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Dung beetles show metabolic plasticity as pupae and smaller adult body size in response to increased temperature mean and variance

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ABSTRACT

Though organisms may use thermal plasticity to cope with novel temperature regimes, our understanding of plastic responses is limited. Research on thermal plasticity has traditionally focused on the response of organisms to shifts in mean temperatures. However, increased temperature variation can have a greater impact on organismal performance than mean temperature alone. In addition, thermal plasticity studies are often designed to investigate plasticity in response to more extreme temperatures despite the fact that organisms make physiological adjustments to diurnal temperature fluctuations that they experience. Using pupae of the dung beetle *Onthophagus taurus*, we investigated the potential for plasticity in response to increasing temperature mean and variance using thermal regimes that were well within the species critical thermal limits. We reared 40 beetles from egg to pupae (n = 20) or adults (n = 20) at one of nine incubation treatments, including all combinations of three mean temperatures (22, 24, 26 °C) and three amplitudes of fluctuation (±2, ±4, ±8 °C). To measure thermal plasticity of pupae, we quantified CO_2 production across a range of temperatures (i.e., 15, 20, 25, and 30 °C) for 20 beetles per treatment. The relationship between CO_2 production and temperature provides an estimate of energetic costs at a given temperature (i.e., using the intercept) and thermal sensitivity (i.e., using the slope). We reared the remaining *O. taurus* in each treatment (n = 20) to adulthood and then recorded mass (g) to determine body size, a proxy for fitness. Pupae exhibited thermal plasticity in response to the additive and interactive effects of temperature mean and variance. Pupae reared in the warmest and most variable treatment (26 ± 8 °C) showed the greatest decrease in overall metabolism compared to all other treatments, and adult beetles from this treatment (26 ± 8 °C) were also significantly smaller than adult beetles from any other treatment. We found that both temperature mean and variance contributed to thermal plasticity of pupae and had consequences for adult body size, a trait related to dung beetle fitness. Importantly, the temperatures we used in our treatments are not extreme and are likely well below the critical thermal maxima of the species, demonstrating that organisms can make adjustments to temperatures they experience across diurnal or seasonal timescales.

1. Introduction

Temperature profoundly affects the metabolism, growth, and fecundity of ectotherms (Angilletta, 2009; Vasseur et al., 2014). For small insects with limited thermal inertia and reduced capacity to maintain their body temperature, physiological adjustments in response to temperatures changes may be key to persistence (Chown and Terblanche, 2006). Specifically, thermal plasticity could allow insects to better regulate physiological rates and reduce the costs associated with living in fluctuating environments. Despite the potential importance of plasticity in species responses to temperature change, we have limited understanding of how physiology is altered across a broad range of temperatures or whether early life stages demonstrate plasticity to compensate for concurrent changes in temperature mean and variance.

Thermal plasticity of metabolism may play a key role in insect responses to fluctuating environments. The metabolic rate of ectotherms increases as temperature increases due to the thermodynamics of the underlying molecular reactions (i.e., Arrhenius, 1915). This type of metabolic plasticity can be categorized as "passive plasticity" since it is constrained by biophysical laws and not directly regulated by the individual (Havird et al., 2020; Schulte et al., 2011; Whitman and Agrawal, 2009). However, overall metabolic rates as well as the thermal

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sensitivity of metabolism (i.e., the slope of the temperature-metabolism function) can shift in response to the environment in a manner that constitutes "active plasticity" (i.e., acclimation) (Havird et al., 2020; Whitman and Agrawal, 2009). Active metabolic plasticity is subject to selection and may aid responses to rapidly changing thermal conditions. Insects have shown significant active metabolic plasticity following exposure to shifts in mean temperature (Frazier et al., 2001; Lann et al., 2011; Nespolo et al., 2003; Niitepold, 2010) and fluctuating temperatures of varying amplitudes (Paaijmans et al., 2013; Bozinovic et al., 2013; Williams et al., 2012); however, studies rarely consider the effects of shifts in temperature mean and variance concurrently despite the ecological relevance of doing so (Niehaus et al., 2012; Vasseur et al., 2014). In addition, most studies on metabolic plasticity utilize a limited number of acclimation treatments or measure metabolism across a narrow range of temperatures (Kielland, et al., 2017; Seebacher and Wilson, 2006; Sokolova and Hans-Otto, 2002). Concurrent study of physiological responses to a wide range of temperature means and variances is needed to facilitate an ecologically-relevant understanding of how organisms respond to fluctuating conditions.

