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Thermal Variation, Thermal Extremes and the Physiological Performance of Individuals

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REVIEW

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ABSTRACT

In this review we consider how small-scale temporal and spatial variation in body temperature, and biochemical/physiological variation among individuals, affect the prediction of organisms' performance in nature. For 'normal' body temperatures – benign temperatures near the species' mean – thermal biology traditionally uses performance curves to describe how physiological capabilities vary with temperature. However, these curves, which are typically measured under static laboratory conditions, can yield incomplete or inaccurate predictions of how organisms respond to natural patterns of temperature variation. For example, scale transition theory predicts that, in a variable environment, peak average performance is lower and occurs at a lower mean temperature than the peak of statically measured performance. We also demonstrate that temporal variation in performance is minimized near this new 'optimal' temperature. These factors add complexity to predictions of the consequences of climate change. We then move beyond the performance curve approach to consider the effects of rare, extreme temperatures. A statistical procedure (the environmental bootstrap) allows for long-term simulations that capture the temporal pattern of extremes (a Poisson interval distribution), which is characterized by clusters of events interspersed with long intervals of benign conditions. The bootstrap can be combined with biophysical models to incorporate temporal, spatial and physiological variation into evolutionary models of thermal tolerance. We conclude with several challenges that must be overcome to more fully develop our understanding of thermal performance in the context of a changing climate by explicitly considering different forms of small-scale variation. These challenges highlight the need to empirically and rigorously test existing theories.

KEY WORDS: Thermal performance curve, Jensen's inequality, Scale transition theory, Extreme events, Environmental bootstrap, Spatial variation, Thermal biology

Introduction

Many of the ground-breaking developments in biochemical adaptation address two related questions: (1) how have biochemistry and physiology evolved across generations and among species in response to the environment? (2) Within a generation, how do inducible responses indicative of physiological plasticity differ among species from different environments? These questions have yielded a wealth of mechanistic insights into how organisms cope with environments that differ in average conditions (e.g. tropical versus polar) and environmental variability (Hochachka and Somero, 2002). In the quest for answers to these overarching questions, physiologists

and biochemists have focused on the mean of performance, often glossing over variation (Bennett, 1987). However, this focus draws attention away from the fact that contemporary patterns in biochemistry and physiology are products of selection on the variation that existed in the ancestors of present-day organisms. When confronted with changes in their environment, different individuals within a species can be quite variable in their response (Crawford and Oleksiak, 2007; Krebs and Feder, 1997; Nikinmaa and Waser, 2007; Williams, 2008). The sources and consequences of this variation are pivotal to understanding species' potentials to cope with changing environments (Whitehead and Crawford, 2006). As Steven Jay Gould (1985) eloquently put it:

> In short, we view means and medians as the hard 'realities,' and the variation that permits their calculation as a set of transient and imperfect measurements of this hidden essence. … But all evolutionary biologists know that variation itself is nature's only irreducible essence. Variation is the hard reality, not a set of imperfect measures for a central tendency. Means and medians are the abstractions.

A growing literature in behavioral ecology (Dingemanse et al., 2010), climate change biology (Estay et al., 2014), chronobiology (MacDougall-Shackleton et al., 2015), sex differences in human physiology and medicine (Mendelsohn and Karas, 2005), disease ecology (Raffel et al., 2013), ecology (Ruel and Ayres, 1999), community ecology (Violle et al., 2012) and endocrinology (Williams, 2008) supports this perspective, showing that there is much to be gained by embracing the sources and consequences of variation.

Here, we identify several key opportunities and challenges for incorporating the role of variation into our understanding of organismal thermal biology and its ecological and evolutionary consequences. We focus on three forms of variation that interactively contribute to differences in organisms' abilities to perform the physiological tasks necessary for survival and reproduction: (1) temporal variation in an individual's body temperature; both 'normal' variation (fluctuations that individuals encounter day to day in a typical lifetime) and extreme events (rare fluctuations that impose severe stress on individuals and possibly decimate populations); (2) spatial variation in body temperature among individuals; (3) inter-individual variation in the biochemical and physiological capacities to cope with thermal variation.

Although we focus our examples on the effects of variation in body temperature, analogous arguments apply to other types of variation; for example, variation in oxygen availability, pH, salinity or water availability. The three forms of variation listed above manifest at spatial and temporal scales relevant to individuals, but explicit consideration of all three types of small-scale variation is crucial for developing a robust ability to predict large-scale responses to the environment and for addressing the pressing issue of anthropogenic global change.

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We begin with an analysis of thermal performance curves, the traditional approach to thermal variation, and discuss how variation in body temperature can affect their interpretation in the context of climate change. Next, we move beyond the performance-curve approach to consider the effects of extreme thermal events. We then review the importance of considering both spatial variation in how individuals experience their environment and inter-individual variation in the biochemical and physiological capacities to cope with thermal stress, and we finish with a discussion of challenges and opportunities for future research.

Performance curves: quantifying the effects of variation in sublethal temperature

Body temperature has profound effects on physiological processes at the level of molecules, cells and whole organisms (Dell et al., 2011; Hochachka and Somero, 2002). These myriad effects can be described by a performance curve (Fig. 1A), where the *x*-axis characterizes the organism's body temperature and the *y*-axis indicates some quantitative measure P of the individual's
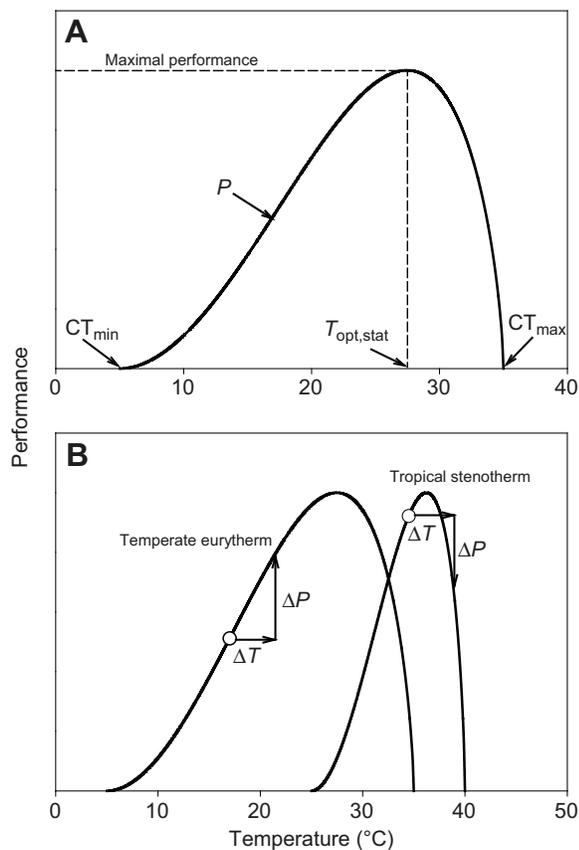

