Developmental temperature and repeatability of metabolic rate across temperatures

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Abstract

Phenotypic plasticity is an important mechanism that allows populations to adjust to changing environments. Theory predicts that plastic responses induced early in life experiences can have lasting impacts on how individuals respond to environmental variation later in life (reversible plasticity). Developmental cues can also influence variability of reversible plastic responses thereby altering the capacity for reaction norms to respond to selection. This may allow reaction norms of threatened populations to evolve in the face of warming climate. Here, we compared thermal reaction norms of metabolic rate in lizards (*Lampropholis delicata*) that were incubated at two developmental temperatures (ncold = 26, nhot = 25). We repeated assayed individual reaction norms across six acute temperatures to estimate the repeatability of the slope and repeatability of average metabolic rate (nobs = 3,818). The elevation and the slope of the thermal reaction norm were not susceptible to changes in developmental temperatures. The slope of the reaction norm was repeatable (*R* = 0.43) suggesting that individuals were moderately consistent in their response to acute temperature variation, however consistency did not depend on early developmental temperature. Repeatability of average metabolic rate was stable across acute temperatures and did not differ between developmental temperatures. Our results suggest that selective processes may be able to operate on consistent expression of metabolic rate as well as thermal plasticity. Moreover, thermal extremes in natural nest temperatures promotes constancy in thermal plasticity. Understanding how environments at different life stages impact plasticity and its capacity to evolve will become increasingly more relevant for terrestrial ectotherms.

Keywords

reaction norm, repeatability, metabolic rate, incubation temperature, thermal performance curve, thermal sensitivity, phenotypic flexibility

Introduction

A substantial amount of variation in an individual’s phenotype is determined by formative processes that occur during embryonic development. Environmental perturbations during this critical period can have persistent effects on an individual’s physiology, morphology, behaviour and life history (Eyck et al., 2019; Noble et al., 2018; O’Dea et al., 2019). Developmental shifts may be adaptative if it allows organisms to better cope in similar environments experienced later in life (Beldade et al., 2011). However, environment-phenotype mismatches can occur when developmental cues fail to predict later life environments (Auld et al., 2010; Bonamour et al., 2019). A multitude of traits throughout an animal’s life is labile; reversibly responding to environmental change. Reversible plasticity in phenotypic traits allow individuals to adjust to moment-to-moment changes in their surroundings (Piersma & Drent, 2003). Reversible plasticity can be broadly classified into two categories, namely acclimation and phenotypic flexibility (Havird et al., 2020; Piersma & Drent, 2003). Acclimation is generally a slower form of plasticity and involves remodelling of physiological systems over longer periods with chronic exposure to an environmental cue (Seebacher, 2005). Whereas, phenotypic flexibility describes short-term changes in traits induced by acute exposure to an environmental cue, such as changes in metabolic rate in response to acute temperature (Piersma & Drent, 2003; Piersma & Lindström, 1997).While it is widely known that cues experienced during early stages of development can have a profound impact on the mean trait value, recent evidence suggests that the same cues also affect trait plasticity later in life (reviewed in Beaman et al., 2016).

Reversible plasticity may be able to alleviate costs associated with phenotype mismatches induced by early life environments (Angilletta Jr et al., 2003; Ghalambor et al., 2007). Cues experienced during development not only convey information about average changes in the environment, such as decreases in precipitation, but also its variability (i.e. how often rainfall occurs) (Bonamour et al., 2019). When environments shift predictably, flexibility in the average phenotype would be advantageous because individuals can compensate the effects of the prevailing conditions to avoid discrepancies between the environment and the phenotype (Botero et al., 2015). For example, seasonal variation in temperature during development reliably predicts dispersal strategies in adult spiders (*Erigone atra*), possibly in response to changes in resource availability (Bonte et al., 2008). The interaction of early- and later life plasticity is supported by a few studies that show developmental differences in plasticity of a variety of traits including mitochondrial function (Shama et al., 2014), metabolic rate (Seebacher et al., 2014) and locomotor performance (Kazerouni et al., 2016). However, these studies solely focus on developmental effects on acclimation, whereas the influence on phenotypic flexibility is largely unknown. Moreover, these studies have neglected to consider potential confounds such as local adaptation (Amarillo-Suárez & Fox, 2006; Stillwell & Fox, 2009) and parental effects (Bentz et al., 2013; Polačik et al., 2017) that could also affect the shape as well as the variability of plastic responses later in life (George et al., 2017).

