected log pointwise predictive density should be compared. If the standard error of the differences are much larger than the point estimate of the difference then the model closer to zero is preferred ([Sivula *et al*, 2020](#_ENREF_76)). The difference in LOO between models can be used for model selection, and in our case, gave similar results to model selection using Watanabe–Akaike Information Criterion (WAIC) (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. Three models were equally supported, the first included a random linear and quadratic slope for *G* and *M* and *PE*. (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. The same top model selected was similar no matter whether we used the full data or only the data subset for individuals incubated in cold or hot developmental treatments.

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 (), 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 following ([Gavrilets and Scheiner, 1993](#_ENREF_33); [Schielzeth and Nakagawa, 2022](#_ENREF_73)):

where is a specific age. Age-specific maternal effect was calculated using the same formula 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.

As the mean body mass increases over time, the variance may also increase concurrently due to scale effects and potentially bias estimates of quantitative genetics parameters ([Wilson *et al*, 2005b](#_ENREF_93)). 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 age2 (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 ( = 125, = 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. The average age for hot incubated lizards was 335.82 (range: 0 – 711) and for cold incubated lizards it was 384.8 (range: 0 – 707). On average, a lizard had 11.5 measurements (SD = 4.71).

Overall, additive genetic variance and heritability () 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 (nlizards = 126) and cold (nlizards =136) developmental treatment group averaged across age. Point estimates and 95% credible intervals are presented in Table S3. There were no significant differences in variance components between developmental temperature treatments.

*The influence of developmental temperature on genetic and non-genetic variance across age*

Treatment groups did not differ in how the relative contributions of and 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. S1). 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 (nlizards = 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.

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 () (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 decreased soon after hatching, however it increased slightly again from six months of age (Fig. 3). The and matrices for each treatment group are presented in Table S4-S5.



**Figure 3** Heritability (*h2,* **A**) and the proportion of total variance explained by maternal effect variance (*M*2, **B**) across age (days) for the hot developmental treatment (nlizards = 125, red) and the cold developmental treatment (nlizards = 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.

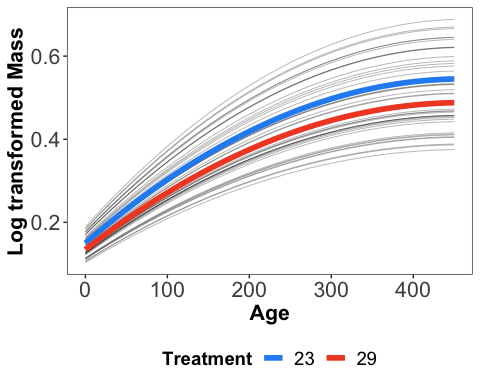
Developmental plasticity in growth trajectories in response to temperature

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 (Age2) did not differ significantly between the two developmental temperature treatments in any of the models containing interactions (Table S7). Irrespective of treatment, lizard mass increased by 1.65 g for every 1 SD unit increase in age.

**Table 1** Comparisons of LOOIC values of four models ( = 2926) with different combinations of treatment interactions with age parameters. represents the expected difference (on a log scale) in predictive density for a new dataset estimated from cross-validation. Age measured in days was z-transformed (mean = 361.34, SD = 185.16)

|  |  |  |  |
| --- | --- | --- | --- |
| Formula of Fixed Effects | LOO |  | Std. Error |
| Treatment + Age + Age2 + Treatment Age2 | -3,245 | 0.00 | 0.0 |
| Treatment + Age + Age2 + Treatment Age + Treatment Age2 | -3,244 | -0.65 | 1.9 |
| Treatment + Age + Age2 | -3,240 | -2.36 | 2.6 |
| Treatment + Age + Age2 + Treatment Age | -3,235 | -4.74 | 2.4 |

Developmental temperature did, however, influence 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). *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. nobs = 2,926. Age measured in days was z-transformed (mean = 361.34, SD = 185.16). *G* andM matrices for this model are presented in Table S6.