Fig. 1. Thermal performance curves based on a modified beta function. (A) Performance curves typically have a characteristic, unimodal shape exemplified by the thermal reaction norm: performance as a function of body temperature. Often the reaction norm's peak is shifted to the right of center, such that performance increases relatively slowly up to T_{opt}, but decreases rapidly above T_{opt}. Although performance curves are generally modeled as functions, performance is typically only measured at a small number (4–8) of discrete temperatures in empirical studies. (B) A given increase in temperature (ΔT) results in an increase in nominal performance (ΔP) for a temperate eurytherm, but the same temperature increase results in a decrease in nominal performance for a tropical stenotherm. Starting temperatures are based on typical mean body temperatures reported in the literature (see, e.g. Deutsch et al., 2008), with mean body temperature much closer to T_{opt} for stenotherms.

performance (Huey and Stevenson, 1979). Performance in a static environment, which we refer to as the 'nominal' performance function $P(T)$, peaks at some 'optimal' temperature ($T_{opt,stat}$). Below some minimum critical temperature (CT_{min}) the organism cannot maintain the functions necessary to survive and reproduce, and above some maximum critical temperature (CT_{max}) performance is similarly compromised. Typically, the curve is asymmetric with a relatively gentle rise to the peak followed by a steep decline. Eurythermal organisms have a relatively large thermal breadth ($CT_{max}-CT_{min}$), stenothermal organisms have a narrower breadth (Fig. 1B). The magnitude of performance at any point along the curve can vary from organism to organism. The generality of unimodal responses to temperature was recently corroborated by two broad surveys: one of a variety of traits at different levels of biological organization across a wide range of taxa (Dell et al., 2011) and the other of thermal dependency of growth rates across the three domains of life (Corkrey et al., 2012). Later, we raise concerns regarding the applicability of performance curves in variable environments, but given their widespread use in the literature they warrant a detailed review.

Despite the assumption by the metabolic theory of ecology that the underlying parameters controlling the shape of the performance curve are universally constrained (Brown et al., 2004; Gillooly et al., 2001), considerable variation exists in the shape of performance curves among taxa and among performance traits (Ehnes et al., 2011). Consequently, a variety of mathematical functions have been used to fit experimental performance data (Angilletta, 2006). For biochemical and physiological rate data, the ascending leg of the performance curve is often assumed to result from thermodynamic effects that increase reaction rates with increasing temperature (according to the Boltzmann–Arrhenius equation), while deceleration near the peak and on the descending leg is assumed to result from destabilizing effects of high temperatures on rate-limiting enzymes (Dell et al., 2011; Johnson and Lewin, 1946; Knies and Kingsolver, 2010; Ratkowsky et al., 2005). In aquatic organisms, recent attention has focused on the roles of limitations in oxygen-carrying capacity and resulting oxidative stress in setting thermal performance bounds (Pörtner, 2002, 2010). For 'integrative' traits (such as running speed, fecundity, survivorship or population growth rates), the Boltzmann–Arrhenius family of functions often do not fit well to available data (Knies and Kingsolver, 2010) and other functions (e.g. Gompertz×Gaussian, polynomial, beta) or statistical approaches (such as generalized additive models) have been used. As we discuss below, differences in the shape of the performance curve, particularly in the nature of its first and second derivatives, have important implications for predicting the effects of thermal variability.

Despite the importance of the shape of performance curves, relatively few studies have measured performance at a large enough number of temperatures to effectively distinguish between possible underlying shapes (Knies and Kingsolver, 2010). Typically, organisms are exposed to fewer than five discrete body temperatures (Dell et al., 2011). Often, these temperatures are clustered near the central region of the performance curve, and our understanding of performance near the extremes remains relatively undeveloped. It is also likely that the shape of thermal performance functions varies for the same organism at different levels of organization [e.g. mitochondria versus whole organism (Schulte et al., 2011)], for different performance traits (Huey, 1982) and for different ontogenetic stages (Kingsolver et al., 2011).

Thermal performance models have been used to infer a variety of organismal- and population-level responses to temperature change,

from effects on individual processes such as metabolic rate (Schulte et al., 2011) to potentially complex consequences that might lead to range shifts (Sunday et al., 2012) and influence ecological interaction strengths (Rall et al., 2012). For example, thermal biologists have quantified performance curves for species from different latitudes, often concluding that species near the tropics – where temperatures are generally high and relatively stable – tend to live closer to their thermal optimum than do species living in temperate latitudes (Deutsch et al., 2008; Morley et al., 2012; Stillman and Somero, 2000), where short-term and seasonal variation is greater. A common corollary to these conclusions is that tropical species – many, but by no means all, of which are stenotherms – are at greater risk of population decline or extinction in the context of global warming (Bonebrake and Deutsch, 2012; Deutsch et al., 2008; Huey et al., 2012), whereas temperate species may benefit from warming as a result of their position on the ascending arm of the performance curve (Fig. 1B).

In the next section, we explore how consideration of short-term variation in body temperature may modify the interpretation of thermal performance curves.

Temporal variation in body temperature and its effects on performance

Average performance and Jensen's inequality

For ectotherms and regional endotherms, body temperature can fluctuate rapidly, with concomitant effects on an organism's performance. Often, the consequences of the variability in performance are quantified by integrating performance over time to calculate average performance (e.g. average metabolic rate) or net performance (e.g. reproductive output).

However, several recent studies have highlighted important complications inherent in calculating the average (or net) of a nonlinear function such as a thermal performance curve (Estay et al., 2014; Martin and Huey, 2008; Vasseur et al., 2014). In each, the authors use Jensen's inequality (Jensen, 1906; Ruel and Ayres, 1999) to show that, when temperature varies, mean performance – the average over a portion of the nominal performance function, $\overline{P(T)}$ – does not equal the performance at the mean temperature (the function's value at the average temperature, $P(\bar{T})$). Building on Jensen's inequality, scale transition theory (e.g. Chesson et al., 2005) estimates the difference between the average of the function and the function of the average (Estay et al., 2014). To a first approximation:

$$\overline{P(T)} \approx P(\bar{T}) + \frac{1}{2}P''(\bar{T})\sigma_T^2, \tag{1}$$

where $P''(\bar{T})$ is the performance curve's second derivative at the average temperature $\bar{T}$, and σ_T is the standard deviation of body temperature encountered by the organism. When $P''(\bar{T})$ is positive, average performance in a variable environment is greater than the nominal performance at the average temperature; when $P''(\bar{T})$ is negative, average performance is less than the nominal performance. Eqn 1 is accurate when σ_T is a small fraction of the thermal breadth. For larger variations, $\overline{P(T)}$ must be calculated using numerical simulations in which temperatures are drawn at random from a distribution (usually Gaussian); the calculated $P(T)$ values are averaged to give $\overline{P(T)}$ (Benedetti-Cecchi, 2005).

