Heritability and developmental plasticity of growth in an oviparous lizard

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Abstract

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

32

Keywords

34 Body mass, growth rate, additive genetic variance, incubation temperature, maternal effects,

35 temperature-size rule, cryptic genetic variation

36

Introduction

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

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

69 evolutionary change will be expected to change making it difficult to predict genetic

70 adaptation.

71

72   Comparative studies have shown that the influence of environmental stress on genetic

73 variance during development is not straightforward (Charmantier & Garant, 2005; Hoffmann

74 & Merilä, 1999; Rowiński & Rogell, 2017). In lab studies, elevated developmental stress has

75 been shown to increase the heritability of morphological traits (Hoffmann & Merilä, 1999),

76 whereas wild, non-domestic populations tend to have higher heritability in favourable

77 environments (Charmantier & Garant, 2005). Lack of consensus may be related to increased

78 environmental heterogeneity in wild populations, making them more difficult to compare

79 with lab studies. It has been suggested that responses to different developmental stressors

80 (e.g. heat shock vs. starvation) may be associated with disparate patterns of gene expression

81 making broad comparisons more variable (Charmantier & Garant, 2005; Dahlgaard &

82 Hoffmann, 2000). Importantly, environmental comparisons of heritability have been

83 criticised as the ratio nature of its calculations can mask changes in the relative contributions

84 of non-genetic and genetic variance (Rowiński & Rogell, 2017). For example, a meta-

85 analysis found that heritability of life history traits which has been argued to be more

86 important to fitness, did not change between control and stressful conditions (Rowiński &

87 Rogell, 2017). The same pattern was observed for morphological traits (Fischer et al., 2020).

88 Upon closer inspection, both additive genetic and environmental variance of life history traits

89 increased under stressful conditions whereas the opposite was true for morphological traits

90 (Rowiński & Rogell, 2017). The dynamics of both genetic and non-genetic sources of

91 variation under different developmental environments can thus influence the evolutionary

92 potential of fitness related traits.

93

94   Body size is fundamental to fitness and is both heritable and environmentally

95 responsive (Noordwijk et al., 1988; Stillwell & Fox, 2009). Developmental environments,

96 such as temperature and nutritional stress can drive substantial variation in body size, largely

97 through shifts in how organisms grow (Eyck et al., 2019; Noble et al., 2018). Maternal

98 investment in offspring are also important sources of body size variation (Noble et al., 2014;

99 Wilson & Réale, 2006). Variation among mothers in egg investment, nest site selection or

100 timing of birth (Mitchell et al., 2018; Shine & Harlow, 1996; Uller & Olsson, 2010) are

101 expected to contribute the most to offspring body size early in development (Mousseau &

102 Fox, 1998). However, these effects have shown to decline with age as maternal investment

103   subside (Krist, 2010; Wilson, Kruuk, et al., 2005). Additionally, environmental factors such

104   as shared habitats or long-term seasonal effects can also account for a substantial proportion

105   of variability in body size (Kruuk, 2004). For example, permanent environmental effects that

106   varied across years explained 26% – 35% of body size variation in bighorn sheep (Réale et

107   al., 1999). Similarly, 56% of variation in body mass was attributed to nest boxes shared

108   among siblings in blue tit chicks (Charmantier et al., 2004). As such, the various sources that

109   influence body size variation (genetic, environmental, maternal) are predicted to vary across

110   ontogeny and temporal approach is therefore needed in order to evaluate when evolutionary

111   potential of body size is greatest.

112

113   Here we investigated the impact of developmental temperature on body size (mass)

114   and growth in an oviparous skink (*Lampropholis delicata*) – two traits that are critically

115   important to fitness. We also test how developmental environments affect evolutionary

116   potential in these traits. Growth trajectories ($n_{observations}$ = 3,002) for lizards that hatched from

117   two incubation treatments (n_{hot} = 126, n_{cold} = 136), were measured over the first 16 months of

118   life (nearly half their life). Using 8,433 single nucleotide polymorphic (SNP) markers, we

119   derived a genomic relatedness matrix to estimate quantitative genetic parameters. Using these

120   data, we address two key questions: 1) How does developmental temperature affect the rate

121   and shape of growth trajectories (initial mass, growth rate and curvature of growth

122   trajectory)? and 2) How does developmental temperature affect genetic and non-genetic

123   variance across age? According to the 'temperature-size rule', we expect lizards experiencing

124   cold developmental temperatures to have larger initial masses and slower growth rates –

125   possibly resulting in lizards reaching sexual maturity at a later age compared to lizards

126   experiencing hot developmental temperatures (Angilletta Jr et al., 2017). In addition, we

127   predicted greater amount of genetic variance under higher developmental temperatures, after

128   controlling for non-genetic sources of variance. We expected maternal effects and permanent

129   environment effects to manifest early in development and dissipate over time.

