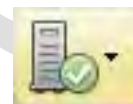


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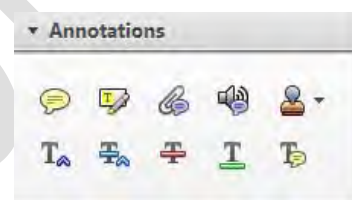


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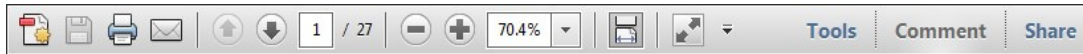


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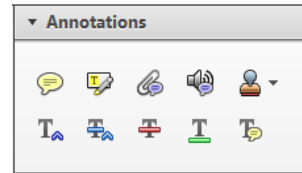
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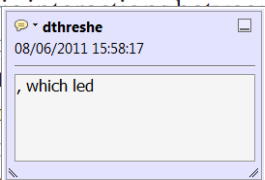


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standard framework for the analysis of microeconomic activity. Nevertheless, it also led to the development of a number of strategic approaches. The number of competitors in an industry is that the structure of the industry is a main component. At the industry level, are externalities important? (Mankiw henceforth) we open the 'black b



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there is no room for extra profits as mark-ups are zero and the number of firms (net) values are not determined by market structure. Blanchard and ~~Kiyotaki~~ (1987), perfect competition in general equilibrium. The effects of aggregate demand and supply shocks in a classical framework assuming monopolistic competition. An exogenous number of firms

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dynamic responses of mark-ups consistent with the VAR evidence

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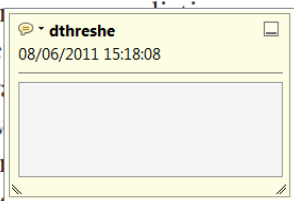


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and supply shocks. Most of the time, the number of firms in an industry is determined by market structure. The effects of aggregate demand and supply shocks in a classical framework assuming monopolistic competition. An exogenous number of firms



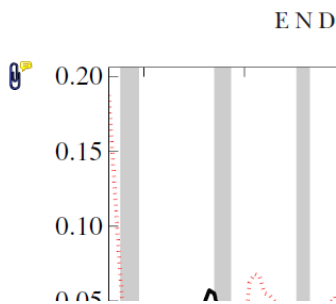
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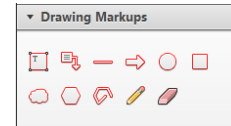
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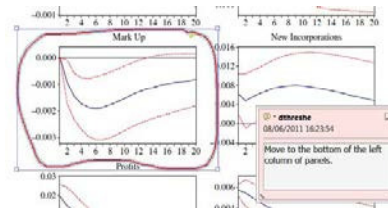


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## SHORT COMMUNICATION

# Ontogenetic thermal tolerance and performance of ectotherms at variable temperatures

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## Keywords:

*Drosophila melanogaster*;  
environmental variability;  
global warming;  
ontogeny;  
performance;  
thermal tolerance.

## Abstract

Early experience and environmental conditions during ontogeny may affect organismal structure, physiology and fitness. Here, we assessed the effect of developmental acclimation to environmental thermal variability on walking speed in *Drosophila melanogaster* adults. Our results showed a shift in the performance curve to the right. Thus, upper and lower thermal limits exhibited developmental plasticity. Additionally, in constant and variable climatic scenarios, flies shifted to the right the optimum temperature but the maximum performance decreased only in flies reared on high temperatures and high thermal variability. Overall, we showed that environmental cues during ontogeny might help to construct phenotypic variation, which supports the hypothesis of ontogenetic dependence of thermal tolerances.

## Introduction

Studies of phenotypic flexibility are central to contemporary evolutionary biologist. Flexibility should be heritable and appears to evolve through natural selection (Schlichting & Pigliucci, 1998). Reversible phenotypic flexibility has been studied by ecological and evolutionary physiologists under the paradigm of environmental acclimation and/or acclimatization (Piersma & Drent, 2003). The environmental modification of organism's physiology in the field or acclimatization is often hypothesized to be responsible for allowing organisms to adjust to fluctuating environmental conditions, through increases in performance (Huey & Berrigan, 1996). Acclimation to varying thermal environments depends on the temporal pattern of environmental changes and the physiological tolerance of each phenotype (Levins, 1968; Futuyma, 1998). Huey & Kingsolver (1993) hypothesized four possible changes in the thermal tolerance of ectotherms as response to increased performance at high temperatures. The first model proposed that selection will favour increased performance at a new temperature in comparison with the ancestral