Changes in temperature during insect development, when many physiological systems are maturing, can impact organismal phenotype with lasting consequences into adulthood (Emlen and Nijhout, 2000; Kellermann et al., 2017; Slotsbo et al., 2016; Telemeco et al., 2017). Active thermal plasticity may allow developing offspring to compensate for stressful conditions (Chidawanyika and Terblanche, 2011; Mitchell et al., 2011; Seebacher and Wilson, 2006). However, even when insects show plasticity, they may suffer fitness declines (Williams et al., 2012). Currently, we lack an understanding of how early life stages respond to shifts in both temperature mean and variance and whether plasticity early in ontogeny elicits a trade-off with fitness later in life.

We used the dung beetle *Onthophagus taurus* to investigate the potential for an early life stage to exhibit active plasticity to temperature changes that are well within the species critical thermal limits. We exposed developing offspring in brood balls to a series of incubation treatments representing increases in both temperature mean and variance which better simulates natural environments (Bauerfeind and Fischer, 2014; Paaijmans et al., 2013; Sheldon and Dillon, 2016; Vasseur et al., 2014). To quantify plasticity, we measured thermal sensitivity of metabolism of pupae from different temperature treatments. Active thermal plasticity can reduce metabolic rates and increase energy efficiency in offspring exposed to variable temperatures, potentially compensating for stressful conditions. Alternatively, active plasticity may come at a cost to fitness (Ghalambor et al., 2007; Williams et al., 2012). Thus, we also examined how temperature changes during development affect adult body size, which is a proxy for fitness in dung beetles (Emlen, 1997; Hunt and Simmons, 2000; Kingsolver and Huey, 2008). Our approach allowed us to examine 1) whether pupae of *O. taurus* show active metabolic plasticity in response to increased temperature mean and variance, 2) whether pupae alter overall metabolic rate, the thermal sensitivity of metabolism, or both in response to these temperature changes, and 3) whether increased temperature mean and variance during development impact adult body size, a proxy for fitness. As our investigation focuses on active plasticity, unless otherwise noted, our use of the term plasticity below refers specifically to active plasticity.

2. Methods

2.1. Study species

We established two lab colonies of *O. taurus* from beetles collected in June 2018 on a cattle farm in Kings Mountain, North Carolina (35°15′53.7″ N, 81°21′18.6″ W). For each colony, we placed ~30 adult beetles (even sex ratio) in cylindrical plastic containers (0.02 m^3) filled with a moist soil mixture (4:1 parts soil to sand) and fed them *ad libitum* with autoclaved cow dung. For 4 weeks, we allowed colonies to breed, collecting brood balls every 2–3 days. Brood balls were housed individually in 74 mL plastic cups filled with the soil mixture and sealed with a lid with air holes. Beetles were reared to adulthood in cups at a constant temperature (24 °C) and then divided into new breeding colonies of ~30 beetles. This process was repeated until F_3 brood balls (n = 360) were produced.

We weighed brood balls from the F_3 generation and then individually housed them in 74 mL plastic cups filled with the soil mixture. We randomly assigned 40 brood balls to one of nine temperature treatments. Specifically, we used a full-factorial design that included three average temperatures (22, 24, 26 °C) and three amplitudes of fluctuation (±2, ±4, ±8 °C). Treatment temperatures fluctuated in a 24-hr diel pattern with temperatures changing hourly. Temperatures were ramped from the previous temperature to the next point over the course of 60 min, passing the treatment's mean temperature twice in a 24-hr period. We selected treatment temperatures based on soil temperatures in the field. *O. taurus* brood balls are buried at depths ranging from 2 to 25 cm below the soil surface, and developing dung beetles experience diurnal temperature variation of ~ 10.8 °C at the shallowest burial depth to ~ 1.0 °C at the deepest burial depth (Carter and Sheldon, unpublished data). Based on a long-term soil dataset from Knoxville, TN (NCEI, 2018), local soil temperatures at a depth of 10 cm range from 12.2 °C (T_{min}) to 31.04 °C (T_{max}) in months that beetles are active, and average 22 °C during the time of year that beetles develop underground. Thus, our 22 ± 8 °C treatment approximates current conditions for offspring in brood balls buried at shallower depths. Our remaining temperature treatments represent temperatures that would be experienced by offspring in brood balls buried further beneath the soil surface (22 ± 4, 22 ± 2 °C), as well as offspring in brood balls that, depending on burial depth, would be experiencing increasing temperature means (22, 24, 26 °C). Importantly, the minimum (14 °C) and maximum (34 °C) temperatures experienced in any treatment are well within the critical thermal limits of temperate dung beetles like *O. taurus* (Sheldon and Tewksbury, 2014).