Materials and Methods

130

131   *Lizard collection and husbandry*

132   From 2015 – 2017, we established a breeding colony of adult *L. delicata* ($n_{females}$ =

133   144, n_{males} = 50) using wild individuals collected across five sites throughout the Sydney

134   region between August and September 2015. Using a half-sib breeding design, we paired

135   three females with a single male in opaque plastic enclosures measuring 35cm × 25cm

136   × 15cm (L × W × H). Enclosures were kept under UV lights (12L:12D) in a temperature-

137   controlled room set to 24ºC. Lizards were given access to a heat lamp that elevated

138   temperatures to between 28-32 ºC. Each enclosure was lined with newspaper and lizards had

139   constant access to water. Tree bark was used as refuge. Adult lizards were fed medium sized

140   crickets *ad libitum* (*Acheta domestica*) dusted with calcium powder and multi-vitamin every

141   two days. From the beginning of the egg laying season (October of each year), we replaced

142   newspaper lining with garden potting mix and placed an opaque plastic box (12 cm × 17.5

143   cm × 4.3 cm) containing moistened vermiculite in each enclosure for females to oviposit

144   their eggs. During this time, enclosures were sprayed with water every second day to

145   maintain a relatively humid environment. From October to November, egg boxes were

146   checked every day. Tail tissue samples (~1 mm) were taken from adults that were from

147   enclosures producing eggs for DNA extraction (see below). All tissues were stored in 70%

148   ethanol. Animal collection was approved by the New South Wales National Parks and

149   Wildlife Service (SL101549) and all procedures were approved by the Macquarie University

150   Ethics committee (ARA 2015/015) and University of New South Wales Animal Care and

151   Ethics committee (ACEC 15/51A).

152

153   *Developmental Temperature Manipulations*

154   Eggs were collected between October to March, over two reproductive seasons from

155   2016 and 2017. As soon as eggs were found, they were weighed using a digital scale to the

156   nearest 0.01g (Ohaus Scout SKX123). We also measured egg length (distance between the

157   furthest points along the longest axis of the egg) and egg width (distance between the widest

158   points along the axis perpendicular to the longest axis of the egg) using digital callipers to the

159   nearest 0.01mm. Following measurements, each egg was placed in a plastic cup (80ml)

160   containing three grams of vermiculite and four grams of water. Each cup was then covered

161   using cling wrap and secured using an elastic band. We used a split-clutch design where eggs

162   from single clutch were pseudo-randomly assigned to one of two developmental temperature

163   treatments. We used two incubators to precisely control the temperature of eggs (LabWit,

164   ZXSD-R1090). The 'hot' treatment was exposed to a mean temperature of 29ºC whereas the

165   'cold' treatment was exposed to a mean temperature of 23ºC. Both incubators fluctuated +/-

166   3ºC over a 24-hour period around these mean temperatures to simulate natural nest site

167   temperature variability. These treatments represent the temperature extremes of natural nest

168 sites for *L. delicata* (Cheetham et al., 2011). Egg cups were rotated within each incubator

169 weekly to avoid uneven heat circulation within incubators. Incubators were also checked

170 daily for hatchlings.

171

172 *Quantifying Growth Rate*

173   Newly emerged hatchlings were weighed to the nearest 0.01g and a small tail tip

174 clipping (~2mm) was taken for genetic analyses. Ventral photographs were taken for digital

175 measurement (Nikon Coolpix A900). For the first two months, photographs of hatchlings

176 were taken approximately every 14 days. After which, hatchlings were photographed at

177 approximately a 35-day interval. From six months onwards, we manually measured hatchling

178 SVL using a clear ruler to the nearest ~0.5mm. We also recorded the mass of the individual

179 each time photographs or SVL measurements were taken. Growth measurements continued

180 until we had approximately 16 measures per individual (mean = 11.5, SD = 4.71). By the end

181 of the study, the mean age for hot incubated lizards was 335.82 (range: 0 – 711) and for cold

182 incubated lizards it was 384.8 (range: 0 – 707) which is approximately 40 – 50% of their total

183 lifespan (Chapple et al., 2014). From the photographs, we extracted snout-vent-length (SVL;

184 from tip of snout to the beginning of the cloaca opening) using ImageJ software (Rueden et al.,

185 2017). For the first initial nine months, hatchlings were housed individually in opaque plastic

186 enclosures (32.3cm x 18.5cm x 6cm) lined with newspaper. Hatchlings were fed the same

187 number of crickets every second day and had constant access to a tree bark refuge and water.

188 Hatchling enclosures were placed in a temperature control room under the same conditions as

189 described above for the adult colony. For logistical reasons, at approximately nine months,

190 hatchlings were housed in groups of five in opaque bins with the same measurements as the

191 adult enclosures. We pseudo-randomised individuals to each shared enclosure while

192 maintaining a similar number of individuals from each treatment.

