The Mean and Variance of Environmental Temperature Interact to Determine Physiological Tolerance and Fitness

Francisco Bozinovic[1,2,*]
Daniel A. Bastías[1]
Francisca Boher[1,2]
Sabrina Clavijo-Baquet[1]
Sergio A. Estay[1,3]
Michael J. Angilletta Jr.[4]
[1]Center for Advanced Studies in Ecology and Biodiversity, Departamento de Ecología, Pontificia Universidad Católica de Chile, Santiago 6513677, Chile; [2]Laboratorio Internacional en Cambio Global, Departamento de Ecología, Pontificia Universidad Católica de Chile, Santiago 6513677, Chile; [3]Instituto de Ecología y Evolución, Facultad de Ciencias, Universidad Austral de Chile, Valdivia 6513677, Chile; [4]School of Life Sciences, Arizona State University, Tempe, Arizona 85287

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ABSTRACT

Global climate change poses one of the greatest threats to biodiversity. Most analyses of the potential biological impacts have focused on changes in mean temperature, but changes in thermal variance will also impact organisms and populations. We assessed the combined effects of the mean and variance of temperature on thermal tolerances, organismal survival, and population growth in *Drosophila melanogaster*. Because the performance of ectotherms relates nonlinearly to temperature, we predicted that responses to thermal variation ($\pm 0°$ or $\pm 5°$C) would depend on the mean temperature (17° or 24°C). Consistent with our prediction, thermal variation enhanced the rate of population growth (r_{max}) at a low mean temperature but depressed this rate at a high mean temperature. The interactive effect on fitness occurred despite the fact that flies improved their heat and cold tolerances through acclimation to thermal conditions. Flies exposed to a high mean and a high variance of temperature recovered from heat coma faster and survived heat exposure better than did flies that developed at other conditions. Relatively high survival following heat exposure was associated with low survival following cold exposure. Recovery from chill coma was affected primarily by the mean temperature; flies acclimated to a low mean temperature recovered much faster than did flies acclimated to a high mean temperature. To develop more realistic predictions about the biological impacts of climate change, one must consider the interactions between the mean environmental temperature and the variance of environmental temperature.

* Corresponding author; e-mail: fbozinovic@bio.puc.cl.

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Introduction

The rapid and ongoing warming of our planet, caused largely by human activities (Solomon et al. 2008), has caused biologists to focus on the potential impacts on populations and ecosystems. Climate change has already been associated with spatial and temporal shifts in the distributions of species, as well as loss of community diversity and ecosystem services (Lavergne et al. 2010). Although biologists widely recognize the potential impacts of warming, less attention has been paid to changes in thermal variation on a scale that pertains directly to organisms (Helmuth 2002; Folguera et al. 2009, 2011; Estay et al. 2010; Helmuth et al. 2010; Bozinovic et al. 2011). Yet, theoretical approaches (Katz et al. 2005) and empirical observations (Easterling et al. 2000) indicate that global warming impacts not only the mean temperatures of local environments but also the magnitude of diel and seasonal variation in temperature. Burroughs (2007) proposed three scenarios of climate change related to the mean and variance of temperature. In the first scenario, an increase in the mean temperature results from fewer cold events and more hot events, with a high probability that future temperatures will exceed previously recorded maxima. In the second scenario, an increase in the variance of temperature without a change in the mean will result from a greater frequency of hot and cold events, with a high probability that future temperatures will break current records for both extremes. In the third scenario, both the mean temperature and the variance of temperature will increase because of greater frequency and intensity of hot events. Although each scenario could have pervasive effects for life, we cannot predict these effects without understanding how the mean and variance of temperature interact to determine the fitness of individuals (Stillman 2003; Calosi et al. 2008; Folguera et al. 2009).

Recent models indicate that thermal variance could have as much (or more) of an impact on fitness as does the mean temperature. The impact of thermal variance stems from the asymmetric relationship between body temperature and organismal performance. Generally, the optimal temperature for performance, or thermal optimum, lies many degrees above the lower limit of thermal tolerance but only a few degrees below

