Integrative and Comparative Biology

Integrative and Comparative Biology, volume 56, number 1, pp. 98–109
doi:10.1093/icb/icw004

Society for Integrative and Comparative Biology

SYMPOSIUM

How Extreme Temperatures Impact Organisms and the Evolution of their Thermal Tolerance

Lauren B. Buckley[1] and Raymond B. Huey

Department of Biology, University of Washington, Seattle, WA 981951800, USA

From the symposium "Beyond the Mean: Biological Impacts of Changing Patterns of Temperature Variation" presented at the annual meeting of the Society for Integrative and Comparative Biology, January 3–7, 2016 at Portland, Oregon.

[1]E-mail: lbuckley@uw.edu

Synopsis

Synopsis Understanding the biological impacts of extreme temperatures requires translating meteorological estimates into organismal responses, but that translation is complex. In general, the physiological stress induced by a given thermal extreme should increase with the extreme's magnitude and duration, though acclimation may buffer that stress. However, organisms can differ strikingly in their exposure to and tolerance of a given extreme temperatures. Moreover, their sensitivity to extremes can vary during ontogeny, across seasons, and among species; and that sensitivity and its variation should be subject to selection. We use a simple quantitative genetic model and demonstrate that thermal extremes—even when at low frequency—can substantially influence the evolution of thermal sensitivity, particularly when the extremes cause mortality or persistent physiological injury, or when organisms are unable to use behavior to buffer exposure to extremes. Thermal extremes can drive organisms in temperate and tropical sites to have similar thermal tolerances despite major differences in mean temperatures. Indeed, the model correctly predicts that Australian *Drosophila* should have shallower latitudinal gradients in thermal tolerance than would be expected based only on gradients in mean conditions. Predicting responses to climate change requires understanding not only how past selection to tolerate thermal extremes has helped establish existing geographic gradients in thermal tolerances, but also how increasing the incidence of thermal extremes will alter geographic gradients in the future.

Introduction

Extreme weather events can have broad biological significance. From a physiological perspective, such events can trigger immediate physiological stress, reduce reproduction, or even cause death (Easterling et al. 2000; Somero 2010; Dowd et al. 2015). From an ecological perspective, heat waves cause population die-offs in both terrestrial and marine ecosystems (e.g., mussels, Tsuchiya 1983; Harley and Paine 2009; coral reefs, Hughes et al. 2003; desert birds, McKechnie and Wolf 2009; trees, Allen et al. 2010). From an evolutionary perspective, extreme events may serve as major selective factors that influence—and perhaps even dominate—the evolution of physiological capacities and resistances (Denny et al. 2009; Hoffmann 2010; Kingsolver et al. 2011; Denny and Dowd 2012).

Extreme thermal events are typically identified statistically as temperatures exceeding a given threshold of the distribution (e.g., $\ll$1%) for a given duration of time (Coumou and Rahmstorf 2012). Statistically defined events often do show strong correlations with human and organismal mortality (Easterling et al. 2000). But they are at best a first approximation for organism-centric estimates of extremes, mainly because they essentially ignore the organism, its behavior, and its physiology (Kearney et al. 2009; Huey et al. 2012). An ecological definition might require statistically rarity and as well as a biological response (Smith 2011). Identifying thresholds for biological responses can be difficult (Williams et al. this issue), particularly as they vary, as described below (Dowd et al. 2015).

Advanced Access publication April 28, 2016

Identifying thresholds for stress (in terms of survivorship, growth, and reproduction) may be more tractable (Williams et al. this issue). A related issue is whether biologically relevant extremes need be unpredictable. If stresses are predictable, selection could potentially lead to adaptations that alleviate stress for subsequent generations, but this evolutionary potential is likely heavily constrained, especially if events do not happen in most generations. Defining biologically relevant extremes as those that are both stressful and rare will require a broader initiative, but we outline some of the underlying complications germane to deriving a biological index. Then we suggest some methodologies that can be applied to defining extremes for particular organisms. Finally, we develop a simple genetic model to explore how thermal extremes alter the evolution of organismal thermal tolerances.

Why meteorological estimates are inadequate

Any statistically-defined extreme must be an inadequate predictor of the complex interaction between organisms and their physical environment. Here we justify that assertion.

Air temperature can be a poor predictor of body temperatures

The heat budget of an organism depends on convective heat exchange with air, and also on radiation, conduction, evaporation, and metabolism (Bakken 1992). Consequently, equilibrium body temperatures (operative temperatures T_e) of organisms in natural environments can differ strikingly from local air temperatures (T_a) (Bakken 1992; Helmuth et al. 2010); micro-scale variation in operative temperatures can be substantial (Sears et al. 2011); and even operative temperatures of different animals (size, color, shape, skin wetness) in the same spot can differ markedly. Therefore, analyses of heat waves should be based on operative temperatures, and on how those temperatures are distributed spatially, temporally, and on an organism-specific scale.

Many organisms are not thermoconformers

Many animals and even some plants use behavior and physiology to gain some control over body temperatures (T_b), such that their T_b can be somewhat independent of T_a. Thus whether a given organism experiences thermal extremes—defined either by air or by operative temperature—will depend in part on its behavioral capacity to evade or blunt extreme temperatures (Huey et al. 2003; Kearney et al. 2009; Huey et al. 2012).

Physiological sensitivity to temperature is distinctly non-linear

Air temperature patterns and extremes are computed on a linear scale, but physiology shifts non-linearly with temperature (Savage 2004). For example, metabolic rates increase exponentially (to a point) with temperature, such that small temperature increases at high temperature can have relatively large physiological effects (Savage 2004; Dillon et al. 2010; Dell et al. 2011). Further, performance (fitness) traits show distinct unimodal responses with temperature (Gilchrist 1995; Dell et al. 2011). Thus, physiological responses may bear little or no relationship to statistically defined extremes.

Organismal thermal sensitivity changes during ontogeny and varies by process

As organisms grow and mature, their thermal sensitivity can change (Brett 1971; Coyne et al. 1983; Kingsolver et al. 2011). Moreover, different ontogenetic stages sometimes live in different thermal microenvironments (Kingsolver et al. 2011; Woods 2013). Thus, thermal extremes that are stressful to an egg or larvae might not be to an adult (and vice versa). Further, different physiological traits can have somewhat different thermal sensitivities (Dell et al. 2011).

Physiological resistance to extreme temperatures can change over multiple time scales

An organism's thermal sensitivity isn't constant but often shifts in response to acute heat shocks (heat hardening) or to seasonal conditions (acclimatization). Thus, a T_b that is stressful in one season may not be so in another (Dillon et al. this issue). For example, a warm spell in winter—though still very cold compared with summer conditions—can be very detrimental to hibernating ectotherms, either by draining metabolic reserves or bringing them out of diapause (Marshall and Sinclair 2010; Williams et al. 2014). Variable temperatures can drain metabolic reserves more rapidly than constant conditions due to the non-linear sensitivity of metabolic rate to temperature (see below, Williams et al. 2012). Thermal sensitivity in nature can differ markedly from that measured under constant conditional in the laboratory (Kingsolver and Woods 2016).

