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RESEARCH ARTICLE

Life stages differ in plasticity to temperature fluctuations and uniquely contribute to adult phenotype in *Onthophagus taurus* dung beetles

Amanda W. Carter* and Kimberly S. Sheldon

ABSTRACT

Adaptive thermal plasticity allows organisms to adjust their physiology to cope with fluctuating environments. However, thermal plasticity is rarely studied in response to thermal variability and is often measured in a single life stage. Plasticity in response to thermal variability likely differs from responses to constant temperature or acute stress. In addition, life stages likely differ in their plasticity, and responses in one stage may be affected by the experiences in a previous stage. Increasing the resolution with which we understand thermal plasticity in response to thermal variation across ontogeny is crucial to understanding how organisms cope with the thermal variation in their environment and to estimating the capacity of plasticity to mitigate costs of rapid environmental change. We wanted to know whether life stages differ in their capacity for thermal plasticity under temperature fluctuations. We reared *Onthophagus taurus* dung beetles in either low or high temperature fluctuation treatments and quantified thermal plasticity of metabolism of pupae and adults. We found that adults were thermally plastic and pupae were not. Next, we tested whether the plasticity observed in the adult life stage was affected by the thermal conditions during development. We again used low and high temperature fluctuation treatments and reared individuals in one condition through all egg to pupal stages. At eclosion, we switched half of the individuals in each treatment to the opposite fluctuation condition and, later, measured thermal plasticity of metabolism in adults. We found that temperature conditions experienced during the adult stage, but not egg to pupal stages, affect adult thermal plasticity. However, temperature fluctuations during development affect adult body size, suggesting that some aspects of the adult phenotype are decoupled from previous life stages and others are not. Our data demonstrate that life stages mount different responses to temperature variability and uniquely contribute to the adult phenotype. These findings emphasize the need to broadly integrate the life cycle into studies of phenotypic plasticity and physiology; doing so should enhance our ability to predict organismal responses to rapid global change and inform conservation efforts.

KEY WORDS: Acclimation, Body size, Coleoptera, Development, Dung beetle, Metabolism, Ontogeny, Temperature fluctuation

INTRODUCTION

Thermal plasticity allows organisms to adjust their physiology to suit the current conditions and is thought to be especially important in fluctuating environments (Kingsolver and Huey, 1998; Lande, 2014; Woods and Harrison, 2002). Paradoxically, thermal plasticity is rarely measured in response to fluctuating temperatures, and instead is often measured following exposure to constant temperature or acute thermal stress (Niehaus et al., 2012). This is problematic because temperature fluctuations can dramatically alter performance (e.g. metabolic rate) (Jensen, 1906; Ruel and Ayres, 1999; Williams et al., 2012) and may trigger plasticity via different suites of mechanisms compared with constant temperature (Sørensen et al., 2016). Understanding plastic responses to temperature variation is crucial to evaluating how most animals cope with thermal variation in their environment and to deciphering the capacity of plasticity to mitigate costs associated with rapid environmental change.

Thermal plasticity is not likely uniform across an individual's life. Life stages can experience unique selection pressures or exhibit distinct behaviors and physiologies that alter thermal plasticity (Fischer et al., 2014). For example, life stages inhabiting environments with high temperature variation may experience selection for high thermal plasticity (i.e. climate variability hypothesis) (Colinet et al., 2015; Sheldon et al., 2018; Woods, 2013). In contrast, life stages with greater mobility may evade selection pressures from fluctuating conditions and exhibit reduced thermal plasticity (i.e. Bogert effect) (Bogert, 1949; Marais and Chown, 2008; Mitchell et al., 2013). Despite this, the bulk of thermal biology research considers a single life stage at a time, most often the adult stage (Chiu et al., 2015; Kingsolver, 2009; Kingsolver et al., 2011; Radchuk et al., 2013). Knowing whether and how life stages vary in thermal plasticity may facilitate significant strides in the accuracy of climate change modeling; estimates not based on the most critical life stages may over- or under-estimate persistence (Chiu et al., 2015; Kingsolver et al., 2011; Levy et al., 2015; Pincebourde and Casas, 2015; Radchuk et al., 2013).

In addition to independent plastic responses in each life stage, temperatures in an earlier life stage may alter the phenotype of a later life stage, including the ability to be plastic. Temperatures early in ontogeny can cause organizational shifts in growth and development trajectories that may permanently impact adult phenotype (i.e. developmental plasticity) (Beldade et al., 2011; Uller, 2008; West-Eberhard, 2003). For example, over 80% of surveyed ectotherms exhibit larger body sizes following cooler developmental temperatures (Atkinson, 1994), which can have outsized effects on adult physiology and fitness (Kingsolver and Huey, 2008; Stillwell and Fox, 2005). In addition to affecting mean trait value, developmental conditions may also modify the capacity for plasticity later in life, an area of research that has received surprisingly little attention (Beaman et al., 2016). For example, development at cooler or warmer temperatures enhances the capacity for thermal acclimation in adult zebra fish (*Danio rerio*) compared with development at an intermediate temperature (Scott and Johnston, 2012). Further complicating life stage–temperature

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interactions is the diversity of life history patterns among ectotherms. Unlike vertebrate ectotherms where growth and development are incremental, ectotherms with modular life cycles, like insects, may decouple the thermal conditions and physiological responses from one life stage to the next [‘life cycle modularity hypothesis’ (Potter et al., 2011); ‘adaptive decoupling hypothesis’ (Moran, 1994; Stoks and Cordoba-Aguilar, 2012; Gray, 2013; Kingsolver et al., 2011)].

