Heritability and developmental plasticity of growth in an oviparous lizard

Fonti Kar[1], Shinichi Nakagawa[1,2], Daniel W.A. Noble[1,3]
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

Abstract
Selective processes act on phenotypic variation although the evolutionary potential of a 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 (n_{hot} = 125, n_{cold} = 136) over 16 months ($n_{observations}$ = 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

37 # Introduction

38 Developmental plasticity plays a key role in generating phenotypic variation
39 (Ghalambor *et al*, 2007; Noble *et al*, 2018; West-Eberhard, 2003). The complex interplay
40 between an individual's genotype, and the developmental environment in which that
41 genotype finds itself, means that a range of different phenotypes can arise (Monaghan, 2008;
42 West-Eberhard, 2003). Phenotypic changes resulting from distinct early life experiences can
43 have persistent effects on individual fitness (Monaghan, 2008; Noble *et al*, 2018). For many
44 oviparous (egg-laying) organisms, early life stages are particularly sensitive periods because
45 many species do not provide parental care that would shelter embryos from environmental
46 insults. Changes induced by developmental environments may result in a better match
47 between the adult phenotype and the subsequent selective environment. However, in some
48 cases, maladaptive phenotypes can arise if there is a mismatch between later-life
49 environments and those experienced early in development (Beaman *et al*, 2016; Ghalambor
50 *et al*, 2007). Regardless, phenotypic plasticity represents a promising immediate solution for
51 threatened populations by allowing them to better track adaptive optima and persist providing
52 the population experiences environmental conditions they have experienced in the past
53 (Beldade *et al*, 2011; Chevin, 2010; Noble *et al*, 2019; West-Eberhard, 2003). Understanding
54 the consequences of developmental environments on phenotypes and fitness is therefore
55 critical to predict how populations will survive in stressful conditions (Botero *et al*, 2015;
56 Reed *et al*, 2010).
57
58 A population's capacity to evolve depends not only on the strength of selection but
59 also on the underlying standing genetic variation (Lynch and Walsh, 1998). It has long been
60 recognised that both selection and genetic variation change across environments (Falconer
61 and Mackay, 1996). As such, a great deal of effort has been put towards understanding the
62 circumstances under which genetic variation may change with the environment and the
63 magnitude of those changes (Charmantier and Garant, 2005; Fischer *et al*, 2020b; Hoffmann
64 and Merilä, 1999; Noble *et al*, 2019; Rowiński and Rogell, 2017; Wood and Brodie, 2015).
65 Genetic variance in novel environments may increase due to relaxation of selection pressures
66 combined with higher mutation rates (Hoffman and Parsons, 1991; Hoffmann and Merilä,
67 1999). An increase in genetic variance is also expected when buffering mechanisms
68 breakdown triggering a release of 'cryptic genetic variation' (Paaby and Rockman, 2014).
69 However, other mechanisms, such as low cross-environment genetic correlations or
70 condition-dependence of gene expression can also affect the amount of genetic variance in
71 different environments (Charmantier and Garant, 2005; Coltman *et al*, 2001). Under the same
72 selection pressure, should genetic variation change with the environment, the speed of
73 evolutionary responses can be impacted making it potentially difficult to predict genetic
74 adaptation.
75
76 Comparative studies have shown that the environmental impacts on genetic variance
77 is not straightforward (Charmantier and Garant, 2005; Hoffmann and Merilä, 1999; Rowiński
78 and Rogell, 2017). In lab studies, elevated developmental stress has been shown to increase
79 the heritability of morphological traits (Hoffmann and Merilä, 1999), whereas wild, non-
80 domestic populations tend to have higher heritability in favourable environments
81 (Charmantier and Garant, 2005). Lack of consensus may be related to increased
82 environmental heterogeneity in wild populations, making them more difficult to compare
83 with lab studies. It has been suggested that responses to different developmental stressors
84 (e.g. heat shock vs. starvation) may be associated with disparate patterns of gene expression
85 making broad comparisons more variable (Charmantier and Garant, 2005; Dahlgaard and
86 Hoffmann, 2000). Importantly, environmental comparisons of heritability have been

87 criticised because they mask changes in the relative contributions of non-genetic and genetic
88 variance (Hansen *et al*, 2011; Rowiński and Rogell, 2017). For example, a meta-analysis
89 found that heritability of life history traits, which have been argued to be more important to
90 fitness, did not change between control and stressful conditions (Rowiński and Rogell, 2017).
91 The same pattern was observed for morphological traits (Fischer *et al*, 2020b). Upon closer
92 inspection, both additive genetic and environmental variance of life history traits increased
93 under stressful conditions whereas the opposite was true for morphological traits (Rowiński
94 and Rogell, 2017). The expression of genetic variation under different developmental
95 environments can thus influence the evolutionary potential of fitness related traits.
96
97 Body size is fundamental to fitness and is both heritable and environmentally
98 responsive (Noordwijk *et al*, 1988; Stillwell and Fox, 2009). Developmental environments,
99 such as temperature and nutritional stress can drive substantial variation in body size, largely
100 through shifts in how organisms grow (Eyck *et al*, 2019; Noble *et al*, 2018). Maternal
101 investment in offspring are also important sources of body size variation (Noble *et al*, 2014;
102 Wilson and Réale, 2006). Variation among mothers in egg investment, nest site selection or
103 timing of birth (Mitchell *et al*, 2018; Shine and Harlow, 1996; Uller and Olsson, 2010) are
104 expected to contribute the most to offspring body size early in development (Mousseau and
105 Fox, 1998). However, these effects have been shown to decline with age as maternal
106 investment subsides (Krist, 2010; Wilson *et al*, 2005b). Additionally, environmental factors
107 such as shared habitats or long-term seasonal effects can also account for a substantial
108 proportion of variability in body size (Kruuk, 2004). For example, permanent environmental
109 effects that varied across years explained 26% – 35% of body size variation in bighorn sheep
110 (Réale *et al*, 1999). Similarly, 56% of variation in body mass was attributed to nest boxes
111 shared among siblings in blue tit chicks (Charmantier *et al*, 2004). As such, the various
112 sources that influence body size variation (genetic, environmental, maternal) are predicted to
113 vary across ontogeny and temporal approaches are needed in order to evaluate age-specific
114 evolutionary potential of body size – higher genetic variation at a given age would imply that,
115 if selection were to operate, it would be more likely to lead to an evolutionary response.
116
117 Here we investigated the impact of developmental temperature on growth and mass in
118 an oviparous skink (*Lampropholis delicata*) – two traits that are critically important to
119 fitness. We also test how developmental environments affect evolutionary potential in these
120 traits. Growth trajectories ($n_{observations}$ = 3,002) for lizards that hatched from two incubation
121 treatments (n_{hot} = 125, n_{cold} = 136), were measured over the first 16 months of life (lifespan is
122 ~3-4 years). Using 8,433 single nucleotide polymorphic (SNP) markers, we derived a
123 genomic relatedness matrix to estimate quantitative genetic parameters. Using these data, we
124 address two key questions: 1) How does developmental temperature affect the rate and shape
125 of growth trajectories (initial mass, growth rate and curvature of growth trajectory)? and 2)
126 How does developmental temperature affect genetic and non-genetic sources of phenotypic
127 variance across age? According to the 'temperature-size rule', we expect lizards experiencing
128 cold developmental temperatures to have larger initial masses and slower growth rates –
129 possibly resulting in lizards reaching sexual maturity at a later age compared to lizards
130 experiencing hot developmental temperatures (Angilletta Jr *et al*, 2017). In addition, we
131 predicted greater amount of genetic variance under higher developmental temperatures, after
132 controlling for non-genetic sources of variance, as higher temperatures may release 'cryptic
133 genetic variation' (Rowiński and Rogell, 2017). We expected maternal effects and permanent
134 environment effects to manifest early in development and dissipate over time.