phenotype, shifting its thermal sensitivity quickly enough to track increasing temperature. The second model predicts an increase in performance (i.e. upper thermal limit and breadth of the performance) at high temperatures; an increased breadth of the performance curve may evolve if there is no trade-off between physiological performances at higher and at lower temperatures. The third model posits that the entire performance curve (upper and lower thermal limits) may be shifted by selection if there is a trade-off between performances at higher and at lower temperatures. Finally, the fourth model suggested that selection for performance at high temperatures also increases performance at low temperatures, that is the stress resistance hypothesis (Hoffmann & Parsons, 1989), but maximal performance may be reduced.

Nevertheless, recently Araújo *et al.* (2013) and Sunday *et al.* (2014) reported that tolerance to heat is largely invariant across lineages, but tolerance to cold varies (Addo-Bediako *et al.*, 2000; Boher *et al.*, 2010). In other words, heat tolerance of ectotherms seems to be conserved with little variation across latitude and environmental temperatures, but cold tolerance decreases significantly with environmental temperatures and latitude and exhibits a large variability (Araújo *et al.*, 2013; Bozinovic *et al.*, 2014).

Several studies have evaluated the effect of thermal fluctuation on life-history traits, including hatching

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success (Ji *et al.*, 2007) and developmental time (Ragland & Kingsolver, 2008) and also have studied the effect on parameters linked to population dynamics of the organisms (Orcutt & Porter, 1983; Estay *et al.*, 2011). Recently, Colinet *et al.* (2015) reviewed how adult insects perform in fluctuating thermal environments and indicated that fluctuating temperatures that remain within tolerant physiological ranges usually improve performance. Nevertheless, those which cover to extreme temperatures may have both positive and negative impacts. For instance, Kingsolver *et al.* (2015) quantified the effects of diurnally fluctuating temperatures on larval growth rates of *Manduca sexta* and found that larvae reared on fluctuating environments had higher optimal temperatures and maximal growth than larvae reared at constant temperatures (see also Estay *et al.*, 2014).

Nevertheless, there is an ontogenetic dependence of thermal sensitivity that must be taken into account (Denver, 1997; Gilbert & Epel, 2009; Hossack *et al.*, 2013; Manenti *et al.*, 2014). Bowler & Terblanche (2008) claim that basal thermal tolerance and acclimation responses are influenced by ontogeny and may confound studies of temperature responses if unaccounted for. This is important in the current era of global warming because it seems necessary to test not only the effect of different climatic scenarios of global warming on adults' acclimation capabilities (Bozinovic *et al.*, 2011; Estay *et al.*, 2014; Field & Van Aalst, 2014; Vázquez *et al.*, 2015) but also during ontogeny, because the varied thermal environments experienced by animals during ontogeny shape thermal tolerances and performance. In this vein, Cooper *et al.* (2012) posits that when thermal variation among generations exceeds that within generations, natural selection should favour developmental plasticity, which may alter the responses of organisms to new climatic scenarios.

Consequently, here we experimentally test the effect of developmental acclimation in thermal performance and tolerance of fruit flies (from eggs to adults) to environmental thermal variability. Specifically, we tested the hypothesis of ontogenetic dependence of thermal sensitivity within scenarios of thermal variability. We used as an animal model, the fruit fly *Drosophila melanogaster*. This species constitutes a good model to test hypotheses about the impacts of climate change, because its phenotypic responses to environmental temperature are well known (Hoffmann, 2010).

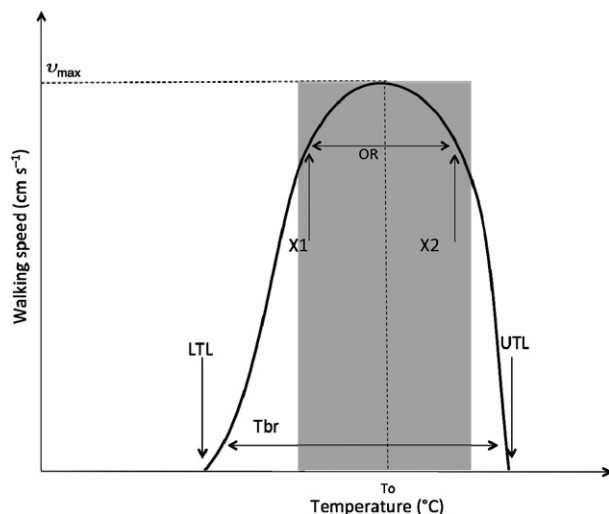
## Materials and methods

Individuals were collected in Santiago, central Chile (33°26'S; 70°39'W), identified and reared at 24 °C in 250-mL glass vials with Burdick culture medium (Burdick, 1954). Flies were maintained under controlled conditions at 24 °C and LD = 12 : 12. F3 flies were