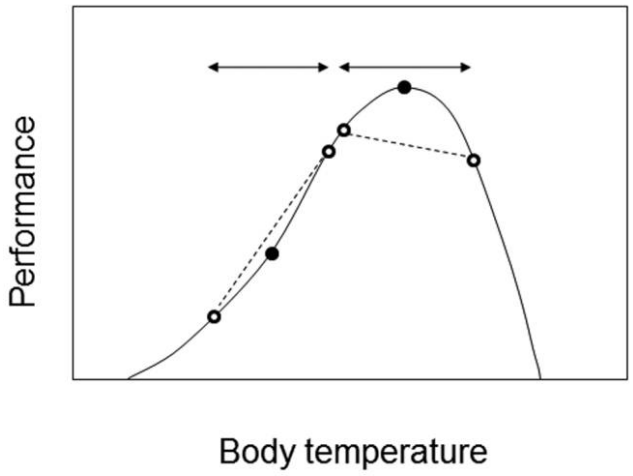

Figure 1. Given a nonlinear performance curve, mean performance differs between individuals in constant and fluctuating thermal environments. Filled circles denote the performance of individuals kept at either a low constant temperature or a high constant temperature. Open circles denote performances of individuals kept at thermal cycles following a step function. Arrows denote the range of the step function. When the mean temperature lies below the thermal optimum, thermal variation can either enhance or reduce the mean performance. By contrast, when the mean temperature equals or exceeds the thermal optimum, thermal variation generally reduces the mean performance.

the upper limit (Fig. 1). Therefore, near the optimum temperature, variation in body temperature can easily cause animals to exceed their upper thresholds (Deutsch et al. 2008). This danger might favor the evolution of thermoregulatory behaviors that minimize the risk of thermal stress (Martin and Huey 2008). However, costs of and constraints on thermoregulation can prevent certain organisms from avoiding extreme temperatures. For example, small organisms such as fruit flies have low thermal inertia and thus limited capacity to thermoregulate (Huey and Stevenson 1979; Huey et al. 1999). Other organisms, such as tropical ectotherms, possess relatively narrow thermal limits and would suffer great loss of performance when body temperature exceeds the optimum (Deutsch et al. 2008). In these cases, an increase in thermal variation might have a profound impact on the fitness of individuals and the persistence of populations (Lamb 1961; Angilletta et al. 2002; Pascual et al. 2009).

On the basis of the typical shape of the relationship between temperature and performance, we expect the impact of thermal variation to depend on the mean temperature (see Fig. 1). If the mean temperature is close to the thermal minimum, brief warming enhances performance more than brief cooling impairs performance. By contrast, if the mean temperature equals the thermal optimum, both warming and cooling impair performance. Therefore, the mean and variance of temperature should interact predictably to determine fitness in a variable environment (Siddiqui and Barlow 1972; Siddiqui et al. 1973). In this regard, experiments that quantify not only the effect of mean temperature but also the effect of thermal variation on fitness are important for inferring ecological and evolutionary responses in natural environments (Travis and Futuyma 1993).

Most studies of the effects of thermal variation have focused on life-history traits, such as developmental time (Dallwitz 1984; Worner 1992; Ragland and Kingsolver 2008), hatching success (Ji et al. 2007), offspring quality (Pétavy et al. 2004; Ji et al. 2007; Folguera et al. 2009), mortality, or fecundity (Siddiqui and Barlow 1972; Siddqui et al. 1973). In particular, Lamb (1961) suggested that fluctuating temperatures have a distinct effect on insect development compared to constant temperatures (see also Siddiqui and Barlow 1972; Dallwitz 1984; Hilbeck and Kennedy 1998, Hercus et al. 2003). Recent studies have assessed the impacts of thermal fluctuations on stress resistance (Terblanche et al. 2010; Folguera et al. 2011). Daily fluctuations in temperature can also affect the contraction and transmission of diseases (Paaijmans et al. 2010). In general, these studies indicate that the magnitude of thermal fluctuations can have important effects on phenotypes, which in turn could influence fitness.

Here, we experimentally test the effects of mean temperature and thermal variance on the physiological performance and Darwinian fitness of *Drosophila melanogaster*. This species constitutes a good model to test hypotheses about the impacts of thermal change, because its phenotypic responses to environmental temperature are well known (reviewed in Pétavy et al. 2004; Ragland and Kingsolver 2008; Hoffmann 2010). After rearing flies at either constant or fluctuating temperatures, we quantified population growth rate, which is a common index of fitness. On the basis of the assumptions described above, we predicted that the mean and variance of temperature would interact strongly to determine fitness. Because fitness responses might be complicated by thermal acclimation, we also compared the heat and cold physiological tolerances of adult flies that had developed in our thermal treatments. Although laboratory experiments cannot capture the wide range of thermal conditions in nature, even simple experiments such as this one should better position us to understand the mechanisms by which thermal change impacts organisms. Moreover, these kinds of experiments can shed light on geographic variation in phenotypes, because diel and seasonal patterns of environmental temperature change along latitudinal and altitudinal gradients (Rind 1998; Montgomery 2006; Angilletta 2009).

