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The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine 'winners' and 'losers'

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Summary

Physiological studies can help predict effects of climate change through determining which species currently live closest to their upper thermal tolerance limits, which physiological systems set these limits, and how species differ in acclimatization capacities for modifying their thermal tolerances. Reductionist studies at the molecular level can contribute to this analysis by revealing how much change in sequence is needed to adapt proteins to warmer temperatures – thus providing insights into potential rates of adaptive evolution – and determining how the contents of genomes – protein-coding genes and gene regulatory mechanisms – influence capacities for adapting to acute and long-term increases in temperature. Studies of congeneric invertebrates from thermally stressful rocky intertidal habitats have shown that warm-adapted congeners are most susceptible to local extinctions because their acute upper thermal limits (LT_{50} values) lie near current thermal maxima and their abilities to increase thermal tolerance through acclimation are limited. Collapse of cardiac function may underlie acute and longer-term thermal limits. Local extinctions from heat death may be offset by in-migration of genetically warm-adapted conspecifics from mid-latitude 'hot spots', where midday low tides in summer select for heat tolerance. A single amino acid replacement is sufficient to adapt a protein to a new thermal range. More challenging to adaptive evolution are lesions in genomes of stenotherms like Antarctic marine ectotherms, which have lost protein-coding genes and gene regulatory mechanisms needed for coping with rising temperature. These extreme stenotherms, along with warm-adapted eurytherms living near their thermal limits, may be the major 'losers' from climate change.

Key words: acclimation, acclimatization, adaptation, biogeography, climate change, extinction, genome, global warming, intertidal zone, thermal adaptation.

Introduction

The pivotal role played by environmental temperature in determining the distribution patterns of ectothermic species is manifested by the frequency with which species replacement patterns reflect latitudinal and vertical thermal gradients. Through understanding the underlying mechanistic causes of these temperature-related distribution patterns, we may not only be better able to explain contemporary biogeographic patterning but also able to predict how global warming will affect where species occur and how well they perform in the warmer ecosystems they face. This review focuses on four sets of closely related questions whose answers may provide us with a strong basis for predicting shifts in species distribution patterns in a warming world and, in extreme cases, for projecting when – and why – extinctions will occur.

The first set of questions involves whole-organism phenomena and sets the stage for the subsequent mechanistic analyses. How do thermal tolerance limits differ among species, notably among highly similar congeneric species with different latitudinal or vertical distributions? Which species seem most threatened by warming, i.e. which species currently experience body temperatures close to their lethal limits? How do these species differ in their abilities to increase tolerance of high temperature through acclimatization? Are ecologically similar warm-adapted congeners, notably cryptic species, available to replace more cold-adapted congeners that are negatively impacted by global warming? Do latitudinally separated populations of a species differ genetically in thermal tolerance, such that local extinction of heat-sensitive populations can be offset by their replacement by more heat-tolerant conspecifics? Thus, through these two mechanisms of 'like replacing like', can ecosystems maintain functionality despite shifts in species or population composition?

The second set of questions is more mechanistic and asks what physiological systems are most likely to be instrumental in setting thermal tolerance limits of ectothermic animals. Here, I consider both acute heat death, which occurs within minutes to hours, and longer-term effects that, while not acutely lethal, may nevertheless lead to the disappearance of a species from its ecosystem if fitness is impacted due to diminished physiological or behavioural performance at elevated temperatures. Are the same physiological systems responsible for both temporal patterns of temperature-induced stress? Does the phenotypic plasticity – the acclimatization potential – in these systems differ among species, thereby establishing species' relative degrees of stenothermy and eurythermy and sensitivity to climate change?

The third set of questions examines protein evolution and the issue of whether this adaptive process can 'keep up with' global warming. How much change in protein sequence is required to adaptively modify proteins' thermal sensitivities, to allow conservation of function and stability under new temperatures? Are there many sites within the protein sequence where such adaptive change can occur – or are there few options in this regard? Answers to these closely related questions may provide insights into the potential rate of temperature-adaptive protein evolution in a warming world.

A final set of questions addresses a large-scale genomic issue. How do the contents of genomes – the 'tool kit' of protein-encoding genes and regulatory switching mechanisms required for appropriate modulation of gene expression – affect the capacities of organisms to cope with climate change? How do the gene regulatory capacities of stenothermal and eurythermal species differ with respect to their relative abilities to alter patterns of gene expression in response to temperature change? Have millions of years of evolution in thermally stable habitats like the Southern Ocean led to the loss of protein-encoding genes and regulatory elements that are essential for adapting to rising temperatures?