From Eqn 1, it is clear that the effect of temporal variation (σ_T) on average performance depends on the shape (that is, on the second derivative) of $P(T)$. In some cases (e.g. Boltzmann–Arrhenius-based thermal performance curves, Fig. 2A), the ascending arm of the curve is initially concave up from $\mathrm{CT_{min}}$, an indication that $P''(\bar{T})$
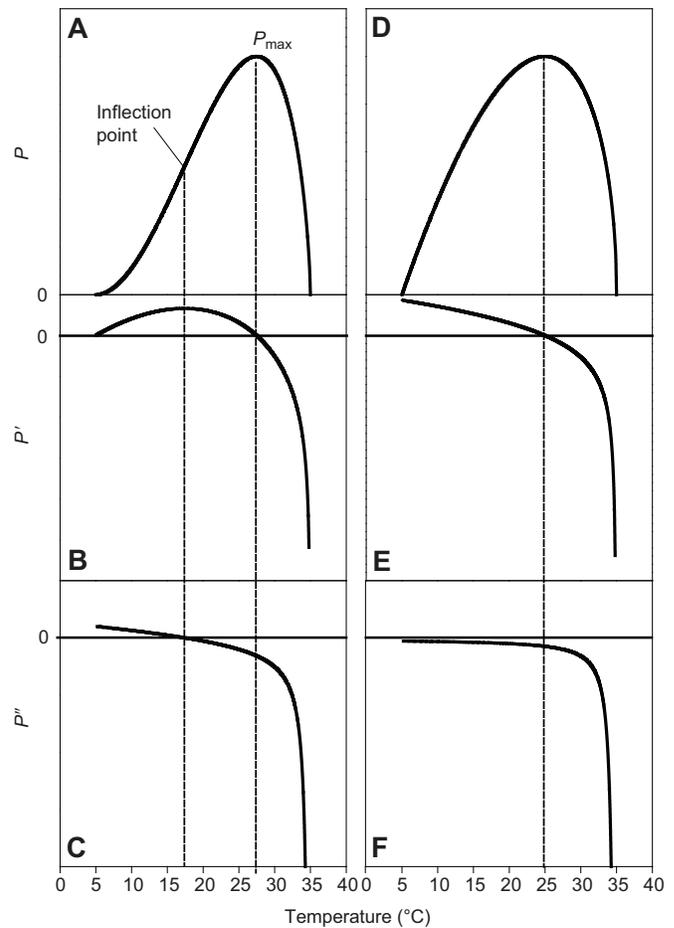

Fig. 2. Different performance curve functions exhibit varying mathematical characteristics. (A–C) A performance curve (A) and its first (B) and second (C) derivatives. This curve, typical of processes such as metabolic rate, has the initial upward concavity typical of curves based on the Arrhenius equation. Note that the zero crossing of the second derivative indicates the inflection point in the ascending leg of the curve. (D–F) A performance curve typical of 'integrative' whole-organism performance; the second derivative is negative throughout (F).

in this portion of the curve is positive (Fig. 2C). The remainder of the curve is concave down [$P''(\bar{T})$ is negative]. In such cases, variation in body temperature produces an average performance curve in which the mean temperature associated with highest average performance ($T_{\mathrm{opt,var}}$) is lower than the $T_{\mathrm{opt,stat}}$ (Estay et al., 2014; Vasseur et al., 2014) (Fig. 3A). Displacement of optimum temperature increases as σ_T increases. For a given σ_T, the difference between $T_{\mathrm{opt,var}}$ and $T_{\mathrm{opt,stat}}$ is a larger fraction of a stenotherm's narrow thermal breadth than for a eurytherm's broad thermal breadth (Martin and Huey, 2008).

The difference between $T_{\mathrm{opt,var}}$ and $T_{\mathrm{opt,stat}}$ has been used to explain why some organisms, such as lizards, choose mean body temperatures in the field below their optimal static temperature in the laboratory ('suboptimal is optimal', Martin and Huey, 2008). However, lizards' choice of preferred temperature cannot be fully explained by Jensen's inequality, and the remaining discrepancy has been at least partially attributed to thermodynamic effects on performance (Asbury and Angilletta, 2010). A suboptimal-is-optimal strategy could also arise from bet-hedging behaviors that reduce the risk of experiencing high temperatures that would push an individual onto the rapidly descending arm of the curve,
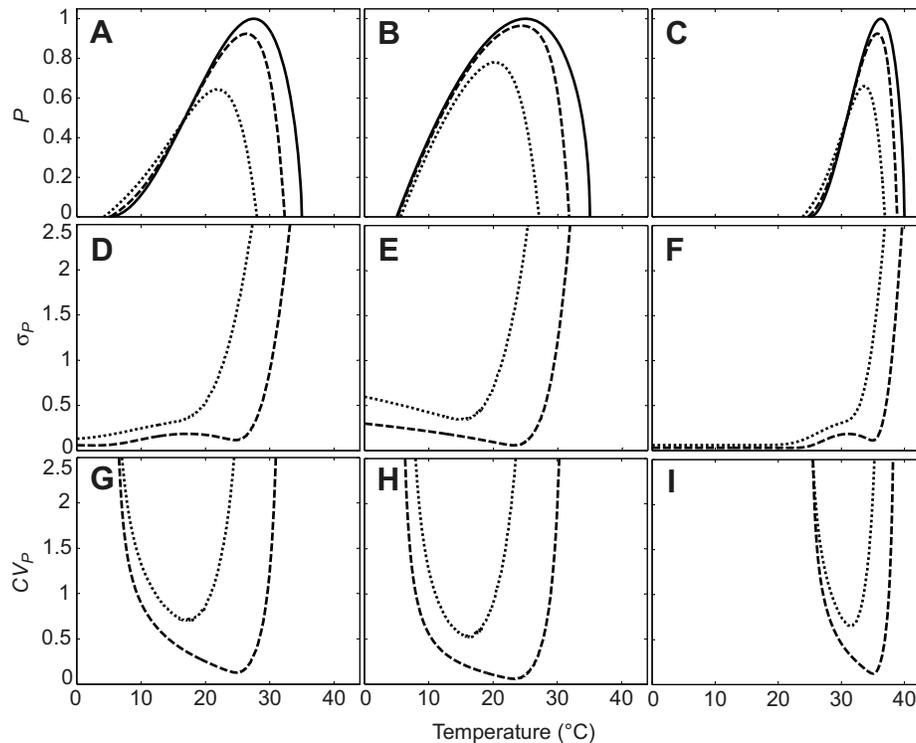
Fig. 3. Small increases in temporal thermal variation (σ_T) have significant effects on mean performance, the temporal variation of an individual's performance (σ_P), and the coefficient of variation of performance (CV_P). The effects of Jensen's inequality on mean performance for a temperate eurytherm characterized by a Boltzmann–Arrhenius-type curve (A), a temperate eurytherm characterized by an integrative curve with negative second derivative throughout (B) and a warm stenotherm (C). In each panel, the performance curve is based on a modified beta function. (D–F) Corresponding values for the standard deviation of performance as a function of mean temperature. (G–I) The CV_P is calculated by dividing σ_P by P for each given temperature. Lines indicate values for the nominal, static performance curve (solid lines; σ_P is zero) and the realized curves when σ_T is 10% (dashed lines) or 20% (dotted lines) of the nominal curve breadth (CT_{max}–CT_{min}). In this example, temporal variation in body temperature produces a local minimum in σ_P near $T_{opt,var}$ that is more pronounced in stenotherms (F) than in eurytherms (D), even though the absolute magnitude of σ_T for the dotted lines is smaller in F (1.5°C) than it is in D (3°C). Even small increases in σ_T lead to dramatic increases in CV_P for stenotherms. These curves could be generated using Eqn 2 (A–C) and Eqn 3 (D–F), but owing to the limitations noted in the text, we used simulations to better account for the curve's behavior near the bounds of the nominal performance curve. In this example, we assume linear costs of temporarily exceeding the bounds of the nominal performance curve (descending the ends of each curve below the x-axis in A–C). The nature of these costs requires further experimental documentation.

particularly when information about variation in the environment is incomplete (Angilletta, 2009; Martin and Huey, 2008).