2.2. Thermal plasticity of metabolism

Of the 40 beetles in each incubation treatment, we reared 20 beetles to pupation for metabolic trials. For metabolic trials, we checked the brood balls every 2–3 days to determine when beetles pupated. Depending on the temperature treatment, beetles pupated within 3–5 weeks, at which point we conducted metabolic trials.

We performed metabolic trials on 20 pupae per incubation treatment. We measured carbon dioxide (CO_2) production of individual pupae using stop-flow respirometry at 15, 20, 25, and 30 °C (Williams et al., 2012). These temperatures are well below the critical thermal maximum of temperate dung beetle species (Sheldon and Tewksbury, 2014) and allowed us to examine plasticity at temperatures beetles often experience in the field. For metabolic trials, we first calibrated the CO_2/H_2O analyzer (LI-7000; Li-Cor, Lincoln, NE) with a calibration gas (CO_2 in N_2 99.50 PPM, Airgas, Knoxville, TN). We then gently removed a pupa from its brood ball, weighed it, and placed it in a 20 mL syringe. Next, we perfused the syringe with zero air. The zero air (Airgas, Knoxville, TN) has CO_2 and water vapor removed, but, as an added precaution, we also ran it through CO_2 and water vapor scrubbers (Ascarite® and Drierite®, respectively). Once we perfused the syringe containing the pupa with zero air, we sealed the syringe and placed it inside an incubator at 15 °C for exactly 30 mins. We included a control syringe with each trial set to account for any potential disruptions in flow rate that might be caused by the injection alone. Following the 30 min incubation, we expelled 1 mL of air from the syringe to remove excess air in the needle and injected 10 mL (0.5 mL/sec) of air from the syringe into tubing connected to the CO_2/H_2O analyzer (LI-7000). Specifically, we continuously pushed zero air in tubing connected to an air tank through the CO_2/H_2O analyzer using an SS4 (Sable Systems International, Las Vegas, NV) at a rate of 120 mL/min. Thus, when we injected the 10 mL of air from the syringe containing the pupa into the tubing, we were injecting the syringe air

directly into the flow-through air connected to the metabolic set-up. We recorded data using Expedata Software and a data acquisition interface (UI-3, Sable Systems International, Las Vegas, NV). We took baseline readings of CO_2 before and after each injections of air from the syringe to provide frequent readings for baseline corrections. We repeated this process at 20, 25, and 30 °C for each pupa to produce CO_2 production rates across a range of increasing temperatures. We held pupae at room temperature (22 °C) while incubators were ramped to the next temperature step. We used a Catmull-Rom correction to account for any drift in baseline CO_2 readings (Catmull and Rom, 1974) and removed data spikes with a Savitsky-Golay filter (Savitzky and Golay, 1964) with an 11-step window. We converted the CO_2 values to ml/min by taking total syringe volume (20 mL), the volume of the pupa (based on mass), injection volume (10 mL), and total trial time (30 min) into account.

2.3. Body size of adult beetles

We reared the remaining beetles in each incubation treatment to adulthood to examine the impacts of increased temperature mean and variance on body size, a proxy for fitness (Hunt and Simmons, 2000). Following adult emergence, we recorded mass of each beetle as well as sex. We then euthanized each beetle and recorded two additional measures of body size, thorax width and body length, using a digital imaging microscope (Zeiss SteREO Discovery V8 with a Canon EOS Rebel T6i). Because wet mass was moderately positively correlated with both thorax width ($r = 0.4$, $p < 0.001$) and body length ($r = 0.6$, $p < 0.001$) we used body mass for all further analyses.