|  |  |  |  |
| --- | --- | --- | --- |
| Parameter | Estimate | Lower | Upper |
| Intercept | **-0.998** | **-1.036** | **-0.961** |
| Treatment | **-0.085** | **-0.118** | **-0.056** |
| Age | **0.496** | **0.458** | **0.534** |
| Age2 | **-0.189** | **-0.223** | **-0.156** |
| Treatment Age | 0.012 | -0.019 | 0.041 |
| Treatment Age2 | 0.025 | -0.006 | 0.055 |

# 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 variance, permanent environment variance 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](#_ENREF_26); [Noble *et al*, 2018](#_ENREF_59); O’Dea *et al*, 2019; [While *et al*, 2018](#_ENREF_90)). While we found that hot lizards were smaller than cold incubated lizards at hatching, we did not show that growth rate differed between developmental temperatures. Some studies have reported increases in growth at higher incubation temperatures ([De Jong *et al*, 2023](#_ENREF_21); [Elphick and Shine, 1999](#_ENREF_25); [Hare *et al*, 2004](#_ENREF_41); Verdú‐Ricoy *et al*, 2014), while others have found either the opposite result or no differences at all ([Andrews *et al*, 2000](#_ENREF_1); [Goodman, 2008](#_ENREF_38)). The directionality of change is highly variable, even among studies of the same species (e.g., Bassiana dupreyi, [Elphick and Shine, 1998](#_ENREF_24); [Elphick and Shine, 1999](#_ENREF_25); [Flatt *et al*, 2001](#_ENREF_31); [Telemeco *et al*, 2010](#_ENREF_81)), and we had more data across life compared with many other studies. Lack of generality may be related to how growth is statistically modelled (e.g., polynomial regression versus Von Bertalanffy growth models). In addition, 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 later 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 provides 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 because they were born heavier ([Dayananda *et al*, 2016](#_ENREF_20); [Downes and Shine, 1999](#_ENREF_23); [Flatt *et al*, 2001](#_ENREF_31); [Goodman *et al*, 2013](#_ENREF_37)). These results support the temperature-size-rule whereby organisms reared in cold temperatures tend to have larger body sizes ([Angilletta Jr *et al*, 2017](#_ENREF_2)). Larger hatching size can be achieved through prolonged development at cooler temperatures during embryonic stages ([Forster and Hirst, 2012](#_ENREF_32)). It is well known that cold developmental temperatures results in longer incubation periods in many reptiles ([Booth, 2006](#_ENREF_7); [Dayananda *et al*, 2016](#_ENREF_20); [Downes and Shine, 1999](#_ENREF_23); [Elphick and Shine, 1998](#_ENREF_24); [Goodman, 2008](#_ENREF_38)). Longer developmental time may allow embryos to assimilate yolk nutrients more efficiently thus increasing mass at hatching ([Storm and Angilletta, 2007](#_ENREF_78)). Indeed, turtle embryos exposed to high temperatures have enhanced mitochondrial metabolism and metabolic enzymic activity which constrained developmental time and reduced overall hatching size ([Ji *et al*, 2003](#_ENREF_46); [Sun *et al*, 2015](#_ENREF_79)). Thermal plasticity in embryonic development may be adaptive for lizards born late in the season when nest temperatures are generally colder ([Warner and Shine, 2008](#_ENREF_87); [While *et al*, 2015](#_ENREF_91)). 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](#_ENREF_15)). 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 and Shine, 1999](#_ENREF_23); [Gifford *et al*, 2017](#_ENREF_36); [Qualls and Shine, 2000](#_ENREF_64)). 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](#_ENREF_27); [Ghalambor *et al*, 2007](#_ENREF_35); [Hoffmann and Merilä, 1999](#_ENREF_43)). Stressful developmental environments are hypothesized to lead to the release of ‘cryptic’ genetic variation ([Fischer *et al*, 2020b](#_ENREF_30); [Noble *et al*, 2019](#_ENREF_58); Rowiński and Rogell, 2017; [Wood and Brodie, 2015](#_ENREF_97)), 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 ([Falconer and Mackay, 1996](#_ENREF_28); [Hoffmann and Merilä, 1999](#_ENREF_43)). 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](#_ENREF_65)), macaques – 0.39 ([Kimock *et al*, 2019](#_ENREF_47)) lizards – 0 to 0.54 – ([Martins *et al*, 2019](#_ENREF_53); [Noble *et al*, 2014](#_ENREF_57))]. 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](#_ENREF_49)). Indeed, when this variance partitioning is done accordingly, heritability estimates are often low ([Noble *et al*, 2014](#_ENREF_57)).

