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W. Wesley Dowd
Loyola Marymount University, wdowd@lmu.edu

M. W. Denny

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REVIEW

Biophysics, environmental stochasticity, and the evolution of thermal safety margins in intertidal limpets

M. W. Denny* and W. W. Dowd[†]

Hopkins Marine Station of Stanford University, Pacific Grove, CA 93950, USA

*Author for correspondence (mwdenny@stanford.edu)

[†]Present address: Department of Biology, Loyola Marymount University, Los Angeles, California, CA 90045, USA

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Summary

As the air temperature of the Earth rises, ecological relationships within a community might shift, in part due to differences in the thermal physiology of species. Prediction of these shifts – an urgent task for ecologists – will be complicated if thermal tolerance itself can rapidly evolve. Here, we employ a mechanistic approach to predict the potential for rapid evolution of thermal tolerance in the intertidal limpet *Lottia gigantea*. Using biophysical principles to predict body temperature as a function of the state of the environment, and an environmental bootstrap procedure to predict how the environment fluctuates through time, we create hypothetical time-series of limpet body temperatures, which are in turn used as a test platform for a mechanistic evolutionary model of thermal tolerance. Our simulations suggest that environmentally driven stochastic variation of *L. gigantea* body temperature results in rapid evolution of a substantial 'safety margin': the average lethal limit is 5–7°C above the average annual maximum temperature. This predicted safety margin approximately matches that found in nature, and once established is sufficient, in our simulations, to allow some limpet populations to survive a drastic, century-long increase in air temperature. By contrast, in the absence of environmental stochasticity, the safety margin is dramatically reduced. We suggest that the risk of exceeding the safety margin, rather than the absolute value of the safety margin, plays an underappreciated role in the evolution of thermal tolerance. Our predictions are based on a simple, hypothetical, allelic model that connects genetics to thermal physiology. To move beyond this simple model – and thereby potentially to predict differential evolution among populations and among species – will require significant advances in our ability to translate the details of thermal histories into physiological and population-genetic consequences.

Key words: stochastic, fitness, climate change, heat-shock response, environmental variability, energetic costs, environmental bootstrap.

Introduction

In traditional perspectives (e.g. Slobodkin, 1961), evolution is assumed to play out over hundreds of thousands of years, whereas ecological processes occur orders of magnitude faster. As a consequence, while ecology certainly affects evolution, this perspective assumes that evolution has little chance to affect the short-term interactions that shape communities. Recent research shows, however, that evolution can be rapid enough to provide feedback to ecological dynamics (Pelletier et al., 2009; Schoener, 2011; Baskett, 2012) – a mechanism that could be increasingly important in the context of global change. In this review and prospective, we explore how the integration of biophysical, physiological and genetic models can be used both to predict the magnitude of thermal 'safety margins' and to monitor their evolutionary dynamics in the face of stochastic environmental fluctuations.

This mechanistic approach to evolutionary thermal biology offers a complement to theoretical considerations regarding the evolution of thermal performance optima and thermal performance breadth (reviewed by Angilletta, 2009). Many of these theories are based on thermal reaction norms, in which the performance of an organism is quantified as a function of its body temperature (Fig. 1). In this context, 'performance' is defined ideally as the fitness of an organism, but it is often quantified operationally by some more easily measured proxy, such as reproductive output, growth rate or even

running speed. Owing in large part to the assumptions and goals of the approach, models using thermal performance curves do not address (or address only implicitly) several considerations we deem important for their application in the context of short-term global change: the physiological costs underlying variation in performance as a function of temperature; the influence of realistic, sublethal environmental variation on these physiological costs and resulting fitness patterns; and the role of chance (e.g. temporal environmental stochasticity) in generating evolutionary patterns. Moreover, the algorithms underlying such models often search for local fitness optima (or equilibria) under relatively 'static' environmental conditions (Angilletta, 2009), whereas (as noted above) recent work has demonstrated that environmental and evolutionary patterns often vary on similar, short time-scales (e.g. Rank and Dahlhoff, 2002). It is exactly this short-term variation that we must consider in the context of global change. We perceive in these characteristics of the response-curve approach opportunities for mechanistic contributions from ecomechanics and physiology to evolutionary thermal biology, and thereby to ecology.

To demonstrate the promise – and current limitations – of this mechanistic evolutionary approach, we here employ biophysical response functions to translate environmental conditions into body temperature of individuals, coupling these functions with a statistical approach to specify how stochastic environmental parameters drive minute-to-minute temporal variability of body
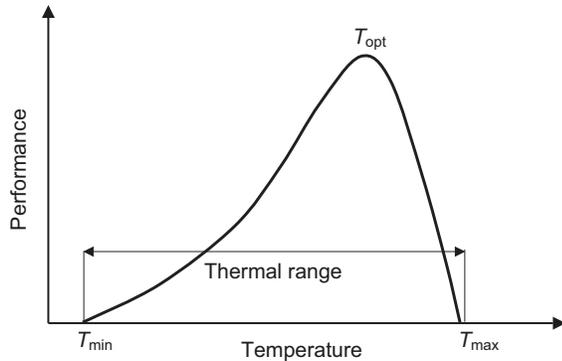
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Fig. 1. The thermal performance reaction norm, which relates some parameter representative of organismal performance (e.g. running speed, feeding rate, growth, reproductive output) to body temperature [adapted from Angilletta (Angilletta, 2009)]. The thermal range (or thermal window) represents the range of tolerable temperatures, and optimal temperature (T_{opt}) denotes the temperature at which performance reaches its peak. The thermal range can vary – for example, between eurytherms and stenotherms – as can the position (both in terms of temperature and level of performance) of T_{opt}. T_{max}, in this context the elevated temperature at which the organism ceases to perform, can differ from T_{lethal}, the temperature at which the organism dies.

temperature. We then use a simple genetic model of thermal physiology (specifically, allelic control of thermal tolerance limits and energetic costs of tolerance) to quantify how stochastic environmental variation impacts evolution of thermal safety margins, which, in turn, might ultimately determine population range and viability. This approach establishes a framework for predicting the (potentially different) evolution of thermal tolerance in species within a community as they experience both environmental variability and directional environmental change.

   We take as our example the intertidal owl limpet, *Lottia gigantea* Sowerby 1834, a co-dominant competitor with the mussel *Mytilus californianus* for space on the rocky substratum of the California coast (Fig. 2) (Morris et al., 1980). This intertidal habitat is exceptionally dynamic, both physically and biologically. *L. gigantea* is territorial and aggressive; each individual defends a 'garden' of open space on the rock from which it grazes microalgae during night-time low tides. During even the warmest daytime low tides, each limpet returns to a home site on the rock, where it remains until the subsequent high tide (Denny and Harley, 2006). Unlike some other intertidal animals (such as crabs and snails), *L. gigantea* does not hide in crevices during times of high temperature; instead, it hunkers down on its home site and waits for the tide to come in, rendering it effectively sessile. Thus, unlike the highly mobile terrestrial organisms that have been the focus of much attention in thermal biology [e.g. lizards, frogs, toads and fruit flies (Angilletta, 2009; Kearney et al., 2009)], behavior contributes relatively little to determining body temperature in *L. gigantea*. Instead, body temperature is governed primarily by the interaction of abiotic environmental factors (e.g. air temperature, wind speed) that are individually well understood. These characteristics make this ectothermic species particularly amenable to predictions of body temperature variation and to a mechanistic approach to the evolution of thermal tolerance.

Predicting body temperature: a heat-budget model
Biophysical models of body temperature are standard tools for exploring thermal biology (e.g. Gates, 1980; Campbell and

Fig. 2. The owl limpet, *Lottia gigantea*, is the large limpet pictured here. The smaller limpet is *Lottia scabra*, which is commonly resident on the shells of *L. gigantea*. Photo by Luke Miller.

Norman, 1998; Helmuth, 1998; Helmuth, 2002; Angilletta, 2009; Kearney et al., 2012). In this tradition, Denny and Harley (Denny and Harley, 2006) constructed a heat-budget model for *L. gigantea*, from which body temperature can be calculated as a function of the environment of the limpet. The model begins by quantifying all the ways in which heat energy can enter or leave the organism. Energy is injected into the limpet as solar or infrared radiation is absorbed, an energy influx independent of the body temperature of the organism. By contrast, the other transport phenomena by which heat is exchanged with the environment depend on the temperature of the limpet. If the limpet is hotter than the surrounding air, heat is lost by convective transfer; if the animal is colder, heat is gained. In either case, convective flux is greater the faster the wind blows. Because the foot of the limpet provides a large area of contact with the substratum, the animal readily exchanges heat with the rock by conduction. If the limpet is hotter than the rock, heat flows out of the body, and, if colder, heat flows in. Finally, the limpet radiates infrared light to its surroundings at a rate that increases with increasing body temperature. In theory, *L. gigantea* could exchange heat with the air through evaporation or condensation of water, but, in practice, this factor does not appear to play an important role (Denny and Harley, 2006).

   The sum of these heat inputs and outflows is the heat budget of the limpet. Because, with the exception of solar influx, all of these transport phenomena are functions of body temperature, it is possible, for a given state of the environment, to calculate the body temperature at which heat influx equals heat efflux. For organisms the size of *L. gigantea* (typically, 2–6 cm shell length), this calculated equilibrium temperature predicts body temperatures in the field to within a fraction of a degree Celsius (Denny and Harley, 2006). Thus, given knowledge of relevant factors in the environment of the limpet (in our model: solar irradiance, air and sea temperature, wind speed, orientation of the substratum and the immersion status of the organism, which depends on the location of the animal and the tides), body temperature can be predicted.

