Title:

Keywords

Abstract

Abbreviations

Introduction

Importance of phenotypic plasticity

Phenotypic plasticity is the ability for an individual to modify their phenotype in response to environmental cues. It has been considered an important mechanism through which animals can cope with changing environments because in some cases, phenotypic adjustments can better match an individual to the environment. Plasticity of a particular trait is typically conceptualised as a reaction norm, where a trait e.g. activity rate is measured across an environmental gradient e.g. predator density. The intercept of this reaction norm represents an individual’s average expression of the trait and the slope represents how flexible the trait is in response to the environmental cue.

Individual differences in reaction norms

There is increasing appreciation for consistent individual differences in both the average expression and plasticity of traits. This is because selection can act on this individual variation, thereby changing the shape of reaction norms. Individual variation is summarised by estimating repeatability i.e. the proportion of total phenotypic variation explained by between-individual variation. Since between-individual variation incorporates genetic and environmental sources of variation, repeatability can determine the upper limits of heritability. Understanding the drivers and constraints of individual variation reaction norms can provide important insights in the evolution of plasticity.

Case for metabolism, eco-evolutionary implications in flexible MR

Metabolic rate – the rate at which food is converted into energy, underpins biological processes at every level of variation. Studies have shown that metabolic rate is highly plastic in response to temperature, food intake and… examples of MR plasticity. Flexibility in metabolic rate has important consequences because this means that the idling cost of living varies according to the environment.

Metabolic rate is linked to organism performance, growth and fitness

Indeed, individuals also show substantial differences in metabolic rate. A few meta-analyses have shown that metabolic rate is indeed repeatable, however this declines with time and varies between experimental conditions. and a few studies have shown that individuals differ in their metabolic reaction norms

Reaction norm insights in MR and eco-evolutionary implications

Some gap….

This study

Phenotypic plasticity {Pigliucci:2006bt}. In some cases, phenotypic plasticity can better match an animal to environmental conditions, allowing the animal to still perform optimally.

Plasticity is typically conceptualised as a reaction norm where some phenotypic trait is measured across an environmental gradient e.g. swimming performance at different water temperatures.

There is increasing interest in how plasticity itself evolves.

Directional natural selection can act on phenotypic variants, which can change the underlying reaction norm of a trait. As selection continues to persist, plasticity is lost, such that environmental stimulus is no longer needed to trigger the expression of a given phenotype i.e. the trait becomes canalised (genetic assimilation) {Pigliucci:2006bt}.

[i.e changes the shape of the reaction norm]

Reaction norm function value approaches – what does it bring to the table

Most organisms live in variable environments where they can experience fluctuations in biotic and abiotic factors on both daily and seasonal time scales.

MR is flexible and changes in response to many variables including food availability and temperature

Reaction norms or thermal performance curves? can also tell us about how flexible at trait is to a particular environmental variable, average trait values for an individual or population as well as, optimum expression of the trait at a given point in the environmental gradient.

Meta-analysis in evolution of reaction norms (Morrissey)

[Intercept and slope trade offs/covariances] - why do they exist?

In order for phenotypic plasticity to evolve, there must be variability between individuals in their reaction norms for selection to act on.

Repeatability and its relevance to the evolution of plasticity

Individuals vary in how they respond to environmental cues, this is important because

individual variation is raw material for selection to act on.

Review White and Ipso and lab vs captive study

Need relevant time scales in repeatability measures in order understand the evolution of plasticity.

Case for energy metabolism

Metabolism represents the idling cost of an individual and imposes constraints on resource allocation to growth, reproduction or maintenance.

Unsurprisingly, many have documented individual differences in metabolic rate across a variety of taxonomic groups.

Some have even argued that metabolism governs life history strategies and even behaviour, which integrates these traits from different levels of biological variation into ‘pace-of-life syndromes’.

[Why is metabolic rate important?]

Metabolic rate is intrinsically sensitive to changes in temperature. By investigating plasticity in metabolism, we can learn about plasticity in other ‘higher level’ traits due to the mechanistic links between metabolism and other whole-organism traits.

[Review of other studies, what is lacking?] 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 plasticity of metabolism using the delicate skink (*Lampropholis delicata*). The delicate skink is a native Australian species and has a widespread distribution throughout south east Australia. Small body size – experience greater fluctuations of temperature. An established model for personality and life history research.

Hypothesis: [Trade off in average rate and slope]

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

L.delicata reproductive season: Mating occurs Aug – Sept maybe? Female in reproductive mode from Sept – Feb.

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 LabAnalyst 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’ {TeamRAla:2013tx}. 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 temperature corrected using the Boltzmann constant {Gillooly:2001cg} .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.

Model fitting

We ran our models in the package ‘MCMCglmm’ {Hadfield:2010ud}. In order to group together individual reaction norms that were measured at different sampling periods, we coded ‘series’ a categorical variable which denotes a unique combination of individual IDs and the sampling period IDs (see {ArayaAjoy:2015ir}). Our final model included: VCO2, mass, measurement temperature and previous temperature as fixed effects. We 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 within individuals across sampling periods. Uninformative parameter expanded priors to improve mixing and convergence. We ran three parallel chains for 7,510,000 iterations, with a burn-in of 10,000 and a thinning interval of 5000 and pooled the posterior distributions. Mixing was checked by visually inspecting trace plots. Gelman and Rubin diagnostic was used to check for convergence. Non-independence between successive samples were checked using the ‘autocorr.diag’ function. We report posterior modes and 95% credible intervals. We also calculated conditional and marginal R2 values for our model following {Nakagawa:KLT2YYdG, Johnson:2014hl}.