It has been long recognised that individuals vary in their plastic responses, with some

responding more flexibly than others (Dingemanse & Wolf, 2013; Nussey et al., 2007). Consistent variation among individuals may be heritable but importantly, provides substrate for selective forces to act on (Araya-Ajoy & Dingemanse, 2017; Nussey et al., 2007). Developmental environments, however, can influence consistent variation possibly via changes to individual condition (Sultan & Stearns, 2005). For example, zebra finches that experienced nutritional stress as nestlings weighed less and had reduced growth rates which may have contributed to increases in consistent among individual differences in metabolism and behaviour (Careau, Buttemer, et al., 2014). Consistent among individual variation in plasticity have been reported in a number of labile traits including aggressiveness in great tits (Araya-Ajoy & Dingemanse, 2017), explorative behaviour in chickadees (Thompson et al., 2018) and metabolic rate in amphipods (Réveillon et al., 2019). Whether developmental cues affect consistent variation in reversible plasticity *per se* is still poorly understood. Identifying the factors that impact the consistency of plastic responses (i.e. repeatability) critical for understanding the evolution of plasticity in fluctuating environments.

Energy metabolism is a key fitness related trait that is both consistently different among individuals and highly labile within individuals (Nespolo & Franco, 2007; Norin & Metcalfe, 2019). All organisms require energy for growth, maintenance and reproduction (Careau, Killen, et al., 2014). Metabolic rate is also strongly linked with other traits such as behaviour (Biro & Stamps, 2010) and life history (Biro & Stamps, 2008) which implies that phenotypic changes in metabolic rate may go on to affect other aspects of the phenotype (Burton et al., 2011; Pettersen et al., 2016). While numerous studies have investigated the influence of various developmental cues such as temperature (Gangloff et al., 2015; Noble et al., 2018), UV exposure (Kazerouni et al., 2016), dietary restriction (Careau, Buttemer, et al., 2014) on *average* metabolic rate, the impacts on the plasticity of metabolic rate is not well established (but see Seebacher et al., 2014). Developmental cues could influence metabolic plasticity possibly through modifications in metabolic enzymes or cellular membrane structure that influence their function in different environments (Angilletta Jr, 2016). Developmentally induced changes in metabolic plasticity implies that tolerance to environmental perturbations may be determined by the developmental environment a given cohort experiences. Furthermore, if repeatability of metabolic plasticity is also affected by developmental cues, then the capacity to respond to selection might also be specific to early life conditions. Understanding how early life environments shapes plastic responses will be important for animals that develop and inhabit in variables environments.

Here we employed a ‘reaction norm approach’ to examine how developmental temperature impacts metabolic plasticity to acute temperature change in an oviparous skink (*Lampropholis delicata*) (Via et al., 1995). Specifically, we were interested in whether developmental temperature affects the shape and repeatability of the thermal reaction norm of metabolic rate. Over 3.5 months, we repeatedly measured routine metabolic rate at six temperatures for lizards (nobs = 3,818) that hatched from two incubation treatments (nhot = 25, ncold = 26) to address the following key questions: (1) How does developmental temperature change the intercept and slope of the thermal reaction norm; (2) How does the repeatability of metabolic plasticity (i.e. slope of the reaction norm) change with developmental temperatures (3) Does developmental temperature differ in their repeatability of average metabolic rate (intercept) at each acute temperature? Given that ectotherms tend to have smaller body sizes when reared in warm environments (temperature-size rule, Angilletta Jr et al., 2017), we expect lizards from the hot developmental temperatures to have higher mass-specific metabolic rates. If developmental changes under high temperatures resulted in more thermally stable enzyme and membrane structures, we expect lizards from hot developmental temperatures to have more shallow reaction norms. Furthermore, early development under high thermal stress might reduce individual condition thereby promoting increases in repeatability in both average metabolic rate and metabolic plasticity. Our experimental approach will provide important insights on how development cues mediate the capacity for ectotherms to respond to thermal variation.