The lack of difference in genetic variation between developmental temperatures environments support findings from recent meta-analyses. Fisher et al. ([2020a](#_ENREF_29)) 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*, 2020a](#_ENREF_29)). 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](#_ENREF_58)) 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](#_ENREF_67)). While our incubation temperatures were selected based on temperature extremes of naturally occurring *L. delicata* nests ([Cheetham *et al*, 2011](#_ENREF_15)), 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](#_ENREF_6)). 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 and Hoffmann, 2004](#_ENREF_74); [Weigensberg and Roff, 1996](#_ENREF_88)).

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

Genetic contributions to body size are expected to vary throughout ontogeny ([Lynch and Walsh, 1998](#_ENREF_51)). 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 and Rowe, 2015](#_ENREF_68)). 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](#_ENREF_65)), soay sheep ([Wilson *et al*, 2007](#_ENREF_94)) and ladybird beetles ([Dmitriew *et al*, 2010](#_ENREF_22)). 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](#_ENREF_22); [Hoffmann and Merilä, 1999](#_ENREF_43)). 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](#_ENREF_11)). Nonetheless, ontogenetic variation in genetic variance implies that potential rates of evolution varies with age ([Houle, 1998](#_ENREF_44)), 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](#_ENREF_16); [Wilson *et al*, 2005b](#_ENREF_93)). In accordance with other studies, maternal effects did in fact decline after hatching ([Dmitriew *et al*, 2010](#_ENREF_22); [Lindholm *et al*, 2006](#_ENREF_50); [Pick *et al*, 2016](#_ENREF_63); [Wilson *et al*, 2005a](#_ENREF_92); [Wilson *et al*, 2005b](#_ENREF_93)). Maternal investment, such as investment in clutch number or egg quality, has been shown to influence hatching size in lizards ([Brown and Shine, 2009](#_ENREF_9); [Noble *et al*, 2014](#_ENREF_57); [Warner and Lovern, 2014](#_ENREF_86)), however, as predicted these effects dissipated post-hatching ([Pick *et al*, 2016](#_ENREF_63); [Réale *et al*, 1999](#_ENREF_65)). 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](#_ENREF_63)). 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](#_ENREF_71); [Salin *et al*, 2019](#_ENREF_72)). 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](#_ENREF_60); [Wilson *et al*, 2007](#_ENREF_94)). 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](#_ENREF_52); [Noble *et al*, 2018](#_ENREF_59)).This means that developmental time decelerates with temperature following an negative exponential function. As a result, hot incubated lizards are more comparable in their development time compared to lizards that were reared a cooler temperature. In 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](#_ENREF_85)). 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](#_ENREF_55)). 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](#_ENREF_8); [Marshall *et al*, 2020](#_ENREF_52); [Reed *et al*, 2010](#_ENREF_66)). 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, 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)

# Acknowledgements

Many volunteers and interns from Lizard Lab: Birgit Szabo, Christine Wilson, Joshua Cunningham, Victor Frichot and Matthieu Monserand. Scott Keogh and Julia Riley for their advice with SNP data.

# References

Andrews RM, Mathies T, Warner DA (2000). Effect of Incubation Temperature on Morphology, Growth, and Survival of Juvenile Sceloporus undulatus. *Herpetological Monographs* **14:** 420--431.

Angilletta Jr MJ, Steury TD, Sears MW (2017). Temperature, Growth Rate, and Body Size in Ectotherms: Fitting Pieces of a Life-History Puzzle. *Integrative and Comparative Biology***:** 1-12.