Material and Methods

Experimental Acclimation

We used flies of Oregon RC strain from a laboratory stock maintained at 24°C for more than 50 years under standard *Drosophila* rearing conditions. This represents more than a thousand generations in laboratory conditions since the colonies were first established from the field. Flies were cultured in 250-mL bottles with 30 mL of Burdick (1954) medium. Each generation, 40 adult flies were transferred to fresh bottles, and these adults were removed after 3 d to prevent overlap between

generations. Seven days after the third generation emerged, groups of 10 males and 10 females were transferred to vials containing fresh medium. Nine vials were randomly assigned to each of four thermal treatments: 17° ± 0°C (low mean, no variance = 17C), 17° ± 5°C (low mean, high variance = 17V), 24° ± 0°C (high mean, no variance = 24C), and 24° ± 5°C (high mean, high variance = 24V). These treatments were based on the assumption that populations of *Drosophila melanogaster* grow fastest at or near 24°C, as was observed by Siddiqui and Barlow (1972). The photoperiod was 12L : 12D. Flies were exposed to their respective treatments for 15 d. Halfway through this period, we transferred the flies to fresh vials to prevent these individuals from mixing with their offspring. After 15 d of exposure to the thermal treatments, flies were removed to assess their heat and cold tolerances, as described below.

Recovery Time and Survival Rate

We used the times required to recover from heat and chill comas as indexes of heat and cold tolerances, respectively. Chill coma recovery time was measured after exposure to −2°C for 120 min, while heat coma recovery time was measured after exposure to 38°C for 30 min. For each assay, five males and five females from each vial in each thermal treatment (90 individuals per treatment) were transferred to 1.5-mL Eppendorf tubes. Tubes were then placed inside an aluminium bath with commercial antifreeze. The bath was set to maintain either −2° or 38°C (±0.5°C). As soon as the tubes had been immersed in the bath for the desired time, they were removed from the bath and placed at room temperature (21° ± 1°C). We considered recovery time as the time taken for an individual to stand on its legs after returning to room temperature. Flies that did not recover within a defined period of observation (1,200 s for heat coma and 2,700 s for chill coma) were censored in our analysis (see below). After recovery times were recorded, the flies were transferred to vials containing medium and were kept at 24°C for 24 h. After this period, we assessed the state of each fly and calculated the survival rate as the number of living flies divided by the total number of flies.

We used a Cox proportional-hazards model to estimate the effects of temperature and sex on the duration of recovery from heat or chill coma. This nonparametric model makes fewer assumptions about the distribution of residuals than do comparable parametric models and accounts for censored individuals (i.e., individuals that failed to recover from thermal coma before the end of our observations). We used the survival library of the R Statistical Package (Therneau and Lumley 2010) to fit a model that included the effects of mean temperature (17° or 24°C), thermal variance (high or low), sex (male or female), and the interactions among these factors. Because the responses of flies acclimated in the same vial were likely correlated, we also included a robust sandwich estimator of the variance attributable to this factor. Median recovery times are reported for comparison of expected times among groups.

We used a generalized linear mixed model to compare the

Table 1: Parameters of the Cox proportional-hazards models fitted to data for recovery from chill coma or heat coma

Effect	Coefficient	Robust SE	*z*	*P*
Chill coma:				
Females at 17° ± 5°C	...	...	...	...
High mean temperature	−5.876	.711	−8.26	<.001
Low thermal variance	1.430	.283	5.05	<.001
Male	−.198	.169	−1.17	.242
High mean, low variance	−.770	.705	−1.09	.274
High mean, male	−.896	.619	−1.45	.148
Low variance, male	−.937	.308	−3.04	.002
High mean, low variance, male	1.949	.816	2.39	.017
Heat coma:				
Females at 17° ± 5°C	...	...	...	...
High mean temperature	1.514	.753	2.010	.044
Low thermal variance	−.967	.836	−1.156	.248
Male	−.185	.271	−.682	.495
High mean, low variance	−3.096	1.328	−2.332	.020
High mean, male	−.106	.328	−.324	.746
Low variance, male	−.064	.656	−.098	.922
High mean, low variance, male	1.092	1.602	.682	.496

Note. For each analysis, only the best model is shown. The models compare mean survivorships of flies exposed to combinations of factors to the mean survivorship of flies exposed to a baseline combination (e.g., females acclimated to 17° ± 5°C).