Different species and individuals have different heat tolerances

Whether a given high temperature is stressful depends on the heat tolerance of a species, and heat tolerances can differ substantially, even among taxa in a local environment (Kaspari et al. 2015).

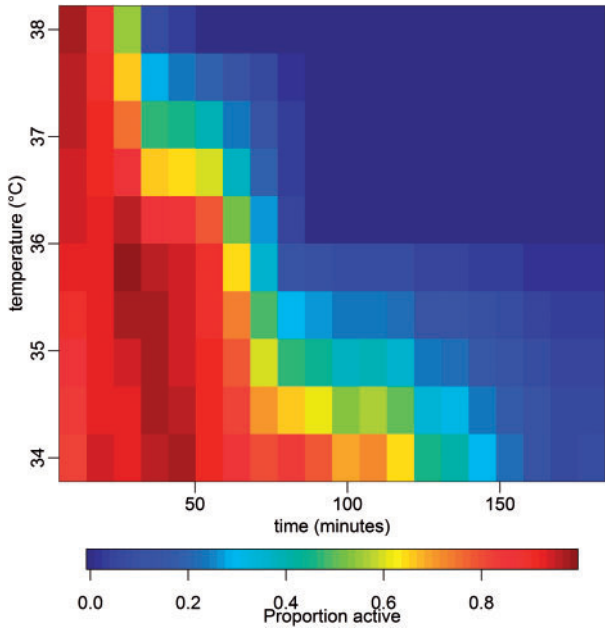

Fig. 1 The proportion of *Drosophila melanogaster* flies that remain active declines with the magnitude (*y*-axis) and duration (*x*-axis) of exposure to extreme temperatures (data from Kjaersgaard et al. 2010). The depicted flies were selected to tolerate heat shocks. See Supplementary Fig. S1 for a comparison with flies selected to tolerate cold shocks.

Individuals within a population can also differ in thermal sensitivity (Logan et al. 2014). Thus not all organisms in a given site will experience a thermal stress event, even if T_a is relatively high for that site.

Inactivity may buffer extreme temperatures

Many organisms (especially in temperate zones) are not active all year: some retreat to hibernacula during winter, or seek shelters during the heat of summer (Kearney et al. 2009; Kingsolver et al. 2011). Such dormant organisms may rarely experience what passes for a heat (or cold) wave above ground (Danks 1987).

Factors influencing the fitness consequences of extremes

(i) Fitness consequences should increase (exponentially) with the deviation of the stress from "normal" environmental levels and with the duration of exposure

The larger impacts of extreme events of greater magnitude and duration have been documented across numerous other taxa. Performance (locomotor activity) of *Drosophila melanogaster* decreases with both the magnitude and duration of exposure to thermal extremes (Fig. 1), but heat-selected flies remain relatively more active at moderately extreme temperatures (Supplementary Fig. S1) (Kjaersgaard et al. 2010). The survival of intertidal mussels (*Mytilus californianus*) declines with increases in the duration and the magnitude of exposure to thermal extremes (Supplementary Fig. S2, Mislan et al. 2014).

Using experimental evolution, studies with *Drosophila* have tested how acclimation, hardening, and selection for survival and reproduction—in both moderate and extreme cold and heat—influence thermal tolerance. Models of thermal adaptation generally assume that selection for thermal tolerance breadth (or cold or heat tolerance) has associated fitness tradeoffs (Gilchrist 1995). Empirical results tend to be more complex. Experiments exposing flies to relatively extreme conditions reveal that resistance is often correlated across stressors (Feder and Hofmann 1999). Potential stress-resistance mechanisms include reduced metabolic rates, accumulation of energy reserves, or enhanced capacity for expressing heat-shock proteins (Bubliy et al. 2012). Each of these mechanisms is expected to induce fitness costs, such as decreased longevity or reduced reproduction, but the fitness costs can be difficult to detect (Bubliy and Loeschcke 2005).

The potential for organisms to evolve resistance to the increase in extreme temperatures associated with climate change may be limited (but see Logan et al. 2014). A *Drosophila* selection study conducted both in the laboratory and field found that the evolutionary potential (heritabilities, genetic variances) of heat resistance declines at higher temperatures (Kristensen et al. 2015). Selection on upper knock-down temperature increased $T_{kd,}$ but only for a few generations, when the response plateaued (Gilchrist and Huey 1999); but no such plateau was evident in flies selected for knock-down time at high temperature (McColl et al. 1996). *Drosophila melanogaster* adapted to fluctuating conditions had decreased tolerance of thermal extremes (Condon et al. 2015).

(ii) Fitness consequences should be inversely proportional to the breadth of the thermal fitness curve

In other words, specialists should be more vulnerable to a given magnitude and duration of thermal stress (Levins 1968). Broad scale data support this pattern: the resilience of European bird populations to a heat wave increased with the thermal breadth of their occupied range (and presumably their thermal tolerance) (Jiguet et al. 2006). Although this principle follows directly from thermal tolerance, responses of organisms via multiple mechanisms to complex patterns of environmental variability considerably complicate this principle. Selection to tolerate warmer temperatures can be accompanied by thermal specialization. Indeed, selection in warmer

moderate (16.5–29 °C) temperatures decreased the thermal breadth of *D. melanogaster* and increased their ability to survive heat (but not cold) shocks (Cavicchi et al. 1995; Gilchrist et al. 1997).

Vertical zonation in the intertidal has provided a primary venue for disentangling long-term responses via adaptive evolution from short-term responses via acclimation because shifts in variability are uncoupled from shifts in means. Resistance to high temperature is strongly correlated with increasing tidal height via selection for tolerance of warm temperatures (Somero 2002). Porcelain crabs (*Petrolisthes* spp.) living high in the intertidal occupy warmer and more thermally variable microhabitats and are relatively tolerant of extreme high temperatures (Stillman and Somero 2000). At the same time, their thermal tolerances acclimate very little (Stillman 2003). A similar result was found for *D. melanogaster* subject to laboratory natural selection at high temperatures (Cavicchi et al. 1995). The relationship between environmental variability and acclimation ability varies across taxa and a broad scale compilation across studies found that plasticity in heat tolerance was unrelated to either latitude or thermal seasonality (Gunderson and Stillman 2015). Organisms, including porcelain crabs, adapted to relatively variable environments often constitutively express high levels of heat shock proteins but induce less expression when facing thermal extremes (Stillman and Tagmount 2009).

Fitness consequences of stress

The fitness consequences of thermal extremes are difficult to quantify because they usually fall somewhere between the worst case of death and the best case of a transient reduction in performance during the event itself. Thus, thermal stress is likely to result in permanent damage or an extended period of recovery. Again, this effect has been most thoroughly quantified for *Drosophila* (David et al. 2005). The time to recover from male sterility after heat stress increased with an increasing magnitude of stress (Rohmer et al. 2004). The recovery time was longer for temperate than for tropical *Drosophila*, which had experienced more selection for tolerating heat stress (Rohmer et al. 2004; see also Vollmer et al. 2004).