Materials and Methods

*Lizard Collection and Husbandry*

We established a breeding colony of adult *L. delicata* (nfemales = 144, nmales = 50) using wild individuals collected across five sites throughout the Sydney region between 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 one side of the enclosure to 28 – 32 ºC. Each enclosure was lined with newspaper and lizards had constant access to water and tree bark was used as refuge. Adult lizards were fed medium sized crickets *ad libitum* (*Acheta domestica*) dusted with calcium powder and multi-vitamin every two days. From the beginning of the egg laying season (October of each year), we replaced 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 other day to maintain a relatively humid environment. From October to November, vermiculite boxes were checked every day for eggs. 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. When eggs were discovered, 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 by an elastic band. Eggs from each clutch were pseudo-randomly assigned to one of two fluctuating incubation 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 30 days (SD = 1.40, range = 27 - 33) days and 47.7days (SD = 5.90, range = 25 - 53) for the ‘cold’ treatment.

*Planned Missing Data and Metabolic Rate at Different Temperatures*

Metabolic measurements commenced in April 2018 and continued until August 2018. At the beginning of measurements, hatchlings were on average 88.68 days old (SD = 23.75, range = 26 - 131). We used closed-system respirometry instead of flow-through respirometry because it was more logistically feasible compared to measure a large number of hatchlings at a range of temperatures. We quantified routine metabolic rate (hereafter referred to as metabolic rate [MR]) as our measurements likely included the energetic costs of random movements (Withers 1992; Mathot & Dingemanse 2015). MR was measured as the volume of CO2 production per unit time ( mL min-1) as CO2 production is less susceptible to fluctuations in water vapour and more feasible to detect in smaller organisms. Nonetheless, CO2 production was strongly correlated with O2 consumption (*r* =0.81, p < 0.05]) with RQ values averaging 0.77. 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).

Metabolic rate was measured at 24ºC, 26ºC, 28ºC, 30ºC, 32ºC and 34ºC in a randomised order. However, at each sampling session we intentionally missed measurements at two randomly selected temperatures using a planned missing data design (Nakagawa, 2015; Noble & Nakagawa, 2018). Missing data was imputed after during analysis (see Statistical 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 to allow body temperatures to equilibrate. 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 being placed back into the incubator for the second measurement temperature (2 temperatures / day) following the same procedure approximately two hours later.

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

We fitted Bayesian linear mixed effect models in *R* (Core Team, 2013) using the package ‘*brms’* (Bürkner, 2017). Our planned missing data design resulted in random missingness across temperatures. The package *brms* is capable of performing model-based data imputation. As such, we performed imputation during model fitting in all of analyses. Model-based imputation not only retains the hierarchical structure of the dataset but also increases statistical power (P. Bürkner, personal communication 25 October 2020, Nakagawa, 2015). Sensitivity analyses suggest that models with imputed data resulted in similar conclusions to complete case analyses. We present results from the imputation analysis in the main text as parameter estimates were more precise and we also wanted to present complete thermal reaction norms for individuals in the figure (See ESM). For all models we used noninformative priors and ran four Markov Chain Monte Carlo (MCMC) chains; taking 800 samples from the posterior distribution after discarding the first 1500 iterations. This gave a total of 3200 samples from the posterior distribution across all chains. We ensured chains were mixing by inspecting trace plots and checked that scale reduction factors were less than 1.01, suggesting all chains had converged. Throughout we report posterior means and 95% credible intervals for all parameters. All data and code to reproduce our results are provided (see Data accessibility).

To test whether developmental temperatures changed the shape of reaction norms, we fitted a full model with MR as the response and temperature, treatment and an interaction between treatment and temperature as fixed effects. The model also included a random intercept for lizard identity and sampling session. We wanted to account for measurement error in all our models as it may conflate parameter estimates (Ponzi et al., 2018). Using the two replicate samples we took to calculate metabolic rate, we estimated measurement error variance by including a nested random effect of lizard identity, sampling session and temperature in all our models (e.g. ID001\_session1\_temp24). This nested random effect (hereafter referred to as measurement error) groups the two replicates together and estimates the variance attributed to differences among replicates. While we show measurement error can vary by temperature (Chapter 2), here we assumed that measurement error was constant across temperatures by fitting it as a random intercept as estimating a random slope for resulted in model convergence issues. Heterogeneous variance across temperatures can also influence parameter estimates (Careau, Buttemer, et al., 2014). However, heterogeneous residual variance was not supported by our data, therefore homogenous variance was used in all models (Table S1).

