

Acclimation to daily thermal variability drives the metabolic performance curve

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ABSTRACT

Background: Among the predictions of the effect of future climate change, the impact of thermal conditions at local levels on the physiological performance of individuals and their acclimation capacities is key to understanding animals' responses to global warming.

Goal: Test for the effect of acclimation to environmental thermal variability, namely $24 \pm 0^\circ\text{C}$, $24 \pm 4^\circ\text{C}$, and $24 \pm 8^\circ\text{C}$, on the metabolic performance curve in the mealworm beetle *Tenebrio molitor*.

Results: Maximum resting metabolism and metabolic breadth were significantly different, but optimal temperature was similar, between treatments. Thus, increases in ambient thermal variability caused a reduction in maximum performance at each level of acclimation, with a decrease of almost 50% between the nil variability and $\pm 8^\circ\text{C}$ daily variability treatments.

Conclusions: If thermal variability changes in any of the directions forecast by climatologists, ecologists will have to use mechanistic and modelling approaches based on physiological and biophysical traits to predict the biodiversity consequences of climate change.

Keywords: fitness, global warming, physiological responses, thermal variability.

INTRODUCTION

Global climate change poses one of the greatest threats to biodiversity (Dawson *et al.*, 2011). Anthropogenic activities are likely to lead to increased frequency of climatic extremes (e.g. heat waves and severe droughts or floods), as well as increased climatic variability in certain regions of the world (Lean and Rind, 2008; Rahmstorf and Coumou, 2011). Theoretical and empirical approaches indicate that global warming impacts both the mean temperatures of local environments, and the magnitude of diel and seasonal variation in temperature (Easterling *et al.*, 2000; Parmesan *et al.*, 2000; Burroughs, 2007; Thompson *et al.*, 2013). Indeed, in addition to increases in mean temperatures on Earth, the Intergovernmental Panel on Climate Change

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(IPCC, 2007) predicts increases in extreme events such as heat waves. The expected increased frequency of extreme environmental conditions is likely to have an even greater impact on biodiversity than the increase in average temperatures (Coumou and Rahmstorf, 2012). Although the potential impacts of warming are widely recognized by ecologists, studies in thermal ecology have focused on the impact of changes in average values. But the variability in these conditions (e.g. extreme temperature events) could be as important as average values, and could also be an important selective force on organisms in the wild (Jentsch *et al.*, 2007; Lalouette *et al.*, 2007; Terblanche *et al.*, 2010; Bannerman *et al.*, 2011; Bozinovic *et al.*, 2011a; Thompson *et al.*, 2013). For instance, during 2010 in Australia, a two-week heat wave led to the death of thousands of birds in the wild. This happened when environmental temperatures exceeded the maximum critical thermal limits of these birds (Miller and Stillman, 2012). Thus, observations and experiments that quantify not only the effect of increases in mean environmental variables but also the effect of environmental variation on physiological traits are important for inferring ecological and evolutionary responses to climate change and the mechanisms by which organisms cope with this variation (Chown and Terblanche, 2006; Terblanche *et al.*, 2008; Bozinovic *et al.*, 2011b; Catalán *et al.*, 2012a, 2012b; DeVries and Appel, 2013).

Several studies have evaluated the effect of thermal variability on life-history traits, including developmental time (Ragland and Kingsolver, 2008), hatching success (Ji *et al.*, 2007), and some phenotypic characteristics of the progeny (Pétavy *et al.*, 2004; Folguera *et al.*, 2009). Williams *et al.* (2012) reported that thermal variability increases the effect of fall (autumn) warming and drives metabolic depression of an overwintering butterfly (see also Paaajmans *et al.*, 2010; Krams *et al.*, 2011). Others have focused on the effect on parameters linked to population dynamics of the organisms (Orcutt and Porter, 1983; Estay *et al.*, 2011). In the wild, ectotherms are continuously exposed to several short-term variations in environmental conditions. Organisms deal with this variability through thermal acclimation and/or acclimatization, affecting current and future survival of natural populations (Terblanche *et al.*, 2010). In this context, the study of the impact of thermal conditions on the performance of individuals and their plastic responses to it, such as lethal and sub-lethal effects, are key to understanding the responses of biota to the environment and to climate change (Pörtner *et al.*, 2006; Somero, 2011).