For sensitivity analysis, we also ran our models using a maximum likelihood framework using the package ‘lme4’ {Bates:2014vy}. These results are presented in the supplementary materials.

Repeatability in reaction norms

We calculated repeatability following {ArayaAjoy:2015ir} by extracting the posterior distribution for the relevant variance components. 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 within individuals, between sampling periods, we calculated short and long-term repeatability of the intercept (eqn 4 and eq5, respectively). The calculation for short-term repeatability includes within individual, between sampling period variability (series) in the numerator. This accounts for short-term consistency between individuals caused by short-term consistency in environmental conditions, whereas, long-term repeatability calculations does not.

Eqn 4:

Eqn 5:

Results

Both body mass and temperature had a significant positive effect on metabolic rate (Table 1). There were significant differences in the individual intercepts and slopes (Table 1). Individual intercepts and slopes were also moderately repeatable within each sampling period (Table 1). In other words, individuals differed in their average metabolic rate as well as, how their metabolic rate changed in response to temperature (Fig.1). As predicted, the repeatability of individual intercepts decreased over time (Table 1). This indicates the variability in average metabolic rate between individuals decreased across sampling periods.

There was a positive relationship between individual intercepts and slope (Table 1, Fig. 2a). This suggests that individuals with high average metabolic rates responded more strongly to temperature changes, while individuals with low average metabolic rates, were less responsive to temperature changes.

We found that within-individual, across sampling periods intercepts and slopes covaried negatively (Table 1, Fig. 2b). This means that over time, within an individual, a high average metabolic rate is less plastic in their response to temperature, whereas a low average metabolic rate is more responsive to temperature (Fig. 3).

Discussion

We found that individual metabolic reaction norms in response to temperature were moderately repeatable. As predicted, repeatability of average metabolic response decreased over time. Our results show that individuals with a high average metabolic rate were also more flexible in their response to temperature. Interestingly, we found that within an individual, thermal plasticity of metabolic rate is constrained by the average metabolic rate such that a high average metabolic rate is associated with a low capacity to respond to temperature.

Repeatability of reaction norms attributes

Our study shows that there were consistent individual differences in intercepts/ average metabolic rate and slopes/plasticity in metabolic reaction norms.

Temporal/seasonal patterns in reaction norm repeatability

We found that repeatability in intercepts/average metabolic rate decreased over time.

Between-individual covariance in mean trait values and plasticity

At the between-individual level, our estimates of covariance between intercept and slope revealed that there is positive relationship between average metabolic rate and plasticity to respond to temperature.

Temporal within-individual covariance in mean trait values and plasticity

Our approach in modelling between sampling period variation in reaction norm allowed us to identify temporal changes in the covariance between intercepts and slopes.

Conclusions

Acknowledgements

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References

Figure legends

Figure 1. Predicted metabolic rate as a function of Boltzmann constant corrected temperature (K) for 42 male *L. delicata* lizards across ten sampling periods. Predictions were made from model in Table 1 after fixing body mass and prior temperature to the mean.

Figure 2a. Predicted intercepts and slopes at the a) between-individual level and b) within-individual, between-sampling periods level, across ten sampling periods for 42 male *L. delicata* lizards across ten sampling periods. Predictions were made from model in Table 1 after fixing body mass and prior temperature to the mean. Horizontal and vertical error bars represent the 95% credible intervals for the intercept and slope, respectively.

Figure 3. Predicted intercepts and slopes at the within-individual, between-sampling periods level for 42 male *L. delicata* lizards across ten sampling periods. Horizontal and vertical error bars represent the 95% credible intervals for the intercept and slope, respectively. Darker blue colours represent earlier sampling periods, lighter blue colours represent later sampling periods.

../output/fig/reaction.norms.pdfFigures

../../../Downloads/Presentation1.pdfFigure 1

Figure 2

../../../Downloads/Presentation2.pdf

Figure. 3

Tables

|  |  |  |  |
| --- | --- | --- | --- |
| Table 1. Posterior modes and variance components of Z-transformed log metabolic rate as a function of corrected temperature, z-transformed ln mass and prior temperature estimated from a Monte Carlo Markov Chain generalised linear mixed effects model with random intercepts of individual (n = 42) and within individual-between sampling period (n = 420) with respect to random slope of temperature. Bolded values are significant. | | | |
| Fixed effects (R2marginal = 0.45) | Estimate | Lower CI | Upper CI |
| Intercept | -0.02 | -0.11 | 0.11 |
| Temperature | **1.39** | **1.29** | **1.48** |
| Z-transformed lnMass | **0.35** | **0.29** | **0.42** |
| Prior temperature | 0.07 | -0.05 | 0.15 |
| Random effects (R2conditional = 0.64) |  |  |  |
| Between-individuals |  |  |  |
| Ind0 | **0.09** | **0.06** | **0.17** |
| Ind1 | **0.04** | **0.01** | **0.10** |
| Cov(Ind0, Ind1) | 0.02 | -0.01 | 0.06 |
| Within-individuals, between-sampling periods |  |  |  |
| Series0 | **0.08** | **0.06** | **0.10** |
| Series1 | **0.04** | **0.01** | **0.12** |
| Cov(Series0, Series1) | **-0.04** | **-0.07** | **-0.02** |
| Residual variance (e) | **0.4** | **0.37** | **0.42** |
| Repeatability |  |  |  |
| Rintercept | 0.59 | 0.42 | 0.71 |
| Rslope | 0.45 | 0.14 | 0.85 |
| Rshort term | 0.32 | 0.25 | 0.40 |
| Rlong term | 0.17 | 0.11 | 0.27 |