As increasing thermal variation in the environment can increase energetic costs (Ruel and Ayres, 1999; Williams et al., 2012), quantifying shifts in metabolism under increased temperature fluctuation is an especially well-suited measure of plasticity. Metabolism is a multifaceted and dynamic process that can provide insight into overall energy budgets of organisms in fluctuating environments with consequences for organismal fitness and population dynamics (Brown et al., 2004; Chown and Gaston, 1999; Chown and Nicolson, 2004; Dillon et al., 2010; Lighton, 2018; Norin et al., 2016; Sibly et al., 2012). Whole-organism metabolism can be measured via respirometry, which directly measures CO_2 and/or O_2 consumption. When respirometry is measured across a range of temperatures, this enables simultaneous estimation of metabolic rate and thermal sensitivity of metabolism (Lighton, 2018). Depending on the experimental design, thermal plasticity can also be quantified by comparing shifts in metabolic parameters among genetically similar groups (e.g. clones or full-siblings) exposed to different environments (Seebacher et al., 2015).

Thermal plasticity can be measured as shifts in thermal sensitivity of metabolism or overall metabolic rate when individuals or cohorts are exposed to different environments. Thermal sensitivity of metabolism is the relationship between temperature and metabolic rate (e.g. slope of the function, Q_{10}) and dictates energy expenditure in fluctuating environments (Lake et al., 2013; Ruel and Ayres, 1999; Williams et al., 2012). Seemingly minimal adjustments in the thermal sensitivity of metabolic rate can rapidly compound to alter whole-organism performance (Burton et al., 2011; Metcalfe et al., 1995; Williams et al., 2012). In the larvae of *Erynnis propertius*, for example, individuals exposed to increased thermal variation decreased their thermal sensitivity of metabolism (i.e. reduced the slope of the temperature–metabolism function) to reduce energetic costs (Williams et al., 2012). Metabolic rate, in contrast, is simply the magnitude of the metabolic response (e.g. intercept) and can be measured with total CO_2 production (Lighton, 2018).

We compared thermal plasticity in response to increased temperature fluctuation in adults and pupae of *Onthophagus taurus* dung beetles. *Onthophagus taurus* is an excellent system for such questions as they are holometabolous insects with a life history that may select for differential thermal physiology across life stages. In particular, eggs, larvae and pupae develop underground within a parentally provisioned brood ball (Halffter and Edmonds, 1982), which buffers young from daily temperature extremes. Conversely, adults experience a broader range of thermal extremes as they fly in open fields in search of dung and inhabit dung pats for foraging and reproduction (Fig. S1). First, we wanted to know whether life stages, specifically pupae and adults, vary in their capacity for thermal plasticity in response to increased temperature fluctuations. As increases in temperature fluctuations can increase energetic costs, organisms should decrease thermal sensitivity of metabolism and/or decrease overall metabolic rate to conserve energy (Ruel and Ayres, 1999). We predicted that adults would exhibit greater plasticity than pupae, as they likely encounter greater temperature variation across microhabitats.

We next wanted to know whether the conditions experienced during early life stages affect adult plasticity. We predicted that adults would exhibit plasticity in response to their current environment, but that the magnitude of the plastic response would be attenuated by developmental conditions. Because developmental temperatures may impact adult phenotype through a myriad of mechanisms, we were also interested in determining whether increased thermal variation affected body size, a trait important to fitness in *O. taurus* (Moczek and Emlen, 1999). As fluctuating environments can be energetically costly, we predicted that beetles reared in the high temperature fluctuation treatment during development would be smaller than those reared in the low fluctuation treatment, regardless of the temperatures experienced as an adult.

MATERIALS AND METHODS

Do life stages differ in thermal plasticity to increased temperature fluctuation?

We trapped *Onthophagus taurus* (Schreber 1759) beetles in June 2018 in Kings Mountain, NC, USA (n=115). We brought adults to the lab, housed them in breeding triads (one male with two females) with *ad libitum* access to autoclaved cow dung, and collected and individually reared offspring from resulting F_1 brood balls. At approximately 4 weeks post-adult emergence, we paired a virgin F_1 female with an unrelated virgin F_1 male, creating 31 families. We collected F_2 brood balls every 3 days. We individually reared F_2 brood balls at either a low fluctuation (24±4°C) or high fluctuation (24±8°C) temperature treatment using a split-family design (Fig. 1). Temperature treatments fluctuated in a near-sinusoidal fashion to simulate daily temperature fluctuations in the field. We chose these temperatures because the mean and variance are within the range of temperatures normally experienced by adults and pupae during breeding as verified with temperature logger data (Fig. S1 and additional unpublished data). Brood balls and adults were housed within individual ∼59 ml containers filled with moist soil in incubators. We verified that the soil within the containers did not significantly insulate individuals (Fig. S2), and that the ambient