For curves adhering to the general shape of Fig. 2A, variation in temperature can augment performance to the left of the inflection point at the low end of an organism's temperature range, and in some cases the operative range may be extended below the nominal CT_{min} (Estay et al., 2014) (Fig. 3A). Meanwhile, performance in a variable environment suffers at the warm end of the range, plummeting at mean temperatures below the nominal CT_{max} (Fig. 3A). These predictions are supported by studies of thermal variability and development in malaria parasites (Blanford et al., 2013).

In other cases for which the performance curve is concave down throughout (typically curves related to integrative, whole-organism functions such as running speed or fecundity, Fig. 2D), average performance in a variable environment is predicted to be less than the nominal value across the entire range of mean temperatures (Fig. 3B), constricting the realized thermal niche (i.e. the breadth of the curve) at both high and low temperatures (e.g. for reproduction of a pseudoscorpion, Zeh et al., 2014).

In summary, application of scale transition theory to performance curves implies that: (1) the highest level of average performance achieved in a variable environment is lower than maximal performance in a static environment; (2) organisms in

varying thermal environments should choose mean conditions below $T_{opt,stat}$ to maximize mean performance; (3) for a given σ_T, the displacement of $T_{opt,var}$ is a greater fraction of total thermal breadth – and the reduction in performance is a greater fraction of nominal peak performance – for stenotherms than for eurytherms; (4) variability may extend the lower range of mean temperatures over which performance is feasible for some performance functions but not others, while it almost certainly reduces the upper bound of mean temperatures at which organisms can perform adequately (Fig. 3A–C).

These conclusions are generally robust to different choices of the function used to describe performance curves, so long as the curve is asymmetrical and left-skewed, including modified Boltzmann–Arrhenius (Dell et al., 2011), modified beta (Niehaus et al., 2012) (Fig. 1A), Gaussian×Gompertz (Martin and Huey, 2008) and Briere three-parameter (Estay et al., 2014). Although previously popular because of their simple parameterization, symmetrical Gaussian performance curves generate dramatically different results and should be avoided for describing performance (Asbury and Angilletta, 2010; Dell et al., 2011; Gilchrist, 1995).

Scale transition theory can be applied to spatial as well temporal variation. Rather than quantifying the mean performance through time of one individual in a temporally variable environment (as above), Eqn 1 can be used to describe the instantaneous mean

performance of a group of individuals distributed in a thermally variable landscape. Furthermore, spatial and temporal variation can be combined. Because an individual's thermal history is determined by its location, which, for motile species, can change through time, it is important to consider these combined effects (Sears et al., 2011). For example, an individual's thermal history as a function of temporal and spatial variability can be used in conjunction with the individual's performance curve to calculate that individual's average performance. [Note that individual organisms sample their environment temporally regardless of whether the underlying variation is perceived by biologists as temporal or spatial (Woods et al., 2014).] The procedure can be repeated for each individual in a population to estimate average performance across the population.

Effects of thermal fluctuations on the variance of performance

As temperature fluctuates, performance fluctuates and these fluctuations may be more detrimental than the reductions in mean performance discussed in the previous section, especially when the costs of instantaneous poor performance are great. To take an extreme example, even if an organism performs well on average, a single fluctuation beyond the limits of the performance curve can be fatal (Kingsolver et al., 2011).

This aspect of performance has received scant attention in the literature. It can be shown (see derivation in Appendix 1) that the variation in an individual's performance, represented here by the standard deviation of performance σ_P, is positively correlated with the magnitudes of both the first and second derivatives of the performance curve at the mean body temperature. Variation in performance also increases nonlinearly with increasing variation in body temperature, σ_T. To a first approximation:

$$\sigma_P \approx \sqrt{[P'(\bar{T})]^2\sigma_T^2 + \frac{1}{4}[P''(\bar{T})]^2(\sigma_T^2)^2}. \quad (2)$$

As with the estimate of $\overline{P(T)}$ (Eqn 1), this estimate of σ_P is accurate when σ_T is a small fraction of the thermal breadth. For larger values of σ_T, σ_P must be calculated by simulation.

Perhaps of greater physiological relevance than the absolute magnitude of σ_P is its magnitude relative to mean performance; that is, the coefficient of variation of performance CV_P:

$$CV_P = \frac{\sqrt{[P'(\bar{T})]^2\sigma_T^2 + \frac{1}{4}[P''(\bar{T})]^2(\sigma_T^2)^2}}{P(\bar{T}) + \frac{1}{2}P''(\bar{T})\sigma_T^2}. \quad (3)$$

CV_P can be large (Fig. 3G-I). For the representative per-formance curve shown in Fig. 3A, when temperature varies with a standard deviation of 10% of the nominal curve breadth (3°C in this example), σ_P is ~15% of the individual's mean performance at $T_{opt,var}$.

CV_P is minimal near $T_{opt,var}$, indicating that performance is most predictable around this optimal temperature. Notably, the minimum of CV_P shifts further to the left of $T_{opt,var}$ as σ_T increases (compare positions of dashed and dotted lines in Fig. 3G-I). Furthermore, the rate of increase in CV_P with a shift away from $T_{opt,var}$ is greater in warm stenotherms than in temperate eurytherms (compare Fig. 3D,F), perhaps helping to explain observed differences between these groups. Warm stenotherms tend to live at mean temperatures close to their nominal thermal optimum. Because their environments tend to vary less in the first place, Eqn 3 suggests that choosing temperatures near the nominal thermal optimum ensures the most

consistent performance through time (Fig. 3I). Meanwhile, temperate eurytherms tend to choose mean temperatures on the ascending arm of their nominal performance curves. In the less predictable environments where most eurytherms live, this strategy mitigates risk of accidentally exceeding CT_{max} with only a small reduction in mean performance (due to the left shift in optimal temperature and the generally broader curve shape) and a relatively small increase in the standard deviation of performance. Organisms' thermal choices may reflect trade-offs between the magnitude and variability of performance on one hand and the risk of exceeding thermal limits on the other (Angilletta, 2009).

