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ORIGINAL PAPER

Thermal tolerance and survival responses to scenarios of experimental climatic change: changing thermal variability reduces the heat and cold tolerance in a fly

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Abstract Climate change poses one of the greatest threats to biodiversity. Most analyses of the impacts have focused on changes in mean temperature, but increasing variance will also impact organisms and populations. We assessed the combined effects of the mean and the variance of temperature on thermal tolerances—i.e., critical thermal maxima, critical thermal minima, scope of thermal tolerance, and survival in *Drosophila melanogaster*. Our six experimental climatic scenarios were: constant mean with zero variance or constant variance or increasing variance; changing mean with zero variance or constant variance or increasing variance. Our key result was that environments with changing thermal variance reduce the scope of thermal tolerance and survival. Heat tolerance seems to be conserved, but cold tolerance decreases significantly with mean low as well as changing environmental temperatures. Flies acclimated to scenarios of changing variance—with either constant or changing mean temperatures—exhibited significantly lower survival rate. Our results imply that changing and constant variances would be just as important in future scenarios of climate change under greenhouse warming as increases in mean annual temperature. To develop more realistic predictions about the biological impacts of climate change, such interactions between the mean and variance of environmental temperature should be considered.

Introduction

Climate change occurs at various spatial and temporal scales (IPCC 2014). Global warming can alter both the mean annual temperature of local environments and the magnitude of diel and seasonal variations in temperature (Easterling et al. 2000; Burroughs 2007; Coumou and Rahmstorf 2012; Wang and Dillon 2014). The direct and indirect effects of environmental variability have been recognized by ecologists and evolutionary biologists (Borgman and Wolf 2016; Bozinovic and Pörtner 2015; Vazquez et al. 2015; Vasseur et al. 2014), yet, studies have mostly focused on the ecological impact of changes in the mean. Lawson et al. (2015) suggested to test the effect of temporal fluctuations in the environment in at least six scenarios—with and without environmental variance—and to explore the ecological consequences of changes in environmental variance. In this context, the study of the impact of within and between daily thermal variability (Lawson et al. 2015) on the thermal tolerance of individuals and their responses to it, such as lethal and sublethal effects, is imperative for understanding the responses of organisms to different climatic scenarios (Kern et al. 2015; Estay et al. 2014; Bozinovic et al. 2013; Somero 2011; Richter et al. 2012).

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Bozinovic et al. (2013) reviewed several studies that have evaluated the effect of thermal variability on physiological and life-history strategies, showing that 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). Bozinovic et al. (2013) suggested that if short-time thermal variability changes in any of the directions forecast by climatologists, ecologists will have to use mechanistic and modeling approaches based on physiological and biophysical traits to predict the biodiversity consequences of climate change. Recently, Colinet et al. (2015) reviewed how insects perform in fluctuating thermal environments, and showed that fluctuating temperatures that remain within tolerant physiological ranges usually improve performance. Nevertheless, those which cover to extreme temperatures may have both positive impacts, allowing repair of damage accumulated during exposure to stressful extremes, or negative impacts from cumulative damage during consecutive exposures.

Here, we tested if the effects of environmental variance on the limits of thermal tolerance (i.e., critical thermal maxima (CT_{max}), critical thermal minima (CT_{min}), thermal performance scope ($CT_{max} - CT_{min}$), and survival, depend on whether the mean environmental temperature is constant or changing (see Fig. 1). For instance, the effect of increased or constant thermal variance may have different effects when mean temperatures are higher or lower or changing over time. We experimentally test the effects of the scenarios of climate change predicted in Fig. 1 on the thermal tolerance and survival value of *Drosophila melanogaster*. This species constitutes a good model to test hypotheses about the impacts of climate change, because its phenotypic responses to environmental temperature and other climatic factors are well known (Ohtsu et al. 1992; Ragland and Kingsolver 2008; Hoffmann 2010; Parkash et al. 2013). Thus, scenarios shown in Fig. 1 allow foreseeing how environmental variation will affect—at least in part—organismal responses to global warming at an ecological scale. Consequently, we need to understand its impacts on organismal performances in which mean conditions are constant and in which mean conditions are changing.