Beaman JE, White CR, Seebacher F (2016). Evolution of Plasticity: Mechanistic Link between Development and Reversible Acclimation. *Trends in Ecology & Evolution* **31**(3)**:** 237--249.

Beldade P, Mateus ARA, Keller RA (2011). Evolution and molecular mechanisms of adaptive developmental plasticity. *Molecular Ecology* **20**(7)**:** 1347--1363.

Bérénos C, Ellis PA, Pilkington JG, Pemberton JM (2014). Estimating quantitative genetic parameters in wild populations: A comparison of pedigree and genomic approaches. *Molecular Ecology* **23:** 3434–3451.

Bonamour S, Chevin L-M, Charmantier A, Teplitsky C (2019). Phenotypic plasticity in response to climate change: the importance of cue variation. *Philosophical Transactions of the Royal Society B: Biological Sciences* **374**(1768)**:** 20180178–20180112.

Booth DT (2006). Influence of Incubation Temperature on Hatchling Phenotype in Reptiles. *Physiological and Biochemical Zoology* **79**(2)**:** 274--281.

Botero CA, Weissing FJ, Wright J, Rubenstein DR (2015). Evolutionary tipping points in the capacity to adapt to environmental change. *Proceedings of the National Academy of Sciences* **112**(1)**:** 184--189.

Brown GP, Shine R (2009). Beyond size–number trade-offs: clutch size as a maternal effect. *Philosophical Transactions of the Royal Society B: Biological Sciences* **364**(1520)**:** 1097--1106.

Bürkner PC (2017). brms: An R package for Bayesian multilevel models using Stan. *Journal of Statistical Software* **80(1).** [**https://doi.org/10.18637/jss.v080.i01**](https://doi.org/10.18637/jss.v080.i01).

Chapple DG, Miller KA, Chaplin K, Barnett L, Thompson MB, Bray RD (2014). Biology of the invasive delicate skink (Lampropholis delicata) on Lord Howe Island. *Australian Journal of Zoology* **62**(6)**:** 498–506.

Chapple DG, Miller KA, Kraus F, Thompson MB (2013). Divergent introduction histories among invasive populations of the delicate skink (*Lampropholis delicata*): has the importance of genetic admixture in the success of biological invasions been overemphasized? *Diversity and Distributions* **19:** 134–146.

Charmantier A, Garant D (2005). Environmental quality and evolutionary potential: lessons from wild populations. *Proceedings of the Royal Society B: Biological Sciences* **272**(1571)**:** 1415--1425.

Charmantier A, Kruuk LEB, Blondel J, Lambrechts MM (2004). Testing for microevolution in body size in three blue tit populations. *Journal of Evolutionary Biology* **17**(4)**:** 732--743.

Cheetham E, Doody JS, Stewart B, Harlow P (2011). Embryonic mortality as a cost of communal nesting in the delicate skink. *Journal of Zoology* **283**(4)**:** 234–242.

Cheverud JM (1984). Evolution by Kin Selection: A Quantitative Genetic Model Illustrated by Maternal Performance in Mice. *Evolution* **38**(4)**:** 766--777.

Chevin LM, Lande, R., Mace, G.M. (2010). Adaptation, plasticity, and extinction in a changing environment: Towards a predictive theory. *PLoS Biology,* **8:** e1000357.

Coltman DW, Pilkington J, Kruuk LEB, Wilson K, Pemberton JM (2001). Positive Genetic Correlation Between Parasite Resistance and Body Size in a Free-Living Ungulate Population. *Evolution* **55**(10)**:** 2116--2125.

Dahlgaard J, Hoffmann AA (2000). Stress Resistance and Environmental Dependency of Inbreeding Depression in Drosophila melanogaster. *Conservation Biology* **14**(4)**:** 1187--1192.

Dayananda B, Gray S, Pike D, Webb JK (2016). Communal nesting under climate change: fitness consequences of higher incubation temperatures for a nocturnal lizard. *Global Change Biology* **22**(7)**:** 2405--2414.