To estimate the repeatability of the reaction norm slope (*Rslope*) in each developmental temperature treatment, we fitted separate models for each treatment group with MR as the response and temperature, body mass and age as fixed effects. We included lizard identity, measurement error and a nested random effect of individual identity and sampling session (hereafter referred to as series, Araya-Ajoy et al., 2015). Lizard identity estimates among individual variance, whereas series groups together all the measurements from an individual at a given sampling session and partitions variance within individual, among sampling sessions. A random temperature slope was estimated for lizard identity and series which allowed us to calculate slope repeatability. The repeatability of the slope is calculated as the proportion of total variance in slopes explained by among individual differences:

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

Finally, we tested whether developmental temperature affected the repeatability of average metabolic rate at each acute temperature (i.e. intercept of the reaction norm). Similarly, we fitted separate models for each treatment group with MR as the response and temperature, body mass and age as fixed effects. We included lizard identity, sampling session and measurement error as random intercepts and temperature as a random slope for lizard identity. We calculated among individual variance in metabolic rate at each temperature *It*  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 due to sampling session and is residual variance

Results



**Figure 1.** Predicted thermal reaction norms of metabolic rate for the ‘cold’ developmental temperature group (thick blue line, nlizards = 26) and the ‘hot’ developmental temperature group (thick red line, nlizards = 25). Predictions were made from an imputation model. There was no significant difference among treatments 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 represent model predictions for individual’s mean log metabolic rate. Each panel represents 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 average metabolic rate or its response to acute temperature was influenced by early developmental temperature (Fig. 1, Table 1, Table S2). We therefore refitted the model with just the main effects (Table S3-4). Across all models, temperature and body mass had positive effects on metabolic rate (Table 1, Table S3-4). While it was not logistically possible to measure MR upon hatching, we tested for treatment differences in thermal reaction norms at the first sampling session (~2.5 months of age). However, we did not detect treatment differences in thermal reaction norms (see ESM). Nonetheless, reaction norms slopes were significantly repeatable but repeatability did not depend on developmental temperature treatments (Hot: *Rslope* = 0.44, 95% CI: 0.03 – 0.95; Cold: *Rslope* = 0.42, 95% CI: 0.03 – 0.94; Table S6-9). There were no treatment differences in repeatability in average metabolic rate (i.e., intercept) at each acute temperature and estimates were generally small (Fig. 2, Table 2, Table S5). However, there was a tendency for the cold developmental treatment to have higher repeatability compared to the hot developmental (Fig. 2, Fig S2, Table 2).

**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 nobs = 6000. The intercept is the cold developmental temperature. Mass and MR was log transformed and Age was z-transformed. Bolded estimates are significantly different from zero. Lower and upper bound of estimates represent 95% credible intervals. COV represents covariance. Main effects model without the non-signification interaction is presented in Table S3

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameter** | **Estimate** | **Lower** | **Upper** |
| *Fixed effects* |  |  |  |
| Intercept MR | **-7.618** | **-7.84** | **-7.397** |
| Treatment 29 | 0.135 | -0.069 | 0.344 |
| Temperature | **0.077** | **0.072** | **0.081** |
| Age | -0.035 | -0.078 | 0.009 |
| Treatment 29 Temperature | -0.005 | -0.011 | 0.002 |
| Mass | **0.622** | **0.507** | **0.733** |
| *Random Effects* |  |  |  |
| Lizard Identity |  |  |  |
| Intercept | **0.012** | **0.001** | **0.038** |
| Slope | **4.624e-6** | **4.562e-9** | **4.562e-9** |
| COVIntercept – Slope | -0.000115 | -0.000823 | 6.63e-05 |
| Session |  |  |  |
| Intercept | **0.01** | **0.003** | **0.029** |
| Measurement Error |  |  |  |
| Intercept | **0.044** | **0.04** | **0.049** |
| Residuals | **0.041** | **0.038** | **0.043** |