24-h survivorships following heat or cold coma. Mean temperature, thermal variance, and sex were included as independent variables. Because the dependent variable in each analysis was a proportion, we used a logistic link (logit) function of the binomial family. To control for potentially correlated responses of flies raised in the same vial, we also included a random term that estimated the variance among vials (Zuur et al. 2009). Following Crawley (2007), we dropped terms from the maximal model on the basis of their statistical significance and used the Akaike Information Criterion to confirm the improved fit of the simplified model. All models were fitted using the lme4 library of the R Statistical Package (Bates and Maechler 2010).

Maximal Rates of Population Growth

To quantify the effect of our four thermal treatments on the maximal rate of population growth, we cultured flies under the same thermal conditions used in the acclimation experiment (described above). First, we collected newly emerged, virgin flies from our stock population. Then, we transferred a specific number of males and females to sterilized bottles with 8 g of medium. In a preliminary step, we recorded the generation time for flies in each treatment; a generation was defined as the time between the transfer of flies to a bottle and the emergence of the first adults. After this, four densities were established in each vial, according to the discrete design of Utida (1941; Royama 1992): 2, 4, 8, and 16 flies per bottle with a sex ratio 1 : 1. Each density was replicated four or five times, yielding a total of 71 vials. After one generation, the living and dead progeny in each vial were counted. With these data, we were able to fit a model of population dynamics to evaluate differences between treatments.

To estimate per capita rates of population growth (r_t), we used Ricker's discrete-time model,

$$r_t = r_{\max}\left[1 - \left(\frac{N_{t-1}}{K}\right)^{Q}\right], \tag{1}$$

where $r_{\max}$ is the maximal per capita growth rate, N_{t-1} is the abundance at time $t-1$ (in our case, the initial density of 2, 4, 8, or 16 flies), K is the equilibrium density, and Q is a nonlinearity factor (Berryman 1999). Note that $r_t = r_{\max}$ when $N_{t-1} = 0$. Therefore, assuming this model adequately describes population dynamics, we need only to estimate the y-intercept of the function $r_t = f(N_{t-1})$ to obtain r_t. To do so, we used a cubic-splines algorithm (Estay et al. 2010) to obtain values of $f(N_{t-1})$ and $r_{\max}$. The 95% confidence interval of $r_{\max}$ was obtained by bootstrapping ($N = 1{,}000$).

Results

Recovery from both chill coma and heat coma differed between lines in a manner consistent with our expectations. The best model for chill coma recovery indicated that mean temperature, thermal variance, and sex interacted to affect recovery time (Table 1). The interaction reflected the fact that recoveries of males and females differed for flies exposed to some of the thermal treatments but not for flies exposed to others, but this interaction did not affect the pattern of variation among treatments (Fig. 2). For both males and females, recovery from chill coma was rapid for flies acclimated to either 17C or 17V and was slowest for flies acclimated to 24V (Fig. 2). In fact, several of the flies acclimated to 17C did not enter a chill coma during the 2 h of exposure to −2°C. Greater cold tolerance came at