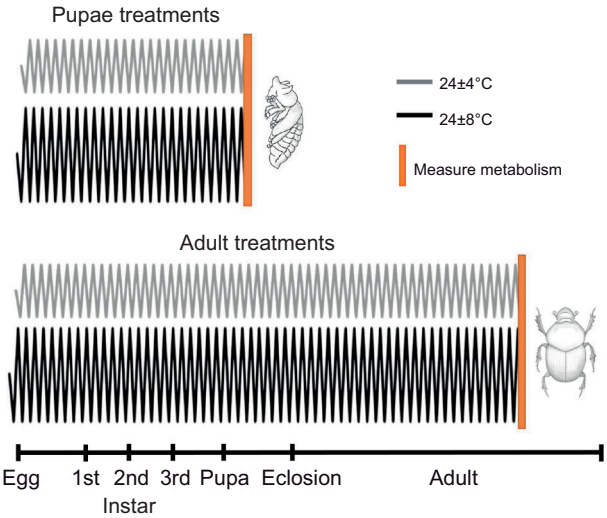

Fig. 1. Do life stages differ in thermal plasticity to increased temperature fluctuation? Using a full-sibling design, *Onthophagus taurus* F_2 brood balls were placed into an incubator running either a low or high temperature fluctuation condition during the egg stage. At either the pupal or adult stage, we used respirometry to test for life stage differences in thermal plasticity, thermal sensitivity of metabolism and metabolic rate.

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temperatures within incubators reflected realized temperatures of experimental individuals. In total, we used 21 families for analyses as some of the original 31 families did not produce enough brood balls. We conducted metabolic trials on F_2 pupae (3 weeks post-egg laying) and adults (3 weeks post-eclosion) using stop-flow and flow-through respirometry, respectively (see 'Respirometry methods', below).

Do temperatures during early life stages affect adult responses to thermal fluctuations?

We reared F_2 individuals in a low fluctuation (24±4°C) or high fluctuation (24±8°C) temperature treatment through all egg to pupal stages (Fig. 2). At eclosion, half of the individuals in each treatment were placed back in their temperature fluctuation treatment, while the other half were switched to the opposite temperature fluctuation treatment. This created four unique fluctuation treatment combinations: low–low, low–high, high–high and high–low. Approximately 4 weeks after adult emergence (which mirrors the duration spent in the developmental treatment), we conducted open-flow respirometry trials on the adults (see 'Respirometry methods', below). This approach allowed us to examine whether thermal fluctuations during development leave a signal on the adult phenotype.

Respirometry methods

We measured CO_2 of each pupa or adult at four sequential trial temperatures: 15, 20, 25 and 30°C. To do this, we used a pump (SS4, Sable Systems International, Las Vegas, NV, USA) to push air free of CO_2 and water vapor ('zero air'; Airgas, Knoxville, TN, USA) through a metabolic set-up at a rate of 120 ml min^{-1}. The zero air was chemically scrubbed of CO_2 and water vapor with Ascarite (Sigma-Aldrich, St Louis, MO, USA) and Drierite (Xenia, OH, USA), respectively, as an additional precaution. As such, we know any CO_2 measured downstream of the beetle was produced exclusively by the beetle. We measured CO_2 with a combination CO_2 and H_2O analyzer (LI-7000; Li-Cor, Lincoln, NE, USA). For pupal trials, we first weighed each pupa and placed them individually in a 20 ml syringe. We perfused the syringe with zero air, sealed it, and placed it in an incubator at the lowest of the four trial temperatures (i.e. 15°C) for 30 min. After exactly 30 min, we removed the syringe from the incubator and injected 10 ml of air from the syringe into the tubing preceding the combination CO_2 and H_2O analyzer at a rate of 0.5 ml s^{-1} to measure respiration rate. We then repeated the procedure at the next trial temperature until the individual had been trialed at all four temperatures. All individuals received the same increasing temperature series across metabolic trails. Though this presents an opportunity for acclimation from previous trial temperatures, measuring thermal sensitivity to a 'ramping' temperature series mimics diurnally increasing temperatures in the field. At the end of the trial, we again recorded pupa mass and noted sex (female, major male, minor male). At the pupal stage, deciphering between minor males and females can be challenging at times, so in the instance that a definitive sex could not be assigned, the datum was omitted.

For measuring respiration in adults, we first weighed an adult and then placed it in a glass chamber connected to tubing in between the flow rate pumps and the combination CO_2 and H_2O analyzer. The chamber was held in the dark to minimize adult activity, as demonstrated in pilot assays. However, we did not restrain beetles, so it is possible that activity may contribute to our results. We held the adult in the glass chamber at the lowest trial temperature (15°C) and passed air continuously over the beetle for 15 min. Data from the last 5 min of this period were used for analyses. We then increased the incubator temperature to the next warmest trial temperature and repeated the respiration measurements. We took baseline CO_2 readings before and after each trial temperature. During baseline readings, CO_2 and H_2O values returned to zero, so any CO_2 measured during a trial reading was from the beetle currently being measured. Baseline readings also allowed us to correct for any drift in the zero air being pushed through the setup and to monitor for contamination or inconsistencies. We also weighed the adult after the metabolic trials.