135 # Materials and Methods

136 *Lizard collection and husbandry*

137 We established a breeding colony of adult *L. delicata* ($n_{females}$ = 144, n_{males} = 50) using wild
138 individuals collected across five sites throughout the Sydney region between August and
139 September 2015. While we collected from five different sites in Sydney, biogeographic data
140 suggests high gene-flow across the Sydney region (Chapple *et al*, 2013). Using a half-sib
141 breeding design, we paired three females with a single male in opaque plastic enclosures
142 measuring 35cm × 25cm × 15cm (L × W × H). We choose a paternal half-sib design
143 because maternal half-sibs are difficult to generate given that females in our colony only
144 produced a single clutch in a year (see below). Enclosures were kept under UV lights
145 (12L:12D) in a temperature-controlled room set to 24ºC. Lizards were given access to a heat
146 lamp that elevated temperatures to between 28-32 ºC. Each enclosure was lined with
147 newspaper and lizards had constant access to water. Tree bark was used as refuge. Adult
148 lizards were fed medium sized crickets *ad libitum* (*Acheta domestica*) dusted with calcium
149 powder and multi-vitamin every two days. From the beginning of the egg laying season
150 (October of each year), we replaced newspaper lining with garden potting mix and placed an
151 opaque plastic box (12 cm × 17.5 cm × 4.3 cm) containing moistened vermiculite in each
152 enclosure for females to oviposit their eggs. During this time, enclosures were sprayed with
153 water every second day to maintain a relatively humid environment. From October to
154 November, egg boxes were checked every day. Tail tissue samples (~1 mm) were taken from
155 adults that were from enclosures producing eggs for DNA extraction (see below). All tissues
156 were stored in 70% ethanol. Animal collection was approved by the New South Wales
157 National Parks and Wildlife Service (SL101549) and all procedures were approved by the
158 Macquarie University Ethics committee (ARA 2015/015) and University of New South
159 Wales Animal Care and Ethics committee (ACEC 15/51A).
160

161 *Developmental Temperature Manipulations*

162 Eggs were collected between October to March, over two reproductive seasons from
163 2016 and 2017. As soon as eggs were found, they were weighed using a digital scale to the
164 nearest 0.01g (Ohaus Scout SKX123). We also measured egg length (distance between the
165 furthest points along the longest axis of the egg) and egg width (distance between the widest
166 points along the axis perpendicular to the longest axis of the egg) using digital callipers to the
167 nearest 0.01mm. Following measurements, each egg was placed in a plastic cup (80ml)
168 containing three grams of vermiculite and four grams of water. Each cup was then covered
169 using cling wrap and secured using an elastic band. We used a split-clutch design where eggs
170 from single clutch were pseudo-randomly assigned to one of two developmental temperature
171 treatments. We used two incubators to precisely control the temperature of eggs (LabWit,
172 ZXSD-R1090). The 'hot' treatment was exposed to a mean temperature of 29ºC whereas the
173 'cold' treatment was exposed to a mean temperature of 23ºC. Both incubators fluctuated +/-
174 3ºC over a 24-hour period around these mean temperatures to simulate natural nest site
175 temperature variability. These treatments represent the temperature extremes of natural nest
176 (~ 2 standard deviations above and below the mean - ~27 ºC) sites for *L. delicata* (Cheetham
177 *et al*, 2011), and this species does not have temperature-sex determination that would
178 possibly bias sex ratios in these two treatments. We chose these temperatures because we
179 expect thermal environments to become more extreme and variable in the future making it of
180 interest in knowing how the expression of genetic variation is likely to manifest in abnormal
181 thermal conditions. While it is challenging to determine if an environment is 'stressful' or not
182 without data on egg mortality (Roelofs *et al*, 2010), we viewed this as atypical of what is
183 commonly encountered in nature. Egg cups were rotated within each incubator weekly to

184 avoid uneven heat circulation within incubators. Incubators were also checked daily for
185 hatchlings.
186

187 *Quantifying Growth Rate*

188 Newly emerged hatchlings were weighed to the nearest 0.01g and a small tail tip
189 clipping (~2mm) was taken for genetic analyses. Ventral photographs were taken for digital
190 measurement (Nikon Coolpix A900). For the first two months, photographs of hatchlings
191 were taken approximately every 14 days. After which, hatchlings were photographed at
192 approximately a 35-day interval. From six months onwards, we manually measured hatchling
193 SVL using a clear ruler to the nearest ~0.5mm. We also recorded the mass of the individual
194 each time photographs or SVL measurements were taken. Growth measurements continued
195 until we had approximately 16 measures per individual (mean = 11.5 , SD = 4.71). By the
196 end of the study, the mean age for hot incubated lizards was 335.82 (range: 0 – 711) and for
197 cold incubated lizards it was 384.8 (range: 0 – 707) which is approximately 25 – 50% of their
198 total lifespan (Chapple *et al*, 2014). From the photographs, we extracted snout-vent-length
199 (SVL; from tip of snout to the beginning of the cloaca opening) using ImageJ software (Rueden
200 *et al*, 2017). For the first initial nine months, hatchlings were housed individually in opaque
201 plastic enclosures (32.3cm x 18.5cm x 6cm) lined with newspaper. Hatchlings were fed the
202 same number of crickets every second day and had constant access to a tree bark refuge and
203 water. Hatchling enclosures were placed in a temperature control room under the same
204 conditions as described above for the adult colony. For logistical reasons, at approximately
205 nine months, hatchlings were housed in groups of five in opaque bins with the same
206 measurements as the adult enclosures. We pseudo-randomised individuals to each shared
207 enclosure while maintaining a similar number of individuals from each treatment. Social
208 housing conditions may result in additive genetic and maternal effects becoming more
209 apparent because of competition and social stress that may drive greater variation among
210 individuals. Our modelling approaches estimate changes in variance components across age
211 and should be able to detect any changes brought about by the release of variation (see
212 below).

213 *Genomic Relatedness Matrix*

214 We derived a genomic relatedness matrix (GRM) using single nucleotide
215 polymorphism (SNP) genotypes for all 262 offspring with growth data (132 putative parents;
216 $n_{females}$ = 69, n_{males} = 63). While our half-sib breeding design allowed us to assign parentage to
217 derive a pedigree, high levels of sperm storage and low levels of multiple paternity (94% of
218 offspring within a clutch had been sired by a single male) meant our pedigree had low
219 resolution to effectively estimate additive genetic variation. Recent studies have shown that
220 GRM derived from SNPs have low error rates (<0.3%) and are able to reconstruct pedigree
221 relationships when at least 200 SNP loci are used (Bérénos *et al*, 2014; Huisman, 2017).
222 Moreover, both relatedness and heritability values estimated from a GRM can be very similar
223 to those inferred using a pedigree (Bérénos *et al*, 2014; Huisman, 2017). GRMs may in fact
224 provide more accurate estimates of genetic relatedness among individuals than was is typically
225 assumed from pedigrees. Single nucleotide polymorphism libraries were designed and animals
226 genotyped using DArTseq™ (Diversity Arrays Technology) methods. For more details on
227 DNA extraction and SNP genotyping see ESM.
228 Prior to deriving our GRM, we filtered our SNPs using the R package *dartR* (Gruber et
229 al., 2018). We filtered loci based on various metrics in the following order: 1) read depth (8 –
230 40); reproducibility (> 0.996); call rate by loci (> 0.97) and then by individual (> 0.80);
231 monomorphic loci; minor allele frequencies (> 0.02); Hamming Distance among loci (> 0.25)
232 and Hardy Weinberg Equilibrium. This clean-up process resulted in a dataset of 8,438 loci with

233 an average call rate of 98.5% (see ESM and provided code). Using these 8,438 loci we derived
234 a GRM, which describes the proportion of the genome that is identical by descent (VanRaden,
235 2008). We calculated a GRM for all hatchlings using the *snpReady* R package (Granato *et al*,
236 2018) following methods described by (VanRaden, 2008):
237

238 $$GRM = \frac{ZZ'}{2\sum p_i(1-p_i)}$$

239
240 where Z is the centered squared matrix of SNP genotypes of all individuals. This is calculated
241 from a matrix of where heterozygote SNP genotypes (AT) were coded as 0, homozygote
242 genotypes for the SNP allele (AA) were coded as 1 and homozygotes for the original allele
243 (TT) were coded as -1. p_i is the frequency of the second locus at locus position *i.* The
244 denominator scales the GRM matrix so that the values approximate a relatedness matrix
245 derived from a pedigree.

246 Statistical Analyses

247 All analyses were performed using *R* (Team, 2023). We checked the data for potential
248 input errors using histograms, scatterplots, and Cleveland plots. We fitted Bayesian linear
249 mixed effects models (LMM) in *brms* which interfaces with Stan (Bürkner, 2017; Gelman *et*
250 *al*, 2015). Mass was log-transformed, and age was z-transformed. For all models we ran 6000
251 iterations with a burn in of 1000, sampling from the posterior distribution every 10 iterations.
252 We ensured proper mixing by inspecting trace plots and checked that scale reduction factors
253 were less than 1.01. We report posterior means and 95% credible intervals for all parameters
254 throughout.