Thermal tolerance limits

Which species are most threatened by climate change – and why?

A powerful approach for examining the evolution of thermal tolerance is to compare the physiologies of congeneric species that have distribution patterns that reflect thermal gradients. These 'apple to apple' comparisons allow adaptive differences among species to be clearly delineated, without ambiguities arising from phylogenetic effects (Stillman and Somero, 2000; Hochachka and Somero, 2002; Somero, 2005). Intertidal animals offer excellent experimental material in this regard. Thermal stress can be extreme in rocky intertidal habitats and is thought to be a prime determinant of latitudinal and vertical distribution patterns (Helmuth et al., 2002; Helmuth et al., 2006; Somero, 2002; Gilman et al., 2006; Helmuth, 2009) and shifts in biogeographic range due to climate change (Barry et al., 1995). For numerous groups of rocky intertidal animals, replacement of one congener by another, highly similar congener is commonly seen along latitudinal and vertical (subtidal-to-intertidal) gradients.

The relationship between habitat temperature and acute thermal tolerance limits found for congeneric porcelain crabs (genus *Petrolisthes*) allows us to address in some detail the first set of questions raised above (Stillman and Somero, 2000; Stillman, 2002; Stillman, 2003). Fig. 1A shows how acute lethal temperature (LT_{50}) varies among congeners native to different latitudes and distributed at various heights along the subtidal-to-intertidal gradient. Adaptive variation is clear. Tropical species are uniformly more heat tolerant than temperate species and, within each latitudinal group, species occurring highest in the intertidal zone have the highest LT_{50} values. To address the question of how species differ in terms of the threats that may be posed by rising temperatures, the LT_{50} data shown in Fig. 1A have been plotted in Fig. 1B against the maximal habitat temperatures (MHT) recorded in the different congeners' habitats. This presentation of the data reveals a striking relationship: for both the tropical and temperate congeners, the most heat-tolerant species are, in general, most threatened by further increases in habitat temperature because current MHTs may reach or exceed the LT_{50}. Furthermore, as discussed below, the most warm-adapted congeners of porcelain crabs are further disadvantaged by possessing a relatively small ability to increase LT_{50} during acclimation (Stillman, 2003).

The pattern seen among different congeners of *Petrolisthes* is mirrored by the differences found among congeners of intertidal and subtidal snails of the genus *Tegula* [now *Chlorostoma* (Tomanek and Somero, 1999; Stenseng et al., 2005)]. Congeners found highest in the intertidal zone are most heat tolerant, yet, because of the similarity of LT_{50} and MHT, they face greater threats from warming than less heat-tolerant subtidal species that rarely, if ever, encounter temperatures near their LT_{50}. Intertidal and subtidal limpets also exhibit this type of relationship between thermal tolerance and relative threats from increasing temperatures (Wolcott, 1973; Dong and Somero, 2009).

The conjecture that the most warm-adapted species within a genus of marine intertidal invertebrates are likely to be most threatened by climate warming agrees with broad conclusions reached in recent analyses of terrestrial ectotherms from different latitudes (Deutsch et al., 2008; Tewksbury et al., 2008). These analyses show that tropical ectotherms are more threatened by climate change than species from mid-latitudes because tropical species live closer to their upper thermal tolerance limits and, in some cases, live at temperatures above those at which physiological processes exhibit their thermal optima. These authors also emphasize that the range of temperatures experienced by an ectotherm may strongly determine its capacity for coping with rising temperatures (Tewksbury et al., 2008). Thus, polar ectotherms, especially marine species living at constant near-freezing temperatures, may share with warm-adapted tropical species a high vulnerability to climate change.