We used the response variable CO_2 production (µl min^{-1}) to examine three parameters: metabolic rate, thermal sensitivity of metabolism and thermal plasticity. Metabolic rate is quantified as overall CO_2 production and gauges total energetic cost. Thermal sensitivity of metabolism is the steepness of the temperature–metabolic rate function and can influence energy expenditure under temperature fluctuations. Finally, thermal plasticity is the shift in thermal sensitivity between treatments. For example, a significant difference in thermal sensitivity of metabolism between adults reared in the high and low fluctuation treatments would indicate thermal plasticity. Though all three metrics are inferred from the same dataset, these are individual traits that can respond independently and may often be under unique selective pressure (Brown et al., 2004).

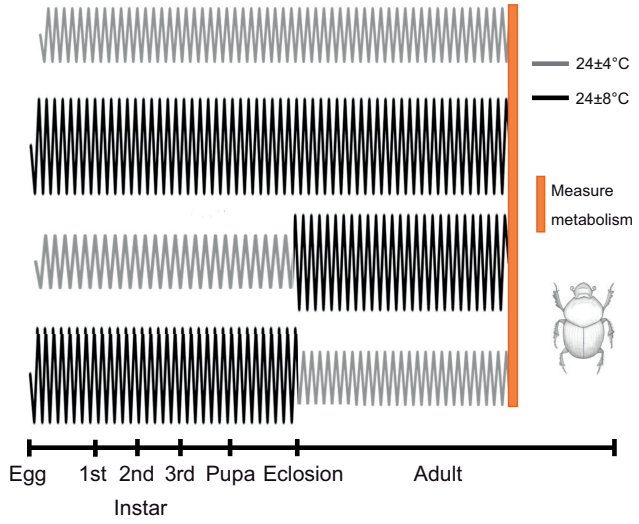

Fig. 2. Do temperatures experienced during early life stages affect adult plasticity and phenotype? We reared the F_2 generation of *O. taurus* individuals from the egg stage to the pupal stage under either a low or high temperature fluctuation condition. At eclosion, half of the individuals in each treatment were switched to the opposite temperature fluctuation condition and remained there for an equivalent duration to that for development (approximately 4 weeks). Then, we used respirometry to test for treatment differences in thermal plasticity, thermal sensitivity of metabolism and metabolic rate of adults. At this time, we also measured adult body mass to determine whether temperature fluctuations during development affect this trait.

Data analysis

Do life stages differ in thermal plasticity to increased temperature fluctuation?

To process metabolic data, we first corrected raw CO_2 data for any drift in baseline readings using a Catmull–Rom spline correction (Catmull and Rom, 1974) and smoothed the data using a Savitsky–Golay filter (Savitzky and Golay, 1964) with an 11-step window. In

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stop-flow trials we accounted for the space within the syringe occupied by the pupa in calculations of CO_2 production using the simplifying assumption that pupae are largely composed of water and 1 ml of water weighs approximately 1 g. We log +1 transformed all data before using linear analyses as doing so improved AIC values. We considered models that characterized metabolic trial temperature as a continuous effect (covariate) or a categorical effect (fixed effect). On the one hand, estimating thermal sensitivity of metabolism as a slope intuits the use of a continuous temperature axis. On the other hand, our static temperature respirometry methods (Lake et al., 2013), where beetles experience four different constant temperatures, make a case for a categorical temperature axis. In the present model, the biological outcomes were similar between the two analyses, so we only present the model where metabolic trial temperature was treated as a covariate. We used a general linear mixed model (Proc Mixed, SAS v9.4) that included the fixed effects of temperature fluctuation treatment and life stage, the covariate of metabolic trial temperature, and all three- and two-way interactions. We also included beetle mass as a covariate, sex as a fixed effect and family as a random effect. We used a repeated statement specifying individual beetle as the subject to account for non-independence among an individual's four CO_2 measurements and specified an unstructured covariance matrix. We used the Satterthwaite method to approximate degrees of freedom and included a solution statement in the model line to print the estimated model coefficients (Tables S1–S3). An outlier datum from one adult's 30°C metabolic temperature trial was removed from final analyses based on a studentized residual greater than an absolute value of 3. Removing the outlier did not change our overall conclusions (i.e. the best fit model remained the same with and without the outlier). Effects were sequentially removed from the final model if they were not significant and doing so improved AIC values by a threshold value of 4. The final model included life stage, thermal fluctuation treatment, metabolic trial temperature, all three-way and two-way interactions, sex, family and mass.

To better disentangle the three-way interaction, we also tested for differences in plasticity due to the temperature fluctuation treatments within a single life stage. These two follow-up models utilized a similar approach to that above and corrected for multiple comparisons.