Whether thermal stress causes short-term performance loss or mortality has major implications for the evolution of thermal performance (or fitness) curves (TPCs) (Kingsolver and Woods 2016). Selection for tolerance of thermal extremes will be much stronger if it causes selective mortality. Theoretical models of the evolution of TPCs require an assumption for how performance or fitness is integrated over time (Angilletta 2009; Asbury and Angilletta 2010). Lynch and Gabriel (1987) assumed fitness (survival) integrates multiplicatively, which selects for thermal generalists. Gilchrist (1995) assumed fitness (reproduction) integrates additively, which generally selects for thermal specialists. Here, we combine additive (reproduction) and multiplicative (survival) components (as in Kingsolver and Buckley 2015) to investigate intermediate evolutionary scenarios. We use a quantitative genetic model to estimate how extremes influence the evolution of thermal tolerance for generalized temperature distributions. We then apply the model to latitudinal gradients in thermal tolerance of Australian *Drosophila*.

Methods: modeling selection for tolerance of thermal stress

We model the evolution of TPCs. First, we estimate the time series of environmental conditions experienced by individuals across their lifespans within a population. We then use the shape of each individual's TPC to integrate performance over time. The individual performances determine fitness and selection on TPC shape.

We use a beta curve to model performance, Z, as a non-linear function of body temperature, T_b (°C):

$$Z(T_b) = \frac{[(T_b-\alpha)/b]^{\gamma/\beta-1}[1-(T_b-\alpha)/b]^{1-\gamma/\beta-1}\Gamma(1/\beta)}{\Gamma(\gamma/\beta)\Gamma[(1-\gamma)\beta]}$$

where α, β, and γ determine the minima, breadth, and skewness of the performance curve, respectively (Asbury and Angilletta 2010). The parameter b determines the maximal breadth. We constrain the parameters to those that generate realistic curves ($-10 < \alpha < 4$, $0.05 < \beta < 0.15$, $\gamma = 0.7$, and $b = 43$; Supplementary Fig. S3), given the large diversity of curves observed among ectothermic animals (see Sunday et al. 2014). The area under the curve is fixed (thus excluding "hotter is better" [Asbury and Angilletta 2010]).

We estimate fitness as the product of fecundity and survival. Fecundity is quantified as the sum of performance across time steps within a generation, and we assume low but non-zero performance outside the critical thermal limits. For those models that include mortality, thermal stress is the sole source of mortality. We assumed that the probability of survival through a thermal stress event declined exponentially to zero between CT_{max} and 60 °C. We confirmed that results were similar if survival

declined linearly. The probability of an individual surviving to the end of its potential lifespan is assumed to be the product of survival across sequential time periods. We initially (questions i and ii) define a generation as 1000 time steps. We subsequently (question iii) vary generation length (in days) as a function of temperature based on empirical data (see below). We ran the model for 200 generations (sufficient to reach equilibrium) for questions i and ii and ~700 generations for question iii. We defined the thermal optimum as the body temperature that yields maximal performance and the critical thermal limits as the body temperatures that yield 1% of maximal performance.

We use a simple quantitative genetic model to predict selection and the evolution of TPCs. We consider how two phenotypes (parameters α: minima and β: breadth) of the performance curve evolve. We use a genetic variance covariance matrix (G matrix) to account for the genetic correlation of the two parameters (vector z), and we model phenotypic evolution as $\Delta z = Gs$, where s is a vector describing selection on each of the traits (Lande and Arnold 1983). We assume genetic variances (heritabilities) of 0.7 and covariances of –0.1. The negative covariance accounts for the observation that organisms with higher thermal tolerances tend to have smaller breadths, but our results are robust to the sign of the covariance. Our estimates of the variances and covariances in the G matrix for TPCs are high and similar, respectively, relative to the limited empirical data available (Kingsolver et al. 2004), but we selected these values to speed evolution in our analysis. We used a sensitivity analysis to confirm that our results are robust to our parameterization of the G matrix.

We initialized our model with TPC minima (α) and breadth (β) that optimized performance in the initial time period in the absence of thermal extremes. We simulated 500 individuals with TPC minima (α) and breadth (β) drawn from a normal distribution with the given phenotypic mean and variance (standard deviations = 1 and 0.02 for α and β, respectively) each generation. We use relative fitness estimates for each individual to estimate the (unstandardized) directional selection gradients and to predict the evolutionary response to selection (change in mean phenotype) in the next generation (Lande and Arnold 1983).

Whether thermal stress occurs depends not only on microclimate variation, but also on whether individuals are able to select their preferred microclimate from the available distribution (Kearney et al. 2009; Sears et al. 2011). We incorporated microclimate variation by drawing a value for thermal heterogeneity from a normal distribution with a standard deviation of 2 °C at each time step for each individual. We incorporated behavioral thermoregulation by assuming that individuals would select the microclimate (from the distribution of available microclimates, specified by the amount of heterogeneity) at each time step that was closest to their thermal optima.

We apply this modeling framework to address three questions:

(i) (How) do extreme temperatures drive the evolution of thermal tolerances?

We started with a truncated normal distribution corresponding to the operative temperatures that a temperate (mean = 20 °C, SD = 5 °C) or tropical (mean = 30 °C, SD = 2.5 °C) organism might experience within a single generation (per year). We truncated the distribution between –10 °C and 60 °C to bound the thermal extremes (R command rTNorm). In (a probabilistically selected) 40% of generations, we include 40 extreme temperatures in the total of 1000 temperatures. The extremes were randomly distributed across the time series of temperatures. Sensitivity analyses confirm that our results are qualitatively robust to the number and annual probability of extreme temperatures. We ran the model for 1200 generations to examine how the TPC responds to changing the incidence of heat events (annual incidences: 0%, 5%, 10%, 20%, 40%, 100%).

We set two scenarios for heat extremes. In the first, "extreme" values are relative to the mean environmental conditions; but in the second, "extreme" values are at a fixed, high temperature. In both cases, the extremes are drawn from a truncated normal distribution with a standard deviation one-fourth that of the initial (tropical or temperate) temperature distribution. In the first scenario, we add thermal extremes centered four standard deviations above the mean environmental conditions. In the second scenario we add thermal extremes with a distribution centered at a fixed position of 42 °C, independent of mean conditions. The latter scenario reflects the observation that extreme temperatures vary less with latitude than do mean temperatures (Ghalambor et al. 2006; Hoffmann 2010). We assumed that thermal stress influences survival as described above.

(ii) (How) does the evolution of thermal tolerance vary with the consequences of experiencing a thermal extreme?

For the temperate site with extremes four standard deviations from the mean, we explored how the

thermal extremes influence the evolution of thermal tolerances. Initially, we assumed a thermal extreme will reduce performance during the extreme but not cause injury or death. Subsequently, we assumed two additional, persistent consequences of encountering a thermal extreme. First, organisms encountering a thermal extreme require a recovery time before resuming normal performance, and so we assumed an additional time step of recovery (with zero performance) for each degree C by which each extreme exceeded the CT_{max}. This roughly corresponds to the observed recovery time of *Drosophila* after a thermal stress (Rohmer et al. 2004). Second, we assumed that the probability of survival through a thermal stress event declined exponentially to zero between CT_{max} and 60 °C.