2.4. Data analysis for thermal plasticity of metabolism

We examined the effect of treatment temperatures on thermal plasticity by comparing the thermal sensitivity of metabolism of pupae developed under different temperature treatments. We first $\log_{10}$ transformed data prior to statistical analyses. We then quantified thermal sensitivity of metabolism as the slope of the relationship between CO_2 and trial temperature and compared among incubation treatments with a general linear model (SAS v 9.4). The model included the average incubation temperature (i.e., 22, 24, and 26 °C), amplitude of temperature fluctuation (i.e., ± 2, 4, 8 °C), metabolic trial temperature (i.e., 15, 20, 25, and 30 °C) and all two- and three-way interactions. The model also included the covariate of pupal mass since body size can affect metabolic rates (Brown et al., 2004). To account for multiple metabolic rate readings for the same individual (i.e., at each metabolic trial temperature), we used a repeated statement identifying individual as the repeated subject. We also included beetle sex and the random effect of colony-of-origin, but neither improved model fit and we removed them from the final model. We used an unstructured covariance matrix, fit models using maximum likelihood estimation, and chose the best model based on Akaike Information Criterion (AIC).

We assessed temperature effects on metabolism using Q_{10} values of beetles reared in different temperature treatments and mass-scaled metabolic rates to account for mass-specific effects on metabolism. We calculated the average CO_2 production of each beetle across all four metabolic trial temperatures (i.e., 15, 20, 25, and 30 °C) and we $\log_{10}$ transformed CO_2 production and body mass before fitting a linear regression between these two factors (Fig. S1). We plotted the residuals of the regression to compare difference in metabolic rate change across treatment group independent of mass-specific effects (Fig. S2). Using the regression summary to establish a mass-scaling exponent (slope) and mass-scaling coefficient ($10^{\text{intercept}}$), we formulated an allometric equation to correct metabolic rates (MR = 1.00009 × Mass^0.0009898) for the potential confounding effects of body mass (Lighton, 2008). Using these corrected CO_2 production rates, we computed Q_{10} values from each pupa's coldest (15 °C) and warmest (30 °C) metabolic trial temperatures. To test for significant differences among incubation treatments, we ran a general linear model and used post-hoc pairwise comparisons with a Tukey's HSD test.

2.5. Data analysis for body size

We tested for the effects of treatment temperature on body mass using a general linear model that included mean incubation temperature (i.e., 22, 24, and 26 °C), amplitude of temperature fluctuation (i.e., ± 2, 4, 8 °C), and their interaction. To test for significant differences among treatments, we ran an ANOVA and then conducted post-hoc pairwise comparisons with a Tukey's HSD test.

3. Results

3.1. Thermal plasticity of metabolism

Onthophagus taurus pupae exhibited plasticity of metabolism in response to temperature mean and variance as demonstrated by the significant three-way interaction in our model (temperature mean × temperature fluctuation × metabolic trial temperature: $F_{12, 177} = 2.26$, $p = 0.011$; Table 1, Fig. 1). Thermal sensitivity of metabolism (i.e., the slope of CO_2 production across metabolic trial temperatures) was affected by mean temperature alone (temperature mean × metabolic trial temperature: $F_{6, 177} = 6.53$, $p < 0.0001$) and was marginally unaffected by temperature variance (temperature fluctuation × metabolic trial temperature: $F_{6, 177} = 1.86$, $p = 0.089$) (Table 1). Overall metabolic rates (i.e., line intercepts) were affected by temperature mean ($F_{2, 177} = 4.98$, $p = 0.008$), temperature variance ($F_{2, 177} = 5.81$, $p = 0.007$), and their interaction ($F_{4, 177} = 6.85$, $p < 0.0001$). Beetle mass significantly affected metabolism ($F_{1, 177} = 61.60$, $p < 0.0001$). Metabolic rates scaled positively with metabolic trial temperature ($F_{3, 177} = 669.64$, $p < 0.0001$). We found pupae reared in the warmest, most variable treatment (26 ± 8 °C) had the lowest overall metabolic rate across metabolic trial temperatures compared with pupae reared in any other treatment.