193 *Genomic Relatedness Matrix*

194   We derived a genomic relatedness matrix (GRM) using single nucleotide

195 polymorphism (SNP) genotypes for all 262 offspring with growth data (132 putative parents;

196 $n_{females}$ = 69, n_{males} = 63). While our half-sib breeding design allowed us to assign parentage to

197 derive a pedigree, high levels of sperm storage and low levels of multiple paternity (94% of

198 females had been sired by a single male) meant our pedigree had low resolution to effectively

199 estimate additive genetic variation. Recent studies have shown that GRM derived from SNPs

200 have low error rates (<0.3%) and are able to reconstruct pedigree relationships in much finer

detail when at least 200 SNP loci are used (Bérénos et al., 2014; Huisman, 2017). Moreover, both relatedness and heritability values estimated from a GRM have been shown to be very similar to those inferred using a pedigree (Bérénos et al., 2014; Huisman, 2017). Single nucleotide polymorphism libraries were designed and animals genotyped using DArTseq™ (Diversity Arrays Technology) methods. For more details on DNA extraction and SNP genotyping see ESM.

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

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

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

Statistical Analyses

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

232 posterior distribution every fifth iteration. We ensured proper mixing by inspecting trace

233 plots and checked that scale reduction factors were less than 1.01. We report posterior means

234 and 95% credible intervals for all parameters throughout.

235 *Impact of Developmental Temperature on Additive Genetic Variance and Maternal Effects*

236 *Across Age*

237 First, we tested whether developmental temperature influenced the overall heritability

238 of mass and the relative contributions of variance irrespective of age. For each treatment

239 group, we fitted intercepts only in the fixed effects with random intercepts for additive

240 genetic variance (*G*), maternal effects (*M*) and permanent environmental effects (*PE*) as we

241 had repeated measures of the same individuals (Wilson et al., 2010). The model also

242 estimated residual variance (*R*). We included our GRM to estimate additive genetic variation.

243 Overall. Heritability (h^2) of mass using this intercept (*I*) model was calculated as:

244
$$h^2 = \frac{G_I}{(G_I + M_I + PE_I + R_I)}$$

245 To then test how *G*, *M* and h^2 change across age, we used model selection to

246 determine the most appropriate random effects structure for our data as we had no *a priori*

247 knowledge of what (or how) variance components change with age (Wilson & Réale, 2006).

248 We fitted seven models with varying complexity in their random effects and compared their

249 Watanabe–Akaike Information Criterion (WAIC) values (Table S1). We fitted random

250 intercepts and random slopes by including either a linear age term or both linear and

251 quadratic age terms to partition variance across age. Two models were equally supported, the

252 first included a random linear and quadratic slope for *G* and *M* and *PE*. (Model 3 - Table S1)

253 and the second included a random linear and quadratic slope for *G* and *M,* respectively, and a

254 random intercept for *PE* (Model 7 – Table S1). To avoid overfitting, we selected the more

255 parsimonious model and used this random effect structure for the remaining analyses unless

256 stated otherwise.

257 Residual variance may be conflated with estimates of other variance components if it

258 changes over time (heterogenous variance) and is not properly accounted for. We therefore

259 explicitly modelled residual variance to verify if this was the case and compared homogenous

260 and heterogenous residual variance models using WAIC. We fitted two models, both of

261 which had the same fixed and random effects structure as Model 7 described above. The first

262 model had homogenous residual variance whereas in the second model we modelled residual

263 variance with a linear slope thereby allowing it to vary with age. The model with
264 heterogenous variance was best supported (Table S2), we therefore modelled heterogenous
265 variance in all subsequent models unless stated otherwise.

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

274
$$G = \begin{bmatrix} G_I & Cov_{I,S} & Cov_{I,C} \\ Cov_{I,S} & G_S & Cov_{S,C} \\ Cov_{I,C} & Cov_{S,C} & G_C \end{bmatrix}$$

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

277
$$M = \begin{bmatrix} M_I & Cov_{I,S} & Cov_{I,C} \\ Cov_{I,S} & M_S & Cov_{S,C} \\ Cov_{I,C} & Cov_{S,C} & M_C \end{bmatrix}$$

278 For each treatment group, we then calculated additive genetic variance at a given age G_x
279 using the random slope terms and their covariances following (Schielzeth & Nakagawa,
280 2020):

281
$$G_x = G_I + (x^2.G_S) + (x^4.G_C) + (2x.Cov_{I,S}) + (2x^2.Cov_{I,C}) + (2x^3.Cov_{S,C})$$

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

286
$$h_x^2 = \frac{G_x}{(G_x + M_x + PE_I + R_I)}$$

287   As the mean body mass increases over time, the variance may also increase
288 concurrently due to scale effects and potentially bias estimates of quantitative genetic

289   parameters (Wilson, Kruuk, et al., 2005). We therefore calculated coefficients of variation

290   (CV) across age for each variance component by dividing variance by the predicted mean

291   mass at a given age. Interpretations using CV estimates did not change our overall

292   conclusions for additive genetic variance or maternal effects, we therefore present the raw

293   estimates of each variance component below (See ESM).