255 *Impact of Developmental Temperature on Additive Genetic Variance and Maternal Effects*
256 *Across Age*
257 First, we tested whether developmental temperature influenced the overall heritability
258 of mass and the relative contributions of additional variance components across age. (i.e.,
259 permanent environmental and maternal effect variance). For each treatment group, we fitted
260 intercepts only in the fixed effects with random intercepts for additive genetic variance (*G*),
261 maternal effects (*M*) and permanent environmental effects (*PE*) as we had repeated measures
262 of the same individuals (Wilson *et al*, 2010). The model also estimated residual variance (*R*).
263 We included our GRM to estimate additive genetic variation. Overall heritability (h^2) of mass
264 at a given age was calculated as:

265 $$h^2 = \frac{G_I}{(V_T)}$$

266 Where, V_T is the sum of the variance components in the model (which could vary
267 depending on the model best supported. We used model selection to determine the most
268 appropriate random effects structure for our data as we had no *a priori* knowledge of what (or
269 how) variance components change with age (Wilson and Réale, 2006). We fitted models with
270 varying complexity in their random effects and used Leave-One Out (LOO) cross validation
271 to compare model fit and select the model with best predictive performance. Using LOO, the
272 expected log pointwise predictive density for a model can be calculated, and these can be
273 used to compare model performance – by calculating the difference between expected log
274 pointwise predictive density of various models. Differences of less than 4 mean that models
275 are comparable (Sivula *et al*, 2020). For differences greater than 4, then the standard error
276 (SE) of the differences in expected log pointwise predictive density should be compared. If

277 the standard error of the differences are much larger than the point estimate of the difference
278 then the model closer to zero is preferred (Sivula *et al*, 2020). The difference in LOO
279 between models can be used for model selection, and in our case, gave similar results to
280 model selection using Watanabe–Akaike Information Criterion (WAIC) (Table S1). We fitted
281 random intercepts and random slopes by including either a linear age term or both linear and
282 quadratic age terms to partition variance across age. Three models were equally supported,
283 the first included a random linear and quadratic slope for *G* and *M* and *PE*. (Table S1) and the
284 second included a random linear and quadratic slope for *G* and *M,* respectively, and a random
285 intercept for *PE* (Table S1). To avoid overfitting, we selected the more parsimonious model
286 and used this random effect structure for the remaining analyses unless stated otherwise. The
287 same top model selected was similar no matter whether we used the full data or only the data
288 subset for individuals incubated in cold or hot developmental treatments.
289 Residual variance may be conflated with estimates of other variance components if it
290 changes over time (heterogenous variance) and is not properly accounted for. We therefore
291 explicitly modelled residual variance to verify if this was the case and compared homogenous
292 and heterogenous residual variance models using WAIC. We fitted two models, both of
293 which had the same fixed and random effects structure as Model 7 described above. The first
294 model had homogenous residual variance whereas in the second model we modelled residual
295 variance with a linear slope thereby allowing it to vary with age. The model with
296 heterogenous variance was best supported (Table S2), we therefore modelled heterogenous
297 variance in all subsequent models unless stated otherwise.
298 To test for treatment differences in variance components, we subset data for each
299 treatment group and fitted an intercept-only model with our best supported random effect
300 structure (Model 7) and heterogenous residual variance. We estimated a genetic variance-
301 covariance matrix for each treatment (G), where the diagonal elements represent the additive
302 genetic variances for the intercept (G_I), slope (G_S) and the quadratic (G_C) across age. The off-
303 diagonal elements are the additive genetic covariances between the growth curve parameters,
304 for example, $Cov_{I,C}$ is the additive genetic variance between the intercept and the quadratic
305 slope.

306 $$G = \begin{bmatrix} G_I & Cov_{I,S} & Cov_{I,C} \\ Cov_{I,S} & G_S & Cov_{S,C} \\ Cov_{I,C} & Cov_{S,C} & G_C \end{bmatrix}$$

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

309 $$M = \begin{bmatrix} M_I & Cov_{I,S} & Cov_{I,C} \\ Cov_{I,S} & M_S & Cov_{S,C} \\ Cov_{I,C} & Cov_{S,C} & M_C \end{bmatrix}$$

310 For each treatment group, we then calculated additive genetic variance at a given age G_x
311 using the random slope terms and their covariances following (Gavrilets and Scheiner, 1993;
312 Schielzeth and Nakagawa, 2022):

313 $$G_x = G_I + (x^2.G_S) + (x^4.G_C) + (2x.Cov_{I,S}) + (2x^2.Cov_{I,C}) + (2x^3.Cov_{S,C})$$

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

318 $$h_x^2 = \frac{G_x}{(G_x + M_x + PE_I + R_I)}$$

319 As the mean body mass increases over time, the variance may also increase
320 concurrently due to scale effects and potentially bias estimates of quantitative genetics
321 parameters (Wilson *et al*, 2005b). We therefore calculated coefficients of variation (CV)
322 across age for each variance component by dividing variance by the predicted mean mass at a
323 given age. Interpretations using CV estimates did not change our overall conclusions for
324 additive genetic variance or maternal effects, we therefore present the raw estimates of each
325 variance component below (See ESM).
326

327 *The Influence of Developmental Temperature on Growth Trajectories*
328 To test how developmental temperatures affect average growth trajectories, we also
329 fitted three models that varied in their fixed effect structure to determine how developmental
330 temperatures affect: 1) initial mass (intercept of curve), 2) linear rate of growth (linear slope)
331 and 3) curvature of the growth trajectory (quadratic term). We also wanted to test for
332 treatment differences in age at which lizards reach their maximum mass by solving for the
333 maxima of quadratic regression equation. We fit mass as the response accounting for the
334 same random effects described above. The first model included the main effect of
335 developmental temperature and the linear and quadratic term for age (Table S1). The other
336 two models differed in their interaction terms between developmental temperature with age
337 and age^2 (Table 2, S3). We then compared WAIC values to select the best model for our data
338 that explained changes in mass across age between the two developmental temperature
339 treatments (Table 1).
340

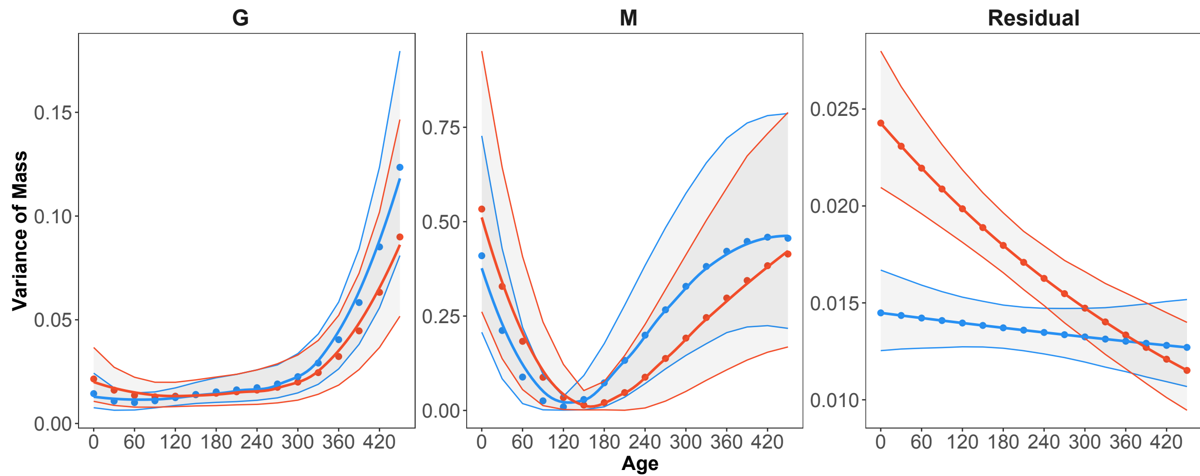
341 # Results

342 Over two years, we collected 3,002 observations of mass data for a total of 261
343 individuals (n_{hot} = 125, n_{cold} = 136). On average, the incubation period for the 'hot'
344 treatment was 29.36 days (SD = 2.17, range = 15 - 49) days and 48.48 days (SD = 4.18, range
345 = 25 - 56) for the 'cold' treatment. The average age for hot incubated lizards was 335.82
346 (range: 0 – 711) and for cold incubated lizards it was 384.8 (range: 0 – 707). On average, a
347 lizard had 11.5 measurements (SD = 4.71).

348

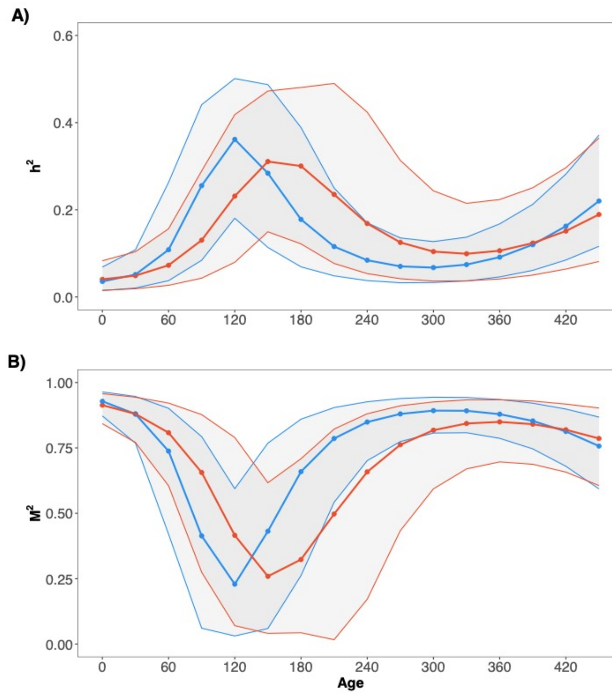
349 *The influence of developmental temperature on genetic and non-genetic variance across age*

350 Models that included random slopes (linear and quadratic) of age for G and M were far moe
351 superior than models without (Table S1). Treatment groups did not differ in how the relative
352 contributions of G and M changed with age as their 95% credible intervals overlapped (Fig.
353 1). Additive genetic variance remained relatively low and constant upon emergence until
354 approximately nine months of age, after which it increased rapidly (Fig. 1). Maternal effects
355 decreased sharply upon hatching and dropped to the minimum at approximately six months
356 before it increased again (Fig. 2). There were some differences among developmental
357 treatments in how residual variance changed with age (Fig. 1). Residual variance in cold
358 incubated lizards had a much lower intercept compared to hot incubated lizard however their
359 residual variance converged by eight months of age (Fig. 1).