Pupae generally showed similar thermal sensitivity of metabolism (i.e., slope), with an average of $Q_{10} = 1.82$ (Fig. S3), which is just below the Q_{10} range of 2.0 – 2.5 found for other insects (Chown, 1997; Forlow and Macmahon, 1988; Nespolo et al., 2003). However pupae from the 24 ± 4 treatments had lower thermal sensitivities than pupae from all but one other treatment ($p < 0.04$ in all but one comparison; Fig. S3).

3.2. Body size of adult beetles

Adult body size of the beetles ranged from an average of 0.10 g in the 24 ± 4 °C treatment to an average of 0.02 g in the 26 ± 8 °C treatment. Adult body size was affected by mean ($F_{2, 136} = 44.12$, $p < 0.0001$) and

Table 1
Results of the general linear model for thermal plasticity of metabolism of *Onthophagus taurus* pupae. The model included the mean temperature (22, 24, and 26 °C) and amplitude of temperature fluctuation (±2, 4, 8 °C) in the incubators, the metabolic trial temperature, and all two- and three-way interactions. The model also included the covariate of pupal mass at the start of the metabolic trials.

Effect	df num, den	*F*
Temperature mean × Temperature fluctuation × Metabolic trial temperature	12,177	2.26 **
Temperature mean × Temperature fluctuation	4,177	6.85 ***
Temperature mean × Metabolic trial temperature	6,177	6.53 ***
Temperature fluctuation × Metabolic trial temperature	6,177	1.86 t
Temperature mean	2,177	4.98 **
Temperature fluctuation	2,177	5.18 **
Metabolic trial temperature	3,177	669.64 ***
Pupal mass	1,177	61.6 ***

†*0.10 > P* > 0.05; **P 0.01; *** *P 0.001; df*, degrees of freedom; num, numerator; den, denominator; *F,* variance ratio.

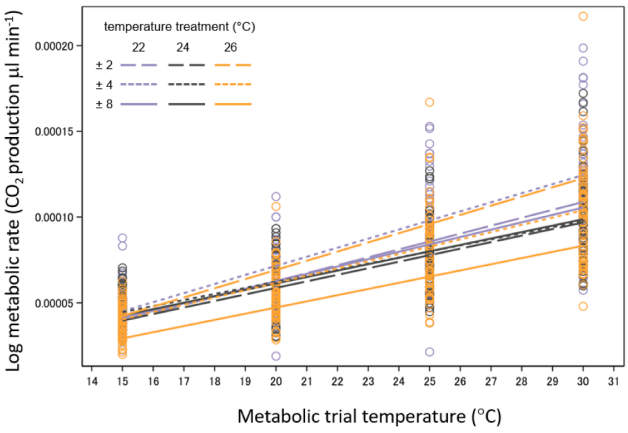

Fig. 1. CO_2 production across a range of temperatures for *Onthophagus taurus* pupae reared in nine incubation treatments. Line colors show CO_2 production for beetles exposed to different mean temperature treatments, including 22 (purple), 24 (black), and 26 (orange) °C. Line types represent CO_2 production for beetles exposed to different amplitudes of fluctuation, including ±2 (dashed), ±4 (dotted), and ±8 (solid) °C. Pupae reared in the warmest, most variable treatment (26 ± 8 °C) had the lowest metabolic rate across temperatures.

variance ($F_{2, 136} = 37.42$, $p < 0.0001$) of incubation treatment temperatures and their interaction ($F_{4, 136} = 19.79$, $p < 0.0001$). Beetles reared in the warmest most variable temperature treatment (26 ± 8 °C) were significantly smaller than beetles from all other temperature treatments ($p < 0.0001$ in all cases; Fig. 2). Based on the best fit model (above), body size impacted overall metabolic rates, however other mass-independent factors influenced overall CO_2 production (Fig. S2). Thus, despite the large reduction in body size in the warmest, most variable treatment, mass alone is an insufficient explanation for the metabolic variation found among beetles from different incubation treatments.