The endemic fauna of the Southern Ocean exhibit striking examples of this vulnerability. Many of these species have evolved for over 15 million years under conditions of extreme and stable cold temperatures. As a consequence of their evolutionary histories – and for the mechanistic factors discussed in the final section of this review – animals of the Southern Ocean are remarkably stenothermal. Many endemic species of the Southern Ocean die of acute heat death at temperatures only a few degrees above their normal habitat temperatures [–1.9°C, the freezing point of seawater, to approximately +1.8°C, the highest temperature currently found in waters around the Antarctic Peninsula (Somero and DeVries, 1967; Peck et al., 2004; Peck et al., 2009; Podrabsky and Somero, 2006)]. Moreover, many of these species have extraordinarily narrow ranges over which acclimation can occur (Peck et al., 2009). A study of 13 marine invertebrates from the Antarctic Peninsula found the

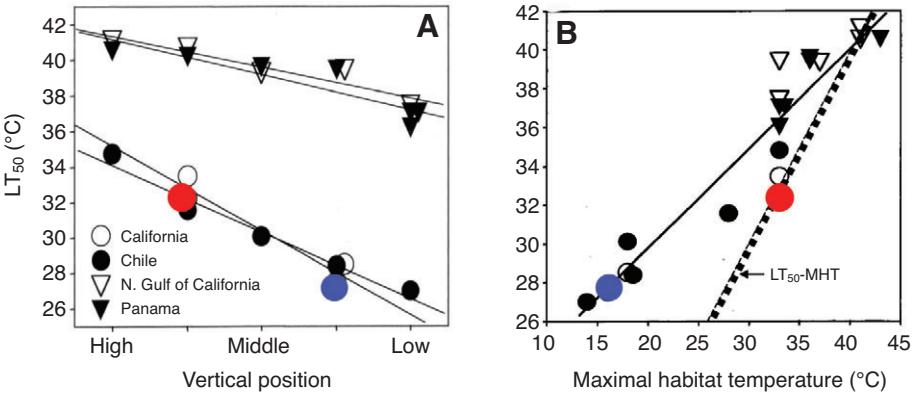

Fig. 1. Thermal tolerance relationships of porcelain crabs (genus *Petrolisthes*) from different latitudes (temperate: California and Chile; tropical/subtropical: Panama and Northern Gulf of California) and vertical positions. (A) Lethal temperature (LT_{50}) as functions of biogeographic and vertical distributions. (B) Relationship of LT_{50} to maximal habitat temperature (MHT). The line of equality (LT_{50}=MHT) allows estimation of risk of heat death under contemporary habitat conditions. Red symbol: *P. cinctipes*; blue symbol: *P. eriomerus* (modified from Stillman, 2002).

highest temperatures of acclimation that could be tolerated over periods of months were 1°C to 6°C (Peck et al., 2009). For some species, for example, the brittle star *Ophionotus victoriae*, acclimation to temperatures of only 2–3°C is not possible (Peck et al., 2009). If these upper temperature limits reflect the genetically fixed abilities of these animals to tolerate high temperatures, some species could be in jeopardy from climate change over the next one or two centuries. The fact that the Southern Ocean is warming as fast as any other region of the seas [temperatures have increased by ~1°C over the past 50 years (Meredith and King, 2005)] and its temperature is projected to rise by another 2°C over the next century (Murphy and Mitchell, 2005) suggests that some of these extreme stenotherms could face local extinction in the most rapidly warming habitats in which they occur. Because different species of fishes and invertebrates exhibit differences in thermal tolerance and capacities to acclimatize, food webs are likely to be impacted because of differential fitness and, thus, mortality in different groups of animals. Furthermore, size and activity level also have a significant effect on thermal tolerance, with smaller individuals of a species having greater heat tolerance than larger individuals, and more active species having higher tolerance than less active or sessile species. Thus, extinctions from warming may lead to altered ecosystem function due to shifts in size and activity levels of the surviving species (Peck et al., 2009). Coupled with extinction events will be the increased entry into Antarctic ecosystems, especially in the Peninsula region, of cold-adapted temperate species that may successfully invade these formerly too-cold habitats. Thus, the Southern Ocean appears to be facing major ecological change due to rising water temperatures.

Local adaptation: intraspecific differences in thermal tolerance and potentials for 'seeding' from better-adapted populations

The patterns of interspecific variation in thermal tolerance observed among congeners from low and high latitudes raise the question of whether ectothermic species that occur over exceptionally wide ranges of latitude, ranges that may be occupied by two or more congeners of less eurythermal species, may comprise a series of locally adapted populations with different genetically determined thermal optima and tolerance limits. Although many studies have examined differences in thermal tolerance among conspecifics from different latitudes or altitudes, e.g. the killifish *Fundulus* (Fangue et al., 2006), kelp crabs (Storch et al., 2009), *Drosophila* (Hoffmann et al., 2003) and whelks (Kuo and Sanford, 2009), few of these studies have raised animals through multiple generations under common garden conditions, to distinguish effects due to developmental plasticity or maternal influences from true genetically based temperature-adaptive differences among populations. Studies of *Drosophila* spp. offer the greatest number of examples of local adaptation (Hoffmann et al., 2003) and illustrate the rigorous experimental approaches needed to document these events.