randomly assigned to four thermal treatments according to Bozinovic *et al.* (2011) in climatic chambers; treatments were as follows: 15 ± 0 °C (low mean, no variance = 15C), 15 ± 4 °C (low mean, high variance = 15V), 28 ± 0 °C (high mean, no variance = 28C) and 28 ± 4 °C (high mean, high variance = 28V). Photoperiod was LD = 12 : 12. In treatments with variance in temperature, during the day, temperature started to increase linearly at 7:00, reached the maximum at 11:00, then stayed constant, and began to decrease at 19:00 and reached the minimum at 23:00 h. The temperature range was set based in the limit of viability of fruit flies, which are 15 and 28 °C (Hoffmann, 2010). After rearing flies at constant or fluctuating temperatures and based on Gilchrist (1996) experimental design, we quantified the effect of temperature on walking speed in adults. Speed is often used as proxy of organismal performance because it is correlated with mating success (Christian & Tracy, 1981) and influences survival (Partridge *et al.*, 1987). Briefly, a total of 128 flies (females and males) were tested. We quantified walking speed (cm s<sup>-1</sup>) by knocking down a fly in a narrow glass test tube (12 × 100 mm) and measuring the time required to walk up the tube to a height of 7.0 cm. Each fly was kept during 10 min at the test temperature following the sequence: 16, 20, 24, 28, 32, 36, 40 and 44 °C. To minimize the effects of acclimation and shock to lower temperatures, all animals remained in a room at 24 ± 2 °C between tests. To achieve the walking speed curve, data were fitted to a polynomial function of second order, then we calculated the upper and lower thermal limits (UTL and LTL, respectively), maximum performance ( $v_{max}$ ), performance breadth ( $T_{br}$ , see Gilchrist (1996)) and the temperature at which performance is maximized ( $T_o$ ). Additionally and to assess the effect of constant and variable temperatures on the optimal range of temperatures, we analysed the performance curve considering the maximum performance (80%) and calculated its limits, namely lower optimal limit ( $X1$ ), upper optimal limit ( $X2$ ) and optimal thermal range, see Fig. 1

## Statistical analysis

To test the potential effects of mean of temperature and daily fluctuation, we performed a linear model, with following variables: sex + mean of temperature \* daily fluctuation. Model selection was performed using Akaike's information criterion for finite sample (AICc); both delta AIC ( $\Delta_i$ ) values, a measure of each model relative to the best model, and model weights ( $w_i$ ), a measure of the evidence supporting a specific model, were used for selections and model comparison. When there were multiple models with  $\Delta_i < 2$ , selection was based on the difference in parameters present in each model, reduction in deviance and log-likelihood values (Burnham & Anderson, 2003). Also, a correlation



**Fig. 1** Theoretical performance curve for a hypothetical ectotherm. Curve parameters are as follows: maximum performance ( $u_{\max}$ ), temperature at which performance is maximized ( $T_o$ ), performance breadth ( $T_{br}$ ), upper and lower thermal limits (UTL and LTL, respectively), lower optimal limit ( $X1$ ), upper optimal limit ( $X2$ ) and optimal thermal range (OR)

matrix of the parameters obtained from each performance curve was conducted. The statistical analyses were carried out using R software environment (R development core team 2012).

## Results

Thermal conditions experienced during ontogeny affected the thermal performance curve (Tables 1 and 2). We did not find effect of sex on performance curve parameters (Tables 1 and S1). Individuals reared at 28V and 28C shifted the  $T_o$  to the right. Only individuals from 28V diminished  $v_{\max}$  (Table 1, Fig. 2). Moreover, at 28C flies decreased cold tolerance, that is shifted LTL to the right. Otherwise, with the increase of daily fluctuation UTL was shifted 4.4 °C to the right, thus becoming more heat tolerant (Fig. 2, Table 1). Limit of maximum performance,  $X1$ , was affected only at constant temperature, and  $X2$  was significantly affected by constant and variable temperatures. In addition,  $X1$  and  $X2$  were positive and significantly correlated (Table 3). We did not find effects of constant neither of fluctuating temperatures on  $T_{br}$  nor OR (Table 1, Fig 2). Finally, we did not find significant correlations among thermal limits (Table 3).