(iii) (How) do extreme temperatures drive latitudinal gradients in thermal tolerances?

We consider a coastal and an interior latitudinal gradient in eastern Australia. We compared our thermal tolerance from the evolutionary model with empirical observations for knock-down temperatures for coastal *Drosophila* (unpublished data courtesy of G. W. Gilchrist and R. B. Huey, see Gilchrist and Huey [1999] for methods). (Note: our comparison is approximate because the critical thermal limits we estimate from the TPCs do not directly correspond to the knockdown metric [temperature at which 50% of individuals lose coordination and fall through an experimental chamber] used to estimate thermal limits in *Drosophila*.)

We accessed daily maximum temperature data from weather stations managed by the Australian Bureau of Meteorology as part of the Australian Climate Observations Reference Network (http://www.bom.gov.au/climate/change/acorn-sat/). We selected weather stations that had nearly complete records since 1962 and that were dispersed along the east coast or located along an interior latitudinal gradient (~145°E) (Supplementary Table S1, Supplementary Fig. S4; map with station information: http://www.bom.gov.au/climate/change/reference.shtml#rcsmap). We linearly approximated temperatures for the few instances of days missing data (R function "na.approx" from the zoo package). We assume that air temperatures at weather station height (1.2 m) approximate the body temperatures experienced by *Drosophila*, an assumption which is reasonable given their small body size and flight above ground (Huey and Pascual 2009).

To estimate generation length we estimated the growing degree days (GDDs) corresponding to a *Drosophila* generation as an exponential function of temperature, T (°C): $\exp(8.4652–0.0669T)$. We fit the relationship using data from Loeb and Northrop (1917). We calculated the cumulative average of temperature to estimate the GDDs required and compared the GDDs required with those available to partition the temperature time series into generations. We initially ran the evolutionary model for 1962–1990, a period approximating the World Meteorological Organization baseline period (of 1961–1990, we started at 1962 due to data limitations). We then extended the run of the evolutionary model across 1991–2010, to examine potential shifts in response to recent climate warming. We assumed that thermal stress increases mortality as described above.

Results

(i) How do extreme temperatures drive the evolution of thermal tolerances?

Model predictions suggest that extreme temperatures can exert a strong influence on the evolution of TPCs (Fig. 2). Thermal extremes exert a stronger influence at temperate sites, where they broaden the TPC and shift it to warmer temperatures. In the tropical sites where environmental temperature distributions are narrow, thermal extremes did not affect the shape of the TPC but shifted it to warmer temperatures.

The magnitude of thermal extremes tend to be fairly constant across latitude (Ghalambor et al. 2006; Hoffmann 2010), even though mean temperatures decline with latitude. Therefore, we examined as well how TPCs evolve when temperature distributions differ but when the extremes are fixed (here 42 °C): this approach thus addresses the relative influence of mean versus extreme temperatures on the evolution of TPCs. In this scenario, fixed extremes exert a stronger influence in the temperate site than in the tropical site, because the TPC in the temperate site is evolved in response to a broad range of temperatures. When extremes are fixed, evolved values of CT_{max} values are similar for temperate and tropical organisms (43.0 °C and 42.6 °C, respectively), but thermal breadth is wider for temperate organisms (35.1 °C vs. 24.6 °C). This finding is consistent with the view that the shallow latitudinal gradient in thermal extremes can exert a strong influence on the evolution of thermal tolerance, regardless of differences in average conditions (Denny et al. 2009; Hoffmann 2010).

Even rare heat waves (e.g., 5% annual incidence) cause the TPC to shift to warmer temperatures and broaden (Fig. 3A). Indeed, the TPC is most

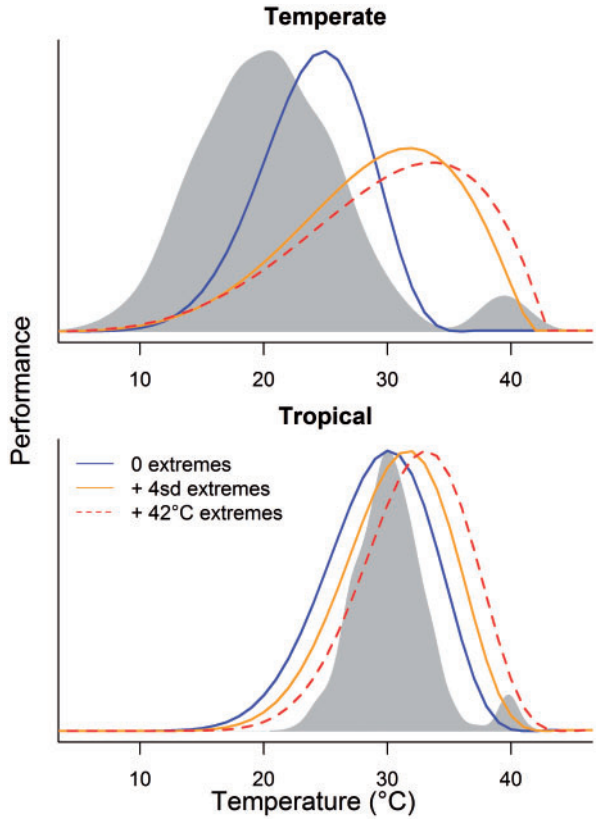

Fig. 2 The evolutionary response to thermal extremes varies between temperate (top) and tropical (bottom) sites. We estimate the thermal performance curves (TPCs) predicted to evolve in idealized environments in the absence of extremes (blue), with thermal extremes centered at four standard deviations from the site's mean temperatures (orange, temperature distribution shown in gray) and thermal extremes centered at 42 °C regardless of the site's mean temperatures (red dashed). We depict (gray shading) the temperature distribution for the second scenario above.

responsive when the frequency of thermal extremes is low (Fig. 3B).

(ii) (How) does this vary with the cost of experiencing a thermal extreme?

When thermal extremes cause only a transient decline in performance during the event, evolutionary change is minor (Fig. 4A). But if the event also reduces performance during a recovery period (1 day per °C that each daily extreme temperature exceeds CT_{max}), the effects are more marked (Fig. 4A). If the event causes mortality, effects are pronounced: TPCs become broader and shift to warmer temperatures. Interestingly, evolution to minimize mortality at high temperatures can result in loss of performance at the most common, intermediate temperatures (Fig. 4B).

(iii) (How) do extreme temperatures drive latitudinal gradients in thermal tolerances?

We next consider whether the responses of TPCs are expected to be similar across latitude when we consider observed temperature distributions for sites along two latitudinal gradients. We consider coastal sites in Australia, which experience thermal buffering, and also interior sites, which have warmer and more variable temperatures (Figs. 5 and 6). The coastal sites at the lowest latitudes tend to experience regular heat waves (Hoffmann 2010) from air masses coming from the interior.