Although well-substantiated examples of local adaptation in marine animals are rare, Kuo and Sanford (Kuo and Sanford, 2009) recently reported that populations of the whelk *Nucella canaliculata* from intertidal sites along the Eastern Pacific coastline from central Oregon to central California differed significantly in upper lethal temperature (Fig. 2). The most heat-tolerant populations came from sites in central Oregon (Fogarty Creek, Strawberry Hill and Cape Arago) where midday low tides in summer expose the snails to more extreme heat stress than is encountered by populations in northern and central California sites, where summer low tides occur during cooler periods of the day. This patterning of thermal tolerance is a clear illustration of how heat stress varies across latitude as a consequence of interactions between temperature *per se* and the timing of the tidal cycle (Helmuth et al., 2002; Helmuth et al., 2006; Helmuth, 2009).

The likelihood that these inter-population differences in *N. canaliculata* were genetically based, rather than consequences of acclimatization, is high because the animals studied were second generation (F2) individuals bred and raised under common garden conditions in the laboratory. The warm-adapted mid-latitude whelks would appear to offer a potential for replenishment or replacement of lower latitude populations that face local extinction from rising temperatures.

The development of these locally adapted populations may be related to the life history of this species, specifically its direct development and limited dispersal capability. In contrast to whelks and other species whose larvae or juveniles have restricted dispersal, species whose larvae spend long periods in the plankton and are distributed over broad ranges of latitude may be less prone to developing locally adapted populations.

Genetic polymorphism and a pool of adaptive variation

The process of local adaptation, whereby fixed genetic differences between populations account for variation in traits like heat tolerance, must be distinguished from the processes in which genetic polymorphism (heterozygosity) generates alternative genotypes that lead to phenotypes with different environmental tolerances, which are then subject to selection in different habitats (Watt and Dean, 2000). If selection favors one homozygous genotype in a certain environment and the alternative homozygote in a different environment, retention of the polymorphism may be

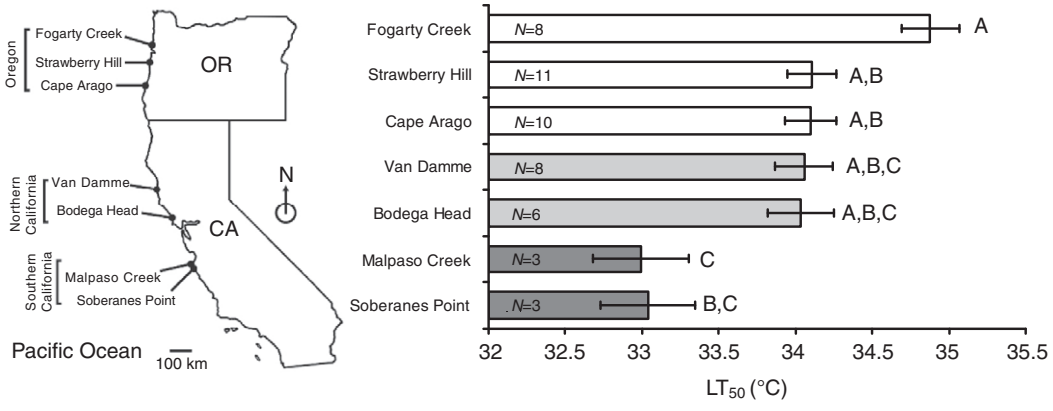

Fig. 2. Thermal tolerance (LT_{50}) of populations of the whelk *Nucella canaliculata* from sites in Oregon and California. Second generation (F2) populations from laboratory-bred lines held under common garden treatment were used to determine LT_{50} (modified from Kuo and Sanford, 2009). Different uppercase letters indicate significant difference.

strongly favored. Among intertidal animals, there are several examples of this type of effect, in which larval/juvenile survival or post-settlement selection leads to different genotypes becoming established in different physical or biotic conditions. For the northern acorn barnacle, *Semibalanus balanoides*, individuals having different allelic variants of the enzyme mannose 6-phosphate isomerase showed significantly different vertical distributions in the intertidal zone, which were also influenced by the presence of a species of alga that provided cover for the animals (Schmidt and Rand, 2001). This genotypic patterning resulted from genotype-specific mortality following larval settlement and may have reflected a combination of physical stress and access to mannose-containing compounds in the barnacle's diet. Other examples of allelic variation across environmental gradients in the intertidal zone include several cases where clines in aspartate and alanine amino transferase allozyme frequencies reflect vertical position and stress from temperature and desiccation (Hull et al., 1999; Panova and Johannesson, 2004).