360
361 **Figure. 1** Scatterplot showing how additive genetic variance (*G*), maternal effects (*M*),
362 residual variance changed with age for the hot developmental treatment ($n_{lizards}$ = 125, red)
363 and the cold developmental treatment (n = 136, blue). Points represent posterior means, thin
364 lines represent the 95% credible intervals, thick lines represent the mean for each treatment
365 group.

366 We investigated whether increases in average mass over time affected variance
367 estimates due to scaling effects between the mean and variance. However, we found that the
368 CV of *G* and *M* similar changes across age to raw variance estimates suggesting that changes
369 in variance were not the result of increasing mean body mass with age (Fig. S1 & S2).
370 After accounting for heterogenous residual variance, we found no treatment
371 differences in heritability, or the proportion of variance explained by maternal effects (M^2)
372 (Fig. 2). Heritability was moderate to low during early growth in *L. delicata* and only began
373 increasing at around 120 days and stayed around an $h^2 \sim 0.15$ (95% CI: 0.06 – 0.28, although
374 it decreased slightly between 240 and 360 days - Fig. 2). As predicted M^2 was a significant
375 contributor to variance in mass ($M^2 \sim 0.72$, 95% CI: 0.50 – 0.88) and decreased soon after
376 hatching, however it increased again from six months of age (Fig. 2). The G and M matrices
377 for each treatment group are presented in Table S4-S5.



378
379
380 **Figure 2.** Heritability (*h^2*, **A**) and the proportion of total variance explained by maternal
381 effect variance (*M^2*, **B**) across age (days) for the hot developmental treatment ($n_{lizards}$ = 125,
382 red) and the cold developmental treatment ($n_{lizards}$ = 136, blue). Points represent estimates
383 generated from the posterior distribution of the variance-covariance matrix, thin lines
384 represent the 95% credible intervals, thick lines represent the mean for each treatment group.

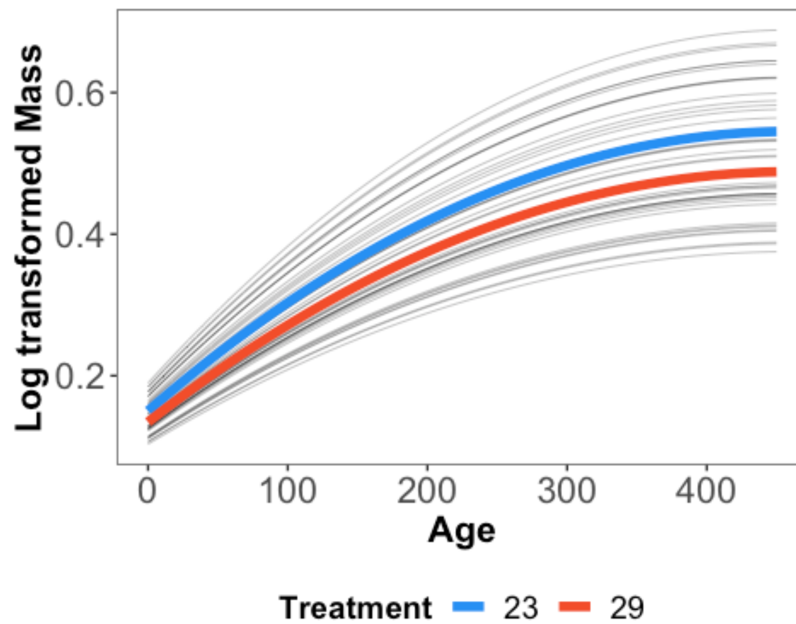
385 *Developmental plasticity in growth trajectories in response to temperature*
386 While the model containing an interaction between treatment and quadratic age was
387 best supported, the improvement in LOO value was marginal (Table 1), with the top model
388 explaining a substantial amount of variation in mass (R^2 = 0.95, 95% CI: 0.94 to 0.95). Mass
389 increased significantly over age with growth not differing significantly between the
390 temperature treatments (both for the linear and curvature parameters in the growth curve, see
391 full model in Table 2). Irrespective of treatment, lizard mass increased by 1.65 g for every 1
392 SD unit increase in age.

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

Formula of Fixed Effects	LOO	$\Delta ELPD$	Std. Error $\Delta ELPD$
Treatment + Age + Age^2 + Treatment × Age^2	-3,245	0.00	0.0
Treatment + Age + Age^2 + Treatment × Age + Treatment × Age^2	-3,244	-0.65	1.9
Treatment + Age + Age^2	-3,240	-2.36	2.6
Treatment + Age + Age^2 + Treatment × Age	-3,235	-4.74	2.4

397
398 Developmental temperature did influence hatching mass (Table 2, Fig. 3). Lizards
399 from the ‘cold’ treatment were on average 0.03 g (0.011g – 0.051g) heavier compared to

400 lizards from the 'hot' treatment (Table 2). *G* and *M* matrices from this model, along with
401 other variance components, are presented in Table S6.



402

403 **Figure 3.** Model predictions of log-transformed mass over age from the two developmental
404 temperatures. We randomly subset 40 lizards (20 from each treatment) to plot their individual
405 growth curves. Points represent mean estimates for each lizard from the hot developmental
406 treatment (hot) and the cold developmental treatment (blue). Thick lines represent average
407 growth curve for each treatment. Faint grey lines are each individual's growth curve. Model
408 predictions were generated from the full model where interaction terms between treatment
409 and both the linear component and quadratic component were included.
410
411 **Table 2** Coefficient estimates from full model testing the effects of developmental treatment
412 on mass and how mass changes with age. Bolded estimates are significantly different from
413 zero. n_{obs} = 2,926. Age measured in days was z-transformed (mean = 361.34, SD = 185.16).
414 *G* and M matrices for this model are presented in Table S6.

Parameter	Estimate	Lower	Upper
Intercept	**-0.998**	**-1.036**	**-0.961**
Treatment (Hot)	**-0.085**	**-0.118**	**-0.056**
Age	**0.496**	**0.458**	**0.534**
Age^2	**-0.189**	**-0.223**	**-0.156**
Treatment × Age	0.012	-0.019	0.041
Treatment × Age^2	0.025	-0.006	0.055

415

416 # Discussion

417 Early development at hot temperatures resulted in smaller body sizes compared to
418 development at cold temperatures. Growth trajectories, however, were not significantly
419 impacted by early thermal environments – lizards from both temperatures grew at the same
420 rate despite cold animals remaining larger throughout life. We found low to moderate
421 heritability (h^2~ 0.15) and high maternal effects (M^2~0.70) with both varying across age. As

422 we predicted, maternal effects on offspring mass declined in the first few months, presumably
423 because maternal non-genetic contributions were less influential on mass over time.
424 Unexpectedly, maternal effects increased again at approximately six months possibly from
425 maternal genetic factors affecting mass. In contrast, heritability increased with age, peaking
426 around 130-180 days before decreasing slightly. Part of these changes reflected differences in
427 the residual variance that changed across ag.