Predicting thermal variability: the environmental bootstrap
Because body temperature is governed by multiple environmental factors that vary through time, limpets experience dramatic variations in body temperature over temporal scales from minutes to centuries (Denny et al., 2009). Shifts in body temperature at any of these scales potentially affect the ability of individuals to perform and survive. For example, natural selection can act
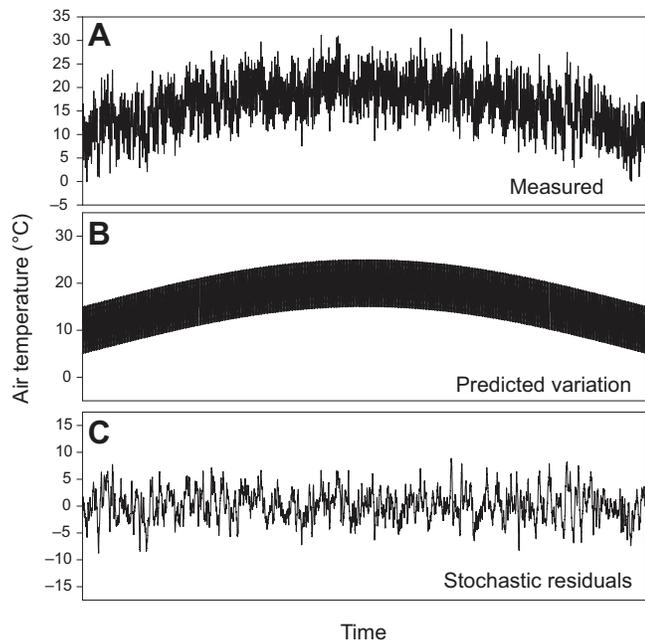
Fig. 3. Processing of time-series data for the environmental bootstrap procedure. (A) A hypothetical year-long series of measurements for a relevant environmental parameter (e.g. air temperature). The series of measurements can be partitioned into two components: (B) predictable cyclical variations of various periods (e.g. the day–night and seasonal cycles shown here) and (C) stochastic residuals, the difference between the measured and predicted values.

instantaneously – heat-tolerant individuals survive an episode of high temperature, whereas heat-sensitive individuals die – but the tolerance of an individual is presumably controlled in part by its genetic make-up, the result of a random sampling of the gene pool of the population during reproduction. Over time, population genetics integrates both the lethal and sublethal consequences of environmental challenges, including reduced reproductive output in stressed individuals. Thus, while selection can happen rapidly, effects of selection on gene frequencies typically play out over longer periods. Because body temperature and thermal tolerance have the potential to interact over scales from minutes to generations, understanding the dynamics of evolution of thermal tolerance requires a detailed understanding of environmental variability at all temporal scales.

Unfortunately, empirically measured records of body temperature with sufficient detail and duration to address issues of evolution are scarce in any system. For limpets, the longest available time-series is that of relevant environmental factors recorded every 10 min for 7 years at a site where *L. gigantea* is common (Denny et al., 2009). When coupled with the heat-budget model of the limpet, the temporal 'grain' of this record is sufficient to provide a picture of the short-term variation in body temperature. Denny and colleagues (Denny et al., 2009) devised a statistical technique – the environmental bootstrap – to use the details of this short-term variation to generate realistic environmental time-series of any desired length. In brief, the empirical record of each environmental factor (e.g. air temperature; Fig. 3A) is decomposed into a predictable component (the average daily and seasonal changes; Fig. 3B), and, by subtraction, the remaining, stochastic residuals (Fig. 3C). Through a simple normalization procedure (for details, see Denny et al., 2009), this random component is then

rendered statistically stationary, such that the mean, standard deviation, skew and autocorrelation function of the resulting normalized residuals are constant across time.

Because these residuals have thus been 'cut loose' from time, it is legitimate to choose random blocks of normalized stochastic data and recombine them with the predictable aspects of the environment to create a hypothetical (but realistic) time-series of how a component of the physical environment might unfold. A heuristic example is shown in Fig. 4. Here, a 13-day block of normalized air-temperature residuals has been chosen at random to combine with the first 13 days of predicted air temperatures, producing a realistic 13-day time-series of air temperature. Another 13-day block is then chosen at random (with replacement) to combine with the next 13 days of predicted temperatures, and so forth, in a type of moving-block bootstrap (see Carlstein, 1986; Künsch, 1989; Efron and Tibshirani, 1993; Bühlmann and Künsch, 1995; Bühlmann and Künsch, 1999; Paparoditis and Politis, 2003).

The example outlined in Figs 3 and 4 relates to air temperature, but the same procedure can be applied to all factors involved in regulating limpet body temperature. There are two additional components to this multiple-factor resampling scheme. First, the same length is used for blocks of all factors, a length sufficient to include all relevant temporal autocorrelation for all factors (13 days in our model for factors contributing to body temperature). Second, when a random starting time is chosen for a block of residuals for one factor, the same starting time is used for all factors. This simultaneity across factors ensures that any cross-correlation among them is retained in the hypothetical time-series (Denny et al., 2009).

Given time-series for each environmental factor that influences body temperature, this environmental ensemble can then be 'played' through the *L. gigantea* heat-budget model to provide a hypothetical time-series of body temperature of any length desired. For example, Denny and colleagues (Denny et al., 2009) used 1000-year records to ask how long a limpet would have to wait (on average) between experiencing days in which, by chance, its body temperature approached or exceeded lethal limits. These random stressful events were predicted to occur at intervals of 1.6 to 8.1 years, depending on the definition of 'stress' (for details, see Denny et al., 2009). Miller and colleagues (Miller et al., 2009) coupled predictions of this sort with measurements of the location of *L. gigantea* in the field to suggest that these rare, random stressful events might control the vertical range of *L. gigantea*. The environmental bootstrap can thus provide the sort of detailed, long-term time-series required to explore the effect of fluctuating body temperature on the spatial distribution of an important component species in an intertidal community. Here, we use the same approach to explore the evolution of thermal tolerance.

An allelic model of thermal tolerance
We employ a simple, allelic model (*sensu* Angilletta, 2009) of how the energetic (and, thus, reproductive) cost of thermal tolerance might be tied to individual genotypes, and we track allele frequencies in hypothetical populations of limpets subjected to the realistic variation in environmentally driven body temperature described above.

Time-series of body temperatures in stochastic, 'average' and global change scenarios
We used 7 years of environmental monitoring data from Hopkins Marine Station (HMS, Pacific Grove, CA, USA; 36°37′15″N, 121°54′15″W) to calculate hypothetical 2000-year-long
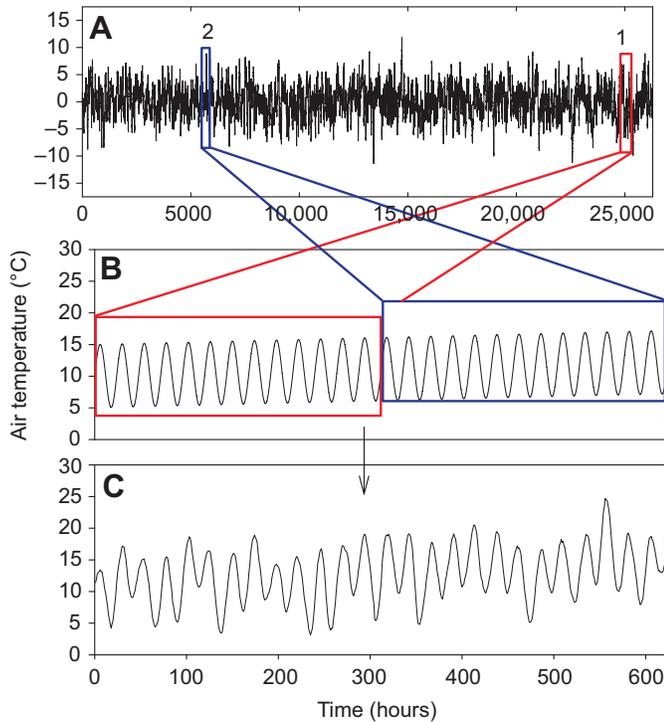
Fig. 4. The environmental bootstrap procedure in practice. Blocks of the multi-year series of normalized stochastic residuals for a given environmental parameter (A) are selected (red, then blue boxes in this example) and combined sequentially with the time-series of average, predictable value (B) to build up a hypothetical realization of how this aspect of the environment might play out (C). Note the change in *x*-axis scale from A to B and C.

Fig. 5. (A) Cumulative probability function for maximum annual limpet body temperatures (T_{max}) experienced by a population of intertidal limpets, calculated from 10 replicate 'stochastic' 2000-year time-series. Lower temperatures are encountered at the north-facing site (blue curve) than at the south-facing site (red curve). (B) Probability density curves derived from data in A.

bootstrap time-series of the body temperature of *L. gigantea*. Specific details of the environmental measurements have been published elsewhere (Denny et al., 2009). We assumed that the representative limpet to which the heat-budget model was applied had a shell length of 3.5 cm, and we carried out calculations for a limpet of this size at two representative sites. The first was on a south-facing shore with a slope of 36 deg relative to horizontal, 1.5 m above mean lower low water (MLLW). This height and orientation are among the more thermally extreme at HMS (see Denny et al., 2009; Miller et al., 2009). In addition, we calculated body temperatures for limpets on a vertical, north-facing shore, 1 m above MLLW, a height and orientation among the least thermally stressful at HMS. Body temperature was calculated every 10 min through the 2000 years of simulated time. This procedure was repeated 10 times using different random numbers in the bootstrap resampling procedure, providing an ensemble of replicate hypothetical time-series of body temperature. We refer to this ensemble as the 'standard scenario'.