Interpreting thermal performance curves in the context of climate change

The theoretical effects outlined above add complexity to previous predictions, such as a global analysis of insect vulnerability to climate change (Deutsch et al., 2008). In particular, Eqns 1–3 predict more-exaggerated consequences of thermal variation for stenotherms than for eurytherms. Because the narrow curves of stenotherms are more intensely concave than the broad curves of eurythermal organisms (Fig. 1B), stenothermal species typically have second derivatives that are more negative in the vicinity of $T_{opt,stat}$. Thus, even though tropical stenotherms may experience less thermal variation than mid-latitude organisms (i.e. σ_T is relatively small), the large magnitude of $P''(\bar{T})$ suggests that their average performance might be more heavily impacted by even small increases in temporal variation. Indeed, recent studies suggest that increases in temperature variability may pose a larger threat to ectotherms than the shift in average temperature as the planet warms (Paaijmans et al., 2013; Terblanche et al., 2010; Vasseur et al., 2014). [Increased temperature variation may also have negative fitness consequences for endotherms such as birds (Pendlebury et al., 2004).]

Our analysis of the temporal variation in an individual's performance may also have important implications for predicting organisms' responses to climate change. For example, even though many tropical species may experience less temperature variability than their extra-tropical cousins – and therefore a smaller difference between $T_{opt,stat}$ and $T_{opt,var}$ – even a small increase in mean temperature could lead to a considerable increase in the temporal variation in performance (σ_P; moving rightward along the dashed line in Fig. 3F). Simultaneous increases in thermal variability in the tropics would compound this effect (Eqn 2). Indeed, climate data show that both mean temperature and daily temperature variation in the tropics have increased by similar amounts over recent decades, though these changes are smaller than those observed in polar and temperate regions (Wang and Dillon, 2014). It is an open and complex question as to how the effects of changes in the mean and variation of temperature might manifest themselves in macro-scale phenomena such as the range limits and persistence of species.

Caveats regarding the application of thermal performance curves

There are several additional caveats regarding the interpretation of performance curves. It is necessary to acknowledge the possibility that organisms can adjust their performance when exposed to thermal variation. In our analysis thus far we have assumed that an individual's performance curve does not change during the period over which performance is averaged. This is probably reasonable for the short-term fluctuations associated with hourly and perhaps daily variation, at least in long-lived organisms. However, fluctuations at lower frequencies (e.g. seasonal fluctuations in temperature)

provide sufficient time for organisms to acclimatize, which has the potential to change CT_{min}, CT_{max}, $T_{opt,stat}$, and the shape of the nominal curve (Angilletta, 2009). Consequently, caution must be exercised when applying Eqns 1–3 or the analogous simulations. Indeed, Estay et al. (2014) characterized the predictions generated by scale transition theory as 'null' models to which the realized performance of individuals and populations in variable environments can be compared: any deviation from the null model implies the existence of biochemical or physiological mechanisms that dynamically adjust performance in response to variation. Similarly, Schulte et al. (2011) divided physiological responses to thermal variation into passive responses due to the shape of the performance curve alone and active forms of plasticity due to compensatory biochemical processes that depend on thermal history. To accurately discern how performance deviates from the passive response, more experimental data are needed for organisms subjected to different combinations of mean temperature and temperature variation.

The nature of physiological and biochemical costs imposed when organisms temporarily move below CT_{min} or above CT_{max} also requires due consideration. For example, if one assumes that individuals can tolerate conditions below CT_{min} for short periods, the positive $P''(T)$ for Boltzmann–Arrhenius type curves near CT_{min} can result in a leftward extension of the organism's functional temperature range (Estay et al., 2014). By contrast, if any excursion below CT_{min} is very costly or lethal, as predicted by the theory of oxygen and capacity-limited thermal tolerance (Pörtner, 2002, 2010), increasing thermal variation narrows the functional range of mean temperatures. It remains to be seen which of these two scenarios – expansion or contraction of thermal breadth under temperature variability – is more representative of real-world performance at mean temperatures near the lower performance bounds, but in an ecological context, the answer may be irrelevant. Species seldom live at average temperatures near the lower bounds of their performance curves. Only in extenuating ecological circumstances would this be likely (e.g. displacement competition, sudden weather changes) and then probably only for short periods of time. Ultimately, brief extreme events near (or beyond) the nominal performance bounds may have greater effect on overall performance (Hoffmann, 2010) (see next section).

Finally, and perhaps most importantly, it is apparent that the pattern of thermal variation (i.e. thermal history, the order of events in time) matters. During and after challenging events, mechanisms are activated (e.g. changes in gene, microRNA and protein expression) to repair damage and, in some cases, to prepare the organism for subsequent insults. Depending on the timing and intensity of the next challenge, the consequences of thermal history might include acclimation or acclimatization (e.g. Buckley et al., 2001; Roberts et al., 1997), latent effects (Pechenik, 2006), hormesis or preconditioning (Calabrese et al., 2007), intensified stress responses and increased energetic costs (e.g. Petes et al., 2007) or even death (e.g. Dowd and Somero, 2013). In short, an individual's response to the variation in the timing, intensity, duration and interval between stressful events can alter its performance. Thus, a nominal performance curve generated under constant conditions in the laboratory may not adequately characterize performance in nature in all but the most predictably variable environments (i.e. those with moderate standard deviations and strictly rhythmic patterns of temporal variation). Indeed, predictions derived from nominal performance curves do not always match with data obtained under variable conditions (Niehaus et al., 2012). Organismal performance has evolved in the face of

constant variation, and only when measured in the presence of realistic variation are performance curves likely to be accurate representations of reality.

The importance of the order of thermal experience has been noted by theoreticians (e.g. Asbury and Angilletta, 2010), although such factors are rarely considered in modeling efforts. In cases where the time-course of events affects overall performance, an expansion of performance-curve theory might prove valuable. For example, Schulte et al. (2011) propose that performance curves should be multi- (rather than 2-) dimensional; the extra dimensions could be used to account for the pattern in which temperature is varied and for other factors such as duration of exposure to extreme conditions (Rezende et al., 2014).

The physiological importance and temporal distribution of extreme events

So far, we have discussed the 'normal' variations that induce sublethal responses. However, in the evolution of at least some aspects of thermal performance (such as CT_{max} and $T_{opt,stat}$), extreme (that is rare and potentially lethal) thermal events may be the driving factor for many taxa (Clusella-Trullas et al., 2011; Hoffmann, 2010). This primacy of discrete episodes has been emphasized in the global change literature ['events, not trends'(Jentsch et al., 2007; Parmesan et al., 2000; Wethey et al., 2011)] and it garners support from modeling studies of thermal adaptation. For example, we have shown that mean thermal tolerance in intertidal limpets appears to be set by rare events of elevated body temperature that are unlikely to occur within any individual's lifespan (Denny and Dowd, 2012). Similarly, others have concluded that acute thermal tolerance may be more relevant to survival in natural environments than responses to chronic exposures (Angilletta, 2009). Unfortunately, because extreme events are by definition rare, they are difficult to observe in the field, forcing us to rely on statistical inference. However, the distribution of inter-event intervals derived from these statistical approaches has important implications.