De Jong MJ, Alton LA, White CR, O’Bryan MK, Chapple DG, Wong BBM (2023). Long-term effects of incubation temperature on growth and thermal physiology in a small ectotherm. *Philosophical Transactions of the Royal Society B: Biological Sciences* **378:** 20220137, <https://doi.org/20220110.20221098/rstb.20222022.20220137>.

Dmitriew C, Blows MW, Rowe L (2010). Ontogenetic Change in Genetic Variance in Size Depends on Growth Environment. *The American Naturalist* **175**(6)**:** 640--649.

Downes SJ, Shine R (1999). Do incubation-induced changes in a lizard's phenotype influence its vulnerability to predators? *Oecologia* **120**(1)**:** 9–18.

Elphick MJ, Shine R (1998). Longterm effects of incubation temperatures on the morphology and locomotor performance of hatchling lizards (Bassiana duperreyi, Scincidae). *Biological Journal of the Linnean Society* **63**(3)**:** 429--447.

Elphick MJ, Shine R (1999). Sex differences in optimal incubation temperatures in a scincid lizard species. *Oecologia* **118**(4)**:** 431--437.

Eyck HJF, Buchanan KL, Crino OL, Jessop TS (2019). Effects of developmental stress on animal phenotype and performance: a quantitative review. *Biological Reviews* **94**(3)**:** 1143--1160.

Falconer DS (1952). The Problem of Environment and Selection. *The American Naturalist* **86**(830)**:** 293–298.

Falconer DS, Mackay TFC (1996). *Introduction to Quantitative Genetics*, 4 edn. Pearson Education.

Fischer K, Kreyling J, Beaulieu M, Beil I, Bog M, Bonte D *et al* (2020a). Species-specific effects of thermal stress on the expression of genetic variation across a diverse group of plant and animal taxa under experimental conditions. *Heredity***:** 1–15. <https://doi.org/10.1038/s41437-41020-40338-41434>.

Fischer K, Kreyling J, Beaulieu M, Beil I, Bog M, Bonte D *et al* (2020b). Species-specific effects of thermal stress on the expression of genetic variation across a diverse group of plant and animal taxa under experimental conditions. *Heredity***:** 1--15.

Flatt T, Shine R, Borges-landaez PA, Downes SJ (2001). Phenotypic variation in an oviparous montane lizard (Bassiana duperreyi): the effects of thermal and hydric incubation environments. *Biological Journal of the Linnean Society* **74**(3)**:** 339--350.

Forster J, Hirst AG (2012). The temperature-size rule emerges from ontogenetic differences between growth and development rates. *Functional Ecology* **26**(2)**:** 483--492.

Gavrilets S, Scheiner SM (1993). The genetics of phenotypic plasticity. vi. theoretical predictions for directional selection. . *Journal of Evolutionary Biology* **6:** 49–68.

Gelman A, Lee D, Guo J (2015). Stan: A Probabilistic Programming Language for Bayesian Inference and Optimization. *Journal of Educational and Behavioral Statistics* **40**(5)**:** 530--543.

Ghalambor CK, McKay JK, Carroll SP, Reznick DN (2007). Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology* **21**(3)**:** 394–407.

Gifford ME, Robinson CD, Clay TA (2017). The influence of invasive fire ants on survival, space use, and patterns of natural selection in juvenile lizards. *Biological Invasions* **19**(5)**:** 1461--1469.

Goodman BA, Schwarzkopf L, Krockenberger AK (2013). Phenotypic Integration in Response to Incubation Environment Adaptively Influences Habitat Choice in a Tropical Lizard. *The American Naturalist* **182**(5)**:** 666--673.

Goodman RM (2008). Latent effects of egg incubation temperature on growth in the lizard Anolis carolinensis. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology* **309A**(9)**:** 525--533.

Granato ISC, Galli G, de Oliveira Couto EG, e Souza MB, Mendonça LF, Fritsche-Neto R (2018). snpReady: a tool to assist breeders in genomic analysis. *Molecular Breeding* **38**(8)**:** 102.