## Discussion

Our key finding was that the different thermal environments experienced by fruit flies during ontogeny shaped the thermal performance curve. Furthermore, our results support the hypothesis of ontogenetic

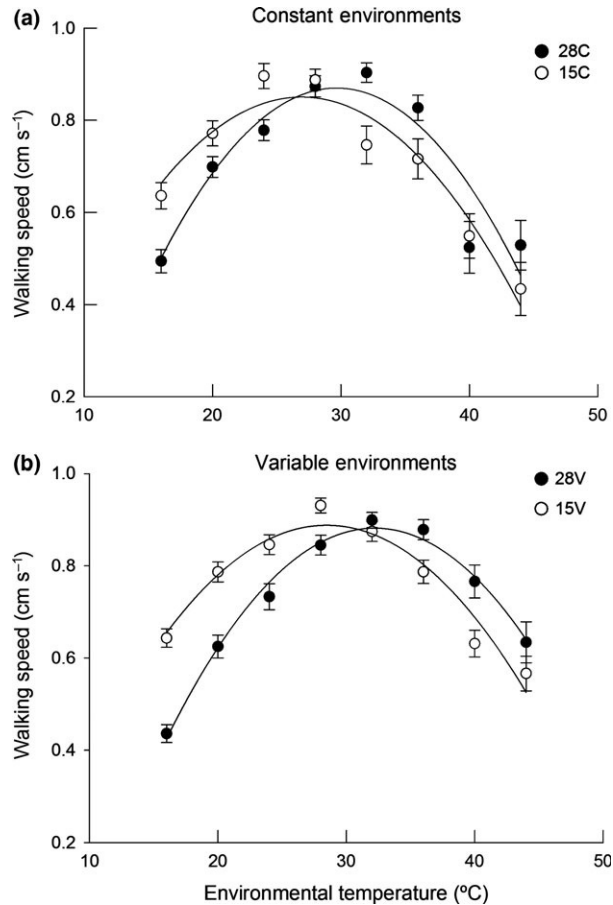
**Table 1** Coefficients of the linear model fitted to data for walking speed. Maximum performance ( $v_{\max}$ ), optimal temperature ( $T_o$ ), performance breadth ( $T_{br}$ ), upper and lower thermal limits (UTL and LTL, respectively), lower optimal limit ( $X1$ ), upper optimal limit ( $X2$ ) and optimal thermal range (OR). For each analysis, only the best model is shown.

Effect	Coefficient	SE	T	P
$T_o$				
Intercept ( $15 \pm 0$ °C)	26.38	0.60	43.84	< 0.0001
High mean temperature (28C)	4.41	0.67	6.5	< 0.0001
High thermal variance ( $\pm 4$ °C)	2.13	0.67	3.13	0.002
$u_{\max}$				
Intercept ( $15 \pm 0$ °C)	2.03	0.10	18.85	< 0.0001
High mean temperature (28C)	0.24	0.14	1.65	0.1
High thermal variance ( $\pm 4$ °C)	0.02	0.14	0.14	0.88
28C: $\pm 4$ °C	-0.42	0.20	-2.03	0.04
$T_{br}$				
Intercept ( $15 \pm 0$ °C)	17.04	0.63	27.05	< 0.0001
High mean temperature (28C)	1.31	0.71	1.85	0.07
High thermal variance ( $\pm 4$ °C)	-1.21	0.71	-1.71	0.09
LTL				
Intercept ( $15 \pm 0$ °C)	3.89	0.61	6.40	< 0.0001
High mean temperature (28C)	5.89	0.72	8.16	< 0.0001
UTL				
Intercept ( $15 \pm 0$ °C)	49.46	1.01	44.63	< 0.0001
High mean temperature (28C)	2.12	1.25	1.76	0.07
High thermal variance ( $\pm 4$ °C)	4.4	1.25	3.57	0.0004
OR				
Intercept	13.88	0.46	29.83	< 0.0001
$X1$				
Intercept ( $15 \pm 0$ °C)	20.79	0.49	42.13	< 0.0001
28 $\pm 0$ °C	4.16	0.69	5.98	< 0.0001
$X2$				
Intercept ( $15 \pm 0$ °C)	33.75	0.69	48.75	< 0.0001
High mean temperature (28C)	3.92	0.78	5.03	< 0.0001
High thermal variance ( $\pm 4$ °C)	2.08	0.78	2.66	< 0.01

**Table 2** Parameters of the performance curve of *Drosophila melanogaster* reared in variable (V) and constant (C) temperatures. Parameters are as follows: maximum performance ( $v_{\max}$ ), optimal temperature ( $T_o$ ), performance breadth ( $T_{br}$ ), upper and lower thermal limits (UTL and LTL, respectively), lower optimal limit ( $X1$ ), upper optimal limit ( $X2$ ) and optimal thermal range (OR).