Although the broader importance and the relative occurrences of local adaptation and polymorphism-based local selection in marine animals remain to be established, both processes have the potential for replenishing populations of conspecifics that face local extinction (or at least suboptimal performance) in warming environments. In terrestrial habitats too, both forms of adaptation to environmental variation have been reported in numerous studies of plants (Savolainen et al., 2007) and animals (Hoffmann et al., 2003). Species with adequate genetic variation to generate phenotypes with different thermal tolerances and optima may, other things being equal, prove to be 'winners' in a warming world.

What are the underlying causes of acute thermal death?

Although a number of physiological systems might be instrumental in causing acute thermal death, any physiological system that collapses rapidly at an organism's lethal temperature is clearly a sufficient cause of death, whether or not other systems are collapsing simultaneously at these same high temperatures. An obvious candidate for a cause of acute thermal death is cardiac function, which for a number of animals has been shown to decrease precipitously as acutely lethal body temperatures are reached (Hochachka and Somero, 2002).

This relationship is evident for congeners of *Petrolisthes* that differ in heat tolerance (Fig. 3). As experimental temperature is increased, the heart rate initially rises steadily, showing a typical Q_{10} relationship (blue filled symbols in Fig. 3A). As temperature is further increased, the heart rate shows two distinct shifts in response. Initially, the Q_{10} begins to decrease (open symbols in Fig. 3A). Then, further increases in temperature lead to a precipitous fall in heart rate (green filled symbols) at what is termed the critical thermal maximum (CT_{max}). For porcelain crabs, recovery of heart function after CT_{max} is reached is not observed. The finding that the CT_{max} of cardiac function is the same as the LT_{50} of the whole organism provides strong evidence for a mechanistic connection between whole-animal thermal tolerance and collapse of function of a specific organ (Fig. 3B) (Stillman and Somero, 1996; Stillman, 2002; Stillman, 2003).

Thermal tolerance often can be modified through acclimation or acclimatization, so it is important to examine how different congeners compare in their abilities to increase heat tolerance when acclimated to higher temperatures. Warm-adapted congeners of *Petrolisthes* again appear to face greater challenges than cold-adapted species. Field-collected porcelain crabs were acclimated to a temperature 10°C higher than the habitat temperature at the time of collection and increases in CT_{max} of heart function were examined post-acclimation (Stillman, 2003). The most warm-adapted congeners, two tropical species, *Petrolisthes gracilis* (LT_{50}=41°C for field-acclimatized specimens) and *P. hirtipes* (LT_{50}=39°C for field-acclimatized specimens), were only capable of increasing CT_{max} by 0.3°C and 0.9°C, respectively. Two temperate species, the mid-intertidal *P. cinctipes* (LT_{50}=32.5°C, field-acclimatized) and the low-intertidal/subtidal *P. eriomerus* (LT_{50}=28°C, field-acclimatized), increased CT_{max} by 1.2°C and 2.2°C, respectively. Thus, the most warm-adapted species, those from low latitudes and from high sites along the subtidal–intertidal gradient, are most threatened by further increases in temperature for two reasons: proximity of CT_{max} and, therefore, LT_{50} values to contemporary thermal maxima in their habitats and a limited ability to further increase CT_{max} and LT_{50} through acclimation.

Other comparisons of heart function at extremes of temperature provide data in support of the conclusions reached for porcelain crabs; namely that cardiac function is an important determinant of thermal tolerance and warm-adapted species live nearer their thermal limits than cold-adapted species. Thermal tolerance of *Tegula* snails found highest in the intertidal zone, e.g. *T. funebralis*, is several degrees higher than that of lower-occurring congeners (Tomanek and Somero, 1999). However, for the higher-occurring, more heat-tolerant *T. funebralis*, body temperatures above those at which heart function begins to decrease (~31°C) are reached commonly during midday low tides on hot days, whereas for two

