Developmental temperature, repeatability of metabolic rate at different temperatures

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Abstract

Keywords

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Introduction

A substantial amount of variation in an individual’s phenotype is determined by formative processes that occur during embryonic development. As such, environmental perturbations during this sensitive period can result in persistent changes to an individual’s physiology, morphology, behaviour and life history (Eyck et al., 2019; Noble et al., 2017; O’Dea et al., 2019). Development plasticity allows embryos to prime themselves to environments they will eventually survive in, provided that early cues are predictive of later life conditions(Beldade et al., 2011). These responses have significant impacts such as changing population dynamics and facilitating the evolution of novel traits for selection to act upon (Forsman, 2014; Moczek et al., 2011; West-Eberhard, 2003). Despite its importance for persistence under changing environments, we still have a limited understanding on how developmental cues can influence an individual’s capacity to adjust to environment (Ghalambor et al., 2007).

After birth, an individual’s phenotype can also exhibit reversible plasticity in response to environmental variation (I x E). Such flexibility may confer adaptive benefits, allowing individuals to compensate for environmental changes however, in some cases, plasticity may be result in maladaptive outcomes (Angilletta Jr et al., 2003; Ghalambor et al., 2007). Reversible plasticity can be broadly classified into two categories, namely acute plasticity and acclimation. Acute plasticity describes the sensitivity of labile traits induced by short-term exposure to an environmental cue such as heart rate in response to hypoxia. This form of plasticity can be represented by a reaction norm across different environments (Via et al., 1995). Acclimation, on the other hand, requires chronic exposure to an environmental cue and remodelling of physiological systems and results in shifting of the reaction norm optimum (Seebacher, 2005; Seebacher et al., 2015). Traditionally, developmental plasticity and reversible plasticity are considered as separate biological processes, yet recent evidence suggests that early developmental cues may actually underpin the capacity for phenotypes to change later in life (Beaman et al., 2016; Seebacher et al., 2014).

Environment-phenotype mismatch occurs when developmental cues fails to predict later life environments. This negatively influences fitness because individuals would express suboptimal traits relative to the environment. However, reversible plasticity modulated by developmental cues may alleviate the costs of mismatches by allowing further fine tuning of the phenotype to track environmental variability (Beaman et al., 2016). For example, intertidal gastropod from different shorelines exhibited varied abilities to acclimate

flexibility in heat tolerance was determined by development at high temperatures only, a result that was consistent two different populations of an intertidal copepod (Healy et al., 2019).

Much of current research on the impacts of developmental environments focuses on changes in the phenotypic mean. However, in order to understand the evolutionary implications of developmental responses we need to also consider its influence on phenotypic variability. In order to evolutionary change to occur, there must be consistent variation among individuals that selection can operation on. Such variation is represented by repeatability which is the proportion of total variance that is attributed to consistent individual differences. Thus, one of the first steps of understanding whether developmental responses can impact evolution is to quantify repeatability across different developmental contexts. [Studies that show variation x devo plasticity – Rose and refs within, limited to certain taxa].[Need more estimates in wide range of taxa to see if the capacity to be selected on is altered by developmental experiences] [ Why would devo environments change variance at different environments, [Mechanisms: developmental stress may undo stabilising agents in the DNA, revealing cryptic genetic variation which manifests as variation in the phenotype.]

]

Metabolic rate is highly labile and previous work have shown that is it repeatable/heritable trait. At the individual level, metabolic rate determines energy budgets which has important consequences on resource allocation and life history evolution] strongly integrated with other fitness related traits. Across broad scales, metabolic rate have been shown to underpin community structures. [Devo x MR] [Indeed, metabolic rate and its variability have been shown to change with temp (), XX () and XX], but how that interacts with developmental environments still needs to be established.

Here we examined how developmental temperature impacts individual responses to acute temperature change in an oviparous skink (*Lampropholis delicata*). Specifically, we were interested in whether developmental temperature affects the overall thermal reaction norm of metabolic rate, as well as the repeatability of metabolic rate at different temperatures and phenotypic correlations of metabolic rate among temperatures. Over XX months, we repeatedly measured routine metabolic rate at six temperatures for lizards (nobs = 3818) that hatched from two incubation treatments (nhot = 25, ncold = 26) to address the following key questions: (1) How does developmental temperature change: (1) the overall thermal reaction norm of metabolic rate (elevation and slope); (2) temperature-specific repeatability and (3) the repeatability of the slope of the reaction norm? We expect lizards that hatched from the hot developmental temperature would have on average higher metabolic rates . [Predictions about slope]. Moreover, we expect increases in repeatability as well as heritability under high thermal stress. Our experimental approach will provide important insights of how changing thermal regimes can affect the capacity for metabolic rate to undergo section as well its evolutionary potential.