If we assume that the probability p of encountering an extreme event in a given interval of time is small and constant (an assumption we return to below), intervals between events (i.e. return times) adhere to the Poisson interval distribution (Berg, 1983; Denny et al., 2009). Specifically, the probability that the next extreme event will occur between time t and $t+dt$ after the last event is:

$$Prob(t) = pe^{-pt}dt. \qquad (4)$$

This relationship (a probability density function, Fig. 4A) bears some counter-intuitive messages. The most probable inter-event intervals are the shortest, while long intervals are rare. As a result, the mean interval between events (the average return time, $1/p$) is hardly characteristic of what organisms experience. Approximately 63% of intervals are shorter than the mean, their brevity being balanced by the relatively rare intervals much longer than the mean. Thus, for randomly occurring extreme events, a typical time series consists of clumps of events delivered in quick succession (e.g. heat waves), interspersed with long periods of benign circumstances (Fig. 4B). If, as suggested above, thermal history plays an important role in determining performance, it will be necessary to take into account the random, episodic pattern of extreme events when designing experiments and when predicting the physiological consequences of thermal variation. It is also likely that climate change will alter the probability of extreme events (Parmesan et al., 2000).
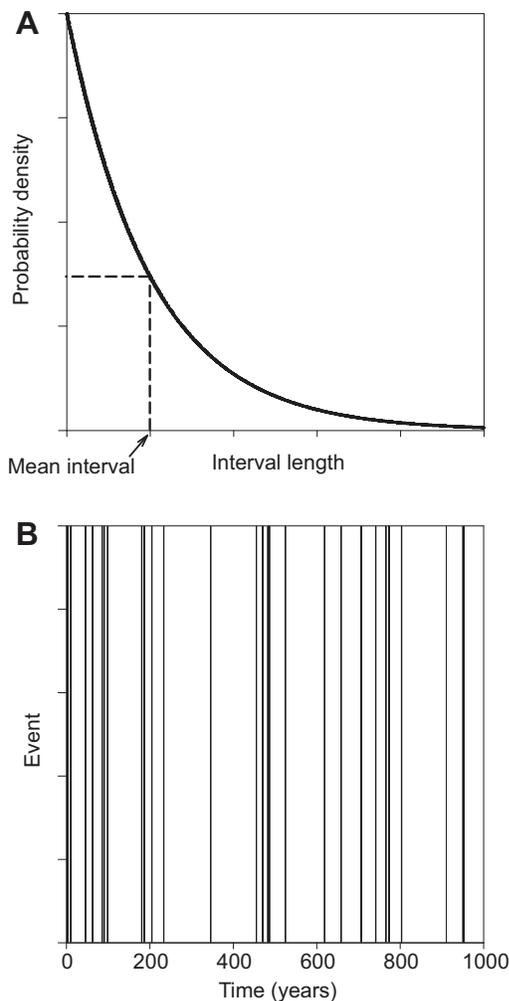
Fig. 4. A Poisson interval distribution for the return times of extreme events. (A) The Poisson probability density function. Note that intervals less than the mean are much more probable than intervals greater than the mean. (B) An example of Poisson-distributed events. Events, indicated by vertical lines, tend to occur in clusters.

As noted above, the Poisson interval distribution rests on the assumption that the probability of encountering an extreme event is small and constant: an assumption made likely by the manner in which extremes arise. Environmentally driven extreme events are often caused not by an excessive value of any single aspect of the physical environment (e.g. air temperature), but rather by the savage alignment of multiple factors that are individually benign (Denny et al., 2009). Consequently, there is a low probability that the requisite values of all parameters will arrive in synchrony to generate extreme conditions. Indeed, estimated return times of extreme events in the rocky intertidal zone, which depend on the confluence of numerous environmental parameters (low tide, bright sun, low wind speed, no wave splash), conform well to the predictions of Eqn 4 (Denny and Dowd, 2012; Denny et al., 2009). Analogous mechanics apply in other aspects of environmental physiology, suggesting that a constant probability of extreme stress (and thereby a Poisson distribution of return times) may be common.

A useful tool in this context is the environmental bootstrap, which resamples relatively short time series (e.g. a 7-year time series of the environmental parameters that determine the body temperatures of intertidal organisms) to generate realistic hypothetical time series of any desired length. These time series allow one to estimate the probability of encountering extreme thermal events, which in turn allows the distribution of inter-event intervals to be calculated (see above). The details of the environmental bootstrap can be found in Denny et al. (2009) and we have recently reviewed the salient features of the approach (Denny and Dowd, 2012).

The environmental bootstrap is particularly useful when combined with biophysical models that calculate time series of body temperatures based on the stochastically varying environmental conditions (an ecomechanical approach, Denny and Gaylord, 2010). For example, we used a 2000-year hypothetical time series of environmental conditions (data for every 10-min interval) generated by the environmental bootstrap as input to a species-specific heat-budget model to simulate the time course of body temperature in a population of intertidal limpets. From these data, we then modeled the effects of environmental stochasticity on the evolution of limpets' thermal tolerance (Denny and Dowd, 2012). Our simulations suggest that this realistic, stochastic variation of limpet body temperature drives the evolution of a substantial 'safety margin', a difference of 5–7°C between the average lethal temperature in a population and the average annual maximum temperature. This predicted safety margin approximates the results of thermal tolerance studies on wild-caught limpets from our study site.

Spatial variability among individuals in how they experience the environment

Although it may seem intuitively obvious that different individuals experience the environment in different ways, this type of variation is frequently overlooked in theoretical and empirical studies. Indeed, the majority of biological studies of spatial variation in temperature address latitudinal, altitudinal or similar large-scale gradients that span scales of 10s to 10,000s of meters, orders of magnitude larger than the scales experienced by most individuals (Potter et al., 2013). For example, a recent study of the effects of spatial variation on insect warming tolerance considered data from grid cells that span 0.5 deg of latitude by 0.5 deg of longitude (Bonebrake and Deutsch, 2012). There is conclusive evidence from this and many other studies that mean conditions do vary in systematic ways over such large spatial extents. However, numerous studies have demonstrated that variation over very small scales can rival or even exceed mean differences observed over much larger scales (e.g. Bartlett and Gates, 1967; Denny et al., 2011; Elvin and Gonor, 1979; Miller et al., 2009; Pincebourde and Woods, 2012; Seabra et al., 2011). For example, in the rocky intertidal zone, the difference in body temperatures between the warmest and coolest mussels over an area of a few square meters (up to 15°C on any given day) rivaled and sometimes greatly exceeded the expected difference in body temperatures along ~1600 km of the western coastline of North America (Denny et al., 2011).