In this study, we experimentally tested the effect of acclimation to environmental thermal variability on the metabolic rate (SMR) performance curve in the mealworm beetle *Tenebrio molitor* (Coleoptera: Tenebrionidae). We hypothesized that the net effect of thermal variability on SMR would depend on the relative advantages (or disadvantages) of being exposed to low and high extreme temperatures. We address two main questions:

- Is thermal variability a modulator of the maximal SMR of those organisms acclimated to the same average temperature?
- How does thermal variability modify optimum temperature in those individuals acclimated to the same average temperature?

MATERIALS AND METHODS

Experimental design

We used larvae of *T. molitor* reared under laboratory conditions with a light/dark cycle of 12:12 h and fed a mixture of flour and oats *ad libitum*. Water was supplied *ad libitum* as wet sponges. We used larvae because they are extremely sensitive to environmental conditions

(Min *et al.*, 2006; Morales-Ramos *et al.*, 2010). We assigned individuals randomly to one of three thermal treatments in climatic chambers. In one of the treatments, we maintained the temperature at 24°C ($\delta = 0$, the $\delta 0$ group, i.e. the constant temperature regime). In the other two treatments (i.e. the alternating temperature regimes), temperature varied during the day, reaching a maximum during daylight and a minimum during the night: 28/12°C ($\delta = 8$, the $\delta 8$ group) and 24/16°C ($\delta = 4$, the $\delta 4$ group). These thermal scenarios represent predictions of climate change for central Chile (see http://www.dgf.uchile.cl/PRECIS/#Escenarios_Climaticos).

To test for the effect of our thermal treatments, we measured SMR as carbon dioxide production ($\dot{V}CO_2$) at the end of the acclimation period (3 weeks) and within a closed system as previously described (Lighton, 2008). Three weeks after the beginning of temperature acclimation, we placed larvae individually inside glass syringes (2 mL) fitted with three-way valves and completely sealed for 4 h at a controlled temperature of 12, 20, 24, 28, 34, 38, 40 or 42°C ($n = 5$ individuals per treatment). Then air from the syringe (1 mL) was slowly injected into a short Tygon tube connected to a glass tube (10 cm long) to avoid any loss before taking the CO_2 readings (Lighton, 2008). The ambient air injected was passed through a Drierite column to scrub the CO_2 before injection. Samples passed directly to the CO_2 analyser (Fox-Box, Sable system) with a 350 mL \cdot min⁻¹ flow rate. We transformed the data from percentages to volume per hour. Then, using the program EXPEDATA (Sable Systems), we calculated the total CO_2 production per animal as the integral of the curve.

Statistical analyses

We began by evaluating the effect of variability on three specific parameters. We performed a parametric curve fitting procedure of the SMR/temperature relationship under each scenario of thermal variability (Fig. 1). For each acclimation/thermal variability treatment, we fitted the relationship using a Gaussian three-parameter curve:

$$SMR = SMR_{\max} \times e^{-0.5 \left(\frac{T_a - T_o}{SMR_b} \right)^2}$$

where SMR_{\max} is the maximum SMR value, SMR_b is a measure of thermal performance breadth, T_a is average environmental temperature, and T_o is the optimal temperature, i.e. the temperature at which SMR_{\max} is attained. Fitting this curve to each treatment allowed us to compare the parameter values and evaluate the effect of the variability of the thermal acclimation regime on SMR.

Next, we obtained a global picture of the effect of variability. To obtain a global response function of SMR to temperature and the variability of the thermal regime, we performed a non-parametric regression analysis using a generalized additive model (GAM) incorporating both variables. Generalized additive models can detect non-linear relationships because they are piecewise. We determined the form of the partial functions related to each variable by fitting cubic regression splines to the data. We determined the complexity of the curve (the number of degrees of freedom) and the smoothing terms using penalized regression splines and generalized cross-validation (GCV) to avoid over-fitting (Wood, 2006). To evaluate the importance of thermal variability in the acclimation regime, we used cubic regression splines with shrinkage (Wood, 2006). This technique allows for extra penalty to be added in the model, and if the penalty is high enough, it shrinks all smoothing coefficients to zero. In this

case, the effective degrees of freedom of the variable(s) are so small that they can be removed and the model fitted again without them (Wood, 2006).

RESULTS

Estimated parameters in the parametric regression show that SMR_{max} and SMR_b were significantly different between treatments, but T_o was not. These results suggest that increases in thermal variability in the acclimation treatments caused a reduction in maximum SMR (Table 1a, Fig. 1a).