Even on the relatively stressful south-facing shore, *L. gigantea* body temperature in the standard scenario is usually benign (Fig. 5). In an average year, maximum body temperature reached by a limpet (30.5°C) is below that required to kill half the limpets present (32–38°C) and well below temperatures at which all limpets die (36–42°C) (Miller et al., 2009). However, on rare occasions, environmental factors come into phase, and limpets experience body temperatures near their lethal limit. The entire distribution of annual maximum temperatures is shifted to lower temperatures for limpets on the north-facing shore (Fig. 5). Because the environmental bootstrap procedure ensures that the
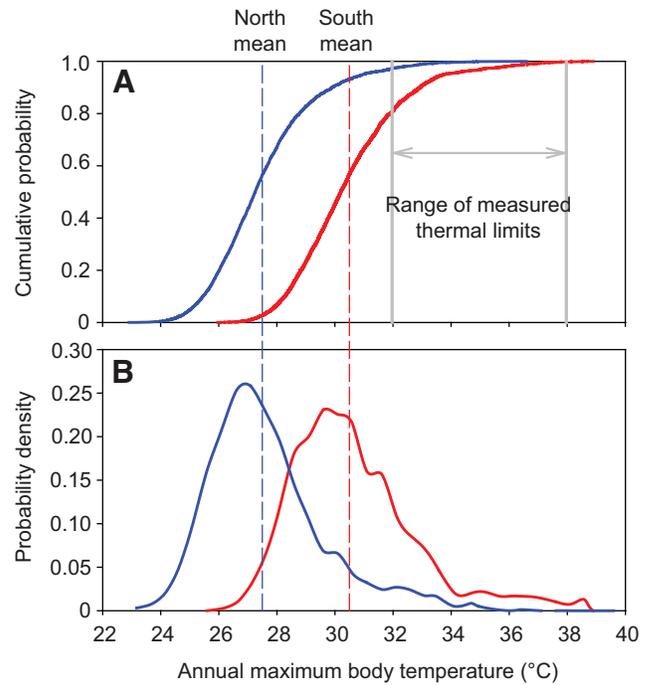
(characteristically low) probability of encountering exceptionally high body temperatures is equal for all years, intervals between these events follow a Poisson interval distribution (Denny et al., 2009), characterized by an abundance of short intervals balanced by a few exceptionally long intervals (Fig. 6). Intervals between impositions of a given extreme body temperature (35°C in this example) are longer at the north-facing than for the south-facing shore.

For purposes of our evolutionary model, the pertinent data from each replicate time-series are, for each year, (1) maximum body temperature reached (T_{max}), for comparison with the thermal tolerance limit of each limpet in an experimental population; (2) the number of 10-min intervals (N_1) below T_{th}, the threshold body temperature at which limpets begin to mount a response to thermal stress; (3) the number of 10-min intervals (N_2) for which body temperatures $T_b > T_{th}$, and (4) the actual body temperatures, $T_b(i)$, during these excursions above T_{th} (here i=1…N_2). Threshold body temperature was set at 25°C based on the induction profile of the molecular chaperone Hsp70 in *L. gigantea* (Miller et al., 2009). Similar values are common among other limpets (Dong et al., 2008) and intertidal snails (Tomanek and Somero, 2000). Although heat-shock proteins (HSPs) can be constitutively expressed and serve numerous functions (Feder and Hofmann, 1999), this stress threshold seemed a reasonable first approximation.

To examine the evolution of thermal tolerance in the absence of realistic stochastic variation, we also created 'average-environment' standard time-series of body temperature. In these scenarios, the maximum body temperature reached in each of 2000 years is set equal to the mean annual maximum body
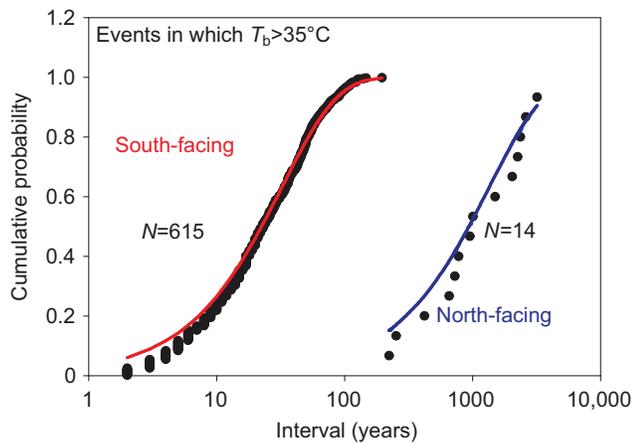
Fig. 6. Cumulative probability density curves for the time interval (years) between thermal events in which body temperature exceeds 35°C in the 10 replicate, stochastic-environment body temperature time-series. Red lines indicate theoretical Poisson interval distributions for mean values equal to those of the bootstrap data. Intervals associated with a given probability are much shorter at the south-facing site than at the north-facing site.

Fig. 7. 'Average-environment' (red) and a representative stochastic (black) time-series of annual maximum body temperature (T_{max}) in our climate-change model scenarios. (A) Data for the south-facing site. (B) Data for the north-facing site. In each case, mean air temperature was increased by 6.4°C between years 1800 and 1900 of the simulation, followed by an equally rapid decrease between years 1900 and 2000.

temperature observed in the calculations described above (30.5°C for south-facing shores, 27.5°C for north-facing shores), and the numbers of intervals both below (N_1) and above (N_2) the threshold of 25°C are set to the averages for the calculations described above (52,278 and 282, respectively, for the south-facing shore; 52,520 and 40 for the north-facing shore).

To assess the ability of the mean thermal tolerance of the population to evolve in response to anthropogenic climate change, an additional scenario was explored for both south- and north-facing shores. In this 'climate-change' scenario, the ensemble of stochastic 2000-year time-series was recalculated to include a linear rise in mean air temperature of 6.4°C over the century from years 1800–1900, followed by an equally steep decline in mean air temperature in years 1900–2000, simulating an optimistic reversal of current climate projections (Fig. 7). This rate of temperature change is the steepest predicted for global average air temperature over the next century by the Intergovernmental Panel on Climate Change (Intergovernmental Panel On Climate Change, 2007). Note that we changed only mean air temperature; any other effects of climate change (e.g. changes in sea-surface temperature, solar irradiance due to changing patterns of coastal fog, or wind speed) were not modeled. As above, we then calculated additional 'average-environment' climate-change scenarios for comparison with each of the 'stochastic' time-series of climate change. In these average-environment climate-change scenarios, each year from 1800 onward has an annual maximum temperature (red lines in Fig. 7) and number of intervals above and below threshold that follow the trend of the stochastic calculations, without the unpredictable variations. For the average-environment scenarios, a temperature equal to the average of T_{th} and T_{lethal} was used when computing the cost of above-threshold temperatures in our physiological model.

Physiological model of the energetic costs of thermal tolerance
To explore the evolution of thermal tolerance in *L. gigantea*, we assume that there is a genetically determined energetic cost associated with thermal tolerance and that the cumulative cost of tolerance is deducted from the energy available to produce gametes,
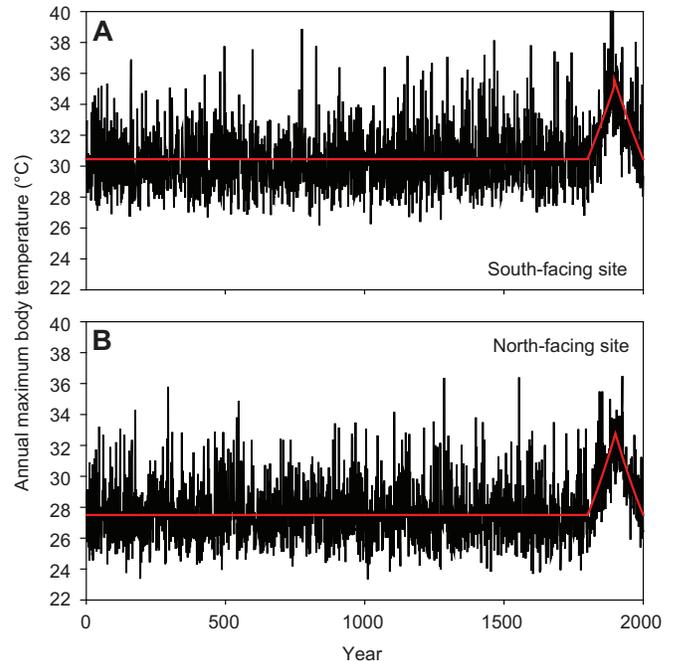
such that the more thermal stress the individual experiences, the fewer gametes produced. Of course, individuals produce gametes only if they are alive, and the more tolerant the individual, the greater the likelihood that it will survive to reproduce. Thus, in our model, the trade-off between residual reproductive output and probability of survival guides the evolution of thermal tolerance.

We implement this conceptual model as follows. As a compromise between computational tractability and biological reality – and where empirical data are scarce – we make several simplifying assumptions, noting each as we progress.

We assume (arbitrarily) that, in the course of a year, each individual limpet acquires enough energy from food to produce 100 gametes. The cost of thermal tolerance is then debited against this potential reproductive output. Given the current dearth of knowledge regarding the costs of thermal tolerance, we do not know whether these costs in *L. gigantea* constitute a large or small fraction of potential reproductive output. Rather than make a guess, we introduce a parameter into our calculations that specifies the maximum fraction of potential reproductive output that could be drained away by expenditures on thermal tolerance. This parameter, F_{max}, can have any value between 0 and 1; for simplicity (although perhaps not realistically), we assume that F_{max} is the same for all individuals in the population and is not under genetic control.