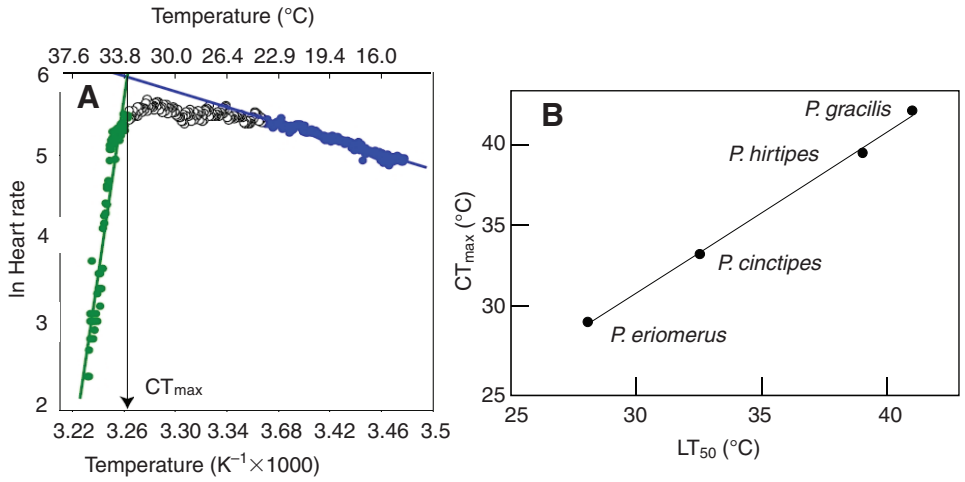

Fig. 3. Thermal sensitivity of cardiac function in porcelain crabs. (A) Arrhenius plot of heart rate (beats min^{-1}) of *P. cinctipes*. The critical thermal maximum (CT_{max}) was calculated by regression analysis, using the low temperature (blue symbols) and high temperature (green symbols) data shown (from Stillman, 2003). (B) Relationship between LT_{50} and CT_{max} for four congeners of *Petrolisthes* (from Stillman, 2003).

lower-occurring, less heat-tolerant congeners, *T. brunnea* and *T. montereyi*, body temperatures seldom if ever reach values high enough to reduce cardiac function (Stenseng et al., 2005). Furthermore, in the two lower-occurring congeners, *T. brunnea* and *T. montereyi*, warm-acclimation increased heat tolerance of heart function by 6.6°C and 4.0°C, respectively, whereas in *T. funebralis*, only a 1.6°C increase in heat tolerance could be induced (Stenseng et al., 2005). Thus, as in the case of porcelain crabs, the most heat-tolerant congener of *Tegula* exhibits the least ability to acclimate to higher temperatures.

For mussels of the genus *Mytilus*, CT_{max} values again reflect the local habitat temperatures of different species. For blue mussels, the northeastern Pacific native species, *M. trossulus*, exhibits a critical temperature for cardiac function near 26°C, whereas the more warm-adapted *M. galloprovincialis*, an invasive species from the Mediterranean Sea that has replaced *M. trossulus* along the southern half of the California coastline, has a CT_{max} near 31°C (Braby and Somero, 2006). Differences in thermal responses of the native and invasive mussel may account, at least in part, for the success of the invader in replacing the native species over the southern reach of its former biogeographic range. These data support the conjecture of Stachowicz and colleagues (Stachowicz et al., 2002) that global warming could be a boon for invasive species in marine ecosystems.

Cardiac limitations in longer-term thermal tolerance

The importance of cardiac function in establishing thermal optima and tolerance limits extends beyond the acute effects emphasized here to longer-term effects that entail a reduction in aerobic scope (Pörtner, 2002; Pörtner and Knust, 2006; Pörtner and Farrell, 2008). The responses of porcelain crab hearts to heating (Fig. 3A) show a fall in Q_{10} of several degrees before the sharp collapse in heart rate at the CT_{max}. The decrease in Q_{10} initiated near ~25–26°C may represent a pejus temperature effect (Pörtner and Knust, 2006). While not acutely lethal, the impairment of oxygen supply that begins around this temperature could contribute to the loss of aerobic scope and follow-on effects like local extinction, if the population is sufficiently impaired by shortfalls in aerobic capacity (Pörtner and Knust, 2006). As in the case of CT_{max} and acute heat death, the most warm-adapted congeners of porcelain crabs (Stillman, 2003) experience body temperatures closest to the putative pejus temperatures for aerobic function.