Hansen TF, Pélabon C, Houle D (2011). Heritability is not Evolvability. *Evolutionary Biology* **38:** 258–277.

Hare KM, Longson CG, Pledger S, Daugherty CH (2004). Size, Growth, and Survival Are Reduced at Cool Incubation Temperatures in the Temperate Lizard *Oligosoma suteri* (Lacertilia: Scincidae). *Copeia* **2004(2):** 383–390. <https://doi.org/310.1643/CP-1603-1084R1642>.

Hoffman AA, Parsons PA (1991). *Evolutionary genetics and evolutionary stress*. Oxford University Press.

Hoffmann AA, Merilä J (1999). Heritable variation and evolution under favourable and unfavourable conditions. *Trends in Ecology & Evolution* **14**(3)**:** 96--101.

Houle D (1998). How should we explain variation in the genetic variance of traits? *Genetica* **102**(0)**:** 241.

Huisman J (2017). Pedigree reconstruction from SNP data: parentage assignment, sibship clustering and beyond. *Molecular Ecology Resources* **17**(5)**:** 1009--1024.

Ji X, Chen F, Du W-G, Chen H-L (2003). Incubation temperature affects hatchling growth but not sexual phenotype in the Chinese soft-shelled turtle, Pelodiscus sinensis (Trionychidae). *Journal of Zoology* **261**(4)**:** 409--416.

Kimock CM, Dubuc C, Brent LJN, Higham JP (2019). Male morphological traits are heritable but do not predict reproductive success in a sexually-dimorphic primate. *Scientific Reports* **9**(1)**:** 19794.

Krist M (2010). Egg size and offspring quality: a meta-analysis in birds. *Biological Reviews* **86**(3)**:** 692–716.

Kruuk LEB (2004). Estimating genetic parameters in natural populations using the ‘animal model’. *Philosophical transactions of the Royal Society of London Series B, Biological sciences* **359**(1446)**:** 873–890.

Lindholm AK, Hunt J, Brooks R (2006). Where do all the maternal effects go? Variation in offspring body size through ontogeny in the live-bearing fish Poecilia parae. *Biology Letters* **2**(4)**:** 586--589.

Lynch M, Walsh B (1998). *Genetics And Analysis Of Quantitative Traits*. Oxford University Press.

Marshall DJ, Pettersen AK, Bode M, White CR (2020). Developmental cost theory predicts thermal environment and vulnerability to global warming. *Nature Ecology & Evolution* **4**(3)**:** 406--411.

Martins F, Kruuk LEB, Llewelyn J, Moritz C, Phillips B (2019). Heritability of climate-relevant traits in a rainforest skink. *Heredity* **122**(1)**:** 41--52.

Mitchell TS, Hall JM, Warner DA (2018). Female investment in offspring size and number shifts seasonally in a lizard with single-egg clutches. *Evolutionary Ecology* **32**(2)**:** 231--245.

Monaghan P (2008). Early growth conditions, phenotypic development and environmental change. *Philosophical Transactions of the Royal Society B: Biological Sciences* **363**(1497)**:** 1635–1645.

Mousseau TA, Fox CW (1998). The adaptive significance of maternal effects. *Trends in Ecology & Evolution* **13**(10)**:** 403--407.

Noble DWA, McFarlane SE, Keogh JS, Whiting MJ (2014). Maternal and additive genetic effects contribute to variation in offspring traits in a lizard. *Behavioral Ecology* **25**(3)**:** 633--640.

Noble DWA, Radersma R, Uller T (2019). Plastic responses to novel environments are biased towards phenotype dimensions with high additive genetic variation. *Proceedings of the National Academy of Sciences* **116**(27)**:** 13452--13461.

Noble DWA, Stenhouse V, Schwanz LE (2018). Developmental temperatures and phenotypic plasticity in reptiles: a systematic review and meta-analysis. *Biological Reviews* **93**(1)**:** 72–97.

Noordwijk AJV, Balen JHV, Scharloo W (1988). Heritability of body size in a natural population of the Great Tit (Parus major) and its relation to age and environmental conditions during growth. *Genetical Research* **51**(2)**:** 149--162.