Materials and Methods

*Lizard Collection and Husbandry*

Since 2015, we established a breeding colony of adult *L. delicata* (nfemales = 144, nmales = 50) using wild individuals collected across five sites throughout the Sydney region between 28 August and 8 September 2015. Three females were housed with a single male in opaque plastic enclosures measuring 35cm 25cm 15cm (L W H). Enclosures were kept under UV lights (12L:12D) in a temperature-controlled room set to 24ºC. Lizards had access to a heat lamp that elevated temperatures on side of the enclosure to 28-32 ºC. Each enclosure was lined with newspaper and lizards had constant access to water and tree bark as refuge. Adult lizards were fed medium sized crickets *ad libitum* (*Acheta domestica*) dusted with calcium powder and multi-vitamin every two days. From the beginning of egg laying seasons (October of each year), we replaced the newspaper lining with garden potting mix and placed an opaque plastic box (12 cm 17.5 cm 4.3 cm) containing moistened vermiculite in each enclosure for females to oviposit their eggs. During this time, enclosures and vermiculite boxes were sprayed gently with water every second day to maintain a relatively humid environment. From October to November, vermiculite boxes were checked every day for eggs. Tail tissue samples (~1 mm) were taken from adults that were from enclosures producing eggs for DNA extraction (see below). All tissues were stored in 70% ethanol. Animal collection was approved by the New South Wales National Parks and Wildlife Service (SL101549) and all procedures were approved by the Macquarie University Ethics committee (ARA 2015/015) and University of New South Wales Animal Care and Ethics committee (ACEC 15/51A).

*Developmental Temperature Manipulations*

Eggs were collected over October 2017 – March 2018. As soon as eggs were found, they were weighed using a digital scale to the nearest 0.01g (Ohaus Scout SKX123). We also measured egg length (distance between the furthest points along the longest axis of the egg) and egg width (distance between the widest points along the axis perpendicular to the longest axis of the egg) using digital callipers to the nearest 0.01mm. Following measurements, each egg was placed in a plastic cup (80ml) containing three grams of vermiculite and four grams of water and covered using cling wrap which was secured using an elastic band. Each clutch was pseudo-randomly assigned to one of two fluctuating developmental temperature treatments. We used two incubators to precisely control the temperature of eggs (LabWit, ZXSD-R1090). The ‘hot’ treatment was exposed to a mean temperature of 29ºC whereas the ‘cold’ treatment was exposed to a mean temperature of 23ºC. Both incubators fluctuated +/- 3ºC the mean temperature over a 24-hour period. These treatments represent the temperature extremes of natural nest sites of *L. delicata* (Cheetham et al., 2011). Egg cups were rotated within each incubator weekly to avoid uneven heat circulation within incubators. Incubators were also checked daily for hatchlings. On average, the incubation period for the ‘hot’ treatment was 29.36 days (SD = 2.17, range = 15 - 49) days and 48.48 days (SD = 4.18, range = 25 - 56) for the ‘cold’ treatment (Kar et al unpublished – Chapter 3).

*Planned Missing Data and Metabolic Rate At Different Temperatures*

Metabolic measurements commenced in April 2018 and went on till July 2018. At the start of measurements, hatchlings were approximately on average 88.68 days old (SD = 23.75, range = 26 - 131). Given the scope of our experiment, we used closed-system respirometry. We quantified routine metabolic rate (hereafter referred to as metabolic rate [MR]) as our measurements included the energetic costs of random movements that we were not able to control for (Withers 1992; Mathot & Dingemanse 2015). MR was measured as the volume of CO2 production per unit time ( mL min-1) as CO2 production is more sensitive to change in smaller organisms, and is less susceptible to fluctuations in water vapour. Nonetheless, CO2 production was strongly correlated with O2 consumption (*r* =0.81, p = <0.05]). Due to logistical constraints, lizards were randomly assigned to one of two blocks for MR measurements (block 1: n =26, block 2: n = 25). We sampled lizards once a week for two-weeks consecutively and then allowed them to rest for one week before the next week of measurements. Each week of measurements was considered a sampling session (ten sampling sessions in total over the course of 14 weeks). We used the same incubators described above to precisely control the temperature at which MR measurements were taken (+/- 1ºC).