The long-term consequences of this small-scale variation might best be addressed by incorporating it into evolutionary models (Denny and Dowd, 2012; Sears et al., 2011). Small-scale spatial variation in the environment likely contributes to the maintenance of significant functional variation within populations (Schmidt et al., 2000; Stratton, 1994), in a manner analogous to that recently demonstrated among sympatric species of ants inhabiting tropical forests (Kaspari et al., 2014). Therefore, small-scale spatial variation may have profound implications for biological responses to global change. In the short term, such variation creates the potential for

individuals to find local thermal refugia as the climate warms (already crucial for behavioral thermoregulation in terrestrial ectotherms, Dobkin, 1985; Sears et al., 2011; Sunday et al., 2014). Over time, the existence of refugia can relax otherwise strict requirements for range shifts. Spatial heterogeneity could also result in highly divergent thermal histories for individuals within the same population, effectively relaxing thermal selective pressures for some individuals. For example, if a genotypically heat-sensitive individual occupies a thermally benign micro-environment, this allows for transmission of its alleles (which impart little thermal tolerance) to future generations. Over multiple generations, relaxed selection due to spatial variability has the potential to retard adaptive responses, prolonging the time required for optimal physiological performance to evolve (Angilletta, 2009).

Inter-individual variation in biochemical and physiological capacities to cope with thermal variation

Lastly, we consider variation from one individual to the next in their mechanistic abilities to respond to their thermal environment. Substantial functional variation is ubiquitous in physiological studies, being at least partly attributable to genetic variation (Gilchrist, 1996; Krebs and Feder, 1997; Rank et al., 2007). Such intra-population variation in physiology must be considered in empirical and theoretical studies, especially those attempting to forecast the effects of global change.

Two brief examples illustrate the scope of this variation. In the first, swimming performance was determined as a function of temperature in salamanders. Both $T_{opt,stat}$ and maximal performance were normally distributed among individuals, with a greater than two-fold range evident in maximal performance (Young and Gifford, 2013). In the second, critical heart rate temperature (H_{crit}, the temperature at which cardiac function declines) was determined in mussels collected from a range of intertidal sites along the West coast of North America and then maintained under common garden conditions for at least a month before measurement (Logan et al., 2012). Within groups of mussels from each site, the H_{crit} varied significantly, often by as much as 8–9°C among the 20 individuals measured. Overall, there appeared to be more physiological variation among individuals within a site than among populations from different latitudes.

Complicating our interpretation of this inter-individual functional variation is the fact that each individual is subject to unique temporal and spatial variation in the way it experiences its thermal environment (see previous two sections), which can influence the amount of physiological variation present in a given population at a particular time (Ghalambor et al., 2015; Sinclair et al., 2006; Williams and Somero, 1996). These feedbacks among types of variation arise via genotype-by-environment interactions, such as developmental or adult plasticity (Terblanche and Chown, 2006), that are difficult to experimentally tease apart (and to parameterize), but they are crucial to understand for accurately predicting how organisms will fare in new environmental conditions.

Inter-individual biochemical and physiological variation interacts in a nonlinear fashion with spatial and temporal variation in environmental experience, with potentially far-reaching impacts. For example, we have explored the interacting effects of variation in physiological tolerance and spatial variation in environmental experience on survival of extreme thermal events (Denny et al., 2011). In this risk-based model, significant spatial variation in experience (i.e. a large standard deviation of maximum body temperature among individuals) can buffer populations from exposure to even very extreme events (5°C above the mean

thermal tolerance). Increasing the magnitude of inter-individual variation in physiological tolerance of warm temperatures in this model further moderates the population-level effects of extreme events by increasing survival; this is particularly evident for the magnitude of spatial variation observed in intertidal mussel beds (see Fig. 12 in Denny et al., 2011). Further studies that incorporate spatial and temporal variation in experience, in combination with inter-individual variation in physiology (and in behaviors that modulate body temperature, Sears et al., 2011) are urgently needed.

Challenges and opportunities

We see at least five major challenges that must be overcome to improve our understanding of the effects of thermal variability. The first, and most tractable, is the requirement for more comprehensive data sets on environmental variation as it is experienced by individual organisms, a challenge echoed by others (e.g. Woods et al., 2015). This is no simple task, requiring technological advances (e.g. in telemetry) in some cases, and paradigm shifts in experimental design in others (reviewed in Angilletta, 2009 and citations therein). Such data must have sufficient temporal resolution to capture the time scales that are important for organisms (intervals of a few hours or less; Montalto et al., 2014), while also capturing inter-individual variation in organismal experience. Once obtained, these data will allow us to use more realistic, skewed temporal distributions of body temperatures in our models and experiments (e.g. Marshall et al., 2011; Martin and Huey, 2008), rather than the Gaussian distributions implicitly assumed in our earlier sections. Furthermore, it appears that in some systems the standard deviation of body temperatures among individuals tends to increase as the mean body temperature increases (e.g. Denny et al., 2011), but it would be premature to incorporate this pattern in models until it has been further substantiated in the field.

Despite the current limitations in quantifying environmental variation, improvement in the ability to measure and model the types of spatial and temporal thermal variation encountered by organisms has greatly outpaced our ability to predict the physiological consequences of realistic environmental variation (Denny and Helmuth, 2009; Denny and Dowd, 2012; Nikinmaa and Waser, 2007). Contrasting the pattern of temporal variation in an organism's body temperature (Fig. 5A) with the variation imposed during a typical physiological or biochemical experiment (Fig. 5B), it is apparent that most experimental designs are at odds with biological reality. In particular, exposing animals to constant acclimation conditions before exposing the organisms to some controlled 'stress' wipes out potentially co-varying environmental (Tagkopoulos et al., 2008) and/or physiological parameters. Only rarely have biologists attempted to mimic the types of variation evident in the field. Indeed, the task of addressing the complex temporal and spatial phenomena we have described is daunting, and it forms the greatest challenge to progress. For example, we know of few research teams with the resources (or fortitude) to tackle all possible permutations of Poisson interval-distributed stress events, themselves of varying intensities and durations, for each of a range of physiological genotypes/phenotypes. Such experiments are extremely difficult and, as yet, untried in all but the simplest organisms (Tagkopoulos et al., 2008). Fractional factorial designs offer one possibility for reducing this experimental complexity to manageable levels; recent computational advances such as machine learning algorithms may further alleviate the workload (Bonneau et al., 2007; Danziger et al., 2014). For example, application of artificial neural networks has allowed groups to randomly sample
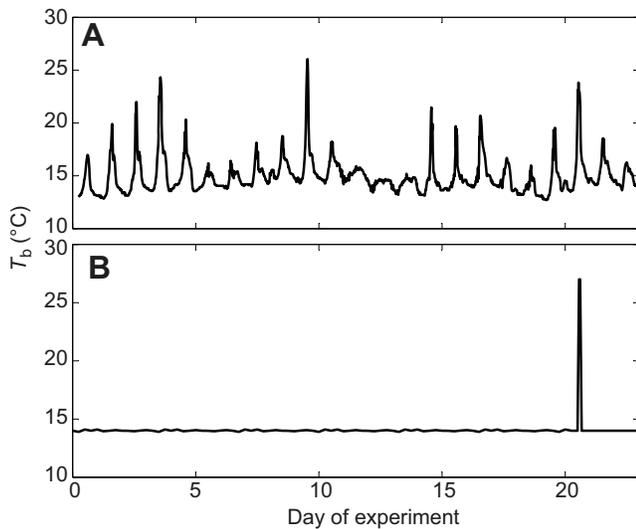
Fig. 5. Temporal variation in body temperature in the field is rarely replicated in biochemical and physiological studies. (A) Average body temperature in a group of intertidal zone mussels, *Mytilus californianus*, over a period of several weeks in the field (Denny et al., 2011). Temperatures were recorded every 20 min. (B) In contrast, laboratory protocols tend to include 'acclimation' phases at nearly constant temperature prior to examining the physiological response to a controlled episode of challenging temperature. This response is typically compared with that of control animals maintained at the acclimation temperature throughout the experiment, but to better replicate natural conditions the control exposure should mimic A.

regions of the parameter space (in this context, this would include all possible combinations of intensity, duration and intervals between events) and to then draw reasonable inferences regarding organismal responses to untested parameter combinations (Bertin et al., 2013). Despite the lack of mechanistic information provided by these algorithms, such approaches could lead to significant advances if properly implemented.