Recently, Vazquez et al. (2015) pointed out the importance of precise definitions of climatic variability and extremes in an ecological and evolutionary framework. Climatic variability can be defined as the variance or by a normalized amount of variability. Defining the frequency of occurrence of extreme climatic events in precise terms is more difficult. Extremes are frequently defined in relation to a climate record over a certain period. This would imply that an increase in the mean temperature, with no change in the distribution or its variance around the mean or shape,
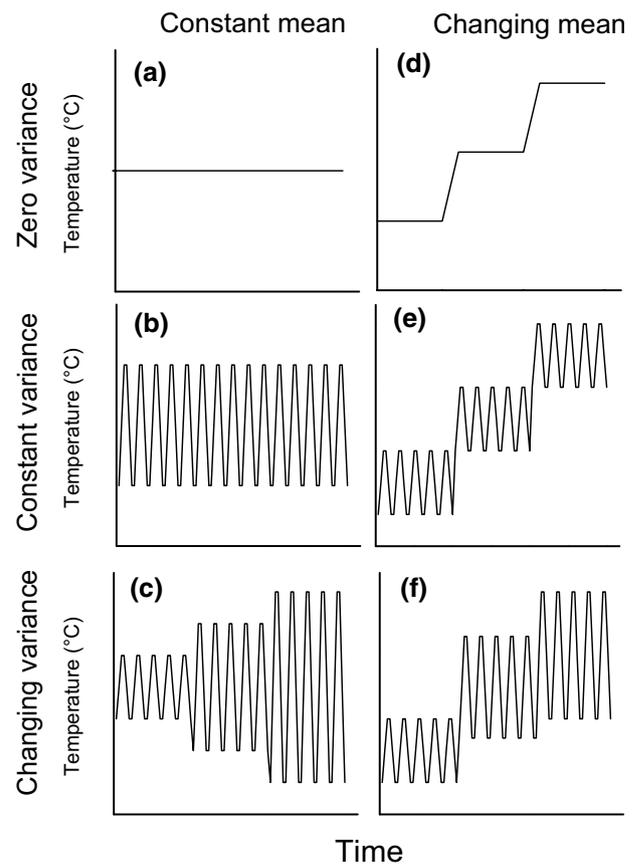

Fig. 1 Experimental environmental change scenarios used in this study to test the effect of environmental thermal mean and variance thermal performance and survival in fruit flies. Time scale among all treatments was: 7 h at minimum temperature; 5 h of upload ramping; 7 h at maximum temperature; and 5 h of download ramping (see text)

would increase the frequency of high extremes and decrease the frequency of low extremes. A change in the variability extremes at both ends, however, would also increase climate (e.g., temperature) variability. Alternatively, extremes can be defined as events that alter the form of the distribution of, for instance, an ambient temperature, without influencing the mean and the variance. Consequently, Vazquez et al. (2015) suggested that an increase in the frequency of extreme events would make the distribution of temperature more leptokurtic, whereas a decrease in the frequency of extreme events would make the distribution more platykurtic, without necessarily changing the mean or the variance of the climatic variable.

Materials and methods

Experimental acclimation

We used more than 200 wild *Drosophila melanogaster* flies collected in Central Chile (33°26′S; 70°39′W at 500 m

a.s.l.) during summer 2015 and 2016 in a nearly 500 m^2 habitat. After capture, adult individuals were identified, bred in ten replicates of nearly ten males and ten females and reared at 24 °C in 250-ml glass vials with Burdick culture medium and maintained under controlled conditions (24 °C and LD = 12:12). After three generations, flies were acclimated to the conditions shown in Fig. 1 (see below).