MR was measured at 24ºC, 26ºC, 28ºC, 30ºC, 32ºC and 34ºC in a randomised order however, at each sampling session we purposely missed measurements at two random temperatures which were imputed during analysis. At ~06:00, lizards were gently encouraged into an opaque respiratory chamber and then weighed. After which, chambers were placed inside preheated incubators set at the randomised temperature for 30 minutes. The lids of the chambers were left ajar during this time to minimise CO2 build up. After 30 minutes, each chamber was flushed with fresh air and sealed. A 3 mL ‘control/baseline’ air sample was immediately taken via a two-way valve to account for any residual CO2 that was not flushed from the chambers. The chambers were left in the incubator at the set temperature for lizards to respire for 90 minutes. After this time, two replicate air samples (3mL) were taken from each chamber in order to estimate measurement error (see Statistical analysis). Chambers were then reopened and flushed with fresh air before placed back into the incubator for the second measurement temperature (2 temperatures / day) following the same procedure.

All air samples were injected into the inlet line of a Sables System FMS (Las Vegas NV, USA) with the flow rate set to 200 mL min-1 to measure and *.* Water vapour was scrubbed from the inlet air with Drierite. Output peaks were processed using the R package ‘metabR’ (<https://github.com/daniel1noble/metabR>). The rate of CO2 produced by an individual was calculated following (Core Team, 2013):

Equation: 1

where %CO2 is the maximum percentage of CO2 in air sample above baseline, which was corrected by subtracting any ‘residual’ CO2 from the initial flush from the larger of the two air samples; Vchamber is the volume of the chamber (70 mL); Vlizard is the volume of the lizard, assuming that the mass of the lizard is the same as its volume, and *t* is the duration of time in minutes after where the chamber has been sealed and the first air sample was taken (90 minutes).

Statistical analysis

All analyses were conducted in *R* (Core Team, 2013)*.* We checked the data for potential input or mechanical errors using density and Cleveland plots, for more details see ESM. MR and mass was log transformed. We fitted linear mixed models in *brms* (Bürkner, 2017). For all models we used noninformative priors with 4000 iterations with a burn in 1500, sampling from the posterior distribution every fifth iteration. We ensured proper mixing by inspecting trace plots and checked that scale reduction factors were less than 1.01. We report posterior means and 95% credible intervals for all parameters throughout.

Since we took two replicate air samples per MR measurement, we wanted to account for its measurement error as it may conflate repeatability and heritability estimates (Tim ref). We did this by fitting a nested random effect of lizard identity, sampling session and temperature in all our models (e.g. ID001\_s1\_temp24). This nested random effect (hereafter referred to as measurement error) groups the two replicates together and partitions out the variance attributed to difference among replicates.

A previous study in the same species using a similar experimental design found that that individual responses to acute temperature change was moderately repeatable (Kar et al unpublished, Chapter 2). The same study also showed that measurement error decreased with temperature (Kar et al unpublished, Chapter 2). We therefore attempted to use model selection to determine the most appropriate random effects structure for our analysis. In all models, temperature and body mass were included as fixed effects. Despite our efforts in running more iterations and setting stronger priors, we encountered convergence issues for estimating random temperature slopes for measurement error. As such, we were unable to use a model selection approach and opted to fit random intercepts for lizard identity, sampling session number and measurement error and a random slope for lizard identity only for all subsequent analyses unless stated otherwise.

Heterogenous residual variance may influence estimates of repeatability. We therefore explicitly modelled residual variance to change over temperature in and verified if it was better supported than our homogenous variance model using WAIC values. Homogenous variance was better supported by our data, as such we did not incorporated heterogenous variance our subsequent models (Table S1).

One benefit of using *brms* is its in-built function to perform data imputation during model fitting using the function *mi* (See Data accessibility). This not only retains the hierarchical structure of the imputed data but also ultimately increases statistical power. We performed imputation during model fitting in all of analyses described below and also performed the same analyses using complete case data which are presented in the ESM. Overall, conclusions matched across imputation and complete case analyses and we therefore present the imputation analysis in the main text

First, we investigated whether developmental temperatures influenced the elevation and slope of the reaction norm. We fitted a model with MR as the response and included an interaction term between treatment and temperature to test for treatment differences in reaction norm shape.