Parameters	15C	28C	15V	28V
$T_o$	26.8 $\pm$ 0.6	30.8 $\pm$ 0.6	28.5 $\pm$ 0.5	32.9 $\pm$ 0.6
$v_{\max}$	2.03 $\pm$ 0.1	2.27 $\pm$ 0.1	2.05 $\pm$ 0.1	1.87 $\pm$ 0.1
$T_{br}$	17.0 $\pm$ 0.6	18.3 $\pm$ 0.6	15.8 $\pm$ 0.6	17.1 $\pm$ 0.6
LTL	3.59 $\pm$ 0.75	9.24 $\pm$ 0.7	3.19 $\pm$ 0.7	9.25 $\pm$ 0.7
UTL	49.46 $\pm$ 1.1	51.6 $\pm$ 1.1	53.9 $\pm$ 1.0	55.6 $\pm$ 1.1
OR	13.4 $\pm$ 0.8	13.1 $\pm$ 0.8	14.3 $\pm$ 0.8	14.6 $\pm$ 0.8
$X1$	20.8 $\pm$ 0.5	24.9 $\pm$ 0.5	20.7 $\pm$ 0.5	24.9 $\pm$ 0.5
$X2$	33.7 $\pm$ 0.6	37.6 $\pm$ 0.6	35.8 $\pm$ 0.6	39.7 $\pm$ 0.6

dependence of thermal sensitivity. Accordingly, in variable environments the upper thermal limit of performance curve shifted to the right, but flies reared in



**Fig. 2** Results of the performance curve of *Drosophila melanogaster* reared in two thermal treatments: (a) variable temperature and (b) constant temperature. Data are reported as mean  $\pm$  EE.

constant environments did not exhibit changes in UTL and the curve did not shift.

Shifting in UTL supports the second model of Huey & Kingsolver (1993), but now at an ontogenetic level. As the model predicted, we did not find a trade-off between performances at higher and lower temperatures. Nevertheless,  $T_{br}$  was not affected by environmental variability. A series of studies have shown that the UTL is conserved in comparison with LTL (Addo-Bediako *et al.*, 2000; Chown & Terblanche, 2006; Araújo *et al.*, 2013; Hoffmann *et al.*, 2013), because of evolutionary (Araújo *et al.*, 2013) and physiological constraints (Hochachka & Somero, 2002; Somero, 2010). However, recent studies had revealed that upper thermal tolerances could be less restricted than previously thought (see Kaspari *et al.*, 2015; Lancaster *et al.*, 2015; Bozinovic *et al.*, 2016). Additionally, it has been proposed that the nature of response to environmental fluctuation is dependent on the scale of the environmental change. Therefore, if the thermal variation among generations is greater than that within

**Table 3** Correlation matrices of parameters of the thermal performance curve of *Drosophila melanogaster* reared on constant (15 and 28C) and variable (15 and 28V) temperatures. Parameters are as follows: maximum performance ( $u_{max}$ ), optimal temperature ( $T_o$ ), performance breadth ( $T_{br}$ ), upper and lower thermal limits (UTL and LTL respectively), lower optimal limit ( $X1$ ), upper optimal limit ( $X2$ ) and optimal thermal range (OR). Significant correlations are in bold ( $P < 0.05$ ).

	X2	OR	$T_o$	$u_{max}$	$T_{br}$	LTL	UTL
Constant temperature							
X1	<b>0.35</b>	<b>-0.50</b>	<b>0.65</b>	0.03	-0.01	<b>0.53</b>	0.08
X2		<b>0.62</b>	<b>0.50</b>	0.18	0.03	<b>0.31</b>	<b>0.59</b>
OR			-0.08	0.14	0.04	-0.15	<b>0.48</b>
$T_o$				0.11	0.03	<b>0.39</b>	0.23
$u_{max}$					-0.01	0.20	-0.02
$T_{br}$						0.01	-0.09
LTL							-0.25
Variable temperature							
X1	<b>0.33</b>	<b>-0.54</b>	<b>0.61</b>	-0.06	0.05	<b>0.62</b>	<b>0.28</b>
X2		<b>0.61</b>	<b>0.58</b>	-0.22	0.23	<b>0.34</b>	<b>0.64</b>
OR			0.00	-0.14	0.16	-0.22	<b>0.33</b>
$T_o$				-0.05	0.24	<b>0.49</b>	<b>0.42</b>
$u_{max}$					0.09	-0.08	0.00
$T_{br}$						<b>0.28</b>	0.02
LTL							-0.08

generations, the phenotypic plasticity is favoured (Gabriel & Lynch, 1992; Angilletta, 2009; Cooper *et al.*, 2012).