Twenty vials with ten adult males each were assigned to each of six thermal treatments: (a) constant mean and zero variance = 20 ± 0 °C (15 days); (b) constant mean and constant variance = 20 ± 5 °C (15 days); (c) constant mean and changing variance = 20 ± 5 °C (5 days), 20 ± 10 °C (5 days), and 20 ± 15 °C (5 days); (d) changing mean and zero variance = 10 ± 0 °C (5 days), 20 ± 0 °C (5 days), and 30 ± 0 °C (5 days); (e) changing mean and constant variance = 10 ± 5 °C (5 days), 20 ± 5 °C (5 days), and 30 ± 5 °C (5 days); and (f) changing mean and changing variance = 10 ± 5 °C (5 days), 20 ± 8 °C (5 days), and 25 ± 10 °C (5 days). Time scale among all treatments was: 7 h at minimum temperature (00:00–07:00 h), 5 h of upload ramping (07:00–12:00 h), 7 h at maximum temperature (12:00–19:00 h), and 5 h of download ramping (19:00–00:00 h). Each treatment started with 5 days old male adults. The two first 1/3 of each treatment was 5 days, and the last 1/3 was of 6 days, because at day 14, we measured the critical thermal minima, and at day 16, we measured the critical thermal maxima. Thus, individual flies rested for 1 day. After visual inspection of the flies stress conditions, under changing mean and changing variance (treatment f), we measured the critical thermal minima and the critical thermal maxima at days 13 and 15, respectively. These treatments assume that populations of *D. melanogaster* grow fastest at or near 24 °C, as observed by Siddiqui and Barlow (1972). Thus, after placing flies in each of the scenarios shown in Fig. 1, we compared the heat and cold physiological tolerances of adult male flies that had acclimated to our treatments and quantified total survival as described below.

Critical thermal maxima (CT$_{max}$), critical thermal minima (CT$_{min}$), and survival

We used CT$_{max}$ and CT$_{min}$ as indices of heat and cold tolerances, respectively. Indeed, CT$_{max}$ and CT$_{min}$ were measured according to Terblanche et al. (2006; 2014). In short, flies were placed in individual 5-mL vials that were submerged into a water bath. The flies were allowed to equilibrate for 10 min at either 19 or 28 °C before either CT$_{min}$ or CT$_{max}$ assessments started, respectively. The cooling and heating rate was 0.1 °C min °C^{-1}. The point of critical thermal minimum (CT$_{min}$) was defined as the temperature of loss of a coordinated muscle function, and critical thermal maximum (CT$_{max}$) was defined as the temperature of
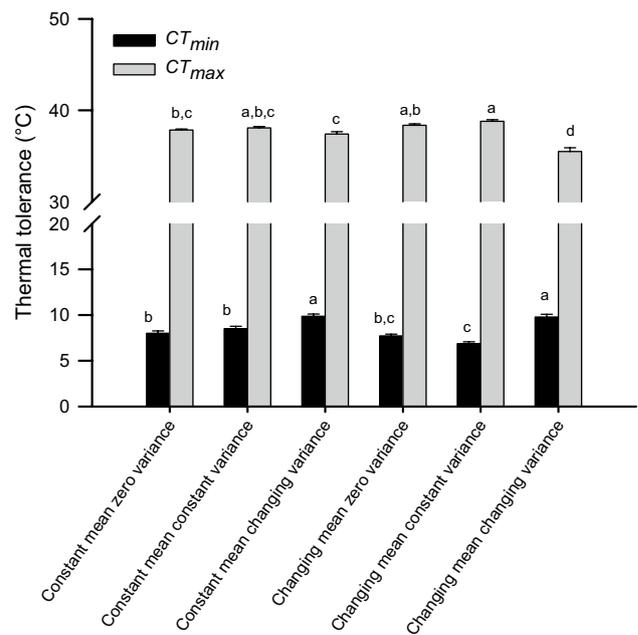

Fig. 2 Effect acclimation to different experimental environmental change scenarios (see Fig. 1) on critical thermal maxima (CT$_{max}$) and critical thermal minima (CT$_{min}$) in fruit flies. *Similar letters* indicated non-significant differences after an a posteriori Tukey test for multiple comparisons. Values are mean ± SD

onset of muscle spasms as suggested by Terblanche et al. (2006). In addition, we individually calculated the scope of thermal tolerance or CT$_{max}$ − CT$_{min}$. To measure survival, eight vials with five 5-days old adult flies were assigned to each climatic scenario. Since our preliminary visual inspections indicated that after 15 days, animals in the changing mean and changing variance treatments were stressed, we measured at day 15 the number of total flies that survived.