428 *Thermal developmental plasticity in growth*
429
430 In ectotherms, temperature plays a pervasive role in phenotypic development (Eyck *et al*,
431 2019; Noble *et al*, 2018; O'Dea *et al*, 2019; While *et al*, 2018). While we found that hot
432 lizards were smaller than cold incubated lizards at hatching, we did not show that growth rate
433 differed between developmental temperatures. Some studies have reported increases in
434 growth at higher incubation temperatures (De Jong *et al*, 2023; Elphick and Shine, 1999;
435 Hare *et al*, 2004; Verdú-Ricoy *et al*, 2014), while others have found either the opposite result
436 or no differences at all (Andrews *et al*, 2000; Goodman, 2008). The directionality of change
437 is highly variable, even among studies of the same species (e.g., Bassiana dupreyi, Elphick
438 and Shine, 1998; Elphick and Shine, 1999; Flatt *et al*, 2001; Telemeco *et al*, 2010), and we
439 had more data across life compared with many other studies. Lack of generality may be
440 related to how growth is statistically modelled (e.g., polynomial regression versus Von
441 Bertalanffy growth models). In addition, very few studies account for individual variation in
442 hatching mass or growth trajectories. We emphasise the importance of partitioning
443 confounding sources of variance such as individual or clutch effects as they can misconstrue
444 conclusions about developmental impacts on later life phenotypes. Moreover, future studies
445 should make use of all repeated measures of mass instead of averaging across individuals as
446 the former approach not only increases statistical power but also provides more accurate
447 estimates of growth.
448
449 Consistent with other squamates, we found that lizards from the cold incubation treatment
450 attained higher hatching mass compared to their hot counterparts because they were born
451 heavier (Dayananda *et al*, 2016; Downes and Shine, 1999; Flatt *et al*, 2001; Goodman *et al*,
452 2013). These results support the temperature-size-rule whereby organisms reared in cold
453 temperatures tend to have larger body sizes (Angilletta Jr *et al*, 2017). Larger hatching size
454 can be achieved through prolonged development at cooler temperatures during embryonic
455 stages (Forster and Hirst, 2012). It is well known that cold developmental temperatures
456 results in longer incubation periods in many reptiles (Booth, 2006; Dayananda *et al*, 2016;
457 Downes and Shine, 1999; Elphick and Shine, 1998; Goodman, 2008). Longer developmental
458 time may allow embryos to assimilate yolk nutrients more efficiently thus increasing mass at
459 hatching (Storm and Angilletta, 2007). Indeed, turtle embryos exposed to high temperatures
460 have enhanced mitochondrial metabolism and metabolic enzymic activity which constrained
461 developmental time and reduced overall hatching size (Ji *et al*, 2003; Sun *et al*, 2015).
462 Thermal plasticity in embryonic development may be adaptive for lizards born late in the
463 season when nest temperatures are generally colder (Warner and Shine, 2008; While *et al*,
464 2015). Indeed, female *L. delicata* have an extended oviposition period (September to
465 February in our population) and nest temperatures during this time can be highly variable in
466 the wild (Cheetham *et al*, 2011). Heavier weight at emergence may result in hatchlings that
467 are in better condition to compete with lizards that hatched earlier or have sufficient body
468 reserves to survive harsher conditions in more seasonal environments (Downes and Shine,
469 1999; Gifford *et al*, 2017; Qualls and Shine, 2000). Understanding how body mass affects

470 survival will be necessary to elucidate the adaptative potential of developmentally plastic
471 responses in the wild.
472

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

475 Adaptative evolutionary responses depend not only on the amount of selection operating on a
476 trait but on also its underlying additive genetic variance (Falconer, 1952; Ghalambor *et al*,
477 2007; Hoffmann and Merilä, 1999). Stressful developmental environments are hypothesized
478 to lead to the release of 'cryptic' genetic variation (Fischer *et al*, 2020b; Noble *et al*, 2019;
479 Rowiński and Rogell, 2017; Wood and Brodie, 2015), possibly increasing the evolutionary
480 potential of a given trait. Higher genetic variation, combined with stronger selection may
481 facilitate rapid evolutionary responses that may allow populations to adapt to novel
482 environments (Falconer and Mackay, 1996; Hoffmann and Merilä, 1999). Contrary to these
483 hypotheses, we found no statistical differences in additive genetic variance for mass between
484 our developmental temperature treatments. In fact, heritability for mass across age in *L*
485 *delicata* was generally low (ranging between 0.04 – 0.36), echoing heritability values for
486 mass in various animal systems [e.g., bighorn sheep – 0.03 to 0.31 (Réale *et al*, 1999),
487 macaques – 0.39 (Kimock *et al*, 2019) lizards – 0 to 0.54 – (Martins *et al*, 2019; Noble *et al*,
488 2014), red squirrels – 0.10, (McAdam *et al*, 2002)]. It should be noted that decoupling
489 additive genetic variances from other non-genetic variance such as maternal effects requires
490 considerable paternal links in the study design and pedigree (Kruuk, 2004). Indeed, when this
491 variance partitioning is done accordingly, heritability estimates are often low (Noble *et al*,
492 2014).
493

494 The lack of difference in genetic variation between developmental temperatures
495 environments support findings from recent meta-analyses. Fisher et al. (2020a) assessed the
496 degree to which stressful thermal environments result in the release of genetic variation. They
497 found that these effects manifested in only a third of the studied cases – in mainly clonal
498 organisms (Fischer *et al*, 2020a). Furthermore, of the 25 cases where genetic variance
499 changed across thermal environments there was no consistent direction (i.e., 11 increased and
500 14 decreased under thermal stress). Noble et al. (2019) also showed that the release of
501 'cryptic' genetic variation depends on the study design – studies not able to partition out non-
502 genetic sources of variation supported a release of genetic variation whereas studies that did
503 showed the opposite pattern. As a caveat, defining an environment as stressful or novel is a
504 difficult task which requires detailed knowledge of a given species' past environmental
505 exposure – information that is often unknown (Roelofs *et al*, 2010). While our incubation
506 temperatures were selected based on temperature extremes of naturally occurring *L. delicata*
507 nests (Cheetham *et al*, 2011), it is nonetheless possible they were not 'stressful' from an
508 evolutionary perspective. Indeed, egg mortality did not differ across incubation treatments
509 which suggests that lizards from both treatments experienced a similar level of thermal stress
510 as embryos (the estimate of treatment difference: 0.80 [-0.04 -1.73]). Furthermore, treatment
511 differences may be harder to detect under realistic fluctuating temperature regimes. As such,
512 lizards were not exposed to extreme temperatures over extended periods which might be
513 more important in orchestrating changes in genetic variation (Bonamour *et al*, 2019). Overall,
514 our results suggest that the thermal extremes experienced by natural nest sites do not modify
515 the evolutionary potential of mass. However this should be interpreted with caution as
516 estimates of quantitative parameters from laboratory studies can differ from wild populations
517 (Sgrò and Hoffmann, 2004; Weigensberg and Roff, 1996).
518

519 *Ontogenetic changes in genetic and non-genetic contributions to body mass*
520
521 Genetic contributions to body size are expected to vary throughout ontogeny (Lynch and
522 Walsh, 1998). Selection pressures on body size are likely to increase at critical life stages,
523 such as at birth or at sexual maturation, thereby reducing genetic variance at certain ages
524 (Rollinson and Rowe, 2015). On the contrary, we found that additive genetic variance of
525 mass was very low upon hatching but slowly increased to a maximum around 120-180 days
526 before stabilising around ~0.15 by the end of the first year. Changes in heritability across age
527 that we observed parallels similar findings seen in big horn sheep (Réale *et al*, 1999), Soay
528 sheep (Wilson *et al*, 2007) and ladybird beetles (Dmitriew *et al*, 2010). While the underlying
529 cause of changes in heritability in mass in our lizards is not well established, it coincided with
530 changes in the social environment (shared housing). This suggests that perhaps competition
531 for resources (basking sites or food) may orchestrate changes in genetic variation (Dmitriew
532 *et al*, 2010; Hoffmann and Merilä, 1999).
533
534 Maternal non-genetic contributions to offspring body size are expected to be highest during
535 early life stages and decline as offspring mature, particularly in precocial species (Cheverud,
536 1984; Wilson *et al*, 2005b). In accordance with other studies, maternal effects did in fact
537 decline after hatching (Dmitriew *et al*, 2010; Lindholm *et al*, 2006; Pick *et al*, 2016; Wilson
538 *et al*, 2005a; Wilson *et al*, 2005b). Maternal investment, such as investment in clutch number
539 or egg quality, has been shown to influence hatching size in lizards (Brown and Shine, 2009;
540 Noble *et al*, 2014; Warner and Lovern, 2014), however, as predicted these effects dissipated
541 post-hatching (Pick *et al*, 2016; Réale *et al*, 1999). Interestingly, maternal contributions
542 increased at a later age and remained moderately low for the remainder of the study. The
543 cause of resurgence in maternal effect variance is unclear. It could be related to intraspecific
544 competition triggering an effect on body size in relation to previously unknown experiences
545 of mothers when offspring were transferred into social housing conditions. Changes in
546 maternal effects across life stages resulting from past maternal experiences have been
547 documented in other taxa (e.g., Marshall, 2008). Alternatively, this pattern may indicate other
548 maternally inherited components such as maternal genetic effects (e.g., mitochondrial genetic
549 variation) that promote variation in body size (Pick *et al*, 2016). Indeed, variation in
550 mitochondrial function has been linked to an individual's metabolic rate and growth –
551 explaining as much as ~50% of the variation in food intake and growth (Salin *et al*, 2016;
552 Salin *et al*, 2019). Therefore, it is likely an important driver of body size variability. Similar
553 to additive genetic variance, the resurgence of maternal effects also cooccurred with changes
554 in the shared environment (housing conditions), suggesting that maternal effects on offspring
555 body size is likely to be environmentally driven.
556
557 Traits under strong selection are expected to show low evolutionary potential as selection acts
558 to remove genetic variation. While low evolutionary potential is at least in part due to
559 reduced levels of additive genetic variance, it is also a result of larger proportions of
560 environmental variance that can impact upon heritability ,slowing evolutionary responses
561 (Charmantier and Garant, 2005). In our study, the environmental component of the phenotype
562 accounted for over 80% of the variation in body mass which is in line with values reported in
563 great tits (53 –74%) and Soay sheep (70 – 96%) (Noordwijk *et al*, 1988; Wilson *et al*, 2007).
564 Interestingly, cool developmental temperatures increased the amount of environmental
565 variance attributed to body mass at an early age. Variation in developmental period between
566 developmental temperatures may explain these differences. In many ectotherms,
567 developmental time exhibits a nonlinear reaction norm with temperature (Marshall *et al*,
568 2020; Noble *et al*, 2018).This means that developmental time decelerates with temperature