4. Discussion

Onthophagus taurus pupae exhibited thermal plasticity in response to changes in temperature mean and variance. We found pupae from the warmest, most variable temperature treatment (26 ± 8 °C) had the largest decrease in metabolism, which could conserve resources in an otherwise energetically demanding environment (Williams et al. 2012). Additionally, we found that the treatment resulting in the greatest decrease in pupal metabolism also resulted in pupae with the smallest adult body size. Finally, pupae exhibited thermal plasticity in response to changes in temperature mean and variance that are likely within the organisms' critical thermal limits.

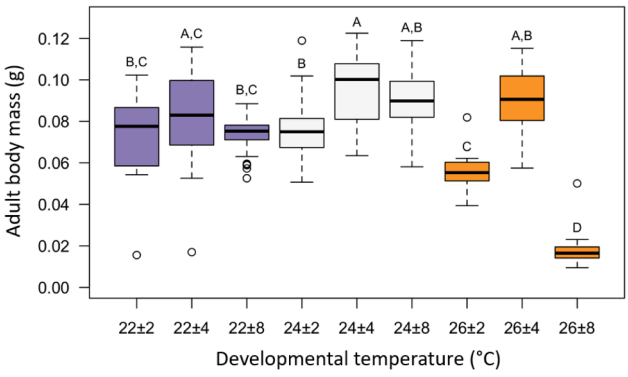

Fig. 2. Beetles reared in the warmest, most variable treatment (26 ± 8 °C) had the smallest adult body size. Boxes show the median and first and third quartiles of beetle mass (g) for all combinations of mean temperature (22, 24, 26 °C) and amplitude of fluctuation (±2, ±4, ±8 °C). Whiskers show the minimum and maximum values of beetle mass, and open circles are outliers. Treatments with different letters denote significant differences based on a Tukey's HSD test.

Our results demonstrate that pupae of *O. taurus* can alter their thermal physiology in response to increased temperature mean and variance. With a few exceptions (e.g. mosquitoes), insect pupae are sessile and cannot use behavioral adjustments to modify the temperatures they experience. Due to this lack of mobility, the pupal life stage is expected to have high temperature tolerance (Huey et al., 2003; Klockmann and Fischer, 2017) and may also exhibit considerable thermal plasticity to temperature variation, especially if they are exposed to extreme temperatures during development (Marshall and Sinclair, 2012). However, dung beetles like *O. taurus* are buried underground from the egg through pupal life stages and are relatively buffered from the extreme temperatures and large diurnal fluctuations that pupae of some insects experience. Yet, we still observed metabolic shifts in this early life stage, potentially suggesting dung beetles experience enough diurnal temperature variation to trigger thermal plasticity of metabolism.

We found that pupae of *Onthophagus taurus* may use thermal plasticity in warmer and more variable environments to alter their metabolic rate in two ways that might allow them to reduce energy expenditure. First, they can lower their overall metabolic rate at a given temperature. In ectotherms, metabolic rate increases with increasing temperature. However, if individuals can lower their metabolic rate, as we observed in pupae from the warmest and most variable treatment, they may reduce the energetic costs of respiration, preserving more energy to be allocated towards growth and development. Second, pupae can reduce the thermal sensitivity of metabolism such that metabolic rate changes less for a given increase in temperature (Williams et al., 2012). Depending on the mean temperature of the environment, this plasticity may allow the insect to reduce energy expenditure in a variable environment (Ruel and Ayers, 1999; Vasseur et al., 2014; Williams et al., 2012).

Pupae in the warmest, most variable temperature treatment (26 ± 8 °C) had a much lower metabolic rate compared to pupae in all other treatments, suggesting there may be a threshold for plasticity of metabolic rate during the pupal life stage. Temperature thresholds are common features of many insect physiological responses, including development rate (Taylor, 1981), thermal tolerance (MacMillan and Sinclair, 2011), and the synthesis of heat-shock proteins (Buckley et al., 2001; Hamdoun et al., 2003). Temperature thresholds have most often been uncovered in response to shifts in mean temperatures (Aghdam et al., 2009; Pakyari et al., 2011). However, we found that a combination of increasing temperature mean and variance was needed to induce plasticity of metabolism. Though the metabolic shifts observed in pupae from the 26 ± 8 °C treatment may indeed stem from thermal plasticity, it's possible that exposure to thermal extremes during development may have caused cellular damage—as a product of increased oxidative stress, for example— that limited the aerobic scope of pupae (Jena et al., 2013; King and Thomas, 2015; González-Tokman et al., 2020). Nonetheless, these findings underscore the importance of incorporating both temperature mean and variance to understand how temperature changes may impact organismal physiology and fitness (Vasseur et al., 2014; Sheldon and Dillon, 2016).