In addition, this pattern is established by the generalized additive model, which shows clearly how thermal variability reduces SMR at any level of temperature (Table 1b, Fig. 1b).

DISCUSSION

Although simple laboratory experiments, such as the one we report here, certainly cannot capture the wide range of conditions in nature, even simple experiments should better position us to understand the mechanisms by which climate change impacts organisms. Indeed, we believe important lessons emerge from our results.

Variability has an obvious detrimental effect on SMR_{max} . It decreases SMR_{max} by almost 50% between the $\delta 0$ and $\delta 8$ treatments. If the pattern of thermal variability changes in any of the directions predicted by Burroughs (2007) and Solomon *et al.* (2008), the performance of ectotherms will be severely impacted, thus modifying geographical distributions, population fluctuations patterns or predator–prey relationships (Estay *et al.*, 2012).

The thermal performance curves of Fig. 1(a) are a suitable descriptor of how a change in temperature influences physiological sensitivity and fitness among ectothermic species and populations (Huey *et al.*, 2012). Low and high temperatures and hence body temperature (T_b) reduce an ectotherm's performance and can be lethal. As observed here, thermal

Table 1. Estimated parameters of parametric and non-parametric regression: (a) Parameter values of parametric regression. Maximal standard metabolic rate or maximum performance (SMR_{max}), metabolic or performance breadth (SMR_b), and thermal optimum (T_o) are indicated. Confidence intervals are in parentheses. (b) Generalized additive model (GAM) for the joint effect of average temperature (Temp) and thermal variability (Var). s_i represents the cubic regression spline for these variables and df is the effective degrees of freedom for each term

	Acclimation treatment (°C)		
	$\delta 0$	$\delta 4$	$\delta 8$
(a) SMR_{max}	1.89 (1.52–2.28)	1.44 (1.15–1.74)	0.93 (0.68–1.20)
SMR_b	4.99 (4.07–6.28)	4.68 (3.78–5.97)	8.28 (5.76–16.57)
T_o	32.55 (31.50–33.60)	32.87 (31.80–34.00)	33.12 (30.74–37.34)
R^2 model	0.66	0.66	0.37
(b) GAM model:	$\text{Ln}(\text{SMR}) = -1.46 + s_1(\text{Temp}, \text{df} = 1.99) + s_2(\text{Var}, \text{df} = 1.14)$		
	Explained deviance, $d = 53.2\%$		

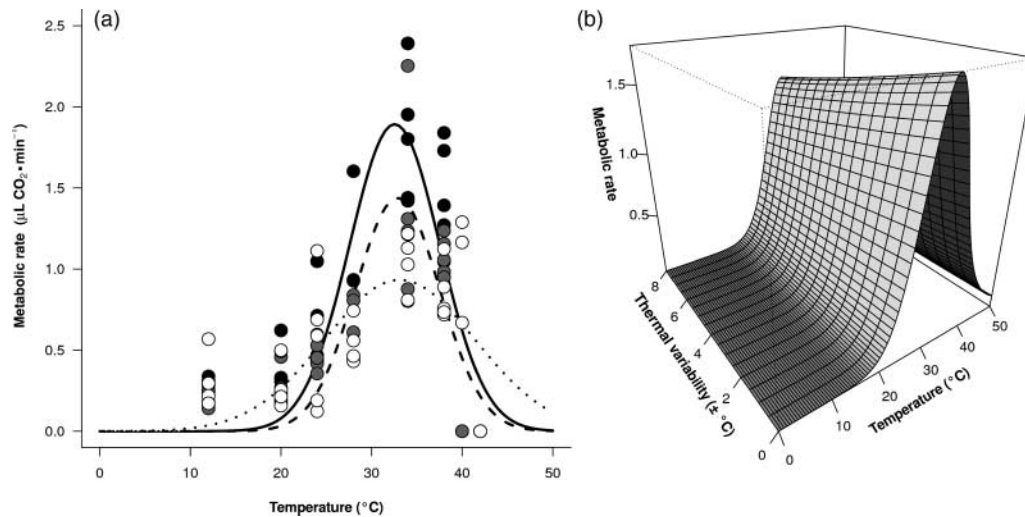


Fig. 1. Performance data fitted with both parametric and non-parametric models. (a) Parametric model fitted to each treatment for the relationship between temperature and metabolic rate. Lines represent fitted models; circles represent observed values. Symbols are: $\delta 0$ treatment (solid line, black circles), $\delta 4$ treatment (dashed line, grey circles), and $\delta 8$ treatment (dotted line, white circles). (b) Generalized additive model fitted to performance data. We show the predicted response surface. Metabolic rate has the same units as (a).

performance curves shift to some extent depending on acclimation to thermal mean and variability.