O’Dea RE, Lagisz M, Hendry AP, Nakagawa S (2019). Developmental temperature affects phenotypic means and variability: A meta-analysis of fish data. *Fish and Fisheries* **20(5)** 1005–1022. <https://doi.org/1010.1111/faf.12394>.

Paaby AB, Rockman MV (2014). Cryptic genetic variation: evolution's hidden substrate. *Nature Reviews Genetics* **15**(4)**:** 247--258.

Pick JL, Ebneter C, Hutter P, Tschirren B (2016). Disentangling Genetic and Prenatal Maternal Effects on Offspring Size and Survival. *The American Naturalist* **188**(6)**:** 628--639.

Qualls FJ, Shine R (2000). Post-hatching environment contributes greatly to phenotypic variation between two populations of the Australian garden skink, Lampropholis guichenoti. *Biological Journal of the Linnean Society* **71**(2)**:** 315–341.

Réale D, Festa‐Bianchet M, Jorgenson JT (1999). Heritability of body mass varies with age and season in wild bighorn sheep. *Heredity* **83** 526–532.

Reed TE, Waples RS, Schindler DE, Hard JJ, Kinnison MT (2010). Phenotypic plasticity and population viability: the importance of environmental predictability. *Proceedings of the Royal Society of London B: Biological Sciences* **277**(1699)**:** 3391–3400.

Roelofs D, Morgan J, Stürzenbaum S (2010). The significance of genome-wide transcriptional regulation in the evolution of stress tolerance. *Evolutionary Ecology* **24**(3)**:** 527--539.

Rollinson N, Rowe L (2015). Persistent directional selection on body size and a resolution to the paradox of stasis. *Evolution* **69**(9)**:** 2441--2451.

Rowiński PK, Rogell B (2017). Environmental stress correlates with increases in both genetic and residual variances: A meta-analysis of animal studies. *Evolution* **71:** 1339–1351. <https://doi.org/1310.1111/evo.13201>.

Rueden CT, Schindelin J, Hiner MC, DeZonia BE, Walter AE, Arena ET *et al* (2017). ImageJ2: ImageJ for the next generation of scientific image data. *BMC Bioinformatics* **18**(1)**:** 529.

Salin K, Auer SK, Anderson GJ, Selman C, Metcalfe NB (2016). Inadequate food intake at high temperatures is related to depressed mitochondrial respiratory capacity. *The Journal of experimental biology* **219**(9)**:** 1356–1362.

Salin K, Villasevil EM, Anderson GJ, Lamarre SG, Melanson CA, McCarthy I *et al* (2019). Differences in mitochondrial efficiency explain individual variation in growth performance. *Proceedings of the Royal Society B: Biological Sciences* **286**(1909)**:** 20191466.

Schielzeth H, Nakagawa S (2022). Conditional repeatability and the variance explained by reaction norm variation in random slope models. *Methods in Ecology and Evolution* **13:** 1214-1223.

Sgrò CM, Hoffmann AA (2004). Genetic correlations, tradeoffs and environmental variation. *Heredity* **93(3):** 241–248. <https://doi.org/210.1038/sj.hdy.6800532>.

Shine R, Harlow PS (1996). Maternal Manipulation of Offspring Phenotypes via Nest-Site Selection in an Oviparous Lizard. *Ecology* **77**(6)**:** 1808–1817.

Sivula T, Magnusson M, Vehtari A (2020). Uncertainty in Bayesian leave-one-out cross-validation based model comparison. *arXiv:200810296*.

Stillwell RC, Fox CW (2009). Geographic variation in body size, sexual size dimorphism and fitness components of a seed beetle: local adaptation versus phenotypic plasticity. *Oikos* **118**(5)**:** 703--712.

Storm MA, Angilletta MJ (2007). Rapid assimilation of yolk enhances growth and development of lizard embryos from a cold environment. *The Journal of experimental biology* **210**(19)**:** 3415–3421.