Our interpretation of the physiological effects of thermal variation are further complicated by the entry of organisms into divergent physiological states when different conditions are encountered. Examples include the predominance of anaerobic (versus aerobic) metabolism in many intertidal invertebrates at low tide when they are likely to encounter elevated body temperatures (Connor and Gracey, 2012; Shick et al., 1986), daily torpor in mammals and birds (Geiser and Ruf, 1995), thermogenesis and elevated metabolic rates in flying (as opposed to resting) insects (Bartholomew and Casey, 1977), insect diapause (Hoffmann, 2010) and metabolic suppression in fish exposed to environmental stress (Richards, 2010). Tools exist that might address these complexities (e.g. dynamic energy budgets and other bioenergetic models; Kooijman, 2010; Nisbet et al., 2012), but their implementation must be informed by additional empirical biochemical and physiological insight.

A fourth challenge, intimately related to the first two, is to determine how individual physiological capacities are distributed in variable environments. In several of our examples, the outcomes of models depend on whether physiological performance is apportioned randomly into a variable environment or if instead physiological phenotypes are sorted (or 'mismatched', Marshall et al., 2010) with environmental conditions. Few data of this kind are currently available at the scale of the individual; see Porlier et al. (2009) for one example.

Lastly, further difficulty in prediction arises from the varying potential for populations or species to evolve rapidly in response to

changes in their environment (Denny and Dowd, 2012; Kellermann et al., 2009; Logan et al., 2014; Pespeni et al., 2013; Schoener, 2011); this is particularly relevant given that the patterns of thermal variation themselves are likely to change in the coming decades. Tools such as the environmental bootstrap, implemented with explicit consideration of individual variation in experience (due to spatial heterogeneity) and in physiology (due to both genetic and environmental contexts) in an evolutionary framework, offer one means of generating null models against which to compare future empirical results.

Conclusions
The past decades of research into large-scale patterns of biochemical adaptation have laid the conceptual and mechanistic foundation for studying the effects of variation at small spatial and temporal scales on the performance of individuals. These individuals are themselves phenotypically variable, and numerous feedbacks exist among types of variation (i.e. genotype×environment interactions) that complicate prediction. Here, we have approached these issues of variation from the disparate backgrounds of biochemical adaptation and ecomechanics, and we conclude that these two fields have much to contribute via reciprocal exchange of ideas. The studies reviewed herein provide important insight into how variation at small scales can generate complex, and perhaps unanticipated, results when scaled up to larger spatial and temporal scales. These phenomena are likely to have important implications for our forecasts of biological responses to global change. However, true integration of the lessons of biochemical adaptation and ecomechanics to study physiological variation requires overcoming several lingering challenges and then developing rigorous, empirical means to test these ideas. Ultimately, individual performance is highly dependent on a multitude of physical factors (temperature being but one, Paganini et al., 2014) and on the specific ecological context (Pörtner et al., 2006; Rosa and Seibel, 2008). This complexity demands concerted efforts from biologists of all stripes to tackle the causes and consequences of variation.

APPENDIX
Calculating the standard deviation of performance
The Taylor expansion of the performance function P is:

$$P(T) = P(\bar{T}) + (T - \bar{T})P'(\bar{T}) + \frac{(T - \bar{T})^2}{2!}P''(\bar{T}) + \cdots \quad (A1)$$

Truncated at the term with the first derivative (a linear approximation):

$$P(T) \approx P(\bar{T}) + (T - \bar{T})P'(\bar{T}). \quad (A2)$$

We know from Eqn 2 that:

$$\overline{P(T)} \approx P(\bar{T}) + \frac{1}{2}P''(\bar{T})\sigma_T^2. \quad (A3)$$

Now, the variance of performance is the average square of deviations from mean performance:

$$\sigma_P^2 = \frac{1}{n}\sum_{i=1}^{n}\left(P(T) - \overline{P(T)}\right)^2. \quad (A4)$$

Substituting in our approximations,

$$\sigma_P^2 \approx \frac{1}{n}\sum_{i=1}^{n}\left(\left[P(\bar{T}) + (T-\bar{T})P'(\bar{T})\right] - \left[P(\bar{T}) + \frac{1}{2}P''(\bar{T})\sigma_T^2\right]\right)^2.$$

(A5)

Simplifying and expanding the square, we find that:

$$\sigma_P^2 \approx \frac{[P'(\bar{T})]^2}{n}\sum_{i=1}^{n}(T-\bar{T})^2 - \frac{P'(\bar{T})P''(\bar{T})\sigma_T^2}{n}\sum_{i=1}^{n}(T-\bar{T}) + \frac{(P''(\bar{T}))^2(\sigma_T^2)^2}{4}.$$

(A6)

Because on average $(T-\bar{T})$ is zero, the second term on the right side of this equation approaches zero as n becomes large, and the equation reduces to:

$$\sigma_P^2 \approx \frac{[P'(\bar{T})]^2}{n}\sum_{i=1}^{n}(T-\bar{T})^2 + \frac{(P''(\bar{T}))^2(\sigma_T^2)^2}{4}.$$

(A7)

But $(1/n)\Sigma_{i-1}^{n}(T-\bar{T})^2$ is σ_T^2, therefore:

$$\sigma_P^2 \approx [P'(\bar{T})]^2\sigma_T^2 + \frac{(P''(\bar{T}))^2(\sigma_T^2)^2}{4},$$

(A8)

$$\sigma_P \approx \sqrt{[P'(\bar{T})]^2\sigma_T^2 + \frac{(P''(\bar{T}))^2(\sigma_T^2)^2}{4}}.$$

(A9)

This is Eqn 2 cited in the text. Note that $P(T)$ is approximated only to P', whereas $\overline{P(T)}$ is approximated to P''. As a result, this approximation of σ_P becomes less and less accurate as σ_T^2 increases relative to the function's breadth (e.g. it is most inaccurate in extreme stenotherms). More accurate approximations can be had by including higher order terms in the Taylor expansion, but their form is considerably less compact.

Competing interests
The authors declare no competing or financial interests.

Author contributions
W.W.D. and M.W.D. reviewed the literature and drafted the manuscript. M.W.D. derived the relationships in the Appendix. F.A.K. performed simulations and drafted sections of the manuscript.

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