**Figure 2.** Adjusted repeatability for average metabolic rate for the ‘cold’ developmental temperature group (blue, nlizards = 26) and the ‘hot’ developmental temperature group (red, nlizards = 25). Estimates were calculated from an imputation model. There were no significant differences among treatment in repeatability estimates (see Table 2). There were no significant differences in repeatability at any temperatures among treatment groups (Table S5). Error bars represent 95% credible intervals.

Discussion

Overall, we found that thermal reaction norms for metabolism, along with metabolic rates at each acute temperature, were repeatable and unaffected by early developmental temperature. Additionally, early developmental temperature did not change the slope or intercept of the population reaction norm. Our results suggest that, while individuals displayed consistent variation in their plasticity (I x E), early thermal environments did not affect how their metabolic rate respond to temperature later in life. Below we discuss the how developmental environments might affect thermal plasticity and implications for the evolution of thermal reaction norms in fluctuating environments

Thermal reaction norms of metabolic rate are robust to developmental temperature

Developmental cues that affect later life plasticity may allow populations to better cope with environmental fluctuations (Beaman et al. 2016). Epigenetic modifications during development that influence the physiological system are likely responsible for shaping plastic responses through complex ways (Hu & Barrett, 2017; McCaw et al., 2020). Developmental changes to enzymes or membrane structures might affect how metabolic processes respond to thermal fluctuations later in life (Angilletta Jr, 2016; Ishihara et al., 2019). However, our results suggest instead that thermal reaction norms of metabolic rate were robust to changes in incubation temperature. Results have been mixed among the few studies that have investigated the effects of pre- and post-hatching temperature on the plasticity of metabolic rate (Table 1, Beaman et al., 2016). For example, wild caught mosquitofish developing under more variable spring conditions exhibited steeper thermal reaction norms for metabolic scope compared to fish born in summer (Seebacher et al., 2014). In contrast, incubation temperature did not affect plasticity in metabolic rate of striped marsh frog tadpoles (Seebacher & Grigaltchik, 2014). Given that our lizards were reared in a common environment post hatching, the lack of difference we observed maybe the result of reversible plasticity via acclimation in metabolic rate. It is possible that acclimation capacities may have overwhelmed any developmental differences in thermal reaction norms. However, we did not detect any treatment differences at the first sampling session where treatment differences is likely to exist. Indeed, studies that have shown a significant interaction between developmental environments and reversible plasticity have used a cross factorial design where late environments are deliberately matched and mismatched with early environmental conditions to disassociate acclimation effects (Kazerouni et al., 2016; Schnurr et al., 2014).

Stable thermal reaction norms of metabolic rate across both developmental temperatures has key evolutionary implications. Our results imply that population reaction norms maybe robust to temperature variation provided that developmental temperatures fall within the thermal range of natural nests (Cheetham et al., 2011). Past thermal regimes encountered by predecessors may have canalized population responses so that they are less sensitive to fluctuations in developmental temperatures (Liefting et al., 2009). Canalization may reduce the costs of reshaping the phenotype during development if environmental variation is predictable across generations (Aubret & Shine, 2010). In support for this, damselflies undergoing range expansion exhibits geographic variation in reversible plastic responses that aligned with past climatic conditions (Lancaster et al., 2015). Population comparisons across environmental gradients might reveal whether local adaptation shapes developmental plasticity of population reaction norms that lead to canalisation (Toftegaard et al., 2015). Developmental cues may play a stronger role in shaping population plastic responses in populations that experience greater thermal variability, such as those in temperate or high elevation regions (Bonamour et al., 2019). Developmental stress is thought to lead to more adverse effects impacting reversible plasticity late in life (Beaman et al., 2016; Chevin & Hoffmann, 2017). While our incubation treatments represent thermal extremes of natural nest sites, they may not have been severe enough to induce changes in the thermal reaction norms. Nonetheless, it has been hypothesised that positive genetic (co)variances between reaction norms in benign and stressful developmental environments can still promote adaptive responses in extreme environments (Chevin & Hoffmann, 2017). Plastic responses may still be able to indirectly respond to selective pressures in benign environments as long as selective forces are consistent in their direction (Chevin & Hoffmann, 2017). Empirical studies that examine cross-environment correlations between tolerable and stressful conditions may provide valuable insight in how populations can persist in extreme environments.