Finally, to verify that our measures of CO_2 correspond to energy expenditure, we used energy equivalents for CO_2 production to calculate energy expenditure in the situation where adults were catabolizing carbohydrates (21.1 kJ l^{-1}) and pupae were catabolizing lipids (27.8 kJ l^{-1}) (Fig. S3) (Walsberg and Hoffman, 2005). Our main conclusions did not change, and, thus, we assume CO_2 is a good proxy for energy expenditure.

Do temperatures during early life stages affect adult plasticity and phenotype?

To answer this question, each individual was exposed to a developmental condition and an adult condition. We analyzed the data using a two-way approach so that we could parse the relative importance of developmental or adult temperature conditions to adult thermal responses. We used a similar model and selection method to that described above. In this dataset, categorizing metabolic trial temperature as a continuous or categorical variable (see above) affected some of the statistical outcomes, so we present both versions of the analysis for full consideration. The final model where metabolic trial temperature was coded as a covariate included metabolic trial temperature, the fixed effects of the pupal and adult treatments and the covariate of mass. The final model where metabolic trial temperature was coded as a fixed effect included metabolic trial temperature and its two-way interactions with the pupal and adult treatments, the fixed effects of the pupal and adult treatments, and the covariate of mass.

We also tested whether the temperature fluctuation treatments affected adult body size. We used a general linear model that tested for the effect of the developmental temperature treatment, adult temperature treatment, and their interaction on adult body size (mass).

RESULTS

Do life stages differ in thermal plasticity to increased temperature fluctuation?

Our goal was to determine whether thermal plasticity differs between pupae and adults. Thermal plasticity was measured as a significant change in the CO_2–temperature function (i.e. a shift in thermal sensitivity) between the high and low fluctuation treatments, within a life stage. In the full model, the significant three-way interaction demonstrated that life stages vary in thermal plasticity (Fig. 3, Table 1). Follow-up analyses based on two separate mixed effects models for each life stage revealed that adult beetles significantly decreased thermal sensitivity of metabolism under the high fluctuation treatment (Fig. 3A, P=0.02), whereas pupae showed no change in thermal sensitivity of metabolism between the two temperature fluctuation treatments (Fig. 3B, P=0.69). Thus, we observed plasticity in thermal sensitivity of metabolism in adult beetles, but not pupae.

In addition to examining stage-specific thermal plasticity via shifts in thermal sensitivity (above), our data also allowed us to examine thermal sensitivity of metabolism itself (i.e. the steepness of the temperature–metabolic rate function). Life stages differed in thermal sensitivity of metabolism; adults exhibited a steeper slope in the CO_2 production–metabolic trial temperature function than pupae (Table 1, Fig. 3; life stage×metabolic trial temperature interaction: $F_{1,74.2}$=146.67, P<0.0001). Additionally, thermal sensitivity of metabolism was lower in the high fluctuation treatment than in the low fluctuation treatment (Table 1; $F_{1,74.2}$=5.29, P=0.024); however, this appears to be driven by treatment differences in the metabolism of adults and not pupae.

Finally, our model also allowed us to examine differences in metabolic rate among groups. Adult metabolic rate (CO_2 production) ranged from a low of 0.53±0.03 µl min^{-1} to a high of 1.68±0.18 µl min^{-1} at trial temperatures of 15 and 30°C, respectively. Pupal metabolic rate was much lower, ranging from a low of 0.12±0.017 µl min^{-1} to a high of 0.23±0.009 µl min^{-1} at 15 and 30°C, respectively (Fig. 3). Not surprisingly, the covariate of body size affected CO_2 production (Table 1).

Do temperatures experienced during early life stages affect adult plasticity and phenotype?

We wanted to understand whether temperatures during development affect adult responses to temperature fluctuations. We found that temperature fluctuations experienced during development did not affect metabolic rate, thermal sensitivity of metabolism or thermal plasticity during adulthood (Table 2, Fig. 4A). Instead, the temperature fluctuations experienced during adulthood triggered differential thermal sensitivity of metabolism (adult treatment×metabolic trial temperature: $F_{3,67}$=3.42, P=0.022; Table 2), where beetles that experienced the high fluctuation treatment during adulthood (regardless of developmental treatment) exhibited lower thermal sensitivity of metabolism than beetles that