We found that adult beetles that had been reared in the warmest, most variable treatment (26 ± 8 °C) were three times smaller than those reared in any other treatment (Fig. 2). Most insects show a negative relationship between mean developmental temperature and body size known as the temperature-size rule (TSR) (Klok and Harrison, 2013). However, this relationship can be complicated by thermal variance in the developmental environment, with larger amplitudes of temperature fluctuation also leading to smaller insect body sizes (Czarnoleski et al., 2013; Kingsolver et al., 2008; Petavy et al., 2001; Pétavy et al., 2004). For the analysis of *O. taurus* body size, we found the effects of

temperature variation depended on mean temperature of the treatment. The interactive effects of temperature mean and variance may have reached a thermal threshold that triggered the large body size reduction in the warmest, most variable treatment (Kingsolver et al., 2008). The exact mechanism of the TSR is not well-understood (Angilletta and Dunham, 2003; Atkinson, 1994) but may involve a tradeoff whereby more energy is allocated to metabolism and self-maintenance at higher temperatures rather than to larger body sizes (Colinet et al., 2015). Ultimately, this tradeoff could affect fitness since larger bodied insects often have a competitive advantage over smaller bodied insects (Kingsolver and Huey, 2008).

If the TSR is driven by a tradeoff between energy invested in metabolism versus body size, a decrease in metabolic rate of pupae should reduce energetic demands and thus increase adult body size at eclosion. In our beetles, we observed the smallest adult body sizes in the pupae showing the greatest reduction in metabolism, suggesting metabolic plasticity could not fully compensate for the energetic demands imposed by the warmest, most variable temperature treatment. However, the TSR may be driven by mechanisms other than energetic tradeoffs. For example, cell differentiation rates increase with warming, and insects may mature faster, and thus be smaller, at warmer temperatures (Colinet et al., 2015). Research also suggests that higher temperatures reduce the ability of insect gas exchange systems to maintain the oxygen supply required for larger individuals, thus reducing insect body sizes at warmer temperatures (Atkinson, 1994; Callier and Nijhout, 2011; Frazier et al., 2001; Woods, 1999). Finally, the temperatures in the warmest, most variable treatment potentially ventured into suboptimal conditions that caused deleterious impacts on growth (Kern et al., 2015). In our study, we cannot pinpoint the mechanism driving smaller body sizes in beetles from the most extreme temperature treatment. Nonetheless, our data demonstrate that the warmest, most variable treatment negatively impacted body size, an important fitness proxy in dung beetles, regardless of whether it is a function of mounting a plastic response.

5. Conclusions

We observed metabolic plasticity of pupae and decreased body size of adult *O. taurus* dung beetles in response to increases in temperature mean and variance. Though insects may be able to adjust their metabolism to better cope with temperature changes, they may still incur fitness costs due to smaller body sizes at warmer, more variable temperatures. We found *O. taurus* pupae altered their metabolism in response to temperatures that are well within their critical thermal limits. This suggests these pupae make physiological adjustments to less extreme temperatures that they experience on a daily basis (Bowler, 2005; Bowler and Terblanche, 2008; Chown and Terblanche, 2006), and not just in response to extreme events or major seasonal changes. Our findings were only revealed by measuring responses between critical thermal limits and using realistic diurnal temperature regimes.

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CRediT authorship contribution statement

J. Morgan Fleming: Conceptualization, Methodology, Investigation, Formal analysis, Visualization, Writing - original draft. **Amanda W. Carter:** Conceptualization, Methodology, Investigation, Formal analysis, Visualization, Writing - original draft. **Kimberly S. Sheldon:** Conceptualization, Methodology, Formal analysis, Visualization, Resources, Writing - original draft, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jinsphys.2021.104215.

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