Sun B-J, Li T, Gao J, Ma L, Du W-G (2015). High incubation temperatures enhance mitochondrial energy metabolism in reptile embryos. *Scientific Reports* **5**(1)**:** 8861.

Team RC (2023). R: A language and environment for statistical

computing. *R Foundation for Statistical Computing, Vienna, Austria*

*URL* [*https://wwwR-projectorg/*](https://wwwR-projectorg/).

Telemeco RS, Radder RS, Baird TA, Shine R (2010). Thermal effects on reptile reproduction: adaptation and phenotypic plasticity in a montane lizard. *Biological Journal of the Linnean Society* **100**(3)**:** 642--655.

Uller T, Olsson M (2010). Offspring size and timing of hatching determine survival and reproductive output in a lizard. *Oecologia* **162**(3)**:** 663--671.

VanRaden PM (2008). Efficient Methods to Compute Genomic Predictions. *Journal of Dairy Science* **91**(11)**:** 4414--4423.

Verdú‐Ricoy J, Iraeta P, Salvador A, Díaz JA (2014). Phenotypic responses to incubation conditions in ecologically distinct populations of a lacertid lizard: A tale of two phylogeographic lineages. *Journal of Zoology* **292(3):** 184–191. <https://doi.org/110.1111/jzo.12091>.

Wallace BP, Sotherland PR, Santidrian Tomillo P, Reina RD, Spotila JR, Paladino FV (2007). Maternal investment in reproduction and its consequences in leatherback turtles. *Oecologia* **152**(1)**:** 37--47.

Warner DA, Lovern MB (2014). The Maternal Environment Affects Offspring Viability via an Indirect Effect of Yolk Investment on Offspring Size. *Physiological and Biochemical Zoology* **87**(2)**:** 276--287.

Warner DA, Shine R (2008). Determinants of Dispersal Distance in Free-Ranging Juvenile Lizards. *Ethology* **114**(4)**:** 361--368.

Weigensberg I, Roff DA (1996). Natural Heritabilities: Can They Be Reliably Estimated in the Laboratory? *Evolution* **50**(6)**:** 2149--2157.

West-Eberhard MJ (2003). *Developmental Plasticity and Evolution*. Oxford University Press.

While GM, Noble DWA, Uller T, Warner DA, Riley JL, Du W-G *et al* (2018). Patterns of developmental plasticity in response to incubation temperature in reptiles. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology* **329**(4-5)**:** 162--176.

While GM, Williamson J, Prescott G, Horvathova T, Fresnillo B, Beeton NJ *et al* (2015). Adaptive responses to cool climate promotes persistence of a non-native lizard. *Proceedings of the Royal Society of London B: Biological Sciences* **282**(1803)**:** 20142638–20142638.

Wilson AJ, Coltman DW, Pemberton JM, Overall ADJ, Byrne KA, Kruuk LEB (2005a). Maternal genetic effects set the potential for evolution in a free-living vertebrate population. *Journal of Evolutionary Biology* **18**(2)**:** 405--414.

Wilson AJ, Kruuk LEB, Coltman DW (2005b). Ontogenetic Patterns in Heritable Variation for Body Size: Using Random Regression Models in a Wild Ungulate Population. *The American Naturalist* **166**(6)**:** E177--E192.

Wilson AJ, Pemberston JM, Pilkington JG, Clutton-Brock TH, Coltman DW, Kruuk LEB (2007). Quantitative genetics of growth and cryptic evolution of body size in an island population. *Evolutionary Ecology* **21**(3)**:** 337--356.

Wilson AJ, Réale D (2006). Ontogeny of Additive and Maternal Genetic Effects: Lessons from Domestic Mammals. *The American Naturalist* **167**(1)**:** E23--E38.

Wilson AJ, Reale D, Clements MN, Morrissey MM, Postma E, Walling CA *et al* (2010). An ecologist's guide to the animal model. *Journal of Animal Ecology* **79**(1)**:** 13–26.

Wood CW, Brodie ED (2015). Environmental effects on the structure of the G-matrix. *Evolution* **69**(11)**:** 2927--2940.