The upper critical thermal limits (CT_{max}) predicted by the evolutionary model are heavily influenced by warm extremes in the tail of the temperature distribution (Fig. 6 and Supplementary Fig. S5). CT_{max} values are predicted to be lower at thermally buffered coastal sites than at inland sites and to be similar across latitudes (Fig. 5). An exception is higher CT_{max} values the lowest latitude coastal sites due to the occurrence of heat waves (Pezza et al. 2012). The absence of a latitudinal gradient corresponds to empirically-measured knockdown temperatures for *Drosophila* populations (Fig. 5). Empirical thermal tolerances for coastal populations fall between our predictions for coastal and interior sites, suggesting that we may have underestimated the ability of the flies to alter their body temperatures through behavior.

The ability to thermoregulate behaviorally, which can buffer thermal extremes (Huey et al. 2003), shifts TPCs to lower values than expected from a non-thermoregulation model at both coastal and interior sites (Fig. 5). Behavior should also maintain a narrow TPC in coastal sites (Figs. 5 and 6), leading to relatively specialized TPCs. The effect of microclimate heterogeneity tends to average out over time and space for thermoconforming organisms that use space randomly. Microclimate heterogeneity can either intensify or alleviate the incidence of extremes depending on the position of the thermal environment relative to the TPC.

Both the means and extremes of the observed air temperature distributions have shifted to warmer temperatures at most sites during recent decades (1991–2010, Supplementary Fig. S6). However, our model does not produce consistent responses in the shape and position of TPCs. This suggests that broad scale gradients in extreme temperatures may have more influence on the evolution of TPCs than do temperature distributions

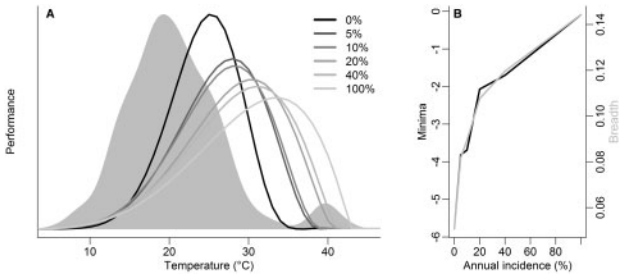

Fig. 3 (**A**) As the annual incidence of heat waves increases from 0% (black) to 100% (light gray), the TPC shifts to warmer temperatures and broadens. (**B**) The TPC (black: minima, gray: breadth) is most sensitive when the incidence of extremes is low.

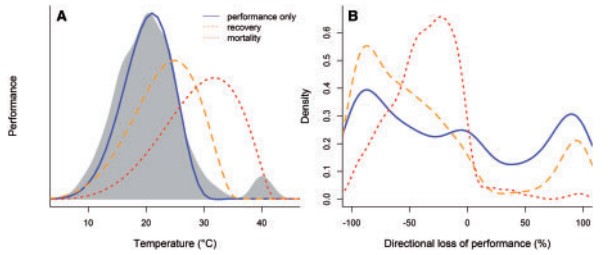

Fig. 4 (**A**) Thermal extremes drive the evolution of TPCs more strongly when they cause mortality (red dotted) than when they only influence short-term performance (blue solid) or when they additionally reduce performance during a recovery period (orange dashed). Temperature distribution shown in gray. (**B**) The extent to which performance is below the optimal due to temperatures below (–) or above (+) the optima. Performance curves evolved to minimize mortality result in the loss of performance at the common, intermediate temperatures. This effect is reduced for the other scenarios.

associated with climate change, which may vary interannually.

Discussion

Our evolutionary model suggests that warm thermal extremes, at least when they cause mortality or persistent physiological damage, exert a larger influence on the evolution of TPCs than do mean conditions. Asymmetries in TPCs may lessen the influence of cold extremes on evolution, but we expect the influence will be qualitatively similar. This finding reinforces the importance of studies that focus on extremes, not merely on means or "normal" variation. We fully appreciate the challenge of designing an experiment that appropriately captures, and measures responses to, the normal and extreme variation experienced in nature, especially for organisms with long generation times and rare extremes (Denny et al. 2009).

A key result of our model, when extremes cause mortality and thermal injury, is that heat tolerance should follow gradients in extreme rather than mean temperatures. This prediction is consistent with a shallow gradient of knock-down temperatures (Fig. 5) of east coast populations of *Drosophila melanogaster* in Australia, with a similar shallow latitudinal gradient in CT_{max} among *Drosophila* species in Australia (Overgaard et al. 2014), and a recent study showing that thermal tolerance better accounts for observed geographic distributions of Australian *Drosophila* than does the thermal sensitivity of population growth (Overgaard et al. 2014). Gene flow among our focal *Drosophila* populations, which was not incorporated in our model, may further reduce geographic variation in thermal tolerance.

The influence of thermal stress events on TPCs also highlights the importance of translating meteorological measures of thermal stress onto the physiological consequences to organisms. Specifically, this requires translating air temperatures and other environmental conditions onto the body temperatures experienced by organisms (Helmuth et al. 2010). That requires both understanding heat transfer processes and considering options for behavioral thermoregulation. Our findings suggest that behavioral thermoregulation can alter the evolution of thermal sensitivity (Kearney et al. 2009; Huey et al. 2012).

Proceeding from body temperature to performance is complex, as thermal sensitivity—and specifically sensitivity to thermal extremes—varies across seasons, ontogeny, physiological processes, energy state (Brett 1971), and species. And different responses of species can change community dynamics (Kingsolver et al. 2011; Levy et al. 2015). Here, we have focused on temperature but note that the most extreme biological responses are often caused by synchronous occurrence of multiple environmental stressors (Denny et al. 2009). For example, mortality of intertidal mussels is rare when only extreme high temperatures occur, but is very high when those temperatures occur on days with low tides and slack winds (Helmuth et al. 2010). Modeling the coincidence of multiple stressors is feasible and can enable predicting such events (Denny et al. 2009).

Our finding that extreme events can exert a strong influence on the evolution of thermal tolerance is consistent with the observation that latitudinal gradients in thermal tolerance are shallower than latitudinal gradients in mean temperatures. Upper thermal limits for plants and both ectothermic and endothermic animals tend to be more constrained across latitude than are lower thermal limits (Huey et al. 2009; Kellermann et al. 2012; Araújo et al. 2013; Hoffmann et al. 2013; Sunday et al. 2014). However, upper

