Title page

Title:

Keywords

Abstract

Abbreviations

Introduction

Importance of phenotypic plasticity

Traits vary with environmental cues

Reaction norms

How does it evolve?

Repeatability and its relevance to evolution plasticity

Individuals vary in their plasticity/responsiveness

Between individual variation is raw material for selection to act on

What do we not know?

Case for energy metabolism

Metabolism represents the idling cost of an individual and imposes constraints on resource allocation.

And it also varies between individuals

Metabolism governs behaviours and life history

Metabolism varies greatly with temperature

Review of other studies: Often measured at one temp, not as a function but animals experience a range of temp a day, repeatability assessed at one temp (but what about Careau).

This study

We investigated the between and within individual variation in thermal reaction norms of metabolism using the delicate skink (*Lampropholis delicata*). The delicate skink is a native Australian species and has an widespread distribution on the south east coast of Australian. Small body size – experience greater fluctuations of temperature

Hypothesis:

Predictions: We predict that repeatability in reaction norms should decrease over time.

Methods

Lizard collection and husbandry

Fourty-two male *L. delicata* were collected across two sites between 28 August and 8 September 2015, across the Sydney region. Lizards were caught by hand or by mealworm fishing and were transported individually in calico bags in an ice-cooler to Macquarie University. Lizards were housed in a temperature control room that warmed up to 24ºC during the day and was switched off in the evening. Each lizard were kept individually in opaque plastic enclosures measuring 35cm 25cm 15cm (L W H). Each enclosure was lined with newspaper and lizards were given access to a water bowl and tree bark as a refuge. Enclosures were placed under UV light (11L:13D). Lizards were fed three - four small crickets (*species*) dusted with calcium powder and multi-vitamin every two days when metabolism measurements were not taking place.

Metabolic reaction norms

Metabolism assays were conducted between 26 December 2016 - 19 March 2017. We measured production of CO2 as standard metabolic rate (VCO2 ml min-1), which is the lowest rate of metabolism at a given temperature in an inactive, post-absorptive ectotherm (REF). Measurements were taken between 22ºC and 32ºC at 2ºC increments across three days (measurement at two temperatures per day) and were repeated every 10 days (10 sampling runs in total). Due to logistical constraints, lizards were randomly assigned to one of two batches for metabolism measurements (batch 1: n = 23, batch 2: n = 22).

We used two incubators (LabWit, ZXSD-R1090) to precisely control the ambient temperature at which measurements were taken (+/- 1ºC). The temperature order was randomly allocated to the incubators across the three days, within each sampling run.

Lizards were fasted for at least 24 hours prior to metabolism measurements, but had access to water in their enclosure between each sampling day. Within each sampling run, lizards were randomly assigned to opaque cylindrical metabolism chambers (volume = 146ml). On the day of measurement, body temperature of each individual was taken using an infrared laser gun (brand) inside their enclosure. Lizards were then gently encouraged into their assigned chambers and then weighed using a digital scale (brand). After which, chambers were maintained in a dark environment inside in the incubators for 30 minutes to settle down. After 30 minutes, each chamber was flushed with fresh air by gently waving ambient air over the chamber and then sealed closed. 3ml of air was then removed of every chamber via two-way valve to account for any residual CO2 that was not flushed out from the chambers. The chamber was then left in the incubator for an additional 90 minutes. After which, two air samples are taken from every chamber and chamber lids unscrewed and placed back into the incubator again for the next measurement temperature. The air sampling process was then repeated again following the same procedure as the first temperature.

After air sampling, each 3ml samples were injected into the inlet line of an open-flow respirometer system to measure VCO2 and VO2*.* The percentage of CO2 and O2 of our samples was analysed by a Field Metabolic System (Sables System, Las Vegas, NV, USA) with flow rate set to 200 ml min-1. Water vapour was scrubbed from the inlet air with Drierite (Brand) prior to the measurement of CO2 and O2. The area of output peaks were integrated using LabAnaylst to calculate percentage of CO2. The total ml of CO2 produced by an individual was calculated as:

Eqn: 1

where %CO2 is the percentage of CO2 in air sample, which was corrected by subtracting any ‘residual’ CO2 from the larger of the two air samples, Vchamber is the volume of the chamber (e.g. 146ml), 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 (e.g. 90 minutes).

Statistical analysis

All statistical analyses were conducted using ‘R’ (REF). VCO2 and body mass were natural log-transformed and then z-transformed to account for the allometric scaling relationship between metabolic rate and body mass. All temperature predictors (measurement temperature, body temperature in home enclosure, or previous temperature) were also natural log-transformed and then z-transformed Collinearity between our response and predictor variables were checked using scatterplot matrices. Pearson correlation coefficients of these are presented in Table SXX. We tested whether there were differences in VCO2 between batches of lizards or incubators by including these variables as covariates in our initial models. Neither of these variables were significant and therefore we removed from our final models.

Carry-over effects

Carry-over effects on VCO2 of previous measurement temperatures and body temperature measured in home enclosure (hereafter ‘body temperature’) were investigated with two methods of how we code ‘previous temperature’ as a covariate variable. The first method, we coded ‘previous temperature’ within each sampling day. For the first measurement temperature, we treated body temperature as the ‘previous temperature’ and the first measurement temperature as the ‘previous temperature’ for the second measurement temperature. The second method, we coded ‘previous temperature’ across each sampling day. For the first day, body temperature was treated as ‘previous temperature’. On the second and third day of measurements, the mean of the two measurement temperatures of the previous day was treated as ‘ previous temperature’. These two variations of ‘previous temperatures’ were then log-transformed and z-transformed and included separately in our final model as covariates. We present our results using ‘previous temperature’ derived from method two, as it yielded a lower AIC, however the output from the model using method one is presented in the ESM.

Repeatability in reaction norms

Repeatability in metabolic reaction norm parameters across our ten sampling series following {ArayaAjoy:2015ir}. We coded ‘series’ a categorical variable which denotes a unique combination of individual IDs and the sampling period IDs in order to group individual reaction norms that were measured at different sampling periods (see {ArayaAjoy:2015ir}). Our final model included: VCO2, mass, measurement temperature and previous temperature as fixed effects. We also fitted random intercept of individual ID and a random slope of measurement temperature in order to quantify variation in the intercept and slope of the reaction norm between individuals. We also fitted a random intercept of series and a random slope of measurement temperature to quantify variation in reaction norm parameters between individuals across sampling periods. We ran our models in the package ‘lme4’ (REF) and extracted the relevance variance components to calculate repeatability as below.We calculated repeatability in intercept (eqn 2) and slope (eqn 3) across all sampling periods using:

Eqn 2:

Eqn 3:

To estimate the proportion of total phenotypic variation in metabolic rate among individuals due to differences in time scales, we calculated short and long-term repeatability of the intercept (eqn 4 and eq5, respectively). [Read more about short term repeatability and elaborate, how is this diff from Rintercept, what does it mean to include Ve0]. Long-term repeatability includes variance among series, which represents short-term consistency between individuals caused by short-term consistency in environmental conditions.

Eqn 4:

Eqn 5:

Results

Discussion

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References