Huey *et al.* (2012) note that the physiological impact of warming depends mainly on an ectotherm's T_b (at the beginning of warming) relative to its optimal temperature (T_o). In fact, Huey and colleagues hypothesize that if pre-warming T_b is not very close to optimal temperature (T_o), then warming-induced increases in T_b will enhance performance. But if pre-warming T_b is close to T_o , a little warming will have a modest impact on performance. Nevertheless, if T_b increases significantly above T_o , warming should have lethal effects because performance quickly decreases when $T_b > T_o$. This kind of sensitivity to global warming may also change depending upon whether a species is a thermal generalist or specialist. In addition, recent models indicate that thermal variance could have as much (or more) of an impact on performance as mean temperature. Indeed, Bozinovic *et al.* (2011b) proposed that if the mean T_b is below T_o under variable thermal scenarios, brief warming will enhance physiological performance more than brief cooling will impair performance. In contrast, if the mean T_b is close to T_o , and thermal conditions are variable, both warming and cooling will impair performance. In general, the mean and variance of temperature should interact predictably to determine performance in a variable environment.

Although physiological and biophysical ecologists widely recognize the potential impacts of global warming, they have paid less attention to changes in thermal variation at a scale that pertains directly to organisms (Helmuth *et al.*, 2005; Lardies and Bozinovic, 2006; Folguera *et al.*, 2007). Also, the lack of a widely accepted theoretical basis to relate arthropod physiology to population processes makes it difficult to understand the role of thermal variability at higher ecological levels. Nevertheless, Lamb (1961) posits that, compared with constant temperatures, fluctuating temperatures have a distinct effect on insect development (see also

Dallwitz, 1984; Hilbeck and Kennedy, 1998; Hercus *et al.*, 2003). For instance, Terblanche *et al.* (2010) and Folguera *et al.* (2011) showed that thermal variation enhanced the rate of population growth at a low mean temperature but depressed this rate at a high mean temperature. In addition, some species fail to acclimate their thermal sensitivity of performance during predictable or stochastic cooling (Schuler *et al.*, 2011). Recently, Kjærsgaard *et al.* (2012) tested the effect of developmental temperature variability of wing traits on stressed locomotor performance in fruit flies, as well as its dependence on heterozygosity. They observed that locomotor performance declined in those flies reared under variable thermal conditions compared with those reared in thermal environments with the same mean. In addition, heterozygosity affected the flies' wing morphology. It would appear that environmental thermal mean and variability interact in a non-additive way to determine animal physiological performance. Thermal extremes – which can act as environmental cues – may also affect the performance curve parameters as observed here. Such changes in animal performance may also produce changes in activity patterns, and in the timing of breeding, and thus may ultimately alter synchronization between trophic levels and species community structure (Ashton *et al.*, 2009).

Altogether, assessing the biodiversity consequences of climate change requires mechanistic and modelling approaches based on physiological and biophysical traits (Chown and Terblanche, 2006; Jentsch *et al.*, 2007). In support of our belief, Huey *et al.* (2012) state that attempts to evaluate organismal vulnerability to global change and to develop management strategies should be based on relevant physiological foundations. Williams *et al.* (2008) developed a framework for assessing traits that promote vulnerability. In fact, the vulnerability of a species depends on its sensitivity to environmental change and variability, its exposure to change, its resilience to recover from ecological perturbations, and its potential to adapt to environmental changes and new scenarios. Physiological information at different hierarchical levels is fundamental here. Also, physiological plasticity may be important not only to deal with the increasing rate of environmental temperature increase, but also with the increasing unpredictability of climatic anomalies. In this vein, adaptive physiological acclimatization, in the context of global warming, may work as a buffer to minimize the coupling of environmental temperature and body temperature. It will be useful to know whether increased environmental variation has selected for increased physiological plasticity. Thus, new approaches linking physiological ecology and evolutionary ecology to climate change may produce important results at different scales. A major outcome will be to provide a solid, mechanistic foundation for predictions about the probability of a life form's success in the face of global change.

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