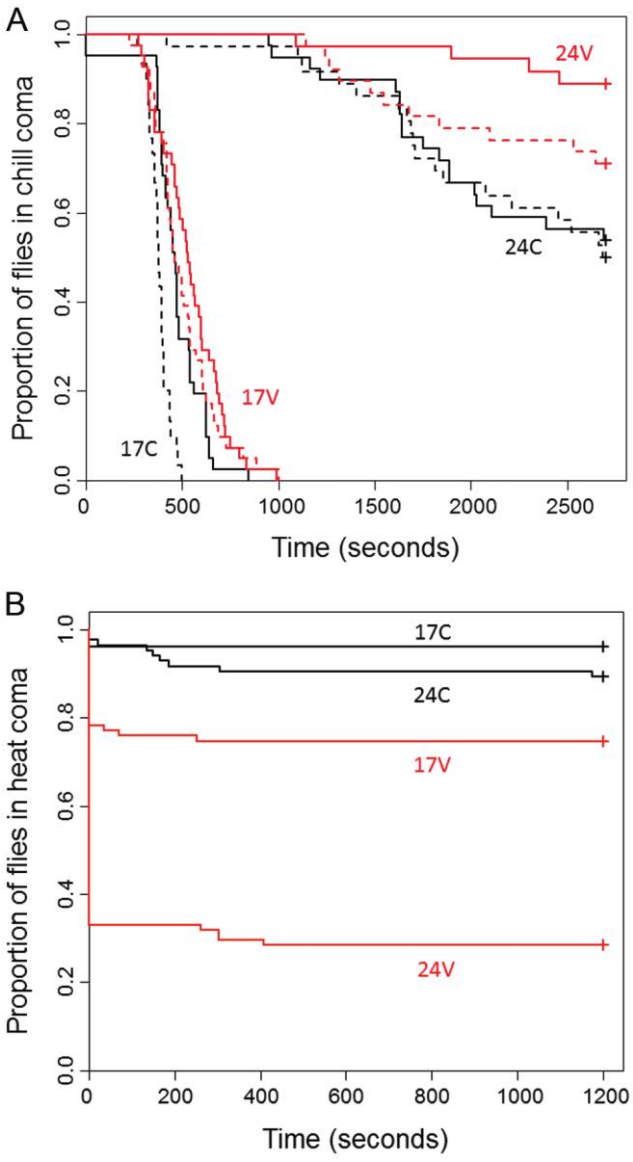

Figure 2. Recoveries of flies from chill coma (*A*) and heat coma (*B*) depended on their thermal history. Thermal treatments were 17° ± 0°C (17C), 17° ± 5°C (17V), 24° ± 0°C (24C), and 24° ± 5°C (24V). Red and black lines represent treatments with and without thermal variance, respectively. Recovery from chill coma differed between males and females. Solid and dashed lines represent males and females, respectively.

Table 2: Parameters of the generalized linear mixed models fitted to data for survivorship following chill coma or heat coma

Effect	Coefficient	SE	Wald z	P
Chill coma:				
Intercept (17° ± 5°C)	3.739	.725	5.158	<.001
High mean temperature	−3.848	.612	−6.292	<.001
Low thermal variance	1.447	.420	−3.442	<.001
Variance among vials	1.876			
Heat coma:				
Intercept (female, 17° ± 5°C)	.0720	.608	.118	.906
High mean temperature	2.426	.498	4.875	<.001
Low thermal variance	−1.379	.449	−3.072	.002
Male	−1.207	.353	−3.420	<.001
High mean, low variance	−4.124	.764	−5.398	<.001
Variance among vials	2.287			

Note. For each analysis, only the best model is shown. The models compare mean times of recovery for flies exposed to combinations of factors to the mean time for flies exposed to a baseline combination (e.g., flies acclimated to 17° ± 5°C).

a cost of lesser heat tolerance, because recovery from heat coma varied among groups in nearly the reverse order (Table 1). Flies acclimated to 24V recovered from heat coma much faster than did flies from other groups; the majority of flies in this group never entered a heat coma during the 30 min of exposure to 38°C (Fig. 2). Sex was not an important factor contributing to variation in heat coma recovery.

The variations in survivorship after cold and heat exposures paralleled the variations in recovery time. The best models indicated that mean temperature and thermal variance were important factors (Table 2). Flies acclimated to either 17C or 17V were very likely to survive for 24 h following cold exposure, whereas those acclimated to either 24C or 24V were less likely survive this period (Fig. 3). Flies acclimated to 24V had very poor survival, which accords with their very long recovery from chill coma. We observed some evidence of a trade-off between survivorship following cold exposure and survivorship following heat exposure (Table 2), similar to the one observed for recovery time: flies acclimated to 24V, which were the least likely to survive cold exposure, were the most likely to survive heat exposure (Fig. 3). Surprisingly, however, flies acclimated to 24C were less likely to survive heat exposure than were flies acclimated to either 17C or 24V (Table 2; Fig. 3). Thus, acclimation to thermal fluctuations, regardless of the mean temperature, improved the chances of surviving a heat shock. Females generally survived heat exposure better than males, but sex did not influence the effect of thermal treatment. Median durations required for flies to recover from chill coma and heat coma after prolonged exposure to different thermal treatments are contained in Table 3.