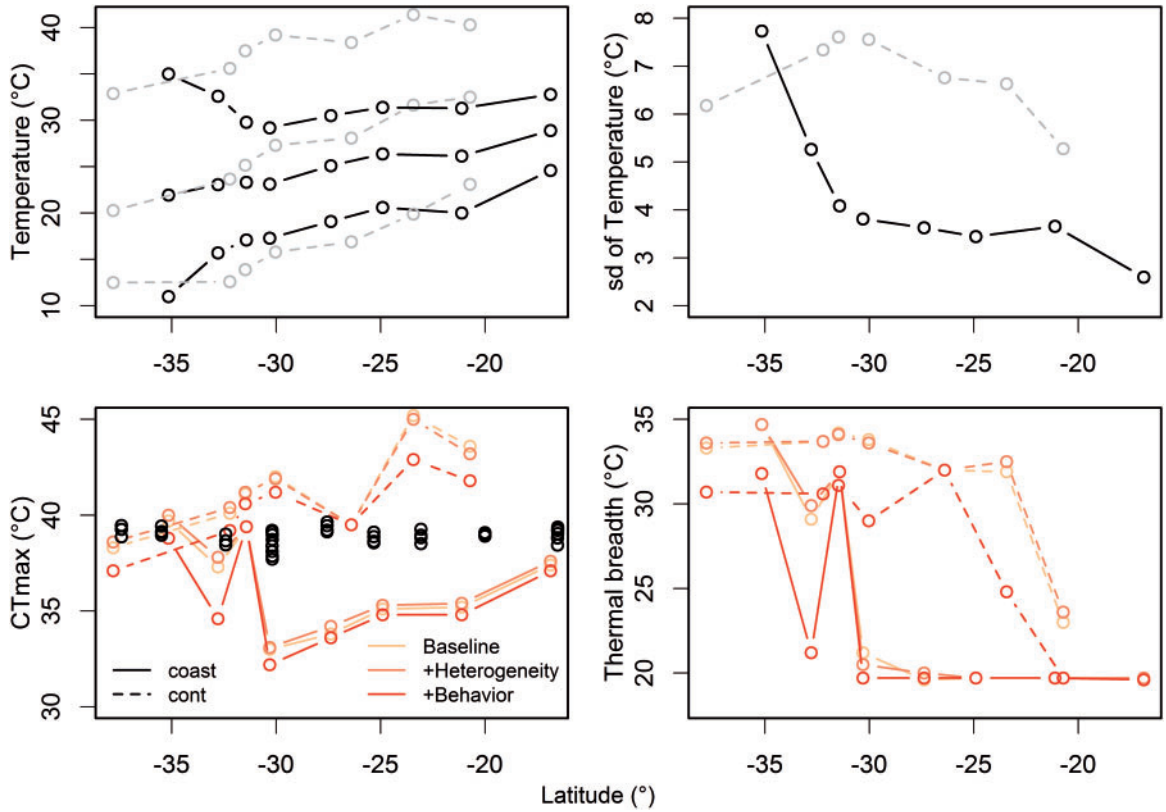

Fig. 5 Coastal sites in Australia are more thermally buffered than interior sites (top left: mean along with 5% and 95% quantiles of temperature, top right: standard deviation of temperature). We predict lower upper critical thermal limits (CT_{max}) values for thermally buffered coastal sites than we do for interior sites (bottom left). We do not predict strong a latitudinal gradient in CT_{max} values. This prediction corresponds to empirically measured Knockdown (50% survival) temperatures for *Drosophila* populations (o). We predict that the breadth of TPCs will be less in coastal sites and when extreme temperatures are buffered by behavior (bottom right).

thermal limits may show a stronger decrease with latitude when accounting for the depression associated with extended exposure time (Rezende et al. 2014).

Evolutionary constraints offer an alternative explanation for the shallow latitudinal gradient in thermal tolerance (Araújo et al. 2013). The physical challenge of countering the destabilization of membranes and proteins at high temperatures is thought to constrain the evolution of higher thermal tolerance (reviewed in Angilletta 2009). Evolutionary constraints are reflected in strong phylogenetic signals in upper thermal limits (Hoffmann 2010; Kellermann et al. 2012; Araújo et al. 2013). Levels of genetic variation lower than that we incorporated in our analyses additionally limit evolutionary potential (Hoffmann et al. 2013). Mortality induced by thermal extremes may cause declines in population size that reduce standing genetic variation and thus limit evolutionary potential (Hoffmann et al. 2013). Even for those systems retaining ample genetic variation for thermal tolerance, selection and heritability experiments suggest that increases in upper thermal limits quickly plateau (Gilchrist and Huey 1999; Hoffmann et al. 2013). We also note that our evolutionary model assumed that the area under the performance curve is fixed. Broadening of the TPC to tolerate warmer temperatures thus trades off with performance at the more frequently encountered intermediate temperatures. However, evidence for such tradeoffs is mixed (reviewed in Angilletta 2009; Kingsolver 2009; see Asbury and Angilletta [2010] for a theoretical assessment). Moreover, our model does not consider a "hotter is better" phenomenon, in which area under the curve increases as optimal temperature increases (Asbury and Angilletta 2010).

Even small shifts in the mean of temperature distributions caused by climate warming will substantially increase the incidence of extreme temperatures (Smith 2011). Understanding the impacts of extreme events is thus paramount to predicting the biological consequences of climate change. Many studies have questioned whether the pace of climate change will be sufficiently fast to preclude evolutionary adaption (Hoffmann and Sgrò 2011). Our analysis suggests that strong selection imposed by mortality associated

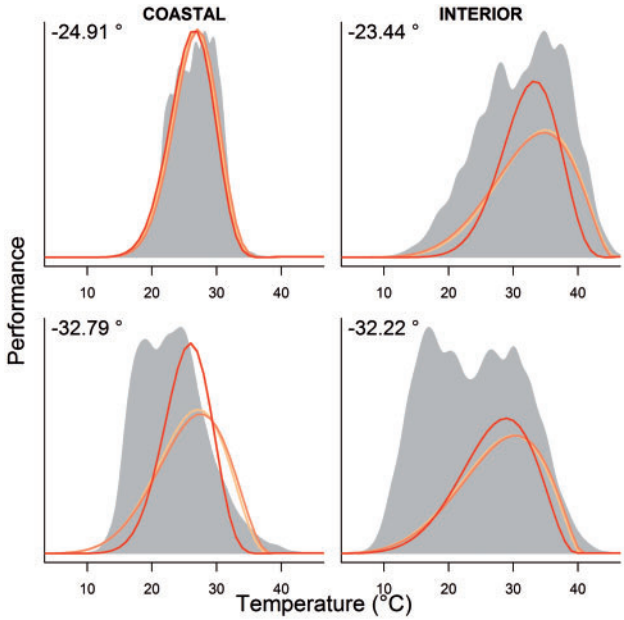

Fig. 6 The breadth and shape of temperature distributions (gray) along latitudinal gradients (latitude depicted) in coastal (left) and interior (right) Australia influence the evolution of TPCs for *Drosophila*. TPCs are narrower and shifted to cooler temperatures when organisms are able to use behavior to avoid thermal extremes (red) compared with the case of a thermoconformer with (orange) or without (peach) microclimate variability. Distributions are depicted for all sites in Supplementary Fig. S5.

with thermal extremes can drive rapid adaptation. However, genetic and selective correlations can slow evolution in response to extremes (Gilchrist 1993). Additionally, increased variability associated with climate change may be sufficient to cause fluctuations in the direction of selection and slow evolution (Kingsolver and Buckley 2015). Acclimation may lessen the selection in response to extremes (Williams et al. this issue). Our analysis contributes to the rapidly expanding recognition that forecasting the ecological and evolutionary responses to climate change will require considering thermal extremes and variability, not just mean conditions.

Acknowledgments

The authors thank M. Dillon, M. Sears, and A. Woods for the opportunity to participate in, and NSF for supporting, the symposium. They thank the members of their laboratory group, M. Denny, G. Gilchrist, J. Kingsolver, and A. Woods for constructive input. They thank G. Gilchrist for providing unpublished *Drosophila* knockdown data. They thank K.A.S. Mislan and A. Kjaersgaard for providing previously published data that they used to create figures.