294

295   *The Influence of Developmental Temperature on Growth Trajectories*

296   To test how developmental temperatures affect average growth trajectories, we also

297   fitted three models that varied in their fixed effect structure to determine how developmental

298   temperatures affect: 1) initial mass (intercept of curve), 2) linear rate of growth (linear slope)

299   and 3) curvature of the growth trajectory (quadratic term). We also wanted to test for

300   treatment differences in age at which lizards reach their maximum mass by solving for the

301   maxima of quadratic regression equation. We fit mass as the response accounting for the

302   same random effects described above. The first model included the main effect of

303   developmental temperature and the linear and quadratic term for age (Table S2). The other

304   two models differed in their interaction terms between developmental temperature with age

305   and age^2 (Table 2, S3). We then compared WAIC values to select the best model for our data

306   that explained changes in mass across age between the two developmental temperature

307   treatments (Table 1).

308

Results

310   Over two years, we collected 3,002 observations of mass data for a total of 261

311   individuals ($n_{hot} = 125$, $n_{cold} = 136$). On average, the incubation period for the 'hot'

312   treatment was 29.36 days (SD = 2.17, range = 15 - 49) days and 48.48 days (SD = 4.18, range

313   = 25 - 56) for the 'cold' treatment.

314   Overall, additive genetic variance, permanent environmental variance and heritability

315   (h^2) of growth appears to be higher in the hot developmental temperature treatment (Fig. 1).

316   However, there were no significant differences among treatment groups (Table S3).
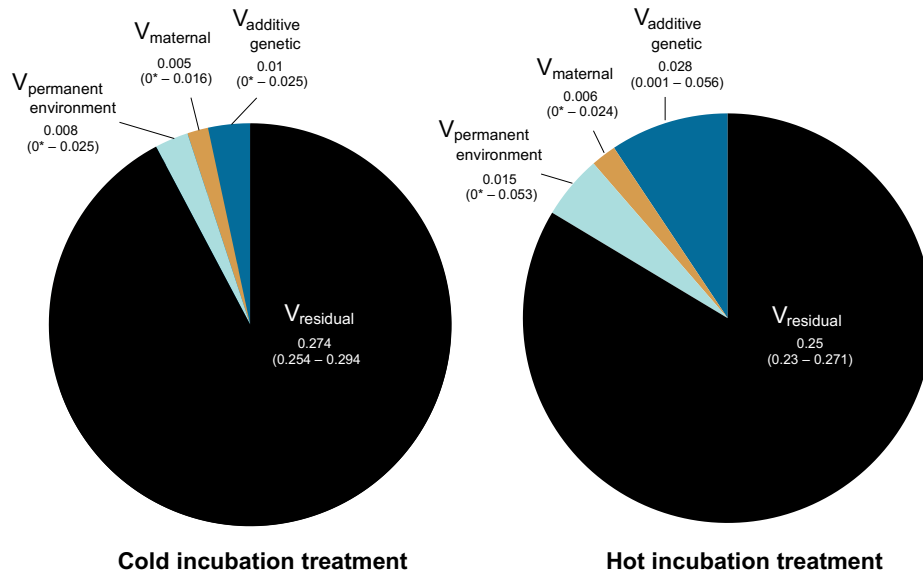
Cold incubation treatment   **Hot incubation treatment**

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

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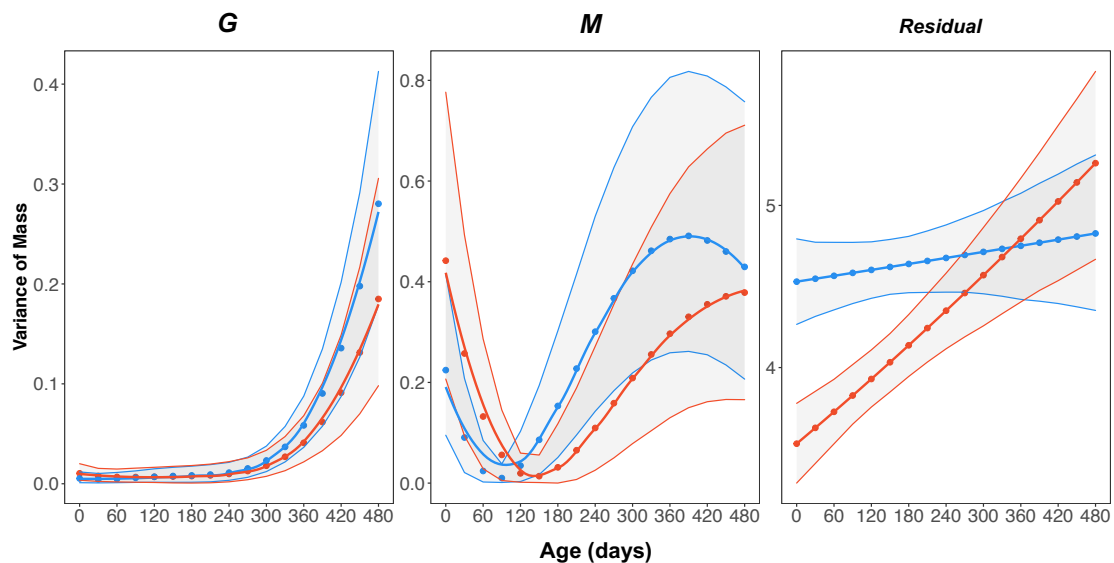
328   There were some differences among developmental treatments in how residual variance