We next assume that there is an absolute limit to the thermal tolerance of the species, T_{lim}, the highest body temperature any individual of the species could potentially survive given biochemical and physiological constraints. In practice in our model, individual limpets die when their body temperature reaches a critical temperature, T_{lethal}, equal to or less than T_{lim} (Fig. 8). We assume that T_{lethal} is set by the genotype of each individual (see below).
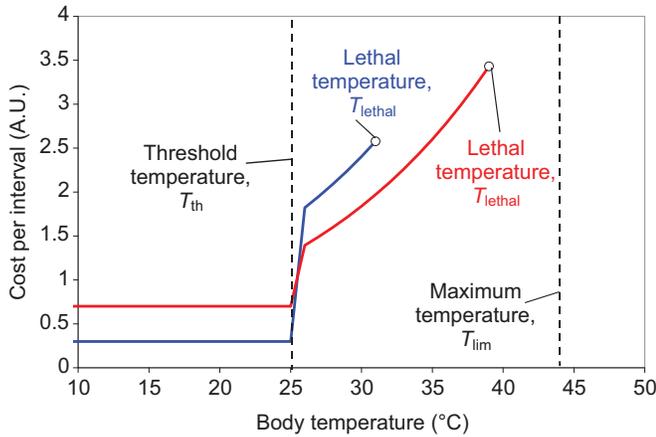
Fig. 8. Schematic representation of the physiological model relating energetic costs of thermal tolerance to reproductive costs, as a function of both body temperature and genotype, in each time interval. In each limpet population model scenario, we assume an absolute maximum temperature tolerance for the species (T_{lim}), as well as a constant temperature threshold (T_{th} =25°C in all simulations) below which the constitutive cost of maintaining thermal tolerance ('readiness') is constant. These constitutive costs are assumed to be genetically determined (red *vs* blue lines). Above T_{th}, energetic cost per interval is modeled as a Q_{10} process (accelerating portion of red and blue lines). Genotypes with higher constitutive costs (i.e. greater thermal 'readiness'; red line) require lower marginal energetic cost to tolerate a given body temperature above T_{th} (note that the red and blue lines cross at T_{th}). Genotypes that maintain a higher state of 'readiness' also enjoy a higher lethal temperature limit (T_{lethal}); T_{lethal} ranges from T_{th} to T_{lim}, depending on the genotype of each individual.

Although T_{lethal} sets the body temperature at which individuals die, we assume that, for each non-lethal time interval, there is an energetic cost to maintaining thermal tolerance. If body temperature T_b is below the threshold temperature T_{th}, we assume that there is a constant, genetically determined, constitutive cost, C_{const}, to maintaining thermal tolerance. This cost accumulates as more time is spent with $T_b<T_{th}$. Furthermore, we assume that the constitutive cost of thermal tolerance at low body temperatures ($T_b<T_{th}$) is linked to the lethal thermal limit of an organism: the more physiological effort the limpet puts into maintaining a constitutive thermal defense when its temperature is low – that is, the higher C_{const} is – the more tolerant a limpet is to exposure to temperatures above T_{th} – in other words, the higher T_{lethal} becomes. (Compare the red and blue lines in Fig. 8. The blue line genotype shows a low cost below T_{th} but a lethal limit of only 30°C; the red line genotype has a relatively high cost below T_{th}, but it can survive to 40°C. See also Eqns 1, 2 below.) Note that, in moderate years, higher thermal tolerance comes at the cost of lower relative reproductive output owing to the cumulative toll of elevated C_{const}. [There is empirical support for such fitness trade-offs for high-temperature 'specialization' (e.g. Willett, 2010), and analysis of the trade-offs has been explored elsewhere (Kearney et al., 2012; Nisbet et al., 2012).] In theory, the costs of thermal defense could be elevated at low, as well as high, temperatures. However, except on rare occasions when low tide, low wave height, low air temperature and clear skies co-occur at night to depress body temperature, the minimum body temperature of *L. gigantea* is set by sea surface temperature, which we expect is benign. We therefore assume that all temperatures below 25°C experienced by these limpets carry the same level of constitutive energetic costs, placing limpets firmly on the 'generalist' side of the 'specialist–generalist' performance spectrum (Angilletta, 2009).

At body temperatures above T_{th}, we assume that the acute cost of thermal tolerance increases with increasing body temperature, a relationship we model as a Q_{10} process, where Q_{10} specifies the multiplicative increase in cost for every 10°C increase in temperature. We also assume that the lower the genetically determined constitutive cost of thermal tolerance, the higher the above-threshold cost for that genotype (note the relatively small increase in cost at T_{th} for the red line in Fig. 8, and the larger increase for the blue line). In essence, we assume that the less energy an individual devotes to maintaining thermal tolerance in the absence of thermal stress, the more the marginal cost incurred when stress is encountered.

Note that our physiological model of the energetic costs of thermal tolerance (Fig. 8) is analogous to the right side of a thermal performance reaction norm (Fig. 1) in that the increased cost at higher temperatures corresponds to decreased reproductive performance.

Genetic control of thermal tolerance

We assume that the thermal tolerance of an individual – and the constitutive and induced energetic costs of this tolerance – are under additive allelic control, determined by a set of $n=10$ genetic loci. For simplicity, each genetic locus in our model has two alleles (+ and 0). p, the total number of + alleles in a diploid organism, determines T_{lethal}:

$$T_{lethal} = T_{th} + \left(T_{lim} - T_{th}\right)\left(\frac{p}{2n}\right). \qquad (1)$$

Here, $p/2n$ is the fraction of + alleles; thus, T_{lethal} can vary between T_{th} and T_{lim}. Similarly, p determines C_{const}:

$$C_{const} = \frac{100 F_{max} p \Delta t}{2 n t_{total}}. \qquad (2)$$

C_{const} is measured as cost per time interval Δt, and t_{total} is the total time between bouts of reproduction. Here, $(F_{max}p)/2n$ is the fraction of the overall reproductive reserve (100 gametes) used for constitutive thermal defense, and the ratio $\Delta t/t_{total}$ converts overall cost to cost per interval. In our model, Δt is 10 min, and t_{total} is one year (5.256×10^5 min). We assume that there is no dominance, mutation or epistasis, and that the n genetic loci assort independently. Consequently, evolution of thermal tolerance in our model proceeds exclusively through selection and drift.

The ensuing relationships between yearly patterns of body temperature and the reproductive output of individuals can be expressed mathematically. Below threshold, T_{th}, the total cost is given by:

$$\text{Below threshold cost} = N_1 C_{const}. \qquad (3)$$

Above threshold, cost per interval is set by three factors: maximum possible constitutive cost:

$$C_{const, max} = \frac{100 F_{max} \Delta t}{t_{total}}, \qquad (4)$$

actual constitutive cost C_{const} and the Q_{10} effect. According to the assumptions of our physiological model, the lower the below-threshold (i.e. constitutive) cost of thermal defense, the higher the above above-threshold cost. We accomplish this by setting the basic above-threshold cost per interval to $C_{const,max}+(C_{const,max}-C_{const})=2C_{const,max}-C_{const}$. This basic above-

threshold cost is then modulated by the Q_{10} effect and summed over all N_2 intervals to give the total above-threshold cost:

$$\text{Above Threshold Cost} = \sum_{i=1}^{N_2}\left[\left(\frac{200F_{max}\Delta t}{t_{total}} - C_{const}\right)Q_{10}^{\frac{T_b(i)-T_{th}}{10}}\right]. \quad (5)$$

The total yearly cost of thermal tolerance is calculated by summing Eqns 4,5, and, if the individual survives the year, this sum (up to 100) is subtracted from the starting potential of 100 gametes the individual could produce. The physiological model 'resets' at the beginning of each year.

We model a fixed, closed population of $Pop=500$ individuals, confined to one shore orientation. These assumptions are likely to be unrealistic for *L. gigantea*, which has planktonic larvae (but see Sanford and Kelly, 2011), but they form a practical simplification for this initial model. Each simulation begins with a random set of genotypes such that, on average, individuals in the population have n positive alleles. The effects of annual temperature variation are then assessed for each individual according to its genotype; for each year, we track whether T_{max} exceeds T_{lethal} and, if the individual survives, we note the combined metabolic cost of maintaining constitutive thermal tolerance and coping with above-threshold thermal stress (from Eqns 4,5).

In addition to death from acute thermal stress, we assume that limpets can die from random acts of predation or from old age. We assign a life span Y, and assume that, regardless of its age, each individual has probability $1/Y$ of dying by chance each year (Hoenig, 1983). If an individual survives for Y years, it reproduces and then dies of old age. For $Y=1$ to $Y=5$ years, the fraction of individuals surviving to die of old age increases rapidly with increasing Y, but, for $Y>5$, this fraction is approximately constant near 0.36 (it asymptotically approaches $1/e$). If an individual dies by any means (overheating, old age or through predation), it is

replaced at the beginning of the next year by drawing two gametes at random (i.e. through lottery recruitment) from the gene pool to which gametes have been contributed by all individuals.

Because we maintain population size at *Pop*, our model does not allow the population to expand (a reasonable assumption for a territorial species in habitat where competition for space is intense both within and among species). Under extreme thermal conditions, all limpets could die, and a population would go extinct. In practice, we arbitrarily designate a population as 'functionally extinct' if the number of gametes in the current gene pool is insufficient to create enough new individuals to maintain the population at its fixed size.

Using these rules, we track the locus-specific genetic composition of the limpet population through time under each of the body-temperature time-series scenarios described above. At the end of each year, we calculate the average, standard deviation and skew of p (the number of + alleles at all loci) for individuals in the population, and average these values among replicates. Using Eqn 1, we convert p values to lethal temperatures.