Funding

This work was supported by the National Science Foundation [DBI-1349865 to L.B.B.; EF-1065638 to L.B.B.; and IB-1038016 to R.B.H.].

Supplementary data

Supplementary data available at *ICB* online.

References

Allen CD, Macalady AK, Chenchouni H, Bachelet D, McDowelle N, Vennetierf M, Kitzbergerg T, Riglingh A, Breshearsi DD, Ted Hoggj EH, et al.. 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. For Ecol Manag 259:660–84.

Angilletta MJ. 2009. Thermal adaptation: a theoretical and empirical synthesis. Oxford: Oxford University Press.

Araújo MB, Ferri-Yáñez F, Bozinovic F, Marquet PA, Valladares F, Chown SL. 2013. Heat freezes niche evolution. Ecol Lett 16:1206–19.

Asbury DA, Angilletta MJ. 2010. Thermodynamic effects on the evolution of performance curves. Am Nat 176:E40–9.

Bakken GS. 1992. Measurement and application of operative and standard operative temperatures in ecology. Integr Comp Biol 32:194–216.

Brett JR. 1971. Energetic responses of salmon to temperature. A study of some thermal relations in the physiology and freshwater ecology of sockeye salmon (*Oncorhynchus nerka*). Am Zool 11:99–113.

Bubliy OA, Kristensen TN, Kellermann V, Loeschcke V. 2012. Plastic responses to four environmental stresses and cross-resistance in a laboratory population of *Drosophila melanogaster*. Funct Ecol 26:245–53.

Bubliy OA, Loeschcke V. 2005. Correlated responses to selection for stress resistance and longevity in a laboratory population of *Drosophila melanogaster*. J Evol Biol 18:789–803.

Cavicchi S, Guerra D, La Torre V, Huey RB. 1995. Chromosomal analysis of heat-shock tolerance in *Drosophila melanogaster* evolving at different temperatures in the laboratory. Evolution 49:676–84.

Condon C, Acharya A, Adrian GJ, Hurliman AM, Malekooti D, Nguyen P, Zelic MH, Angilletta MJ Jr. 2015. Indirect selection of thermal tolerance during experimental evolution of *Drosophila melanogaster*. Ecol Evol 5:1873–80.

Coumou D, Rahmstorf S. 2012. A decade of weather extremes. Nat Clim Change 2:491–6.

Coyne JA, Bundgaard J, Prout T. 1983. Geographic variation of tolerance to environmental stress in *Drosophila pseudoobscura*. Am Nat 122:474–88.

Danks HV. 1987. Insect dormancy: an ecological perspective. Biological Survey of Canada Monograph Series. Entomological Society of Canada. Winnipeg, Manitoba, Canada.

David JR, Araripe LO, Chakir M, Legout H, Lemos B, Pétavy G, Rohmer C, Joly D, Moreteau B. 2005. Male sterility at extreme temperatures: a significant but neglected phenomenon for understanding *Drosophila* climatic adaptations. J Evol Biol 18:838–46.

Dell AI, Pawar S, Savage VM. 2011. Systematic variation in the temperature dependence of physiological and ecological traits. Proc Natl Acad Sci USA 108:10591–6.

Denny MW, Dowd WW. 2012. Biophysics, environmental stochasticity, and the evolution of thermal safety margins in intertidal limpets. J Exp Biol 215:934–47.

Denny MW, Hunt LJH, Miller LP, Harley CDG. 2009. On the prediction of extreme ecological events. Ecol Monogr 79:397–421.

Dillon ME, Wang G, Huey RB. 2010. Global metabolic impacts of recent climate warming. Nature 467:704–6.

Dowd WW, King FA, Denny MW. 2015. Thermal variation, thermal extremes and the physiological performance of individuals. J Exp Biol 218:1956–67.

Easterling DR, Meehl GA, Parmesan C, Changnon SA, Karl TR, Mearns LO. 2000. Climate extremes: observations, modeling, and impacts. Science 289:2068–74.

Feder ME, Hofmann GE. 1999. Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. Annu Rev Physiol 61:243–82.

Ghalambor CK, Huey RB, Martin PR, Tewksbury JJ, Wang G. 2006. Are mountain passes higher in the tropics? Janzen's hypothesis revisited. Integr Comp Biol 46:5–17.

Gilchrist GW. 1993. Specialists and generalists in changing environments: the evolution of thermal sensitivity. Dissertation. University of Washington.

Gilchrist GW. 1995. Specialists and generalists in changing environments. 1. Fitness landscapes of thermal sensitivity. Am Nat 146:252–70.

Gilchrist GW, Huey RB. 1999. The direct response of *Drosophila melanogaster* to selection on knockdown temperature. Heredity 83:15–29.

Gilchrist GW, Huey RB, Partridge L. 1997. Thermal sensitivity of *Drosophila melanogaster*: evolutionary responses of adults and eggs to laboratory natural selection at different temperatures. Physiol Biochem Zool 70:403–14.

Gunderson AR, Stillman JH. 2015. Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. Proc R Soc Lond B Biol Sci 282: 20150401.

Harley CDG, Paine RT. 2009. Contingencies and compounded rare perturbations dictate sudden distributional shifts during periods of gradual climate change. Proc Natl Acad Sci USA 106:11172–6.

Helmuth B, Broitman BR, Yamane L, Gilman SE, Mach K, Mislan KA, Denny MW. 2010. Organismal climatology: analyzing environmental variability at scales relevant to physiological stress. J Exp Biol 213:995–1003.

Hoffmann AA. 2010. Physiological climatic limits in *Drosophila*: patterns and implications. J Exp Biol 213:870–80.

Hoffmann AA, Chown SL, Clusella-Trullas S. 2013. Upper thermal limits in terrestrial ectotherms: how constrained are they? Funct Ecol 27:934–49.

Hoffmann AA, Sgrò CM. 2011. Climate change and evolutionary adaptation. Nature 470:479–85.

Huey RB, Deutsch CA, Tewksbury JJ, Vitt LJ, Hertz PE, Alvarez Pérez HJ, Garland T Jr. 2009. Why tropical forest lizards are vulnerable to climate warming. Proc R Soc B 276:1939–48.

Huey RB, Hertz PE, Sinervo B. 2003. Behavioral drive versus behavioral inertia in evolution: a null model approach. Am Nat 161:357–66.

Huey RB, Kearney MR, Krockenberger A, Holtum JA, Jess M, Williams SE. 2012. Predicting organismal vulnerability to climate warming: roles of behaviour, physiology and adaptation. Philos Trans R Soc B Biol Sci 367:1665–79.

Huey RB, Pascual M. 2009. Partial thermoregulatory compensation by a rapidly evolving invasive species along a latitudinal cline. Ecology 90:1715–20.

Hughes TP, Baird AH, Bellwood DR, Card M, Connolly SR, Folke C, Grosberg R, Hoegh-Guldberg O, Jackson JB, Kleypas J, et al.. 2003. Climate change, human impacts, and the resilience of coral reefs. Science 301:929–33.