Schlichting & Pigliucci (1998) suggested that if the early experience is not used in studies of phenotypic variation, it might underestimate the potential plastic response of organisms. Additionally, phenotypic plasticity could be a solution to heterogeneous environments (Via *et al.*, 1995), when the environmental conditions change in the course of life of organisms. Despite recent interest in understanding the effect of climate variability on organisms, populations and species (Petavy *et al.*, 2004; Oliver & Palumbi, 2011; Kjærsgaard *et al.*, 2013; Clavijo-Baquet *et al.*, 2014; Vasseur *et al.*, 2014; Vázquez *et al.*, 2015), the impact of variability on thermal tolerances has been scarcely explored (Whitman & Agrawal, 2009). For instance, Hoffmann *et al.* (2005) tested the effect of variability in temperature as well as constant temperature on stress resistance in fruit flies. They found that thermal fluctuation does not affect heat resistance. However, the chill coma recovery time was slightly longer in flies reared under constant thermal treatment. Our results, however, revealed that parameters of the thermal performance curve such as  $T_o$ , optimal range boundaries and LTL were plastic. Flies acclimated to high temperatures (in both variable and constant conditions) reached the maximum performance at higher temperatures in comparison with flies acclimated to cold conditions, that results are consistent with the *warmer is better* hypothesis (Frazier *et al.*, 2006). Apparently, and in order to maintain constantly

1 maximum performance, flies right-shifted  $T_0$  as predicted by the first model proposed by Huey & Kingsolver (1993). Also, the limits of the optimal range were plastic and shifted to right at high temperatures. Over the optimal range of temperatures, performance decreased. In addition, UTL was plastic only in flies acclimated to variable thermal conditions. As a consequence, a right shift of the performance curve was detected; thus, ontogenetic thermal tolerance of ectotherms in response to increased performance at variable temperatures rejects the heat-invariant hypotheses in ectotherms (Bozinovic *et al.*, 2014).

2  
3 **11** Contrary to our results, it has been described that  $T_{br}$  is favoured in more stochastic environments (Angilletta, 2009). Our results, however, showed that  $T_{br}$  was not affected by temperature, probably because we used constant thermal fluctuation overtime (from egg to adulthood, see Bozinovic *et al.*, 2016). In that context, the emergency of plastic phenotype could be restricted by possible cost (and limits) include maintenance costs of sustaining the sensory and response pathways that induce plastic responses, genetic costs, in which plasticity genes interact with other genes, developmental instability costs and information acquisition costs that are required to detect changes in the environment (Relyea, 2002; Chevin *et al.*, 2013). In that sense, future studies should test how the pattern and magnitude of environmental variability (or stochasticity) may affect the appearance of generalist/specialist phenotypes during development and also to investigate changes in the overall shape of the thermal performance curve. Models proposed by Huey & Kingsolver (1993), to explain the increase of performance at high temperatures, were designed in a natural selection framework. Now, developmental plasticity acting early during ontogeny could induce the emergence of new phenotypes, reconciling the constraining role of selection in maintaining local adaptation (Young & Badyaev, 2010). Thus, thermal phenotypes would also be a result of selective forces acting on organismal ontogeny (Oster & Alberch, 1982; Bowler & Terblanche, 2008). We support the idea that environments not only select but also generate variation (Schlichting & Pigliucci, 1998; West-Eberhard, 2003; Gilbert & Epel, 2009). Overall, through developmental plasticity, organisms increase heat tolerance in changing environments. Thus, we predict that organisms inhabiting variable environments under new global warming scenarios may exhibit plastic or fixed thermal strategies to survive over a wide range of temperatures.

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## Supporting information

Additional Supporting Information may be found  
online in the supporting information tab for this article:  
**Table S1** Model selection Walking speed, the variables  
included in each model, the degrees of freedom (d.f.),  
Information Criteria for finite samples (AICc), Delta  
AIC (Di) and model weights (wi) are listed.

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