Results of a typical simulation using the standard scenario are shown in Fig. 9, where $T_{lim}=44°C$, $F_{max}=0.7$, $Q_{10}=2.0$ and $Y=20$ years – a set of parameters we use as a benchmark. Several characteristics common to all simulations are apparent in this representative example: the average lethal limit gradually asymptotes, never becoming constant in the 2000 years of the simulation. The effect of rare, extreme thermal events is an abrupt increase in average T_{lethal}, a decrease in the standard deviation of genotypes in the population and a transient positive skew of these genotypes (see the events denoted by the vertical broken lines in Fig. 9). These effects are most clearly seen early in the record, while thermal tolerance is still relatively low and evolving rapidly. Because the average lethal temperature never becomes stationary during the 2000 years of simulations, a statistic was required by which to compare values among the standard scenarios. To this end,
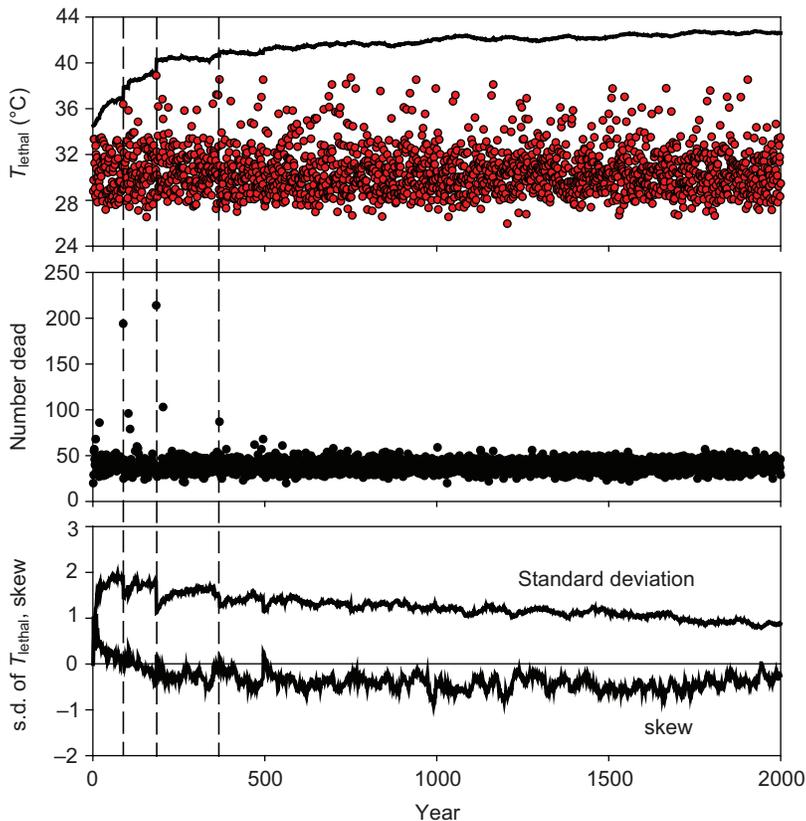

Fig. 9. Results from a typical simulation in the standard stochastic scenario ($T_{lim}=44°C$, $F_{max}=0.7$, $Y=20$ years, $Q_{10}=2$). Red circles denote annual maximum body temperature in each year of the simulation.

we calculated linear regressions of the average, standard deviation and skew of T_{lethal}, each as a function of time for years 1500–2000. The expected value of this regression at year 1750 is then used for comparisons among scenarios. We explore how each parameter of the model (F_{max}, Q_{10}, T_{lim} and Y) affects the evolution of thermal tolerance in each of our environmental scenarios.

Model results and discussion
Evolution of thermal safety margin

When *L. gigantea* is exposed to a standard-scenario stochastic thermal environment, the difference between mean T_{lethal} and average annual maximum body temperature is at least 7°C, a 'safety margin' that applies across all values of T_{lim}, F_{max}, Y and Q_{10} and for limpets on either south- or north-facing shores. Indeed, only at high F_{max} does mean T_{lethal} fall below the absolute maximum body temperature encountered in the 20,000 years of simulated environmental variation (Fig. 10). By contrast, when the environment is the same from one year to the next, safety margin is reduced. As an extreme example, at high F_{max}, the average lethal temperature in the standard average-environment scenario approaches the average yearly maximum temperature (30.5°C and 27.5°C for south- and north-facing populations, respectively; Fig. 10).

These results suggest that the amplitude of environmental variability sets the thermal safety margin for limpets. If this relationship can be generalized, the margin in tropical, polar and subtidal species (for which variation in maximum temperature is likely to be relatively low) should be smaller than that in temperate intertidal species, a prediction in general agreement with theoretical predictions based on thermal performance reaction norms (Asbury et al., 2010), and in rough accord with empirical results. For example, congeneric porcelain crabs exhibit safety margins that increase with increasing latitude or decreasing height on the shore (Stillman and Somero, 2000). Similarly, Deutsch and colleagues (Deutsch et al., 2008) found that warming tolerance (the difference between acute thermal tolerance and mean environmental temperature) increased with latitude for 46 species of insects.

Note that a reduced safety margin in tropical, polar or subtidal regions might not translate to a higher risk of thermal stress because, being less variable, these environments impose less-extreme thermal demands. In this sense, it is not safety margin *per se* but rather the risk of exceeding T_{lethal} that probably matters in the evolution of thermal tolerance (Denny et al., 2011).

Despite methodological differences and perhaps different goals, our approach and those based on thermal performance reaction norms produce qualitatively similar predictions, at least under the simplifying assumptions employed here, a finding that should be reassuring to thermal physiologists and evolutionary thermal biologists alike.

Our results suggest that, barring mutations, immigration of new genotypes or unforeseen ecological consequences of thermal tolerance costs, it should be possible to predict the approximate safety margin for a population from knowledge of the temporal distribution of body temperatures. It is unlikely to be a coincidence that the ~7°C safety margin we predict in our standard stochastic scenarios is equal to the temperature difference between mean annual maximum body temperature for limpets in this intertidal habitat and the infrequent, exceptionally stressful temperatures that have the potential to kill a substantial fraction of the population (Fig. 5). For example, for south-facing limpets, the lowest average T_{lethal} (37.9°C at F_{max}=0.9) – 7.4°C above mean annual maximum body temperature – is exceeded in only 0.19% of years in the
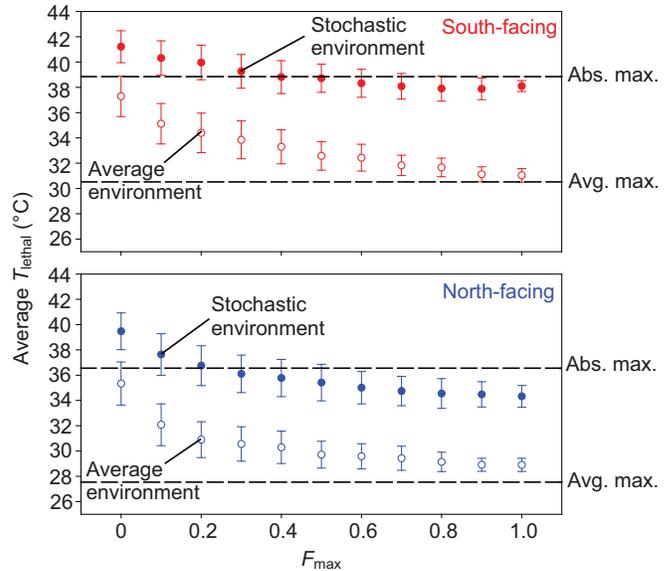

Fig. 10. The effect of varying F_{max}, the maximal fraction of reproductive effort that can be diverted to costs of thermal tolerance (T_{lim}=44°C, Y=20 years, Q_{10}=2). For each shore, results are presented for evolution in either a stochastic environment (filled symbols) or an 'average' environment (open symbols). The results are the expected values of T_{lethal} calculated for year 1750 from a regression through the last 500 years of the 2000-year simulations. Error bars are the expected value of the standard deviations of T_{lethal}, again calculated from a regression through the last 500 years of the 2000-year simulations.

ensemble of time-series; an event with this probability occurs, on average, only every 526 years. On the north-facing shore, the lowest average T_{lethal} (34.5°C at F_{max}=1.0) – again 7.4°C above mean annual maximum body temperature – was exceeded in only 0.24% of years in the overall time-series, corresponding to an average return time of 417 years. Here, as suggested for porcelain crabs (Stillman and Somero, 2000), it appears that rare, extreme thermal events determine the safety margin. Note that calculation of separate lethal temperatures for north- and south-facing shores is based on our assumption that each site is associated with a closed population. A more realistic model that allowed for population mixing would yield quantitatively different results but seems unlikely to change this qualitative conclusion.

Effects of F_{max}

Of all the parameters in our model, F_{max}, the fraction of reproductive energy that can be diverted to constitutive thermal tolerance, had the greatest influence. Average lethal temperature decreases with increased F_{max} (Fig. 10), probably owing to the Poisson-type intermittency of stressful thermal events. In benign years, individuals with low constitutive maintenance costs produce more gametes than those with high costs, and selection against individuals with high maintenance cost increases linearly with F_{max} (see Eqns 4,5). It is only in infrequent, extremely warm years – years in which many individuals with low maintenance costs die because they also have a low thermal limit, whereas others accumulate substantial above-threshold costs – that individuals bearing a thermally tolerant genotype enjoy a fitness advantage.

As noted above, for any given F_{max}, a higher average T_{lethal} is maintained when the environment varies stochastically compared with when the environment is constant. For south-facing populations, the average difference in mean T_{lethal} between the

stochastic standard scenario and the average-environment scenario is 5.8°C (s.d.=0.8°C); for north-facing populations, the difference is 5.4°C (s.d.=0.45°C). Again, note that average annual maximum temperature is the same in both stochastic and average-environment scenarios.

Effects of T_{lim} and Q_{10}

Increasing T_{lim}, the absolute maximum temperature any limpet can survive, has little effect on average lethal temperature (Fig. 11). Limpet populations with T_{lim}=52°C have an average T_{lethal} only 0.8°C higher than limpets with T_{lim} 10°C lower, although this is accompanied by a large decrease in the average number of + alleles (Eqn 1). Similarly, varying T_{lim} does not substantially change the effect of environmental stochasticity on the safety margin; T_{lethal} in standard stochastic environments is always higher than in standard constant environments.

The lack of an effect of T_{lim} is probably due to the range of values we have chosen. Laboratory experiments on a few animals (Denny et al., 2009; Miller et al., 2009) show that, for *L. gigantea* at HMS, T_{lim} must be at least 42°C, and we therefore confined our investigations to values >42°C. Undoubtedly, we would find an effect of T_{lim} on average T_{lethal} if we used a lower value. In the limit, if T_{lim} were set to a temperature less than the highest T_{max} encountered in a given 2000-year times-series (38.9°C and 36.5°C were absolute maxima predicted for south- and north-facing shores, respectively), all individuals would die regardless of their genetic makeup, and the population would become extinct. In short, T_{lim} is likely to play an important role in the evolution of thermal tolerance only for values lower than that which we have measured for

L. gigantea, raising important questions as to what processes and constraints have been overcome to set contemporary absolute thermal tolerance limits.