Statistics

Statistical analyses were performed using the STATISTICA® (2001) version 6.0 statistical package for Windows® operative system. Data were analyzed by one-way ANOVA and the a posteriori Tukey test for multiple comparisons. Data fulfilled the assumptions of the tests. Results are reported as mean ± 1 SD.

Results

Figure 2 shows CT$_{max}$ and CT$_{min}$ values of flies acclimated at the different experimental scenarios shown in Fig. 1. Visual inspection suggests that CT$_{max}$ tends to show little variation among treatments, except perhaps for the lower CT$_{max}$ in the scenario with changing mean and changing variance. One-way ANOVA supported these observations
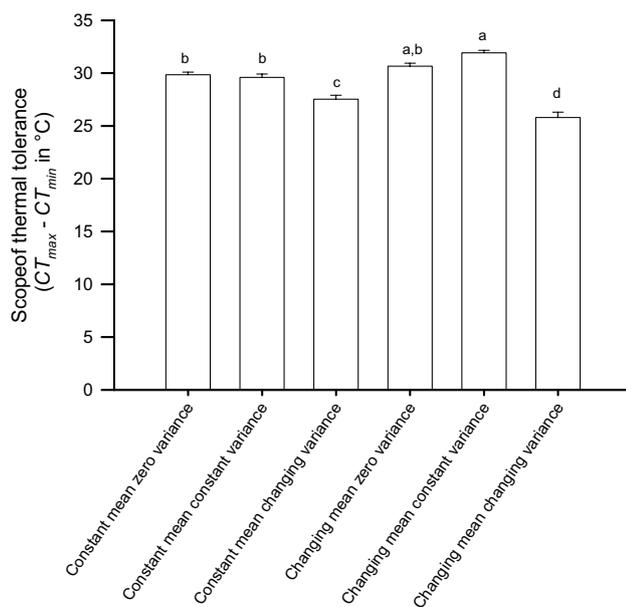
Fig. 3 Effect acclimation to different experimental environmental change scenarios (see Fig. 1) on the scope of thermal tolerance ($CT_{max} - CT_{min}$) in fruit flies. *Similar letters* indicated non-significant differences after an a posteriori Tukey test for multiple comparisons. Values are mean $\pm$ SD
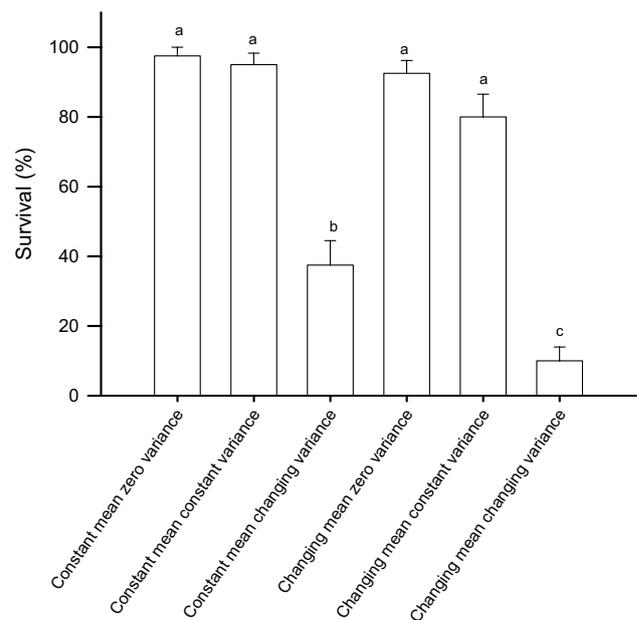
Fig. 4 Effect acclimation to different experimental environmental change scenarios (see Fig. 1) on survival in fruit flies after 15 days. *Similar letters* indicated non-significant differences after an a posteriori Tukey test for multiple comparisons. Values are mean $\pm$ SD

and gave significant results mostly due to that the lower CT_{max} in this scenario ($F = 23.899$, $P < 0.0001$; Fig. 2). Indeed, CT_{max} values were similar among scenarios. The lowest mean CT_{max} was 35.5 ± 0.37 °C in flies acclimated to changing mean and changing variance, whereas the highest mean CT_{max} was 38.8 ± 0.2 °C in flies acclimated to changing mean and constant variance.

