Developmental temperature, repeatability of metabolic rate at different temperatures

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Abstract

Keywords

Introduction

Development is a sensitive period for any organism. Environmental perturbations can result in persistent changes to the phenotype.

Metabolic rate and how it can affect other key life history traits that can change population dynamics. We don’t know how developmental environments can change plasticity of traits. The reaction norm of traits. How animals response to environmental cues.

Repeatability = capacity for selection to act on phenotype, can only evolve if there is underlying genetic variation. We don’t know if developmental stressors can change expression of phenoptyic and genotypic variation

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 = 4000) that hatched from two incubation treatments (nhot = 25, ncold = 26) to address the following key questions. (1) How does developmental temperature change: (1) the thermal reaction norm of metabolic rate (elevation and slope); (2) temperature-specific repeatability; (3) temperature-specific heritability; and finally (4) the phenotypic and genetic correlations of metabolic rate among different temperatures? 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, 26ºCced a 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 equation 4.21 in Lighton 2008:

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).

SNP Analyses And Genomic relatedness matrix

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 was log transformed, mass was z-transformed and then log transformed. We fitted linear mixed models in *brms* (Bürkner, 2017). For all models we used noninformative priors with XXXX iterations with a burn in of XXXX, 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 used model selection to determine the most appropriate random effects structure for our analysis. We compared WAIC values of four different models with varying complexity of random effects (Table SX). In all models, temperature and body mass were included as fixed effects. Despite our efforts in running more iterations and setting 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 and measurement error and a random slope for lizard identity only for all subsequent analyses.

Heterogenous residual variance may also influence estimates of repeatability and heritability. 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 models (Table SX).

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 your sample size and statistical power to test hypotheses. 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 did/did not match across imputation and complete case analyses and we therefore present the imputation analysis in the main text.

Developmental temperature and its impact on thermal reaction norms of metabolic rate

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. We also fitted an interaction term between treatment and body mass as previous studies showed that incubation temperature can affect mass and growth rate. Z-transformed age (measurement date – lizard hatch date) was included as a covariate. [Lizard identity and measurement error as random effects].

Does developmental temperature influence temperature specific repeatability and heritability of metabolic rate?

We ran separate models for each treatment group to test 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 also fitted the same random effects structure describe above.

Temperature-specific repeatability was be calculated from the entire posterior distribution of the random effects. This is following the

The model for temperature-specific heritability (*h2*) has the same structure as the repeatability model, except that we fitted the GRM in the models to estimate additive genetic variance (V*G*). We also included lizard identity twice as a random intercept in the model to partition out permanent environmental variance (V*PE*). Temperature-specific repeatability is calculated in the same way as repeatability as described above, except that we include V*PE* in the denominator of the *h2*:

Results

Developmental temperature and its impact on thermal reaction norms of metabolic rate

Does developmental temperature influence temperature specific repeatability and heritability of metabolic rate?

Discussion

Data accessibility

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

Acknowledgements

We would like to thank Martin Whiting for the use of his facilities at Macquarie University. Numerous Lizard Lab members and interns for husbandry assistance. Special thanks to Christine Wilson.

References