In our model, Q_{10} also had little effect on average T_{lethal}, which differed by only 0.96°C and 0.40°C across the entire Q_{10} range for south- and north-facing shores, respectively (Fig. 12). This result is largely due to the structure of the model used for the cost of thermal tolerance (Fig. 8). We assume that, at all body temperatures below the threshold of 25°C, limpets maintain a constant level of constitutive (as opposed to inducible) defense against thermal insults. As a consequence, any potential role of Q_{10} effects in our model is confined to those times in which body temperature exceeds 25°C. Even if Q_{10} is very large, there are too few of these intervals for their cost to substantially reduce the reproductive energy reserves of individuals.

The effect of life span

Average T_{lethal} increased with increasing life span, but by only 1.1°C for Y between 11 and 51 years (Fig. 13). The example shown here is for F_{max}=0.7, but the effect is similar across the entire range of F_{max}.

Although life span had little effect on mean lethal temperature, it had a substantial effect on the persistence of populations. For life spans of 1 and 6 years, all populations in standard stochastic environments with $F_{max}\geq0.7$ went extinct before the end of the 2000-year time-series on both south- and north-facing shores (Fig. 13). High population turnover with low life span allows the population to evolve rapidly during stretches of 'normal', benign years by reducing the number of positive alleles and, concomitantly, maintenance costs
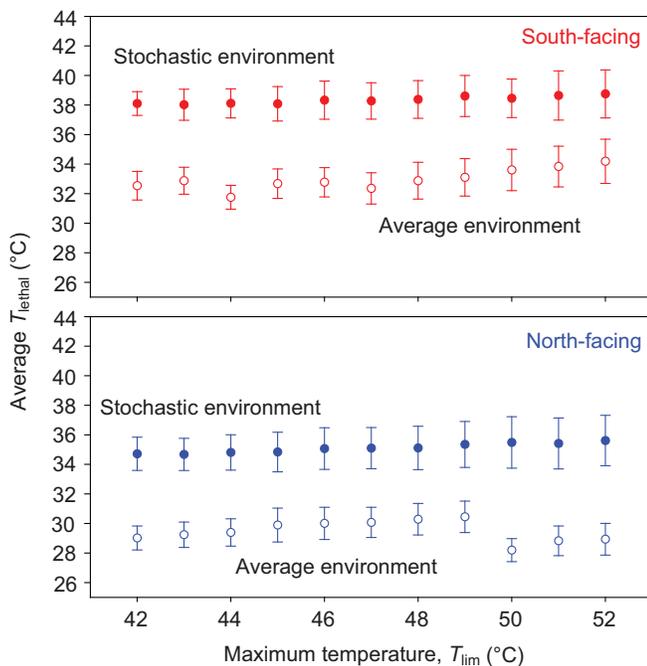

Fig. 11. The effect of varying T_{lim}, the maximum survivable temperature (F_{max}=0.7, Y=20 years, Q_{10}=2). For each shore, results are presented for evolution in either a stochastic environment (filled symbols) or an 'average' environment (open symbols). The results are the expected values of T_{lethal} calculated for year 1750 from a regression through the last 500 years of the 2000-year simulations. Error bars are the expected value of the standard deviations of T_{lethal}, again calculated from a regression through the last 500 years of the 2000-year simulations.

Fig. 12. The effect of varying Q_{10}, the factor governing the temperature dependence of above-threshold thermal cost (T_{lim}=44°C, F_{max}=0.7, Y=20 years). For each shore, results are presented for evolution in either a stochastic environment (filled symbols) or an 'average' environment (open symbols). The results are the expected values of T_{lethal} calculated for year 1750 from a regression through the last 500 years of the 2000-year simulations. Error bars are the expected value of the standard deviations of T_{lethal}, again calculated from a regression through the last 500 years of the 2000-year simulations.
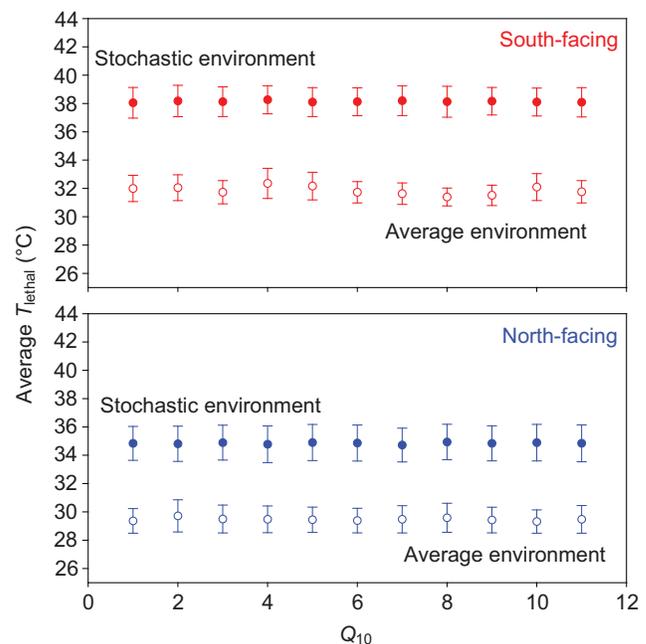
Fig. 13. The effect of varying Y, the life span of the limpet (T_{lim}=44°C, F_{max}=0.7, Q_{10}=2). For each shore, results are presented for evolution in either a stochastic environment (filled symbols) or an 'average' environment (open symbols). The results are the expected values of T_{lethal} calculated for year 1750 from a regression through the last 500 years of the 2000-year simulations. Error bars are expected value of the standard deviations of T_{lethal}, again calculated from a regression through the 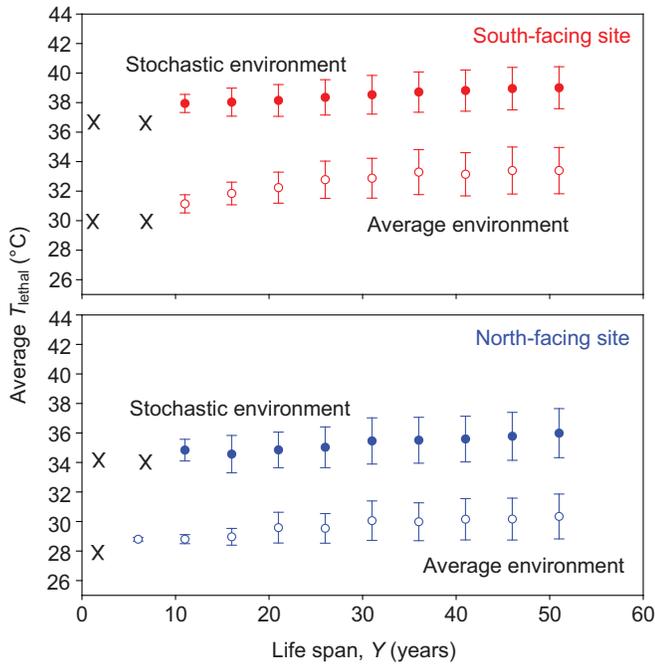last 500 years of the 2000-year simulations. The 'X' symbols denote cases in which populations went functionally extinct in all 10 replicate time-series.
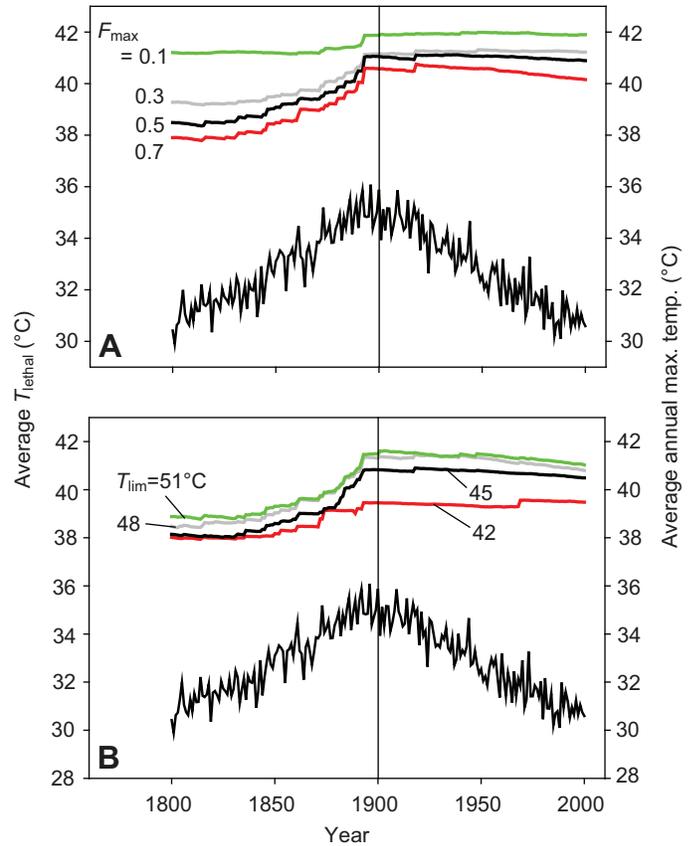

Fig. 14. The effect of trends in air temperature on average T_{lethal}. The average pattern of temperature variation is shown by the lower black line, with its ordinate on the right. The resulting shifts in T_{lethal} are shown for several values of F_{max} (A) and T_{lim} (B).

and lethal temperature. The population then suffers increased mortality during the next extreme year, and, after particularly extreme events, the population goes functionally extinct. Longer life span [for *L. gigantea*, >>8.5years (Denny and Blanchette, 2000; Kido and Murray, 2003)] allows for some genetic 'memory' of extreme thermal events, and the higher lethal limits thereby maintained are sufficient to ensure the persistence of a population in the face of subsequent extreme events.