Second, we ran separate models for each treatment group to test whether developmental temperature may have impacted temperature-specific repeatability of metabolic rate. Each model had MR as the response and temperature, body mass and age as fixed effects. We first calculated among individual variance in metabolic rate at each temperature *It* using the entire posterior distribution. This is was following Schielzeth and Nakagawa (n.d., in review):

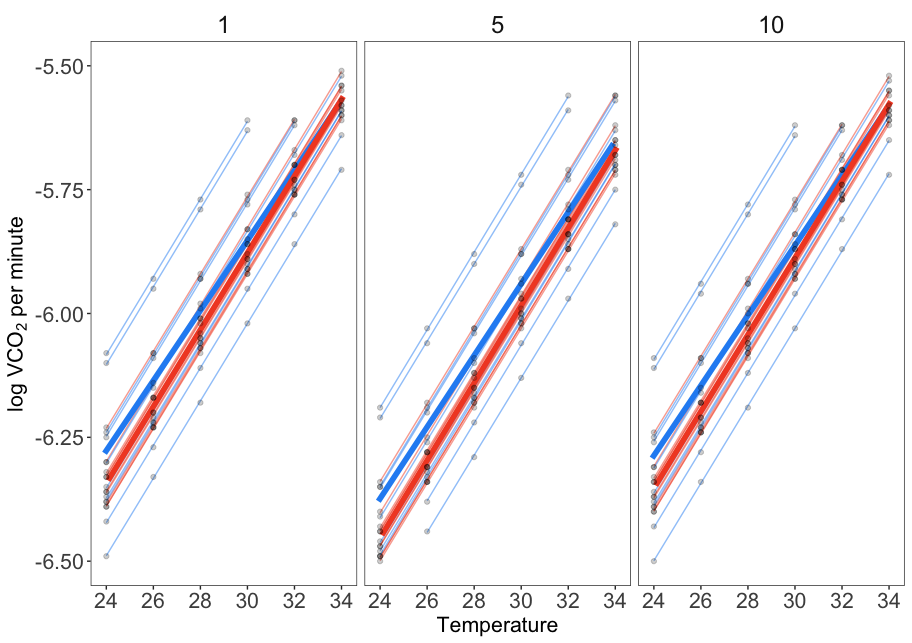
where is the among individual variance in intercepts, is the specific temperature at which repeatability is calculated for, is the among individual and is the covariance between the intercept and slope at the among individual level. Temperature specific repeatability () is then calculated as follows:

where: is the variance among individuals at a particular temperature, is the variance due to sampling session and is residual variance

In order to estimate the repeatability of the slope of the reaction norm (*Rslope*), we fitted another model for each treatment group containing a nested random effect of individual identity and sampling session e.g. id001\_s1 (Araya-Ajoy et al., 2015). We included this random effect (hereafter referred to as series) in place of sampling session random intercept. Series groups all the measurements from an individual at a given sampling session together and allows partitioning variance that is attributed to among sampling sessions, within an individual. We fitted a random slope of temperature for series which allowed the model to estimate an ‘overall’ among sampling session slope. The repeatability of the slope is therefore the proportion of variance in slopes explained among individual differences and is calculated as:

where: is the among individual variance in the temperature slope term and the is the among sampling session within individual variance in the temperature slope

Results



**Figure 1.** Average thermal reaction norms of metabolic rate for the ‘cold’ developmental temperature group (thick blue line, n = 26) and the ‘hot’ developmental temperature group (thick red line, n = 25). There were no significant difference among treatment in the elevation or slope of the reaction norm (see Table 2). Thin lines present individual reaction norms for a subset of 10 individuals from each treatment. Grey points represents model predictions for individual’s mean log metabolic rate. Each panel represents a distinct sampling sessions to illustrate the consistency of individual reaction norms. Note that a slight ‘jitter’ was added to each treatment’s reaction norms to highlight the presence of two reaction norms.

Overall, we found no evidence to suggest that elevation or the slope of the thermal reaction norms of metabolic rate differed between developmental temperatures (Fig. 1, Table 1, Table S2). Both temperature and body mass had positive effects on metabolic rate. Model coefficients for the main effect model is presented in Table S3-4

**Table 1** Model coefficients of full model testing whether developmental temperature affects the elevation and slope of the thermal reaction norm of metabolic rate. This model used an imputed dataset of n = 6000. The intercept is the cold developmental temperature. Note that the imputation model also estimates an intercept and residual variance for mass as it was also missing data. Mass and MR was log transformed and Age was z-transformed. Bolded estimates are significantly different from zero. Values with \* indicate very small values that are still greater than zero.