569 following an negative exponential function. As a result, hot incubated lizards are more
570 comparable in their development time compared to lizards that were reared a cooler
571 temperature. In fact, the cold developmental temperature treatment had much greater variance
572 in incubation duration. With a longer incubation period, embryos can maximise the yolk
573 resources left by their mothers, which can vary considerably within clutches (Wallace *et al*,
574 2007). Our results suggest that thermodynamic effects of development time can give rise
575 greater environmental heterogeneity in hatching mass and may affect the potential for
576 evolution at early life stages.

577 # Conclusion

578 Our work illustrates the pervasive role of developmental temperature on phenotypic
579 variation. The impact of developmental temperature on body mass manifested early and
580 persisted through life (Monaghan, 2008). This has profound implications as developmentally
581 induced variation in body mass may drive life history differences within populations and alter
582 their vulnerability to environmental change (Botero *et al*, 2015; Marshall *et al*, 2020; Reed *et*
583 *al*, 2010). Body size is known to impact survival in lizards with larger animals usually having
584 a survival advantage (Sorci and Clobert, 1999; Warner and Andrews, 2002). As such,
585 environmentally driven changes in body size could have population-wide consequences on
586 recruitment. In contrast, the genetic variance of body mass was robust to thermal extremes
587 experienced by natural nests and suggests that the potential to genetically adapt to warming
588 climate may be limited. However, more stressful incubation temperatures are needed to
589 elucidate the capacity for this species to reveal new genetic material for selection to act on.
590 Non-genetic sources of variance were responsible for most of the variability in body mass,
591 and their dynamics with age means that the effectiveness of evolution is everchanging.
592 Understanding the complexities of adaptive evolution in response to climate change may
593 require intensive long-term studies in wild populations.
594

595 # Author contributions

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

598 # Data accessibility

599 Datasets and code used to generate results of this study is accessible via Open Science
600 Framework (https://bit.ly/2Uy72id)
601

602 # Acknowledgements

603 Many volunteers and interns from Lizard Lab: Birgit Szabo, Christine Wilson, Joshua
604 Cunningham, Victor Frichot and Matthieu Monserand. Scott Keogh and Julia Riley for their
605 advice with SNP data.
606

607 # References

608 Andrews RM, Mathies T, Warner DA (2000). Effect of Incubation Temperature on
609 Morphology, Growth, and Survival of Juvenile Sceloporus undulatus. *Herpetological*
610 *Monographs* **14:** 420--431.
611

612 Angilletta Jr MJ, Steury TD, Sears MW (2017). Temperature, Growth Rate, and Body Size in
613 Ectotherms: Fitting Pieces of a Life-History Puzzle. *Integrative and Comparative Biology*: 1-
614 12.
615
616 Beaman JE, White CR, Seebacher F (2016). Evolution of Plasticity: Mechanistic Link
617 between Development and Reversible Acclimation. *Trends in Ecology & Evolution* **31**(3):
618 237--249.
619
620 Beldade P, Mateus ARA, Keller RA (2011). Evolution and molecular mechanisms of
621 adaptive developmental plasticity. *Molecular Ecology* **20**(7): 1347--1363.
622
623 Bérénos C, Ellis PA, Pilkington JG, Pemberton JM (2014). Estimating quantitative genetic
624 parameters in wild populations: A comparison of pedigree and genomic approaches.
625 *Molecular Ecology* **23:** 3434–3451.
626
627 Bonamour S, Chevin L-M, Charmantier A, Teplitsky C (2019). Phenotypic plasticity in
628 response to climate change: the importance of cue variation. *Philosophical Transactions of*
629 *the Royal Society B: Biological Sciences* **374**(1768): 20180178–20180112.
630
631 Booth DT (2006). Influence of Incubation Temperature on Hatchling Phenotype in Reptiles.
632 *Physiological and Biochemical Zoology* **79**(2): 274--281.
633
634 Botero CA, Weissing FJ, Wright J, Rubenstein DR (2015). Evolutionary tipping points in the
635 capacity to adapt to environmental change. *Proceedings of the National Academy of Sciences*
636 **112**(1): 184--189.
637
638 Brown GP, Shine R (2009). Beyond size–number trade-offs: clutch size as a maternal effect.
639 *Philosophical Transactions of the Royal Society B: Biological Sciences* **364**(1520): 1097--
640 1106.
641
642 Bürkner PC (2017). brms: An R package for Bayesian multilevel models using Stan. *Journal*
643 *of Statistical Software* **80(1).** **https://doi.org/10.18637/jss.v080.i01**.
644
645 Chapple DG, Miller KA, Chaplin K, Barnett L, Thompson MB, Bray RD (2014). Biology of
646 the invasive delicate skink (Lampropholis delicata) on Lord Howe Island. *Australian Journal*
647 *of Zoology* **62**(6): 498–506.
648
649 Chapple DG, Miller KA, Kraus F, Thompson MB (2013). Divergent introduction histories
650 among invasive populations of the delicate skink (*Lampropholis delicata*): has the
651 importance of genetic admixture in the success of biological invasions been overemphasized?
652 *Diversity and Distributions* **19:** 134–146.
653
654 Charmantier A, Garant D (2005). Environmental quality and evolutionary potential: lessons
655 from wild populations. *Proceedings of the Royal Society B: Biological Sciences* **272**(1571):
656 1415--1425.
657
658 Charmantier A, Kruuk LEB, Blondel J, Lambrechts MM (2004). Testing for microevolution
659 in body size in three blue tit populations. *Journal of Evolutionary Biology* **17**(4): 732--743.
660

661 Cheetham E, Doody JS, Stewart B, Harlow P (2011). Embryonic mortality as a cost of
662 communal nesting in the delicate skink. *Journal of Zoology* **283**(4)**:** 234–242.
663
664 Cheverud JM (1984). Evolution by Kin Selection: A Quantitative Genetic Model Illustrated
665 by Maternal Performance in Mice. *Evolution* **38**(4)**:** 766--777.
666
667 Chevin LM, Lande, R., Mace, G.M. (2010). Adaptation, plasticity, and extinction in a
668 changing environment: Towards a predictive theory. *PLoS Biology,* **8:** e1000357.
669
670 Coltman DW, Pilkington J, Kruuk LEB, Wilson K, Pemberton JM (2001). Positive Genetic
671 Correlation Between Parasite Resistance and Body Size in a Free-Living Ungulate
672 Population. *Evolution* **55**(10)**:** 2116--2125.
673
674 Dahlgaard J, Hoffmann AA (2000). Stress Resistance and Environmental Dependency of
675 Inbreeding Depression in Drosophila melanogaster. *Conservation Biology* **14**(4)**:** 1187--1192.
676
677 Dayananda B, Gray S, Pike D, Webb JK (2016). Communal nesting under climate change:
678 fitness consequences of higher incubation temperatures for a nocturnal lizard. *Global Change*
679 *Biology* **22**(7)**:** 2405--2414.
680
681 De Jong MJ, Alton LA, White CR, O'Bryan MK, Chapple DG, Wong BBM (2023). Long-
682 term effects of incubation temperature on growth and thermal physiology in a small
683 ectotherm. *Philosophical Transactions of the Royal Society B: Biological Sciences* **378:**
684 20220137, https://doi.org/20220110.20221098/rstb.20222022.20220137.
685
686 Dmitriew C, Blows MW, Rowe L (2010). Ontogenetic Change in Genetic Variance in Size
687 Depends on Growth Environment. *The American Naturalist* **175**(6)**:** 640--649.
688
689 Downes SJ, Shine R (1999). Do incubation-induced changes in a lizard's phenotype influence
690 its vulnerability to predators? *Oecologia* **120**(1)**:** 9–18.
691
692 Elphick MJ, Shine R (1998). Longterm effects of incubation temperatures on the morphology
693 and locomotor performance of hatchling lizards (Bassiana duperreyi, Scincidae). *Biological*
694 *Journal of the Linnean Society* **63**(3)**:** 429--447.
695
696 Elphick MJ, Shine R (1999). Sex differences in optimal incubation temperatures in a scincid
697 lizard species. *Oecologia* **118**(4)**:** 431--437.
698
699 Eyck HJF, Buchanan KL, Crino OL, Jessop TS (2019). Effects of developmental stress on
700 animal phenotype and performance: a quantitative review. *Biological Reviews* **94**(3)**:** 1143--
701 1160.
702
703 Falconer DS (1952). The Problem of Environment and Selection. *The American Naturalist*
704 **86**(830)**:** 293–298.
705
706 Falconer DS, Mackay TFC (1996). *Introduction to Quantitative Genetics*, 4 edn. Pearson
707 Education.
708
709 Fischer K, Kreyling J, Beaulieu M, Beil I, Bog M, Bonte D *et al* (2020a). Species-specific
710 effects of thermal stress on the expression of genetic variation across a diverse group of plant