329   changed with age (Fig. 2C). Residual variance in cold incubated lizards had a much higher

330   intercept compared to hot incubated lizard however their residual variance converged by

331   eight months of age (Fig. 2).

332   **Figure. 2** Scatterplot showing how additive genetic variance (G), maternal effects (M),

333   residual variance changed with age for the hot developmental treatment ($n_{lizards}$ = 125, red)

334   and the cold developmental treatment (n = 136, blue). Points represent posterior means, thin

335   lines represent the 95% credible intervals, thick lines represent the mean for each treatment

336   group. Note that permanent environmental effects were treated as constant across age.

337    $V_{permanent environment}$ for the hot treatment group was 0.0047 [0.00017 – 0.0096], $V_{permanent}$

338    $_{environment}$ for the cold treatment group was 0.0047 [0.00065 – 0.0085].

339   We investigated whether increases in average mass over time affected variance

340   estimates due to scaling effects between the mean and variance. However, we found that the

341   CV of G and M followed the same pattern as the raw variance estimates suggesting that

342   changes in variance were not the result of increasing mean body mass with age (Fig. S1).

343   After accounting for heterogenous residual variance, we found no treatment

344   differences in heritability or the proportion of variance explained by maternal effects (M^2)

345   (Fig. 3). Heritability was very low for the first year of growth in *L. delicata* and only began

346   increasing at one year of age (Fig. 3). As predicted M^2 decreased soon after hatching,

347   however it increased slightly again from six months of age (Fig. 3). The G and M matrices for

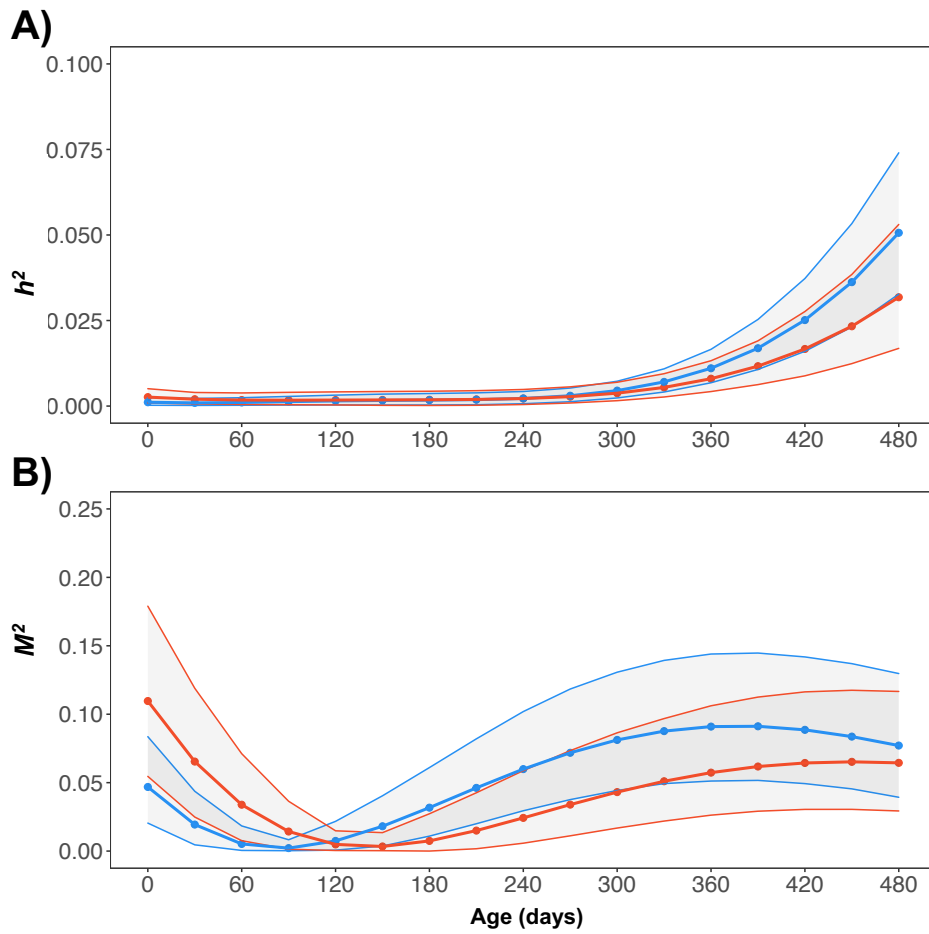348   each treatment group are presented in Table S4-S5.