|  |  |  |  |
| --- | --- | --- | --- |
| Parameter | Estimate | Lower | Upper |
| Intercept MR | **-7.618** | **-7.84** | **-7.397** |
| Intercept Mass | **-1.442** | **-1.449** | **-1.436** |
| Treatment 29 | 0.135 | -0.069 | 0.344 |
| Temperature | **0.077** | **0.072** | **0.081** |
| Age | -0.035 | -0.078 | 0.009 |
| Treatment Temperature | -0.005 | -0.011 | 0.002 |
| Mass | **0.622** | **0.507** | **0.733** |
| VI, Intercept | **0.012** | **0.001** | **0.038** |
| VI, Slope | **0\*** | **0\*** | **0\*** |
| Vsession, Intercept | **0.01** | **0.003** | **0.029** |
| Vmeasurement error, Intercept | **0.044** | **0.04** | **0.049** |
| COVI, Intercept – I, Slope | -0.000115 | -0.000823 | 6.63e-05 |
| Residual MR | **0.041** | **0.038** | **0.043** |
| Residual Mass | **0.043** | **0.041** | **0.045** |

Individual slopes of the thermal reaction norm were repeatable in both treatment groups, however there were no treatment differences (Fig. 1). This result should be interpreted with caution as repeatability of the slope was estimated with a large degree of uncertainty (Hot: Rslope = 0.44 , 95% CI: 0.03 – 0.95; Cold: Rslope = 0.42, 95% CI: 0.03 – 0.94). Coefficients for the models that were used to estimate repeatability of the slope are presented in Table S10-13.

A screenshot of a cell phone

Description automatically generated

**Figure 2.** Adjusted repeatability for average metabolic rate for the ‘cold’ developmental temperature group (blue) and the ‘hot’ developmental temperature group (red). There were no significant differences among treatment in repeatability estimates (see Table 2). Repeatability did not change with acute temperature. Error bars represent 95% credible intervals.

Across both treatment groups, repeatability did not change across acute temperatures (Fig. 2, Table 2). There were no differences in repeatability between developmental temperatures (Fig. 2, Table 2). Although in the complete case analysis, there was a trend for the cold developmental treatment to have on higher repeatability compared to the hot developmental treatment however credible intervals overlapped partially (Fig. S2, Table S5). Moreover, this pattern was not reflected in the more conservative imputation analysis (Fig. 2, Table 2). Model coefficients for each treatment group are presented in Table S6-9.

**Table 2** Temperature specific, adjusted repeatability estimates of log transformed metabolic rate for lizards from two developmental temperatures (nhot = 25, ncold = 26). These values were estimated from an imputation analysis, nobs = 6000. Bolded values are significantly different from zero

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Cold development temperature  n = 26 | | | | Hot development temperature  n = 25 | | |
| Temperature | Repeatability | Lower | Upper | Repeatability | Lower | Upper |
| 24 | **0.15** | **0.09** | **0.24** | **0.15** | **0.08** | **0.24** |
| 26 | **0.16** | **0.09** | **0.24** | **0.15** | **0.09** | **0.24** |
| 28 | **0.16** | **0.09** | **0.23** | **0.15** | **0.09** | **0.23** |
| 30 | **0.16** | **0.09** | **0.24** | **0.15** | **0.09** | **0.23** |
| 32 | **0.16** | **0.09** | **0.24** | **0.16** | **0.09** | **0.24** |
| 34 | **0.16** | **0.09** | **0.25** | **0.16** | **0.09** | **0.25** |

Discussion

Developmental temperature did not influence the elevation or slope of the thermal reaction norm of metabolic rate. Nor did it change repeatability of metabolic rate at each temperature.

[No changes in reaction norm shape because both are acclimated to the same temperature? Thereby aligning reaction norms so may not observe differences. Need a cross factorial acclimation experiment to test this Devo Temp X acclimation X acute temp. Measuring only the linear part of the reaction norm, maybe differences are at extremes. Differences are trait specific.

Variation in developmental cue might be key, both treatments have the same temperature fluctuations, therefore cost and benefit of acclimation is the same and may not result in differences.]

[Potentially affects repeatability, cold higher than hot but this effect dissipates with imputation. Cold temp is actually a bit out of preferred/historical temperature ranges. Novel-ish environments may probably greater repeatability.

Alternatively, cold developmental cues may be actually signalling more variable conditions ahead as it is an unusual nest temperature and that promotes more individual variation and therefore evolutionary potential]

Conclusions

Data accessibility

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

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