711 and animal taxa under experimental conditions. *Heredity*: 1–15.
712 https://doi.org/10.1038/s41437-41020-40338-41434.
713
714 Fischer K, Kreyling J, Beaulieu M, Beil I, Bog M, Bonte D *et al* (2020b). Species-specific
715 effects of thermal stress on the expression of genetic variation across a diverse group of plant
716 and animal taxa under experimental conditions. *Heredity*: 1--15.
717
718 Flatt T, Shine R, Borges-landaez PA, Downes SJ (2001). Phenotypic variation in an
719 oviparous montane lizard (Bassiana duperreyi): the effects of thermal and hydric incubation
720 environments. *Biological Journal of the Linnean Society* **74**(3): 339--350.
721
722 Forster J, Hirst AG (2012). The temperature-size rule emerges from ontogenetic differences
723 between growth and development rates. *Functional Ecology* **26**(2): 483--492.
724
725 Gavrilets S, Scheiner SM (1993). The genetics of phenotypic plasticity. vi. theoretical
726 predictions for directional selection. . *Journal of Evolutionary Biology* **6:** 49–68.
727
728 Gelman A, Lee D, Guo J (2015). Stan: A Probabilistic Programming Language for Bayesian
729 Inference and Optimization. *Journal of Educational and Behavioral Statistics* **40**(5): 530--
730 543.
731
732 Ghalambor CK, McKay JK, Carroll SP, Reznick DN (2007). Adaptive versus non-adaptive
733 phenotypic plasticity and the potential for contemporary adaptation in new environments.
734 *Functional Ecology* **21**(3): 394–407.
735
736 Gifford ME, Robinson CD, Clay TA (2017). The influence of invasive fire ants on survival,
737 space use, and patterns of natural selection in juvenile lizards. *Biological Invasions* **19**(5):
738 1461--1469.
739
740 Goodman BA, Schwarzkopf L, Krockenberger AK (2013). Phenotypic Integration in
741 Response to Incubation Environment Adaptively Influences Habitat Choice in a Tropical
742 Lizard. *The American Naturalist* **182**(5): 666--673.
743
744 Goodman RM (2008). Latent effects of egg incubation temperature on growth in the lizard
745 Anolis carolinensis. *Journal of Experimental Zoology Part A: Ecological Genetics and*
746 *Physiology* **309A**(9): 525--533.
747
748 Granato ISC, Galli G, de Oliveira Couto EG, e Souza MB, Mendonça LF, Fritsche-Neto R
749 (2018). snpReady: a tool to assist breeders in genomic analysis. *Molecular Breeding* **38**(8):
750 102.
751
752 Hansen TF, Pélabon C, Houle D (2011). Heritability is not Evolvability. *Evolutionary*
753 *Biology* **38:** 258–277.
754
755 Hare KM, Longson CG, Pledger S, Daugherty CH (2004). Size, Growth, and Survival Are
756 Reduced at Cool Incubation Temperatures in the Temperate Lizard *Oligosoma suteri*
757 (Lacertilia: Scincidae). *Copeia* **2004(2):** 383–390. https://doi.org/310.1643/CP-1603-
758 1084R1642.
759

760 Hoffman AA, Parsons PA (1991). *Evolutionary genetics and evolutionary stress*. Oxford
761 University Press.
762
763 Hoffmann AA, Merilä J (1999). Heritable variation and evolution under favourable and
764 unfavourable conditions. *Trends in Ecology & Evolution* **14**(3)**:** 96--101.
765
766 Huisman J (2017). Pedigree reconstruction from SNP data: parentage assignment, sibship
767 clustering and beyond. *Molecular Ecology Resources* **17**(5)**:** 1009--1024.
768
769 Ji X, Chen F, Du W-G, Chen H-L (2003). Incubation temperature affects hatchling growth
770 but not sexual phenotype in the Chinese soft-shelled turtle, Pelodiscus sinensis
771 (Trionychidae). *Journal of Zoology* **261**(4)**:** 409--416.
772
773 Kimock CM, Dubuc C, Brent LJN, Higham JP (2019). Male morphological traits are
774 heritable but do not predict reproductive success in a sexually-dimorphic primate. *Scientific*
775 *Reports* **9**(1)**:** 19794.
776
777 Krist M (2010). Egg size and offspring quality: a meta-analysis in birds. *Biological Reviews*
778 **86**(3)**:** 692–716.
779
780 Kruuk LEB (2004). Estimating genetic parameters in natural populations using the 'animal
781 model'. *Philosophical transactions of the Royal Society of London Series B, Biological*
782 *sciences* **359**(1446)**:** 873–890.
783
784 Lindholm AK, Hunt J, Brooks R (2006). Where do all the maternal effects go? Variation in
785 offspring body size through ontogeny in the live-bearing fish Poecilia parae. *Biology Letters*
786 **2**(4)**:** 586--589.
787
788 Lynch M, Walsh B (1998). *Genetics And Analysis Of Quantitative Traits*. Oxford University
789 Press.
790
791 Marshall DJ (2008). Transgenerational plasticity in the sea: context- dependent maternal
792 effects across the life history. *Ecology* **89**(2)**:** 418-427.
793
794 Marshall DJ, Pettersen AK, Bode M, White CR (2020). Developmental cost theory predicts
795 thermal environment and vulnerability to global warming. *Nature Ecology & Evolution* **4**(3)**:**
796 406--411.
797
798 Martins F, Kruuk LEB, Llewelyn J, Moritz C, Phillips B (2019). Heritability of climate-
799 relevant traits in a rainforest skink. *Heredity* **122**(1)**:** 41--52.
800
801 McAdam AG, Boutin S, Reale D, Berteaux D (2002). Maternal effects and the potential for
802 evolution in a natural population of animals. *Evolution* **56:** 846-851.
803
804 Mitchell TS, Hall JM, Warner DA (2018). Female investment in offspring size and number
805 shifts seasonally in a lizard with single-egg clutches. *Evolutionary Ecology* **32**(2)**:** 231--245.
806
807 Monaghan P (2008). Early growth conditions, phenotypic development and environmental
808 change. *Philosophical Transactions of the Royal Society B: Biological Sciences* **363**(1497)**:**
809 1635–1645.