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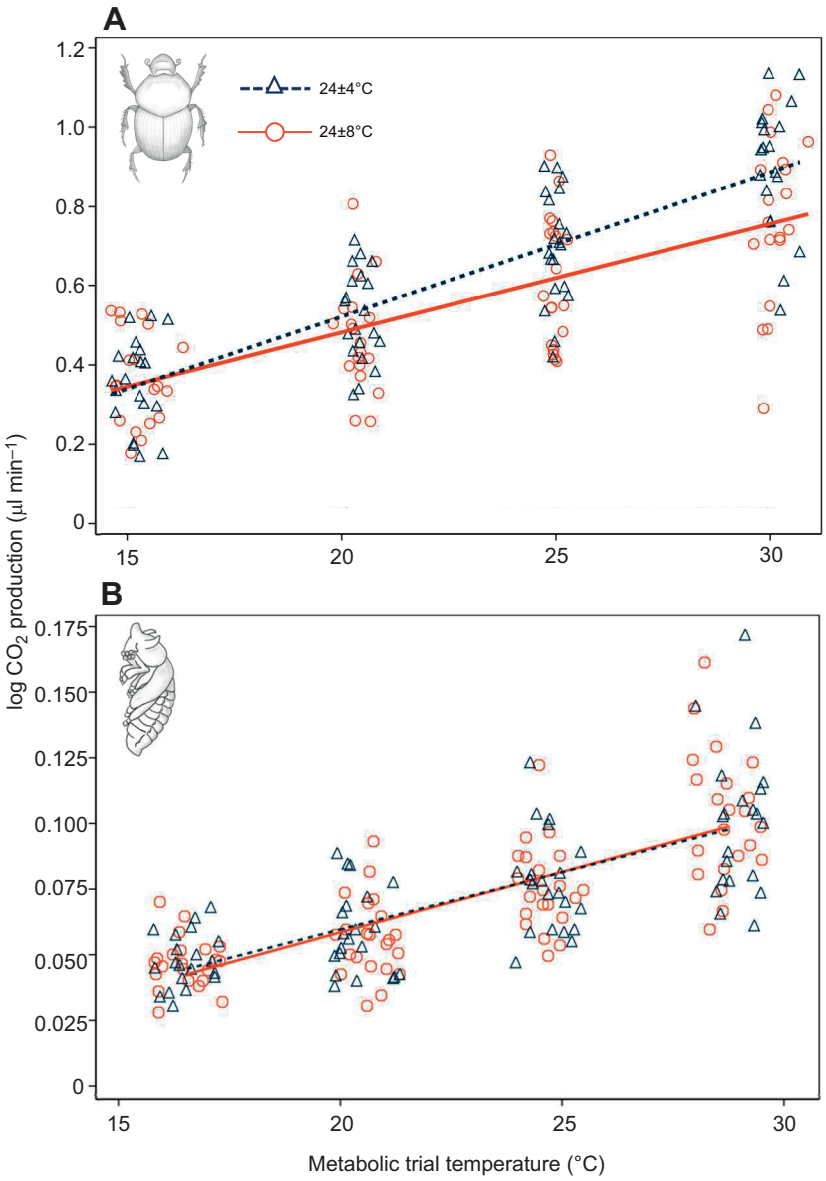

Fig. 3. Life stages differ in thermal plasticity as demonstrated by a significant shift in thermal sensitivity of metabolism between temperature fluctuation treatments in adults, but not pupae. (A) Adult data and (B) pupal data for the low temperature fluctuation treatment (24±4°C) and the high temperature fluctuation treatment (24±8°C). Each point represents the CO_2 production (µl min^{-1}) of an individual at that temperature (see Materials and Methods for details on adult and pupal respirometry) and are jittered along the *x*-axis to better display overlapping points.

experienced the low fluctuation treatment as adults (Fig. 4A). Metabolic rate was also marginally affected by the adult fluctuation treatment ($F_{1,63.4}$=4.09, P=0.048; Table 2). Specifically, adults that experienced the high temperature fluctuations during adulthood (regardless of developmental conditions) had lower overall metabolic rates than beetles that experienced the low fluctuation treatment during adulthood (Fig. 4A). However, these two effects were not evident in both versions of the model (Table 2).

We examined whether temperature fluctuations affect adult mass. On average, beetles from the low temperature fluctuation treatments weighed 0.095±0.003 g, whereas beetles from the high temperature fluctuation treatment were 14% smaller, weighing 0.082±0.003 g.

Table 1. Do life stages differ in thermal plasticity to increased temperature fluctuation?

Effect	d.f. (n,d)	*F*-value	*P*-value
Life stage×temperature fluctuation treatment×metabolic trial temperature	1, 74.2	5.76	0.019
Life stage×metabolic trial temperature	1, 74.2	146.67	<0.0001
Temperature fluctuation treatment×metabolic trial temperature	1, 74.2	5.29	0.024
Life stage×temperature fluctuation treatment	1, 70.5	5.69	0.02
Life stage	1, 93.1	14.01	0.0003
Temperature fluctuation treatment	1, 69.7	4.23	0.043
Metabolic trial temperature	1, 74.2	224.58	<0.0001
Sex	2, 72.2	3.59	0.033
Body size (g)	1, 73.7	15.81	0.0002

Note: the response variable is CO_2 production. Only the model characterizing metabolic trial temperature as a covariate is shown.

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Table 2. Do temperature fluctuations experienced during early life stages affect adult plasticity and phenotype?