The effect of projected trends in air temperature

Both north- and south-facing limpet populations experiencing stochastic environmental variation responded to a century-long drastic increase in air temperature by increasing average T_{lethal} (Fig. 14). Although the rate of increase in mean air temperature is constant in years 1800–1900, the rate of increase in average T_{lethal} accelerates during this period. The amount of increase depends on the model parameters: the higher F_{max}, the greater the response in mean T_{lethal}, but the lower the absolute mean T_{lethal} reached; the higher T_{lim}, the greater the maximum T_{lethal} reached. In all cases, the increase in mean T_{lethal} (<3.5°C) was less than both the increase in mean air temperature (6.4°C) and the increase in mean annual maximum body temperature (4.4°C in both south- and north-facing populations). (Effects of varying Y and Q_{10} were not explored.)

With 1800 years of stochastically varying environment as a prelude to our climate-change scenario, the greater elevation of mean body temperature relative to the increase in mean T_{lethal} resulted in a transient reduction in the safety margin. As air temperature in our

scenario decreased steadily from year 1900 to 2000, average lethal temperature in the population also decreased, but the rate of decrease was much slower than the previous rate of increase. As a result, even though air temperature in year 2000 returned to its pre-year-1800 levels, average T_{lethal} remained elevated on both north- and south-facing shores (Fig. 14). In the unlikely scenario that humans reverse the trajectory of climate change, this 'memory' effect could protract the impacts of climate change on populations. For example, for the lowest T_{lim} of 42°C (closest to values estimated for limpets at HMS), the average T_{lethal} remains near its peak throughout the recovery phase (Fig. 14B), possibly owing to fixation of positive alleles at a number of the genetic loci. Absent mutation or introduction of new alleles through immigration from other populations, the enhanced organismal constitutive costs associated with such a shifted distribution of the genotype of the population, could alter organismal energy budgets, influence species interactions and produce unanticipated ecological consequences in intertidal communities.

Populations subjected to the century-long increase in air temperature went functionally extinct with greater apparent probability than those not subjected to temperature increase (Fig. 15, $P<0.001$). The larger the fraction of overall metabolic energy that can be devoted to thermal defense (i.e. the larger F_{max} is), the greater the probability of extinction ($P<0.001$).

Caveats

There are several caveats to the conclusions discussed above. First, our environmental bootstrap procedure assumes that the
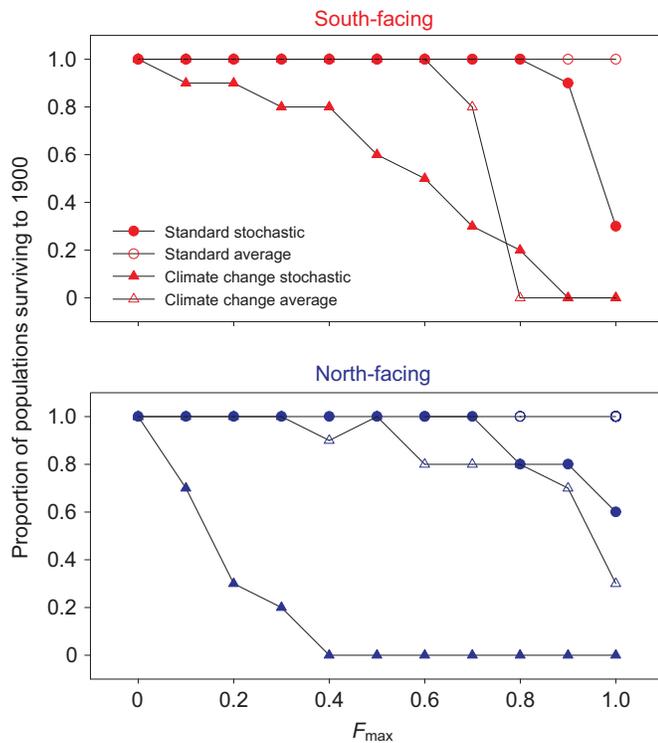
Fig. 15. The likelihood of functional extinction in limpet populations under each of four environmental scenarios as a function of F_{max} (T_{lim}=44°C, Y=20 years, Q_{10}=2). Year 1900 was chosen because it represents the peak of mean body temperature in the climate-change scenarios. In each scenario and for each value of F_{max}, there were 10 replicate simulations (880 data-points overall). The data were analyzed using a quasibinomial (response variable equals extinct or survive) generalized linear model with a logit link function, with F_{max} (P<0.001), population site (P<0.001), climate change (yes or no; P<0.001), and stochastic (yes or no; P<0.001) as additive predictor variables. Owing to the important role of chance in our model, a much larger number of replicate simulations would likely be required to draw definitive conclusions regarding population viability in these scenarios.

statistical characteristics of stochastic variation in each environmental factor are constant through time. It is possible (perhaps likely) that climate change will result not only in shifts in average daily and seasonal variation [e.g. higher minimum air temperatures rather than uniformly warmer temperatures (Easterling et al., 1997)] but also in shifts in the random components of variation as well. It also remains to be seen how the effects of environmental variability might play out in tropical, polar and subtidal sites, where the amplitude of temperature variability could be less than that of our temperate intertidal site. As noted above, we speculate that, although the risk of extreme thermal stress in the absence of climate change might be comparable, the evolved safety margin at such low-variation sites should be smaller. The consequences of imposition of a certain increase in body temperature (as opposed to a certain percentage increase relative to safety margin), in terms of risk of thermal catastrophes, remain to be determined. In our simulations, populations subjected to the same mean increase in body temperature in the average-environment climate-change scenario were less likely to suffer extinction than those populations in the stochastic climate-change scenario (Fig. 15), a preliminary result that requires further examination.

Empirical and predicted limpet thermal tolerance limits: a quantitative comparison

In addition to the qualitative patterns discussed above, our explorations make specific quantitative predictions regarding the thermal tolerance limits of *L. gigantea*. The level of agreement between these predictions and empirical data varies depending on the experimental conditions. When *L. gigantea* from north-facing sites were exposed to increasing body temperatures at 100% relative humidity, the median thermal limit was 36.7°C (Denny et al., 2009) to 37.4°C (Miller et al., 2009), comparable to the mean predicted here for a north-facing site at low F_{max} and approximately 0.5–1°C lower than the lowest mean values predicted for a south-facing population in a stochastic environment (Fig. 10). However, when limpets from north-facing rocks were exposed to elevated temperature under conditions more likely to be encountered in the field (relative humidity of 50–60%, wind speed of 0.5 m s^{-1}), the median thermal limit [32.5°C (Miller et al., 2009)] was 1.8°C lower than the lowest mean values predicted for a stochastic environment (34.3°C).

Several aspects of these earlier experiments complicate these comparisons. Miller and colleagues exposed limpets to elevated temperatures for up to 7 h. Given the complex topography of actual shores and the influence of wave splash, it is unlikely that an entire local population of limpets would ever be emersed under direct sunlight for a period this long. In fact, in our simulations more than 75% of above-threshold (>25°C) body temperature episodes at the more thermally challenging south-facing site, including many in which peak body temperature lies in the 'lethal range' of previous studies, lasted less than 3 h (Fig. 16). Furthermore, a limpet on its home site might be able to seal its shell to the substratum better than limpets used in the experiments, which were placed on a planar aluminum block. A better seal could result in less desiccation and, presumably, higher lethal temperatures. And finally, the thermal limits of limpets from south-facing rocks were not investigated. In sum, although our predictions of limpet thermal limits correlate reasonably well with empirical observations, a number of physiological uncertainties remain.

Prospective: opportunities and challenges for ecomechanical contributions to evolutionary thermal biology

The uncertainties associated with comparing the results of our model with the performance of real limpets highlight several challenges for incorporating the details of thermal variation into a mechanistic understanding of evolutionary thermal biology and, ultimately, for understanding the ecological consequences of thermal variation.

Physiological costs of environmental variation and their genetic basis

No matter how detailed our model of the body temperatures of individual limpets, predictions of tolerance limits (or other aspects of performance) will be constrained until we also have a more detailed understanding of thermal physiology and its genetic determinants. Among the more pertinent considerations, especially given the temporal resolution possible with the environmental bootstrap technique, are the time-dependent consequences of exposure to temperature extremes. For example, we cannot currently say whether a limpet, once stressed, will be more tolerant with lower energetic costs (e.g. owing to prior induction of heat-shock responses and stress 'hardening') or less tolerant with higher energetic costs (owing to accumulated molecular damage from prior episodes) when stressed again. Indeed, the answer probably
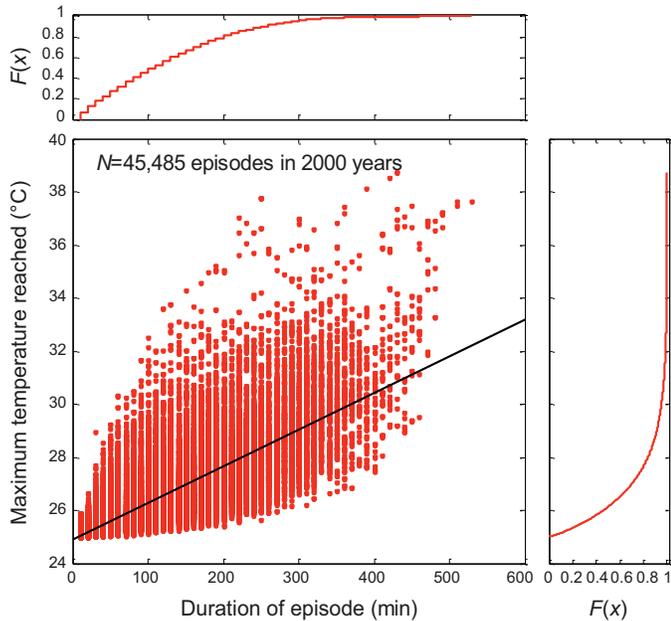
Fig. 16. The duration and maximum body temperature reached for all discrete episodes where $T_b > T_{th}$ in a single, 2000-year simulation for a south-facing limpet population. Curves adjacent to the abscissa and ordinate axes represent Kaplan–Meier estimates of the empirical cumulative distribution functions, $F(x)$, for duration and maximum body temperature, respectively. Analogous distribution functions peaked at significantly lower values for the north-facing population (data not shown). A best-fit regression line through all points (black) is added for reference.

depends on both the intensity of, and interval between, stressful events (Hochachka and Somero, 2002; Stillman and Tagmount, 2009). Lacking information on this subject, we implicitly assume in our model that the cost of thermal tolerance for one 10-min interval is independent of all other intervals (even adjacent ones), a situation that clearly does not hold for organisms in the wild. On longer time-scales, it seems likely that individual limpets adjust their thermal defenses in response to predictable changes in their environment or acclimate to mean conditions over recent experience (e.g. Halpin et al., 2004). Largely owing to uncertainty in their parameterization, these effects have not been taken into account in the current model.