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

Developmental plasticity in growth trajectories in response to temperature

382   While the model containing a full interaction between treatment and linear and

383   quadratic age was best supported, the improvement in WAIC value was marginal (Table 1).

384   Moreover, the linear growth rate (Age) and curvature of the growth trajectory (Age^2) did not

385   differ significantly between the two developmental temperature treatments in any of the

386   models containing interactions (Table S7 - S9). Irrespective of treatment, lizard mass

387   increased by 1.65 g for every 1 SD unit increase in age.

388   **Table 1** Comparisons of WAIC values of four models (n_{obs} = 2926) with different

389   combinations of treatment interactions with age parameters. $\Delta ELPD$ represents the difference

390   in expected log predicted density. Age measured in days was z-transformed (mean = 361.34,

391   SD = 185.16)

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

392

393   Developmental temperature did, however, influenced hatching mass (Table 1, Fig. 3).

394   Lizards from the 'cold' treatment were on average 0.030 g (0.018g – 0.041g) heavier

395   compared to lizards from the 'hot' treatment (Table. 2). Larger initial masses meant that

396   lizards from the 'cold' treatment reached their maximum mass slightly earlier (382.97 days,

397   95% CI: 358.84 – 409.78) compared to lizards from 'hot' treatment (413.04 days, 95% CI:

398   379.70 – 452.34). *G* and *M* matrices from this model, along with other variance components,
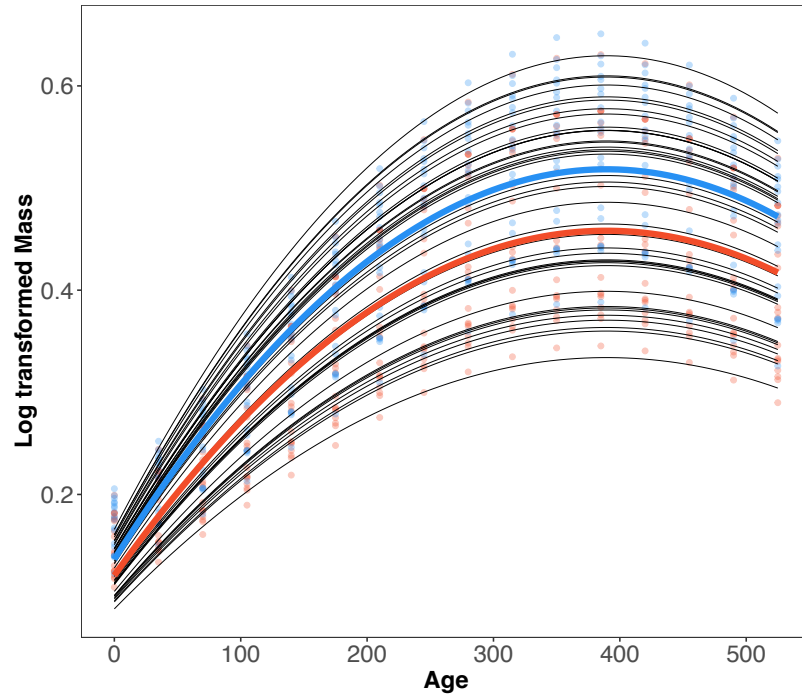
399   are presented in Table S6.

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

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

Parameter	Estimate	Lower	Upper
Intercept	**-0.991**	**-1.01**	**-0.971**
Treatment	**-0.083**	**-0.114**	**-0.05**
Age	**0.5**	**0.476**	**0.526**
Age²	**-0.196**	**-0.216**	**-0.178**
Treatment × Age	0.008	-0.021	0.037

Treatment × Age^2	0.022	-0.007	0.052

414

Discussion

416   Early development at hot temperatures resulted in smaller body sizes compared to

417 development at cold temperatures. Growth trajectories, however, were not significantly

418 impacted by early thermal environments – lizards from both temperatures grew at the same

419 rate despite cold animals remaining larger throughout life. Marginalising over age, we found

420 that developmental temperature did not impact the relative contributions of additive genetic,

421 maternal, permanent environment or residual variance. The environmental component of the

422 phenotype (residual variance) explained most of the variability in body mass. Congruently,

423 heritability of mass was generally low across ontogeny, increasing at one year of age. As we

424 predicted, maternal effects on offspring mass declined in the first few months, presumably

425 because maternal non-genetic contributions were less influential on mass over time.

426 Unexpectedly, maternal effects increased again at approximately six months possibly from

427 maternal genetic factors affecting mass. Upon hatching, the residual variance component of

428 body mass was much higher in lizards that were reared at cold incubation temperatures,

429 suggesting that aspects of development environment played a bigger role in determining their

430 hatching mass.