On the other hand, CT_{min} exhibited a higher variability among scenarios (Fig. 2). The lowest mean CT_{min} was 6.9 ± 0.2 °C in flies acclimated to changing mean and constant variance, whereas the highest CT_{min} was 9.8 ± 0.3 °C in flies acclimated to changing mean and changing variance ($F = 18.106$, $P < 0.0001$). More importantly, in a scenario with changing mean and changing variance seems to reduce cold tolerance, while acclimation to constant mean zero variance, constant mean constant variance and changing mean zero variance increase cold tolerance. Overall, visual inspection of Fig. 3 and one-way ANOVA indicated that thermal performance scope or $CT_{max} - CT_{min}$ increased in flies acclimated to scenarios either with changing mean and zero variance or constant variance (Fig. 3, one-way ANOVA: $F = 34.836$, $P < 0.0001$), whereas it decreased in flies acclimated with constant or changing mean but with changing variance (Fig. 3). Indeed, the lower thermal performance scope was 25.8 ± 0.5 °C in flies acclimated to changing mean and changing variance, and the highest was 31.9 ± 0.2 °C in flies acclimated to an scenario with

changing mean and constant variance, representing a nearly 20 % rise in thermal performance scope.

Finally, total survival was significantly affected by the climatic scenarios (one-way ANOVA, $F = 58.278$, $P \ll 0.0001$). The a posteriori Tukey test revealed that only flies acclimated to scenarios of changing variance—with either constant or changing mean temperatures—exhibit 38 ± 7.0 and 10.0 ± 4 % survival, respectively. Flies in other treatments exhibited mean values of survival ranging from 80 to 98 % (Fig. 4).

Discussion

Environmental variability in time and space imposes selection pressures on organisms (Gould 1985), and drives adaptation to varying thermal environments, depending on the temporal pattern of environmental changes and the tolerance of each phenotype (Levins 1968; Clavijo-Baquet et al. 2014). Theoretically, animals that inhabit variable environments are expected to exhibit plastic strategies that may enable them to survive a broad range of temperatures.

Within this context, our key finding here was that environments with changing thermal variance reduce the scope of thermal tolerance and survival. Furthermore, changing and constant variances would be just as important as increases in mean annual temperature in future scenarios

of climate change under anthropogenic greenhouse gas warming.

We observed that heat tolerance or CT_{max} of fruit flies seems to be more conserved with lower variation among acclimation treatments. Nevertheless, this is not exact in flies acclimated to a scenario of changing mean with changing variance, where flies exhibited significantly lower CT_{max}. On the other hand, cold tolerance or CT_{min} decreases significantly with mean low as well as changing environmental temperatures. The most variable scenario with changing mean and changing variance reduced cold tolerance (highest CT_{min}), and CT_{max} consequently reduces the scope of thermal tolerance with negative effects on survival, indeed values rising near 10 % in the scenario of changing mean with changing variance (Fig. 4). These results seem to support partially the heat and cold tolerance invariant–variant hypotheses in ectotherms (Bozinovic et al. 2014), and indicate that the upper thermal limits of tolerance may be in some grade, evolutionary constrained—except in extremely variable thermal conditions—but not the lower limits. Colinet et al. (2015) nicely discuss the mechanisms underlying these effects. For instance, they indicated that in most cases, chaperone proteins appear to be upregulated more under fluctuating acclimation conditions than under constant ones. These regimens may increase protection of proteins against thermal shock. Nevertheless, there is variation in the degree to which the thermal conditions are stressful and may arise from an attention on basal heat shock protein expression. These authors also showed that the cold tolerance of insects is usually associated with the accumulation of polyols and sugars. Thus, the advance in cold tolerance under fluctuating thermal conditions would be accompanied by increased concentrations of these cryoprotectants. Summarizing higher thermal variability, on the one hand, and higher occurrence of short-time heat waves on the other are expected to accompany with climate change while according to the results of this study, the former may reduce the tolerance against the latter, and thereby, this relationship may constitute an overlooked threat of global warming, at least at organismal scale.