Jiguet F, Julliard R, Thomas CD, Dehorter O, Newson SE, Couvet D. 2006. Thermal range predicts bird population resilience to extreme high temperatures. Ecol Lett 9:1321–30.

Kaspari M, Clay NA, Lucas J, Yanoviak SP, Kay A. 2015. Thermal adaptation generates a diversity of thermal limits in a rainforest ant community. Glob Change Biol 21:1092–102.

Kearney M, Shine R, Porter WP. 2009. The potential for behavioral thermoregulation to buffer ''cold-blooded'' animals against climate warming. Proc Natl Acad Sci USA 106:3835–40.

Kellermann V, Overgaard J, Hoffmann AA, Fløjgaard C, Svenning JC, Loeschcke V. 2012. Upper thermal limits of *Drosophila* are linked to species distributions and strongly constrained phylogenetically. Proc Natl Acad Sci USA 109:16228–33.

Kingsolver JG. 2009. The well-temperatured biologist. Am Nat 174:755–68.

Kingsolver JG, Buckley LB. 2015. Climate variability slows evolutionary responses of *Colias* butterflies to recent climate change. Proc R Soc B Biol Sci 282:20142470.

Kingsolver JG, Ragland GJ, Shlichta JG. 2004. Quantitative genetics of continuous reaction norms: thermal sensitivity of caterpillar growth rates. Evolution 58:1521–9.

Kingsolver JG, Woods HA. 2016. Beyond thermal performance curves: modeling time-dependent effects of thermal stress on ectotherm growth rates. Am Nat 187:283–94.

Kingsolver JG, Woods HA, Buckley LB, Potter KA, MacLean HJ, Higgins JK. 2011. Complex life cycles and the responses of insects to climate change. Integr Comp Biol 51:719–32.

Kjaersgaard A, Demontis D, Kristensen TN, Le N, Faurby S, Pertoldi C, Sørensen JG, Loeschcke V. 2010. Locomotor activity of *Drosophila melanogaster* in high temperature environments: plastic and evolutionary responses. Clim Res 43:127–34.

Kristensen TN, Overgaard J, Lassen J, Hoffmann AA, Sgrò C. 2015. Low evolutionary potential for egg-to-adult viability in *Drosophila melanogaster* at high temperatures. Evolution 69:803–14.

Lande R, Arnold SJ. 1983. The measurement of selection on correlated characters. Evolution 37:1210–26.

Levins R. 1968. Evolution in changing environments. Princeton (NJ): Princeton University Press.

Levy O, Buckley LB, Keitt TH, Smith CD, Boateng KO, Kumar DS, Angilletta MJ Jr. 2015. Resolving the life cycle

alters expected impacts of climate change. Proc R Soc B Biol Sci 282:20150837.
Loeb J, Northrop JH. 1917. On the influence of food and temperature on the duration of life. J Biol Chem 32:102–21.
Logan ML, Cox RM, Calsbeek R. 2014. Natural selection on thermal performance in a novel thermal environment. Proc Natl Acad Sci USA 111:14165–9.
Lynch MJ, Gabriel W. 1987. Environmental tolerance. Am Nat 129:283–303.
Marshall KE, Sinclair BJ. 2010. Repeated stress exposure results in a survival–reproduction trade-off in *Drosophila melanogaster*. Proc R Soc Lond B Biol Sci 277:963–9.
McColl G, Hoffmann AA, McKechnie SW. 1996. Response of two heat shock genes to selection for knockdown heat resistance in *Drosophila melanogaster*. Genetics 143:1615–27.
McKechnie AE, Wolf BO. 2009. Climate change increases the likelihood of catastrophic avian mortality events during extreme heat waves. Biol Lett 6:253–6.
Mislan KAS, Helmuth B, Wethey DS. 2014. Geographical variation in climatic sensitivity of intertidal mussel zonation. Glob Ecol Biogeogr 23:744–56.
Overgaard J, Kearney MR, Hoffmann AA. 2014. Sensitivity to thermal extremes in Australian *Drosophila* implies similar impacts of climate change on the distribution of widespread and tropical species. Glob Change Biol 20:1738–50.
Pezza AB, Van Rensch P, Cai W. 2012. Severe heat waves in Southern Australia: synoptic climatology and large scale connections. Clim Dyn 38:209–24.
Rezende EL, Castañeda LE, Santos M. 2014. Tolerance landscapes in thermal ecology. Funct Ecol 28:799–809.
Rohmer C, David JR, Moreteau B, Joly D. 2004. Heat induced male sterility in *Drosophila melanogaster*: adaptive genetic variations among geographic populations and role of the Y chromosome. J Exp Biol 207:2735–43.
Savage VM. 2004. Improved approximations to scaling relationships for species, populations, and ecosystems across latitudinal and elevational gradients. J Theor Biol 227:525–34.
Sears MW, Raskin E, Angilletta MA. 2011. The world is not flat: defining relevant thermal landscapes in the context of climate change. Integr Comp Biol 51:666–75.
Smith MD. 2011. An ecological perspective on extreme climatic events: a synthetic definition and framework to guide future research. J Ecol 99:656–63.
Somero GN. 2002. Thermal physiology and vertical zonation of intertidal animals: optima, limits, and costs of living. Integr Comp Biol 42:780–9.
Somero GN. 2010. The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine ''winners'' and ''losers.'' J Exp Biol 213:912–20.
Stillman JH. 2003. Acclimation capacity underlies susceptibility to climate change. Science 301:65.
Stillman JH, Somero GN. 2000. A comparative analysis of the upper thermal tolerance limits of eastern Pacific porcelain crabs, genus *Petrolisthes*: influences of latitude, vertical zonation, acclimation, and phylogeny. Physiol Biochem Zool 73:200–8.
Stillman JH, Tagmount A. 2009. Seasonal and latitudinal acclimatization of cardiac transcriptome responses to thermal stress in porcelain crabs, *Petrolisthes cinctipes*. Mol Ecol 18:4206–26.
Sunday JM, Bates AE, Kearney MR, Colwell RK, Dulvy NK, Longino JT, Huey RB. 2014. Thermal-safety margins and the necessity of thermoregulatory behavior across latitude and elevation. Proc Natl Acad Sci USA 111:5610–5.
Tsuchiya M. 1983. Mass mortality in a population of the mussel *Mytilus edulis* L. caused by high temperature on rocky shores. J Exp Mar Biol Ecol 66:101–11.
Vollmer JH, Sarup P, Kaersgaard CW, et al.. 2004. Heat and cold-induced male sterility in *Drosophila buzzatii*: genetic variation among populations for the duration of sterility. Heredity 92:257–62.
Williams CM, Henry HA, Sinclair BJ. 2014. Cold truths: how winter drives responses of terrestrial organisms to climate change. Biol Rev 90:214–35.
Williams CM, Marshall KE, MacMillan HA, Dzurisin JD, Hellmann JJ, Sinclair BJ. 2012. Thermal variability increases the impact of autumnal warming and drives metabolic depression in an overwintering butterfly. PLoS One7:e34470.
Woods HA. 2013. Ontogenetic changes in the body temperature of an insect herbivore. Funct Ecol 27:1322–31.