431 *Thermal developmental plasticity in growth*

432

433 In ectotherms, temperature plays a pervasive role in phenotypic development (Eyck et al.,

434 2019; Noble et al., 2018; O'Dea et al., 2019; While et al., 2018). Contrary to other reptile

435 studies, we did not show that growth rate differed between developmental temperatures.

436 Some researchers reported increases in growth at higher incubation temperatures (Elphick &

437 Shine, 1999; Hare et al., 2004; Verdú-Ricoy et al., 2014), while have others found either the

438 opposite result or no differences at all (Andrews et al., 2000; Goodman, 2008). The

439 directionality of change is highly variable, even among studies of the same species (e.g.

440 Bassiana dupreyi, Elphick & Shine, 1998, 1999; Flatt et al., 2001; Telemeco et al., 2010).

441 Lack of generality may be related to how growth is statistically modelled. Very few studies

442 account for individual variation in hatching mass or growth trajectories. Indeed, if we did not

443 account for among individual variance in our models, significant treatment differences in

444 growth can be detected (Table S10). We emphasise the importance of partitioning

445   confounding sources of variance such as individual or clutch effects as they can misconstrue

446   conclusions about developmental impacts on late life phenotypes. Moreover, future studies

447   should make use of all repeated measures of mass instead of averaging across individuals as

448   the former approach not only increases statistical power but also provide more accurate

449   estimates of growth.

450

451   Consistent with other squamates, we found that the cold incubation treatment group attained

452   higher hatching mass compared to their hot counterparts (Dayananda et al., 2016; Downes &

453   Shine, 1999; Flatt et al., 2001; Goodman et al., 2013). These results support the temperature-

454   size-rule whereby organisms reared in cold temperatures tend to have larger body sizes

455   (Angilletta Jr et al., 2017). Larger hatching size can be achieved through prolonged

456   development at cooler temperatures during embryonic stages (Forster & Hirst, 2012). It is

457   well known that cold developmental temperatures result in longer incubation periods in many

458   reptiles (Booth, 2006; Dayananda et al., 2016; Downes & Shine, 1999; Elphick & Shine,

459   1998; R. M. Goodman, 2008). Longer developmental time may allow embryos to assimilate

460   yolk nutrients more efficiently thus increasing mass at hatching (Storm & Angilletta, 2007).

461   Indeed, turtle embryos exposed to high temperatures have enhanced mitochondrial

462   metabolism and metabolic enzymic activity which constrains developmental time and

463   reduced overall hatching size (Ji et al., 2003; Sun et al., 2015). Thermal plasticity in

464   embryonic development may be adaptive for lizards born late in the season when nest

465   temperatures are generally colder (Warner & Shine, 2008; While et al., 2015). Indeed, female

466   *L. delicata* have an extended oviposition period (September to February in our population)

467   and nest temperatures during this time can be highly variable in the wild (Cheetham et al.,

468   2011). Heavier weight at emergence could mean that hatchlings are in better condition to

469   compete with lizards that hatched earlier or have sufficient body reserves to survive harsher

470   condtions in more seasonal environments (Downes & Shine, 1999; Gifford et al., 2017;

471   Qualls & Shine, 2000). Understanding how body mass affects survival will be necessary to

472   elucidate the adaptive potential of developmentally plastic responses in the wild.

473

474   *Thermal developmental environments and the evolutionary potential of body mass*

475

476   Adaptative evolutionary responses depend not only on the amount of selection operating on a

477   trait but on also its underlying additive genetic variance (Falconer, 1952; Ghalambor et al.,

478   2007; Hoffmann & Merilä, 1999). Stressful developmental environments are hypothesized to

479   lead to the release of 'cryptic' genetic variation (Fischer et al., 2020; Noble et al., 2019;

480   Rowiński & Rogell, 2017; Wood & Brodie, 2015), possibly increasing the evolutionary

481   potential of a given trait. Higher genetic variation, combined with stronger selection may

482   facilitate rapid evolutionary responses that may allow populations to adapt to novel

483   environments (Hoffmann & Merilä, 1999; Falconer and Mackay 1996). Contrary to these

484   hypotheses, we found no statistical differences in additive genetic variance for mass between

485   our developmental temperature treatments. In fact, heritability for mass was overall quite low

486   echoing heritability values for mass in various animal systems [e.g., bighorn sheep – 0.03 to

487   0.31 (Réale et al., 1999), macaques – 0.39 (Kimock et al., 2019) lizards – 0 to 0.54 – (Martins

488   et al., 2019; Noble et al., 2014)]. It should be noted that decoupling additive genetic variances

489   from other non-genetic variance such as maternal effects requires considerable paternal links

490   in the study design and pedigree (Kruuk, 2004). Indeed, when this variance partitioning is

491   done accordingly, heritability estimates are often low (e.g., Noble et al. 2014). In the case of

492   our study, we found relatively low levels of multiple paternity (<1% of clutches were sired by

493   multiple fathers), as such the number of half-sibs were generally low which may have

494   affected our genomic relatedness matrix and estimates of quantitative genetic parameters.