As predicted from a nonlinear reaction norm (Fig. 1), the thermal mean and variance interacted to determine the maximal rate of population growth (r_{max}). When raised at a low mean temperature, populations of flies grew faster in the 14V treatment ($r_{max} = 3.371$, 95% confidence interval [CI] = 2.796–3.768) than they did in the 14C treatment ($r_{max} = 2.179$, 95% CI = 0.887–3.310). The converse was true when populations were raised at a high mean temperature: populations of flies grew faster in the 24C treatment ($r_{max} = 4.056$, 95% CI = 3.772–4.342) than they did in the 24V treatment ($r_{max} = 2.772$, 95% CI = 2.168–3.362; see Fig. 4).

Discussion

To predict responses to climate change, physiological ecologists must understand the patterns of thermal variation and the mechanisms by which animals cope with this variation (Burroughs 2007; Angilletta 2009; Chown et al. 2010; Cooper et al. 2010; Dillon et al. 2010). Anthropogenic impacts on the earth's climate will likely increase the frequency of extremely high temperatures in certain regions (Solomon et al. 2008). Many organisms are expected to suffer a decrement in fitness (Deutsch et al. 2008; Sinervo et al. 2010), but some may preserve their performance through behavioral and physiological responses. Indeed, brief exposure to extreme heat or cold often elicits physiological responses that improve an organism's thermal tolerance (Hoffmann et al. 2003*b*; Zerebecki and Sorte 2011). Therefore, thermal tolerance should depend on the variance of temperature as well as the mean. Consistent with this view, we found that flies exposed to a warm fluctuating treatment (24V) recovered from heat coma faster and survived heat exposure better than did flies exposed to a warm constant treatment (24C) and two cool treatments (17C and 17V). This pattern mirrors increments in heat tolerance that often follow acute exposures to high temperature (Folguera et al. 2007, 2010), probably involving cellular responses such as the upregulation of heat-shock proteins (Feder and Krebs 1998; Feder and Hoffmann 1999; Folguera et al. 2011). However, flies exposed to the cool constant treatment (17C) recovered from

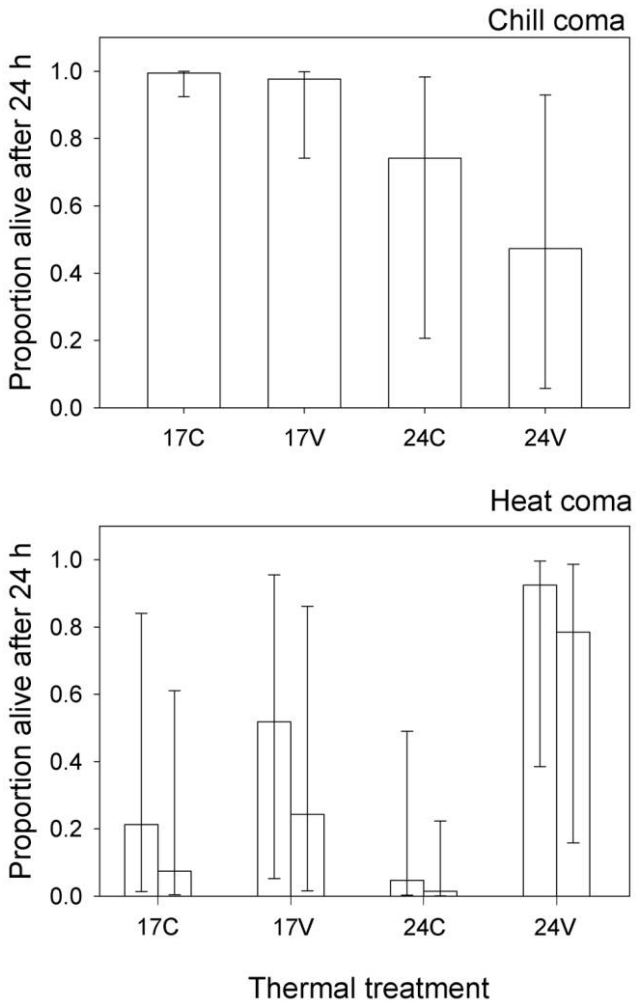

Figure 3. Survivorships of flies following chill coma and heat coma depended on their thermal history. Thermal treatments were 17° ± 0°C (17C), 17° ± 5°C (17V), 24° ± 0°C (24C), and 24° ± 5°C (24V). Error bars denote 95% confidence intervals. Survivorship following chill coma differed between males and females. In the bottom panel, left- and right-hand bars for each thermal treatment represent females and males, respectively.

chill coma faster than did flies exposed to the other three treatments. This pattern suggests that chronic exposure to low temperatures enhances acclimation relative to acute exposure. However, we should note that flies exposed to the cold fluctuating treatment (17V) recovered from chill coma nearly as well and survived cold exposure as well as flies exposed to the cold constant environment (17C).