However, the task of experimentally demonstrating the relationship(s) between variation in individual thermal history (over temporal scales from minutes to life span Y) and variation in organismal performance (ideally, individual fitness) is a massive, arduous and unglamorous one. Consider, for instance, that the relationships between thermal history and performance possibly include several types of physiological 'memory' effects (e.g. time-lags and carryover of molecular damage from one day to the next, acclimation, acclimatization, developmental plasticity, developmental delays, oxidative-stress-mediated reductions in life span, maternal effects) and contingencies [e.g. thermal stress during the reproductive season rather than other times of year, interaction between thermal stress and biological processes such as feeding (Schneider et al., 2010)]. These complexities and the associated logistical challenges in quantifying their effects apply particularly to any 'non-model' organism with a life span longer than a few weeks. In addition, our model does not account for possible coadaptation of the various aspects of thermal tolerance and thermal performance (Angilletta et al., 2006); for example, we

assume the unlikely scenario that all genotypes exhibit the same body temperature threshold for induction of the thermal stress response.

Herein lies an important opportunity for a mechanistic approach such as the current model to help guide integrative thermal biology. Tools such as the environmental bootstrap and heat-budget models, particularly when used in mechanistic evolutionary models of the type developed here, can provide physiologists with reliable, long-term predictions as to how body temperature varies and how thermal tolerances might evolve. Using these predictions, it is incumbent upon physiologists, ecologists and evolutionary biologists to work together to decipher the fundamental mechanisms underlying the relationships between environmental variation, organismal performance and evolution. For example, our analyses suggest that the Q_{10} of above-threshold thermal tolerance costs might not be a fruitful avenue of research (although, if energetic cost of repairing damage caused by above-threshold Q_{10} effects carries over to subsequent below-threshold intervals, Q_{10} effects could become important). By contrast, an examination of the cumulative fraction of reproductive output that can be diverted to thermal tolerance (F_{max}) would be extremely valuable. Finally, both our bootstrap time-series of body temperatures and *in situ* temperature measurements (e.g. Denny et al., 2011) reveal that a substantial fraction (>40%) of episodes exceeding T_{th} occur on successive days, and the frequency of such 'heat waves' is projected to increase with global change (Ganguly et al., 2009). The majority of thermal stress physiology experiments examine responses to a single episode of elevated body temperature or to different constant temperatures, leaving consequences of these unpredictable 'heat waves' largely unexplored (but see Rank et al., 2007; Stillman and Tagmount, 2009).

Several other types of basic information are needed at the level of genetics. For simplicity, our genetic model links the lethal thermal limit to the level of constitutive defense of the individual. There is some indirect evidence to support this assumption in limpets. In a study of four species of intertidal limpets at Hopkins Marine Station, Dong and colleagues (Dong et al., 2008) found that the two species that grow high on the shore maintained higher constitutive levels of Hsp70 than the two low-shore species. Wolcott (Wolcott, 1973) found that the same two high-shore species had higher thermal limits than the two low-shore species. However, this correlation falls well short of demonstrating that constitutive defenses and thermal limits are genetically linked *within* a species. A combination of genomic (allele frequencies), functional-genomic (gene and protein expression patterns), biochemical (protein functional analyses) and organismal approaches are urgently needed in this context. For example, it is possible (perhaps likely) that constitutive and acute thermal tolerance costs are associated with different gene products and pathways or that different levels of thermal stress require different suites of responses (e.g. Logan and Somero, 2010; Logan and Somero, 2011; Whitehead, 2012). Similarly, an investigation of the ratio of induced to constitutive thermal tolerance costs, and how that ratio might vary as a function of genotype and thermal history, would provide crucial information.

We also assume that the contribution of each gene to both thermal tolerance and the cost of maintaining that tolerance has equal and strictly additive effects. In reality, we expect the possibility of genetic linkage and epistatic interactions among genetic loci involved in thermal tolerance (e.g. Perry et al., 2003), which could include molecular chaperones (HSPs), antioxidant defenses, DNA repair enzymes, metabolic enzymes, proteasome

constituents and regulators of membrane composition, among others (Hochachka and Somero, 2002; Rank and Dahlhoff, 2002; Lockwood et al., 2010; Tomanek and Zuzow, 2010). Each of these pathways is subject to a wide variety of regulatory mechanisms (transcriptional, translational, posttranslational modifications), and each bears its own level of ATP costs. There is also some level of redundancy across these pathways, suggesting that there might be multiple genetic solutions to the same end.

Inter-individual variation

As detailed as it is in regards to temporal variability in body temperature, our model takes no account of the inevitable spatial variability among individuals within a population (see, for example, Miller et al., 2009). We recently documented the extreme degree of inter-individual variation in body temperatures among intertidal organisms over very small spatial scales within the intertidal zone, developing a risk-based model to predict the survival of individuals within a population as a function of both variation in exposure to extreme events and variation in the ability to tolerate those events (Denny et al., 2011). This model predicted that high levels of spatial inter-individual variation in environmental experience could buffer populations from extreme thermal events or from climate shifts. However, it assumed a random spatial distribution of thermal tolerances in relation to thermal experience. By contrast, our current results incorporate physiological and genetic considerations and suggest that spatially uniform but temporally variable temperature experience might drive evolution of a mean safety margin sufficient to buffer populations from the effects of projected global change. The conclusions from both of these studies could be affected if the distribution of genotypes within a population interacts with patterns of environmental variation [both spatial and temporal (e.g. Porlier et al., 2009)] to determine population outcomes. For example, it is possible that extreme spatial variability in temperature experience among individuals, by 'relaxing' temperature-dependent selective pressure, could actually reduce mean T_{lethal} and the safety margin and thereby hamper the ability of a population to evolve in response to rapid climate warming.

Demographic and ecological implications

Our simulations on small, isolated populations suggest the potential for important processes acting over larger spatial scales. A population of limpets situated on south-facing rock surfaces, at an angle of 36 deg to the horizontal, 1.5 m above MLLW evolved a higher mean thermal tolerance than a limpet population on north-facing vertical rocks, 1.0 m above MLLW (Fig. 10). Such results imply the chance for demographic and genetic interactions (e.g. rescue effects) to operate within metapopulations, especially where individual populations experience different levels of environmental variation or different rates of environmental change (de Mazancourt et al., 2008). At one extreme, it is possible that populations experiencing relatively little stochastic environmental variation serve as demographic sources for those experiencing a high frequency of lethal events. Alternatively, populations experiencing greater levels of stochastic environmental variation might serve as sources of 'positive' alleles for thermal tolerance to their 'constant' neighbors. Different levels of sublethal environmental variation among populations might also prove important. Given the differences in mean T_{lethal} – and corresponding differences in the physiological costs of maintaining thermal tolerance – that we observed between north- and south-facing populations, we might predict different levels of energetic reserve for fueling other

ecologically important processes such as growth and territory defense. These interactions might become more important in the face of global change, but the outcomes are likely to depend heavily on context-specific details of inter-individual variation, larval exchange and gene flow, competition and other ecological processes. As an extreme example, strong local adaptation combined with robust population segregation in the intertidal copepod *Tigriopus californicus* appears to limit the intra-population potential for further thermal adaptation (Kelly et al., 2011).

Conclusions

A mechanistic approach to the estimation of body temperature – in this case, the combination of a bootstrap calculation of the stochastic fluctuations in relevant environmental factors coupled with an organism-specific heat-budget model – provides an exceptionally detailed picture of the body temperature variation with which *L. gigantea* must cope. This picture in turn provides a platform on which population-level models of evolution can be constructed and tested. The genetic responses we have modeled here occur over time-scales that are short by evolutionary standards, but that match more closely those of ecological processes, particularly in climate-change scenarios. A similar approach can be applied to the other intertidal organisms with which *L. gigantea* interacts. This mechanistic, 'bottom up' approach can thus potentially set the stage for an exploration of the interplay between evolutionary and ecological dynamics in a thermally variable environment. At present, realization of this potential is constrained by our limited mechanistic understanding of the connections among body temperature, thermal physiology and genetics.

List of symbols

C_{const}	constitutive cost of thermal defense
F_{max}	maximum fraction of energy budget devoted to thermal defense
n	population size (=500 individuals)
N_1	number of intervals below threshold temperature
N_2	number of intervals above threshold temperature
Q_{10}	ratio of rates at temperatures separated by 10°C
T_b	body temperature
T_{lethal}	realized lethal temperature
T_{lim}	highest possible lethal temperature
T_{max}	upper temperature limit (Fig. 1), annual maximum body temperature
T_{min}	lower temperature limit (Fig. 1)
T_{opt}	optimum temperature (Fig. 1)
T_{th}	temperature threshold above which an animal is stressed

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