Effect	d.f. (n,d)	*F*-value	*P*-value
Model characterizing metabolic trial temperature as a fixed effect			
Adult treatment×metabolic trial temperature	3, 67	3.42	0.022
Developmental treatment×metabolic trial temperature	3, 67.1	1.35	0.267
Adult treatment	1, 63.4	4.09	0.048
Developmental treatment	1, 67.1	0.96	0.331
Metabolic trial temperature	3, 64.5	208.08	<0.0001
Body size (g)	1, 70	25.79	<0.0001
Model characterizing metabolic trial temperature as a covariate			
Adult treatment	1, 70	2.58	0.113
Developmental treatment	1, 70.1	0.20	0.658
Metabolic trial temperature	1, 68.1	419.30	<0.0001
Body size (g)	1, 70	25.40	<0.0001

Note: the response variable is CO_2 production.

Beetles that experienced high temperature fluctuation conditions during development were significantly smaller than those that developed in the low temperature fluctuation treatment (Fig. 4B; developmental treatment: $F_{1,65}$=8.16, P=0.006). Though structural body size is fixed at eclosion, mass can change slightly as a result of feeding post-eclosion. Thus, we also wanted to determine whether

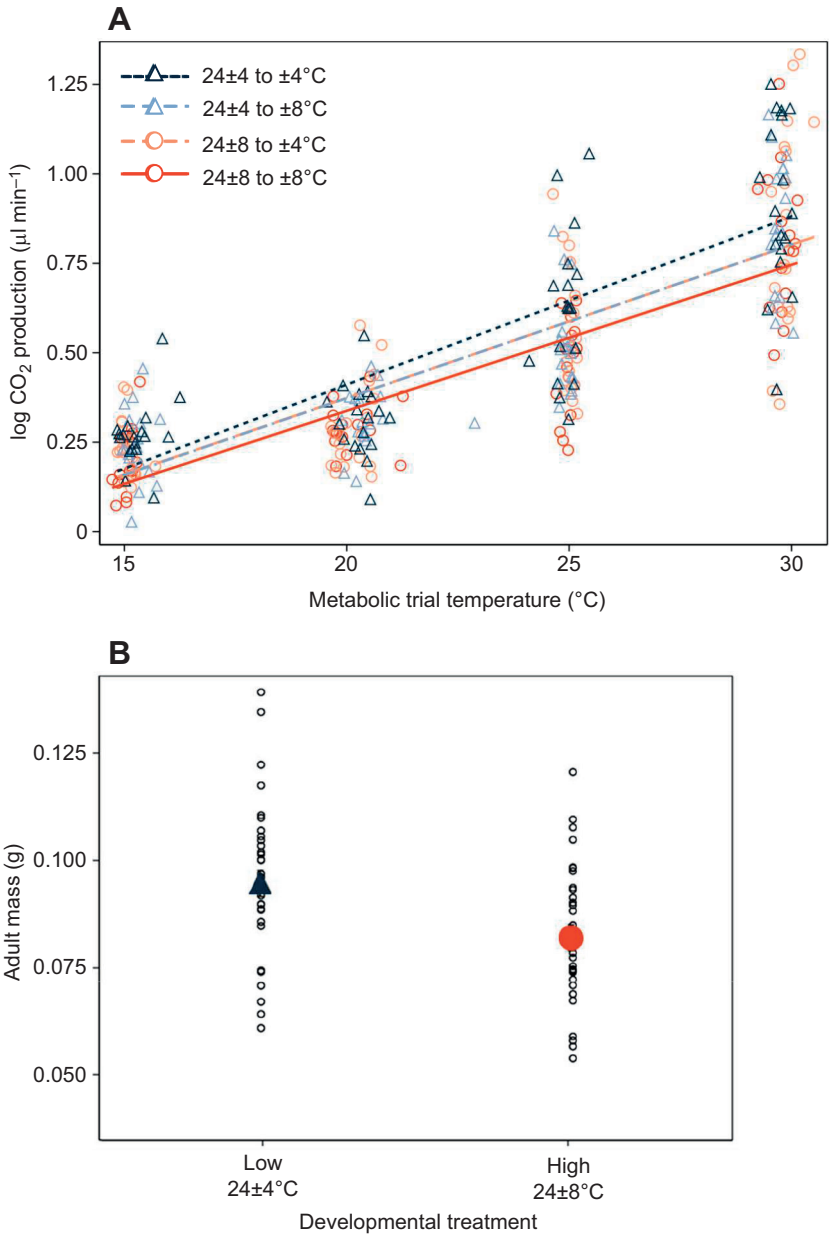

Fig. 4. Temperatures experienced during early life stages affect adult body size but not thermal plasticity. (A) Adult thermal plasticity and (B) body size in relation to different temperature treatments experienced during development. For thermal plasticity (A), the temperature fluctuation treatments included high–high (24±8 to ±8°C), low–low (24±4 to ±4°C), high–low (24±8 to ±4°C) and low–high conditions (24±4 to ±8°C). Adult body mass (B) (least square mean±1 s.e.) is shown as a function of the developmental temperature condition.

the temperature fluctuation treatment experienced during adulthood impacted adult mass. The adult treatment did not affect mass ($F_{1,65}$=0.98, P=0.33) nor did the interaction between adult and developmental treatments ($F_{1,65}$=0.04, P=0.85).