The relationships among thermal environments, thermal tolerance, and population growth might reflect the natural selection of developmental acclimation (Hoffmann 2010). Many researchers have examined geographic variation in thermal tolerance (e.g., Gibert and Huey 2001; David et al. 2003; Hoffmann et al. 2003*a*; Castañeda et al. 2004; Kristensen et al. 2008; Hoffmann 2010). Their studies indicate that genotypes from higher latitudes tolerate low temperatures better and recover from thermal shock more rapidly, which probably provides a fitness advantage (Gibert and Huey 2001). Also, the ability to recover from thermal shock covaries with environmental conditions (e.g., mean minimal temperature), suggesting that temperature acts as a selective agent. Rapid heat hardening has been studied in various species of insects (Chown and Nicolson 2004; Waagner et al. 2010). In *Drosophila melanogaster*, it has been demonstrated that thermal tolerance shifts according to diel variation in temperature (Overgaard and Sørensen 2008). In particular, our results accord with those of Terblanche et al. (2010); these authors found that the critical thermal minimum was slightly higher in flies that had developed at 23° ± 5°C than it was in flies that had developed at 23° ± 1°C. Similarly, thermal variation at a high mean temperature (27°C) increased the critical thermal maximum. Thus, studies to date suggest that we could generalize our conclusions about the effects of thermal variation on thermal tolerance.

Although acclimation to heat or cold improved the thermal tolerance of *D. melanogaster*, this physiological response failed to prevent changes in fitness at thermal extremes. In theory, acclimation of performance without a cost could ensure a constant fitness over a range of thermal environments. In reality, the fitness of an ectotherm such as *D. melanogaster* depends greatly on environmental temperature. This thermal sensitivity of fitness implies that the acclimation of performance either works within limits or imposes some cost. Researchers estimate the thermal sensitivity of fitness by raising related individuals (or similar genotypes) over a range of constant temperatures (see Huey and Berrigan 2001). In *D. melanogaster*, population growth rate has been shown to peak around 24°C and to decrease sharply at higher temperatures. Siddiqui and Barlow (1972) showed that thermal fluctuations, within the range of temperatures favorable for reproduction, enhanced the rate of population growth. Similarly, we found that an increase in thermal variance enhanced population growth when the mean temperature was well below the thermal optimum. By contrast, the effect of thermal variation was reversed when the mean approximately equaled the thermal optimum. Presumably, this interaction stemmed not only from an acute thermal sensitivity (Huey and Stevenson 1979; Huey and Berrigan 1996) but also from limits to or costs of physiological acclimation.

The interaction between the mean and variance of temperatures stems from a mathematical property of nonlinear functions, referred to as Jensen's inequality (Ruel and Ayres 1999). This mathematical law states that, for a sample of x with mean $\bar{x}$ and a nonlinear function $y = f(x)$, $\overline{f(x)} > f(\bar{x})$ if $f(x)$ is accelerating (second derivative >0) and $\overline{f(x)} < f(\bar{x})$ if $f(x)$ is decelerating (second derivative <0). Given Jensen's inequality, the nonlinear relationship between temperature and performance should lead to complex effects of thermal variance (Martin and Huey 2008). Above the thermal optimum, variation in temperature will decrease performance because the mean lies in the descending part of the curve. This phenomenon can explain contrasting results obtained by different studies. For example, Siddiqui and colleagues found that three species had different

Table 3: Median durations required for flies (*Drosophila melanogaster*) to recover from chill coma and heat coma after prolonged exposure to different thermal treatments

	Thermal Treatments			
	17C	17V	24C	24V
Chill coma recovery:				
Male	463 (425–484)	530 (484–597)	>2,700[a]	>2,700[a]
Female	376 (366–403)	463 (424–538)	2,684 (2,031–2,700+)	>2,700[a]
Heat coma recovery	>1,200[a]	>1,200[a]	>1,200[a]	0 (0–0)

Note. Thermal treatments were 17° ± 0°C (17C), 17° ± 5°C (17V), 24° ± 0°C (24C), and 24° ± 5°C (24V). Numbers shown in parentheses are the 95% confidence interval.