495

496   Lack of differences in genetic variation between developmental temperature

497   environments support findings from recent meta-analyses. Fisher et al. (2020) assessed the

498   degree to which stressful thermal environments result in the release of genetic variation. They

499   found that these effects manifested in only a third of the studied cases – in mainly clonal

500   organisms (Fischer et al., 2020). Furthermore, of the 25 cases where genetic variance

501   changed across thermal environments there was no consistent direction (i.e., 11 increased and

502   14 decreased under thermal stress). Noble et al. (2019) also showed that the release of

503   'cryptic' genetic variation depends on the study design – studies not able to partition out non-

504   genetic sources of variation supported a release of genetic variation whereas studies that did

505   showed the opposite pattern. As a caveat, defining an environment as stressful or novel is a

506   difficult task which requires detailed knowledge of a given species' past environmental

507   exposure – information that is often unknown (Roelofs et al., 2010). While our incubation

508   temperatures were selected based on temperature extremes of naturally occurring *L. delicata*

509   nests (Cheetham et al., 2011), it is nonetheless possible they were not 'stressful' from an

510   evolutionary perspective. Indeed, egg mortality did not differ across incubation treatments

511   which suggests that lizards from both treatments experienced a similar level of thermal stress

as embryos (the estimate of treatment difference: 0.80 [-0.04 -1.73]). Furthermore, treatment differences may be harder to detect under realistic fluctuating temperature regimes. As such, lizards were not exposed to extreme temperatures over extended periods which might be more important in orchestrating changes in genetic variation (Bonamour et al., 2019). Overall, our results suggest that the thermal extremes experienced by natural nest sites do not modify the evolutionary potential of mass. However this should be interpreted with caution as estimates of quantitative parameters from laboratory studies can differ from wild populations (Sgrò & Hoffmann, 2004; Weigensberg & Roff, 1996).

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

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

Maternal non-genetic contributions to offspring body size are expected to be highest during early life stages and decline as offspring mature, particularly in precocial species (Cheverud, 1984; Wilson, Kruuk, et al., 2005). In accordance with other studies, maternal effects did in fact decline after hatching (Dmitriew et al., 2010; Lindholm et al., 2006; Pick et al., 2016; Wilson, Coltman, et al., 2005; Wilson, Kruuk, et al., 2005). Maternal investment, such as

545 investment in clutch number or egg quality, has been shown to influence hatching size in

546 lizards (Brown & Shine, 2009; Noble et al., 2014; Warner & Lovern, 2014), however, as

547 predicted, these effects dissipated post-hatching (Pick et al., 2016; Réale et al., 1999).

548 Interestingly, maternal contributions increased at a later age and remained moderately low for

549 the remainder of the study. The cause of resurgence in maternal effect variance is unclear,

550 however this pattern may indicate other maternally inherited components such as maternal

551 genetic effects (e.g., mitochondrial genetic variation) that promote variation in body size

552 (Pick et al., 2016). Indeed, variation in mitochondrial function has been linked to an

553 individual's metabolic rate and growth – explaining as much as ~50% of the variation in food

554 intake and growth (Salin et al., 2016, 2019). Therefore, it is likely an important driver of

555 body size variability. Similar to additive genetic variance, resurgence of maternal effects also

556 cooccurred with changes in the shared environment (housing conditions), suggesting that

557 maternal effects on offspring body size is likely to be environmentally driven.

558

559 Traits under strong selection are expected to show low evolutionary potential as selection acts

560 to remove genetic variation. While low evolutionary potential is at least in part due to

561 reduced levels of additive genetic variance, it is also a result of larger proportions of

562 environmental variance. In our study, the environmental component of the phenotype

563 accounted for over 80% of variation in body mass which is in line with values reported in

564 great tits (53 –74%) and soay sheep (70 – 96%) (Noordwijk et al., 1988; Wilson et al., 2007).

565 Interestingly, cool developmental temperatures increased the amount of environmental

566 variance attributed to body mass at an early age. What mechanisms are comprised in this

567 environmental component? Variation in developmental period between developmental

568 temperatures may explain these differences. In many ectotherms, developmental time

569 exhibits a nonlinear reaction norm with temperature (Marshall et al., 2020; Noble et al.,

570 2018).This means that developmental time decelerates with temperature following an

571 negative exponential function. As a result, hot incubated lizards are more constrained in their

572 development time compared to lizards that were reared a cooler temperature. In actual fact,

573 the cold developmental temperature treatment had much greater variance in incubation

574 duration. With a longer incubation period, embryos can maximise the yolk resources left by

575 their mothers which can vary considerably within clutch (Wallace et al., 2007). Our results

576 suggest that thermodynamic effects of development time can give rise greater environmental

577 heterogeneity in hatching mass and may affect potential for evolution at early life stages.

Conclusion

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

Author contributions

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

Data Archiving

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

Competing Interests

The authors declare no competing interests.

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