DISCUSSION

Life stages may experience varying selective pressures from stage-specific environments and thus thermal plasticity may differ across an individual's lifetime. As predicted, we found that adults mount plastic responses to increased temperature fluctuation and pupae do not. However, pupae were less thermally sensitive (on average) and, not surprisingly, exhibited lower metabolic rates. Coupled with the broad thermal tolerance of pupae reported elsewhere (Klockmann et al., 2017; Moghadam et al., 2019; Pincebourde and Casas, 2015), our data suggest that while adults may rely on thermal plasticity to cope with thermal variation, pupae may alternatively rely on a broad thermal tolerance and low overall metabolic rate to cope with daily temperature variation. It is unclear whether these varying strategies will provide equal protection under future, more variable climates. It is worth noting that, while the amount of exposure to the temperature treatments was similar across developmental and adult stages (i.e. 3 weeks for each), the exposure strictly during the pupal stage was less (~5–7 days). It is possible that differences in stage-specific exposure contribute to differences in thermal plasticity between adults and pupae; however, as we tested mature pupae, our data reflect the maximum acclimation of pupae at these temperatures. Nonetheless, the stage-specific differences herein underscore that the physiology of one life stage should not be used to more generally predict responses across life stages.

The thermal plasticity exhibited by adults is in response to the temperatures experienced during adulthood (i.e. acclimation) rather than during development. Beetles that experienced high temperature fluctuations during adulthood, regardless of developmental conditions, exhibited lower thermal sensitivity of metabolism and lower metabolic rates. This suggests that adult thermal plasticity and metabolism are decoupled from the environment and physiology of egg to pupal stages. This is surprising given the wealth of data linking constant developmental temperatures to variation in adult phenotype in insects (Angilletta, 2009; Atkinson, 1994; Chown and Terblanche, 2006; Gray, 2013). However, it is possible that more extreme fluctuations than those used in our study are required to leave a lasting signal on adult phenotype in *O. taurus*.

We found that adult body size decreased with increasing temperature fluctuations during development, demonstrating that adult phenotype is not wholly independent from the thermal environment of previous stages. Increased temperature fluctuations can increase energetic demands (Ruel and Ayres, 1999; Williams et al., 2012). Though we found no evidence of metabolic compensation in early life stages to reduce these energetic demands, our body size data suggest individuals developing in the high fluctuation treatments had fewer energetic resources available for growth and thus were smaller than individuals that developed in the low fluctuation treatment. Because mass was measured 3 weeks after eclosion, it is possible that differences in the adult stage between treatments, like feeding rates, may contribute to mass differences; however, we did not find any effect of the adult environment on adult mass. Therefore, any systematic differences in feeding in the adult life stage would most likely be triggered by differences in the developmental environment. Our data more broadly suggest that some aspects of adult phenotype may be decoupled from previous life stages while others may not.

We found that adults exhibited thermal plasticity in response to increased temperature fluctuation by decreasing thermal sensitivity of metabolism. Reducing thermal sensitivity should reduce energetic costs under variable temperatures (as a result of Jensen's inequality) (Jensen, 1906; Ruel and Ayres, 1999), helping beetles conserve energy for other energetically costly activities like searching for dung and mates, reproducing, and mounting immune responses. Previous work in insects has shown decreased thermal sensitivity following acclimation to increased thermal variation (Bozinovic et al., 2013; Williams et al., 2012), suggesting this may be a common plastic response. Our findings demonstrate that even though the capacity to reduce thermal sensitivity may be broadly present across insects, it is not necessarily equivalent across each life stage. More research is needed to discern whether the magnitude of adult thermal plasticity can compensate for increased energetic demands under fluctuating environments (Gunderson and Stillman, 2015; Williams et al., 2012).

Integrating life stage variation in thermal biology has important implications for predicting the impacts of global change (Levy et al., 2015). For example, analyses that accommodate thermal variation suggest that climate change may decrease the fitness of tropical insects and increase the fitness of temperate insects (Deutsch et al., 2008). However, when age-dependent thermal tolerance is included in these models, predictions suggest that temperate species should also experience decreased fitness (Kingsolver et al., 2011). While these examples highlight the necessity of considering age-dependent thermal tolerance, few analyses incorporate the potentially ameliorating effects of thermal plasticity (Seebacher et al., 2015), and none in a life stage-dependent manner. Existing models that test whether plasticity will aid in species persistence come to conflicting conclusions (Gunderson and Stillman, 2015; Seebacher et al., 2015). A demographic model that includes thermal tolerance and thermal plasticity in a life stage-dependent manner (Sinclair et al., 2016), though complex, may help resolve climate change predictions across latitude and taxa.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: A.C., K.S.; Methodology: A.C., K.S.; Validation: A.C.; Formal analysis: A.C.; Investigation: A.C.; Resources: A.C., K.S.; Data curation: A.C.; Writing - original draft: A.C.; Writing - review & editing: A.C., K.S.; Visualization: A.C.; Supervision: K.S.; Funding acquisition: A.C., K.S.

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Supplementary information

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