[a]Median value was censored because the majority of flies had not recovered before ceasing observations.

responses to thermal variation; in *Acyrthosiphon pisum*, thermal variation increased fitness at low mean temperatures but increased fitness at high mean temperatures (Siddiqui et al. 1973). In *Anagasta kuehniella*, thermal variation decreased fitness at all mean temperatures (Siddiqui and Barlow 1972). In *D. melanogaster*, adding some thermal variation to any mean temperature increased fitness; however, adding even more thermal variation decreased fitness below that achieved at constant temperatures (Siddiqui and Barlow 1972). These patterns likely result from the combinations of mean and variance relative to the thermal sensitivity of fitness. Similar interactions between the mean temperature and the variance of temperature have been observed for other performances (Dallwitz 1984; Paaijmans et al. 2010). Interestingly, Terblanche et al. (2010) found that thermal variance enhanced fecundity at an intermediate mean temperature but had a lesser effect at a high mean temperature and no effect at a low mean temperature; this result could reflect a complex relationship between temperature and fecundity. Although we observed a positive effect of thermal fluctuations at a mean temperature below the thermal optimum, thermal fluctuations can reduce fitness if temperature drops sufficiently low. Consistent with this idea, flies of *D. melanogaster* exposed to multiple periods of chilling exhibited a lower intrinsic rate of increase compared to flies that underwent a single period of chilling or no chilling (Marshall and Sinclair 2010).

As emphasized by Chown et al. (2010), recent evaluations of climate change indicate that hazardous biological impacts will result from smaller degrees of warming than previously thought. In this vein, Deutsch et al. (2008) showed that warming in the tropics, although relatively small in magnitude, could have a more negative impact than warming in temperate zones. This prediction stems from the fact that tropical insects are relatively sensitive to thermal change and currently experience temperatures close to their optimal temperatures. By contrast, species at higher latitudes have broader thermal tolerances and experience temperatures well below their optimal temperatures; consequently, warming may even enhance the fitness of temperate insects. Subsequently, Dillon et al. (2010) predicted that relatively small changes in temperature in the tropics should cause large changes in the metabolism of ectotherms because of the nonlinear relationship between body temperature and metabolic rate. These conclusions were drawn from reaction norms estimated from measures of performance at several constant temperatures. Such studies would be complemented by experiments that include measures of performance under fluctuating conditions, such as the experiment described here. Still, laboratory experiments cannot incorporate many key factors that influence fitness under natural conditions (see Chown and Terblanche 2007). For example, Kristensen et al. (2008) used a field study to identify costs of cold acclimation that were not evident from a laboratory study. Nevertheless, when interpreted with caution, laboratory studies of performance at fluctuating temperatures reinforce the need to consider changes in the mean and variance of temperature in the context of nonlinear performance curves. Even though changes in thermal variation may not be as drastic as those that have been used in recent laboratory studies (including our study), this work illustrates the potential consequences of thermal

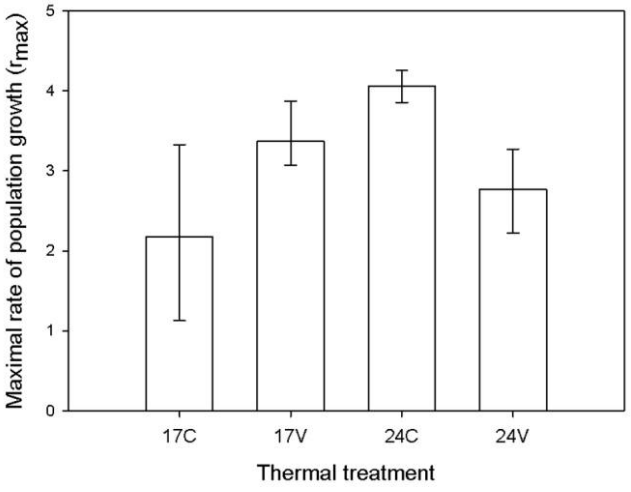

Figure 4. Rate of population growth for *Drosophila melanogaster* depended on the thermal environment. Thermal treatments were 17° ± 0°C (17C), 17° ± 5°C (17V), 24° ± 0°C (24C), and 24° ± 5°C (24V). Error bars denote 95% confidence intervals.

change and should stimulate future studies tailored to climatic scenarios for specific regions.

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