810
811 Mousseau TA, Fox CW (1998). The adaptive significance of maternal effects. *Trends in*
812 *Ecology & Evolution* **13**(10)**:** 403--407.
813
814 Noble DWA, McFarlane SE, Keogh JS, Whiting MJ (2014). Maternal and additive genetic
815 effects contribute to variation in offspring traits in a lizard. *Behavioral Ecology* **25**(3)**:** 633--
816 640.
817
818 Noble DWA, Radersma R, Uller T (2019). Plastic responses to novel environments are
819 biased towards phenotype dimensions with high additive genetic variation. *Proceedings of*
820 *the National Academy of Sciences* **116**(27)**:** 13452--13461.
821
822 Noble DWA, Stenhouse V, Schwanz LE (2018). Developmental temperatures and phenotypic
823 plasticity in reptiles: a systematic review and meta-analysis. *Biological Reviews* **93**(1)**:** 72–
824 97.
825
826 Noordwijk AJV, Balen JHV, Scharloo W (1988). Heritability of body size in a natural
827 population of the Great Tit (Parus major) and its relation to age and environmental conditions
828 during growth. *Genetical Research* **51**(2)**:** 149--162.
829
830 O'Dea RE, Lagisz M, Hendry AP, Nakagawa S (2019). Developmental temperature affects
831 phenotypic means and variability: A meta-analysis of fish data. *Fish and Fisheries* **20(5)**
832 1005–1022. https://doi.org/1010.1111/faf.12394.
833
834 Paaby AB, Rockman MV (2014). Cryptic genetic variation: evolution's hidden substrate.
835 *Nature Reviews Genetics* **15**(4)**:** 247--258.
836
837 Pick JL, Ebneter C, Hutter P, Tschirren B (2016). Disentangling Genetic and Prenatal
838 Maternal Effects on Offspring Size and Survival. *The American Naturalist* **188**(6)**:** 628--639.
839
840 Qualls FJ, Shine R (2000). Post-hatching environment contributes greatly to phenotypic
841 variation between two populations of the Australian garden skink, Lampropholis guichenoti.
842 *Biological Journal of the Linnean Society* **71**(2)**:** 315–341.
843
844 Réale D, Festa-Bianchet M, Jorgenson JT (1999). Heritability of body mass varies with age
845 and season in wild bighorn sheep. *Heredity* **83** 526–532.
846
847 Reed TE, Waples RS, Schindler DE, Hard JJ, Kinnison MT (2010). Phenotypic plasticity and
848 population viability: the importance of environmental predictability. *Proceedings of the*
849 *Royal Society of London B: Biological Sciences* **277**(1699)**:** 3391–3400.
850
851 Roelofs D, Morgan J, Stürzenbaum S (2010). The significance of genome-wide
852 transcriptional regulation in the evolution of stress tolerance. *Evolutionary Ecology* **24**(3)**:**
853 527--539.
854
855 Rollinson N, Rowe L (2015). Persistent directional selection on body size and a resolution to
856 the paradox of stasis. *Evolution* **69**(9)**:** 2441--2451.
857

858 Rowiński PK, Rogell B (2017). Environmental stress correlates with increases in both genetic
859 and residual variances: A meta-analysis of animal studies. *Evolution* **71:** 1339–1351.
860 https://doi.org/1310.1111/evo.13201.
861
862 Rueden CT, Schindelin J, Hiner MC, DeZonia BE, Walter AE, Arena ET *et al* (2017).
863 ImageJ2: ImageJ for the next generation of scientific image data. *BMC Bioinformatics* **18**(1)**:**
864 529.
865
866 Salin K, Auer SK, Anderson GJ, Selman C, Metcalfe NB (2016). Inadequate food intake at
867 high temperatures is related to depressed mitochondrial respiratory capacity. *The Journal of*
868 *experimental biology* **219**(9)**:** 1356–1362.
869
870 Salin K, Villasevil EM, Anderson GJ, Lamarre SG, Melanson CA, McCarthy I *et al* (2019).
871 Differences in mitochondrial efficiency explain individual variation in growth performance.
872 *Proceedings of the Royal Society B: Biological Sciences* **286**(1909)**:** 20191466.
873
874 Schielzeth H, Nakagawa S (2022). Conditional repeatability and the variance explained by
875 reaction norm variation in random slope models. *Methods in Ecology and Evolution* **13:**
876 1214-1223.
877
878 Sgrò CM, Hoffmann AA (2004). Genetic correlations, tradeoffs and environmental variation.
879 *Heredity* **93(3):** 241–248. https://doi.org/210.1038/sj.hdy.6800532.
880
881 Shine R, Harlow PS (1996). Maternal Manipulation of Offspring Phenotypes via Nest-Site
882 Selection in an Oviparous Lizard. *Ecology* **77**(6)**:** 1808–1817.
883
884 Sivula T, Magnusson M, Vehtari A (2020). Uncertainty in Bayesian leave-one-out cross-
885 validation based model comparison. *arXiv:200810296*.
886
887 Sorci G, Clobert J (1999). Natural selection on hatchling body size and mass in two
888 environments in the common lizard (*Lacerta vivipara*). . *Evolutionary Ecology Research* **1:**
889 303-316.
890
891 Stillwell RC, Fox CW (2009). Geographic variation in body size, sexual size dimorphism and
892 fitness components of a seed beetle: local adaptation versus phenotypic plasticity. *Oikos*
893 **118**(5)**:** 703--712.
894
895 Storm MA, Angilletta MJ (2007). Rapid assimilation of yolk enhances growth and
896 development of lizard embryos from a cold environment. *The Journal of experimental*
897 *biology* **210**(19)**:** 3415–3421.
898
899 Sun B-J, Li T, Gao J, Ma L, Du W-G (2015). High incubation temperatures enhance
900 mitochondrial energy metabolism in reptile embryos. *Scientific Reports* **5**(1)**:** 8861.
901
902 Team RC (2023). R: A language and environment for statistical
903 computing. *R Foundation for Statistical Computing, Vienna, Austria*
904 *URL https://wwwR-projectorg/*.
905

906 Telemeco RS, Radder RS, Baird TA, Shine R (2010). Thermal effects on reptile
907 reproduction: adaptation and phenotypic plasticity in a montane lizard. *Biological Journal of*
908 *the Linnean Society* **100**(3)**:** 642--655.
909
910 Uller T, Olsson M (2010). Offspring size and timing of hatching determine survival and
911 reproductive output in a lizard. *Oecologia* **162**(3)**:** 663--671.
912
913 VanRaden PM (2008). Efficient Methods to Compute Genomic Predictions. *Journal of Dairy*
914 *Science* **91**(11)**:** 4414--4423.
915
916 Verdú-Ricoy J, Iraeta P, Salvador A, Díaz JA (2014). Phenotypic responses to incubation
917 conditions in ecologically distinct populations of a lacertid lizard: A tale of two
918 phylogeographic lineages. *Journal of Zoology* **292(3):** 184–191.
919 https://doi.org/110.1111/jzo.12091.
920
921 Wallace BP, Sotherland PR, Santidrian Tomillo P, Reina RD, Spotila JR, Paladino FV
922 (2007). Maternal investment in reproduction and its consequences in leatherback turtles.
923 *Oecologia* **152**(1)**:** 37--47.
924
925 Warner DA, Andrews RM (2002). Laboratory and field experiments identify sources of
926 variation in phenotypes and survival of hatchling lizards. *Biological Journal of the Linnean*
927 *Society* **76:** 105-124.
928
929 Warner DA, Lovern MB (2014). The Maternal Environment Affects Offspring Viability via
930 an Indirect Effect of Yolk Investment on Offspring Size. *Physiological and Biochemical*
931 *Zoology* **87**(2)**:** 276--287.
932
933 Warner DA, Shine R (2008). Determinants of Dispersal Distance in Free-Ranging Juvenile
934 Lizards. *Ethology* **114**(4)**:** 361--368.
935
936 Weigensberg I, Roff DA (1996). Natural Heritabilities: Can They Be Reliably Estimated in
937 the Laboratory? *Evolution* **50**(6)**:** 2149--2157.
938
939 West-Eberhard MJ (2003). *Developmental Plasticity and Evolution*. Oxford University Press.
940
941 While GM, Noble DWA, Uller T, Warner DA, Riley JL, Du W-G *et al* (2018). Patterns of
942 developmental plasticity in response to incubation temperature in reptiles. *Journal of*
943 *Experimental Zoology Part A: Ecological and Integrative Physiology* **329**(4-5)**:** 162--176.
944
945 While GM, Williamson J, Prescott G, Horvathova T, Fresnillo B, Beeton NJ *et al* (2015).
946 Adaptive responses to cool climate promotes persistence of a non-native lizard. *Proceedings*
947 *of the Royal Society of London B: Biological Sciences* **282**(1803)**:** 20142638–20142638.
948
949 Wilson AJ, Coltman DW, Pemberton JM, Overall ADJ, Byrne KA, Kruuk LEB (2005a).
950 Maternal genetic effects set the potential for evolution in a free-living vertebrate population.
951 *Journal of Evolutionary Biology* **18**(2)**:** 405--414.
952
953 Wilson AJ, Kruuk LEB, Coltman DW (2005b). Ontogenetic Patterns in Heritable Variation
954 for Body Size: Using Random Regression Models in a Wild Ungulate Population. *The*
955 *American Naturalist* **166**(6)**:** E177--E192.

956
957 Wilson AJ, Pemberston JM, Pilkington JG, Clutton-Brock TH, Coltman DW, Kruuk LEB
958 (2007). Quantitative genetics of growth and cryptic evolution of body size in an island
959 population. *Evolutionary Ecology* **21**(3)**:** 337--356.
960
961 Wilson AJ, Réale D (2006). Ontogeny of Additive and Maternal Genetic Effects: Lessons
962 from Domestic Mammals. *The American Naturalist* **167**(1)**:** E23--E38.
963
964 Wilson AJ, Reale D, Clements MN, Morrissey MM, Postma E, Walling CA *et al* (2010). An
965 ecologist's guide to the animal model. *Journal of Animal Ecology* **79**(1)**:** 13–26.
966
967 Wood CW, Brodie ED (2015). Environmental effects on the structure of the G-matrix.
968 *Evolution* **69**(11)**:** 2927--2940.
969
970