Developmental temperatures and among-individual plasticity of metabolic rate

Developmental time has important consequences on hatching condition and may contribute to consistent variation in hatchling phenotypes. Developmental time changes with temperature following a negative exponential relationship, such that development times are considerably shorter at hotter temperatures (Marshall et al., 2020; Noble et al., 2018). This could mean that eggs reared in warmer environments may be more constrained in their development rates, thus hatching phenotypes are more likely to be less variable and labile compared to eggs reared in cooler environments. Contrary to these predictions, our study revealed that repeatability of thermal reaction norms did not change with developmental temperature. Our repeatability estimates for the reaction norm slope were consistent with another study of the same species (*R* = 0.23, Chapter 1). Similarly, moderate repeatability of thermal sensitivity of metabolic rate has also been observed in amphipods (*R* = 0.38). Several studies have reported significant among individual variation in thermal plasticity slopes (Briga & Verhulst, 2017; Careau et al., 2014), however repeatability of the slope is rarely estimated as it requires a study design that allows partitioning of within individual variance of slopes (Araya-Ajoy et al., 2015). Assuming that repeatable reaction norm slopes have a heritable basis, our work implies that thermal plasticity can be selected upon and therefore evolve (Falconer, 1952; but see Dohm, 2002). Furthermore, the capacity to undergo selection would not depend on the developmental temperatures of natural nest sites which may facilitate the evolution of plastic responses in populations that live in fluctuating environments. For example, thermal plasticity of cabbage white butterflies has been shown to rapidly evolve and diverge in a variety of thermal environments as they expand their range (Kingsolver et al., 2007; but see Condon et al., 2014)

Consistent individual differences in average metabolic rate were stable across acute temperatures. This result demonstrates that temperatures within the operable range of *L. delicata* maintains consistent individual differences in MR (Matthews et al., 2016). Repeatable average metabolic rate may be an important mechanism that promotes consistent variation in thermal regulation, behaviour and life history (Goulet et al., 2017; Réale et al., 2010; Sæther, 1987). Overall, our estimates for repeatability of MR ranged from 0.09 – 0.22. Our results are in line with a meta-analysis that showed that repeatability decreases with time (White et al., 2013). Indeed, the average repeatability of MR in ectotherms from studies that had a measurement interval that was equal or larger than our study ( 8.5 days) was *R =* 0.33 (SD = 0.21, n = 18). Interestingly, repeatability of average MR in wild caught adult *L. delicata* (*R =* 0.3 – 0.5, Chapter 2) was comparatively larger relative to this study. This is likely due to life stage differences in environmental effects that shape phenotypic variation. As individuals mature, their experiences in different microhabitats (diet, thermal preferences) can promote among-individual variation in traits (Kruuk & Hadfield, 2007). Such common (micro) environment effects could further increase repeatability and may explain differences between lab and wild studies (Auer et al., 2016).

Conclusions

An individual’s ability to adjust in response to environmental change can depend on experiences during embryonic development. In order for plastic responses to evolve, consistent phenotypic variation in reaction norms is required for selection to act on. In this study, we manipulated the temperature at which lizard embryos were incubated and assayed their thermal reaction norms of metabolic rate as juveniles. We demonstrated that thermal plasticity of metabolic rate, as well as the repeatability of reaction norm attributes (intercept and slope) were unfazed by developmental temperature. Understanding the evolution of thermal sensitivity in ectotherms requires a multifaceted approach. Integration of acclimation responses, thermal preferences, past population thermal experiences could reveal important insights on how different aspects of thermal adaptation can shape variation in plasticity and assist threatened ectotherms to persist in warming climate. Our focus should turn to the interactive effect of mean changes as well as variability changes in early life cues to elucidate the conditions that can promote different forms of plasticity.

Data accessibility

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

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