Using a fruit fly experiment, Schou et al. (2014) also show results that demonstrate a limited scope for adaptive evolutionary responses in upper thermal limits (Gilchrist and Huey 1999). One consequence of low variability in CT_{max} by the effect of acclimation to different scenarios of climate change is that estimated niches for cold-adapted species and populations will be likely to underestimate their heat-tolerance limits, so potentially enhancing risk estimations from global warming (Araújo et al. 2013). On the contrary, species whose climatic preferences are close to their upper thermal limits are unlikely to evolve

tolerances to increased global as well as increases variability in temperatures (Sunday et al. 2014).

Several studies have also evaluated the effect of environmental thermal variability on life-history traits, including developmental time, hatching success, and some phenotypic traits of the progeny (Pétavy et al. 2004; Ji et al. 2007; Ragland and Kingsolver 2008; Folguera et al. 2009). Williams et al. (2012) stated that thermal variability increases the effect of fall (autumn) warming and drives metabolic depression of an overwintering butterfly (Paaijmans et al. 2010; Krams et al. 2011). Recently, Kern et al. (2015) tested under different experimental treatments in thermal variability, the thermal sensitivity of physiological traits in three species of tadpoles that differ naturally in their exposure to daily thermal fluctuations. They observed that the daily thermal fluctuations increases upper thermal tolerance limit in a maximum of 1.6 °C. Additional studies have also focused on the effect on parameters related to population dynamics (Orcutt and Porter 1983; Estay et al. 2011). Indeed, Zhang et al. (2015) studied how timing and duration of temperature variability affect demographic rates of the insect pest *Plutella xylostella*. They recorded that adults that experience different temporal patterns of hot periods during their life-cycles frequently had different demography and reproduction. As we pointed here, these results also suggest that it is difficult to predict the effects of current and future climate change on organisms and populations by just focusing on changes in mean temperatures. Heat–cold cyclic events need to be incorporate to explain how organisms and populations will respond to climate change. In the same line, recently, Bozinovic et al. (2016) report that the thermal performance curve of woodlice (*Porcellio laevis*) changes significantly in response to acclimation to different levels of thermal variability, with individuals acclimated to a more variable thermal regime seemingly shifting their thermal optimal to lower temperatures. Even though this shift seems to come with a cost of reduced performance at higher temperatures, there is circumstantial evidence that observed responses may also reflect behavioral modulation rather than the detrimental effect of short-term temperature variability in performance (Rojas et al. 2014).

In addition to the acclimatory plastic responses of animals to different environmental conditions, the diverse thermal environments experienced by animals during ontogeny may also shape thermal tolerances. In fact, Bowler and Terblanche (2008) suggested that thermal tolerance and acclimation responses are influenced by ontogeny and/or age and may confound studies of temperature responses if unaccounted for. For instance, as proposed by Cooper et al. (2012) when thermal variation among generations exceeds that within generations, natural selection

should favor developmental plasticity. Thus, future studies should attempt to determine the importance of development thermal plasticity in scenarios of global warming, because different environmental conditions—in our case environmental variability—may produce different phenotypes, which would lend support to the hypothesis positing that environments not only may select, but also aid in driving variation (West-Eberhard 2003; Hoffmann et al. 2005). Even though responses to climatic variability are often complex and cannot always be generalized, variability represents an important component of climate with potentially profound ecological and evolutionary consequences (Colinet et al. 2015). Finally, in addition, recently, Baldanzi et al. (2015) argued that when considering the interactions between physiology and species geographic ranges, it is not enough to consider mean temperatures, or even temperature variability as pointed out here; instead, the predictability of that variability and vulnerability may also be very important (Kubisch et al. 2016).

Finally, we are aware that our climatic scenarios (Fig. 1) and experiments using animal models and environmental simulations on short-time scales cannot capture the real thermal conditions found in nature. For instance, in both changing thermal variance treatments, the variance was low in the beginning of the treatment and increased with time. We do not know if this design may have an influence on the results when comparing with the setting when variance would have been high in the beginning and would have decreased with progressing experiment. Nevertheless, even simple experiments, such as the ones performed here, will help to increase our understanding of how ongoing climate change—i.e., increases in mean temperatures, variance, and extreme events, such as heat waves—will impact organisms (Bozinovic et al. 2011a, b; Lawson et al. 2015).

Compliance with ethical standards

Conflict of interest Authors declare that they have no conflict of interest.

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