Temperature-adaptive changes in protein sequence

How much change in sequence is required to adapt?

I turn now to an examination of some of the molecular-level phenomena that are instrumental in setting thermal optima and tolerance limits, beginning with a review of adaptation of proteins to temperature. Proteins are a highly temperature-sensitive component of organisms and clear patterns of adaptive variation have been discovered in structural and functional properties of proteins from species adapted to different temperatures (Somero, 2004). With the advent of high-resolution techniques in molecular and structural biology, it is now possible to determine the amount of change in amino acid sequence needed for adaptation, the locations of these changes in the protein's three-dimensional structure and the mechanisms by which substitutions influence stability and kinetic properties.

Studies of orthologous enzymes (orthologs) from congeneric species or conspecific populations adapted to different temperatures have provided especially clear insights into these issues. Dehydrogenase enzymes, notably lactate dehydrogenase-A (LDH-A) and cytosolic malate dehydrogenase (cMDH), which are well studied structurally and undergo large conformational changes during activity, have been an important experimental system. Studies of LDH-A and cMDH have demonstrated that a single amino acid substitution in a protein of approximately 330 residues is sufficient to modify function and stability in a temperature-adaptive manner (Holland et al., 1997; Fields and Houseman, 2004; Johns and Somero, 2004; Fields et al., 2006; Dong and Somero, 2009). Recent data on cMDHs of cryptic congeners of limpets illustrate this phenomenon and emphasize how seemingly minor changes in sequence leading to temperature-adaptive changes in function and stability may be one component of the interspecific differences that underlie shifts in biogeographic distribution as the climate warms (Fig. 4) (Dong and Somero, 2009). Studies of latitudinal distribution ranges of the cryptic limpet congeners *Lottia digitalis* and *Lottia austrodigitalis* have revealed that the southern range limit of the former (northern) species is contracting to the north (Fig. 4A; black arrow indicates loss of range at low latitude) and the northern range limit of the southern species (*L. austrodigitalis*) is expanding northwards (Fig. 4A; black arrow indicates increase in range at higher latitudes) (Crummett and Eernisse, 2007). Consistent with this biogeographic shift, the latter species has a higher thermal tolerance than its cryptic congener (Dong and Somero, 2009). The effects of temperature on the apparent Michaelis–Menten constant K_m of the cofactor NADH differ between the two species in a manner reflective of adaptation to temperature (Fig. 4B); binding of the cofactor is less perturbed by rising temperature in the case of the ortholog of *L. austrodigitalis*, a pattern that reflects broader differences among orthologs of several congeners of *Lottia* (Dong and Somero, 2009) as well as patterns observed for this and other enzymes in a variety of species (Hochachka and Somero, 2002). Thermal stability of the orthologs also differs; the ortholog of *L. digitalis* loses activity more rapidly during incubation at high temperature (42°C) (Fig. 4C). Only a single amino acid difference distinguishes the two orthologs (Fig. 4D); a serine in *L. austrodigitalis* at position 291 replaces a glycine in *L. digitalis*. Molecular modelling analysis suggests that this single substitution is sufficient to increase the number of hydrogen bonds in the warm-adapted ortholog, thus leading to enhanced thermal stability in binding properties and structure.

Where in the 3-D structure is adaptive change localized?

The sites of adaptive changes in LDH-A and cMDH appear to be restricted to regions of the protein that influence conformational mobility (Hochachka and Somero, 2002). The amino acid composition of the active site is fully conserved among orthologs of these two enzymes, even though the kinetic properties of orthologs differ greatly. Because the rate-limiting event in an enzymatic reaction is often the change in conformation that accompanies formation of the enzyme–substrate complex, the energy barriers to these conformational changes may determine how rapidly the enzyme functions, i.e. the value of the catalytic rate constant, k_{cat}. The stability of these same mobile regions of an enzyme also governs its ability to recognize substrates. At any given temperature, a more flexible protein will exist in more non-binding conformations than a more stable protein. Thus binding, as indexed by the apparent Michaelis–Menten constant (K_m) co-varies with k_{cat}: less flexible proteins at any given temperature of measurement have stronger binding (lower K_m) but lower catalytic rates (k_{cat}) (Fields and Somero, 1998; Hochachka and Somero, 2002; Somero, 2004).

The fact that numerous sites in the protein influence the energy changes linked to conformational mobility means that adaptive change has a number of 'targets' to work with. If only one of these

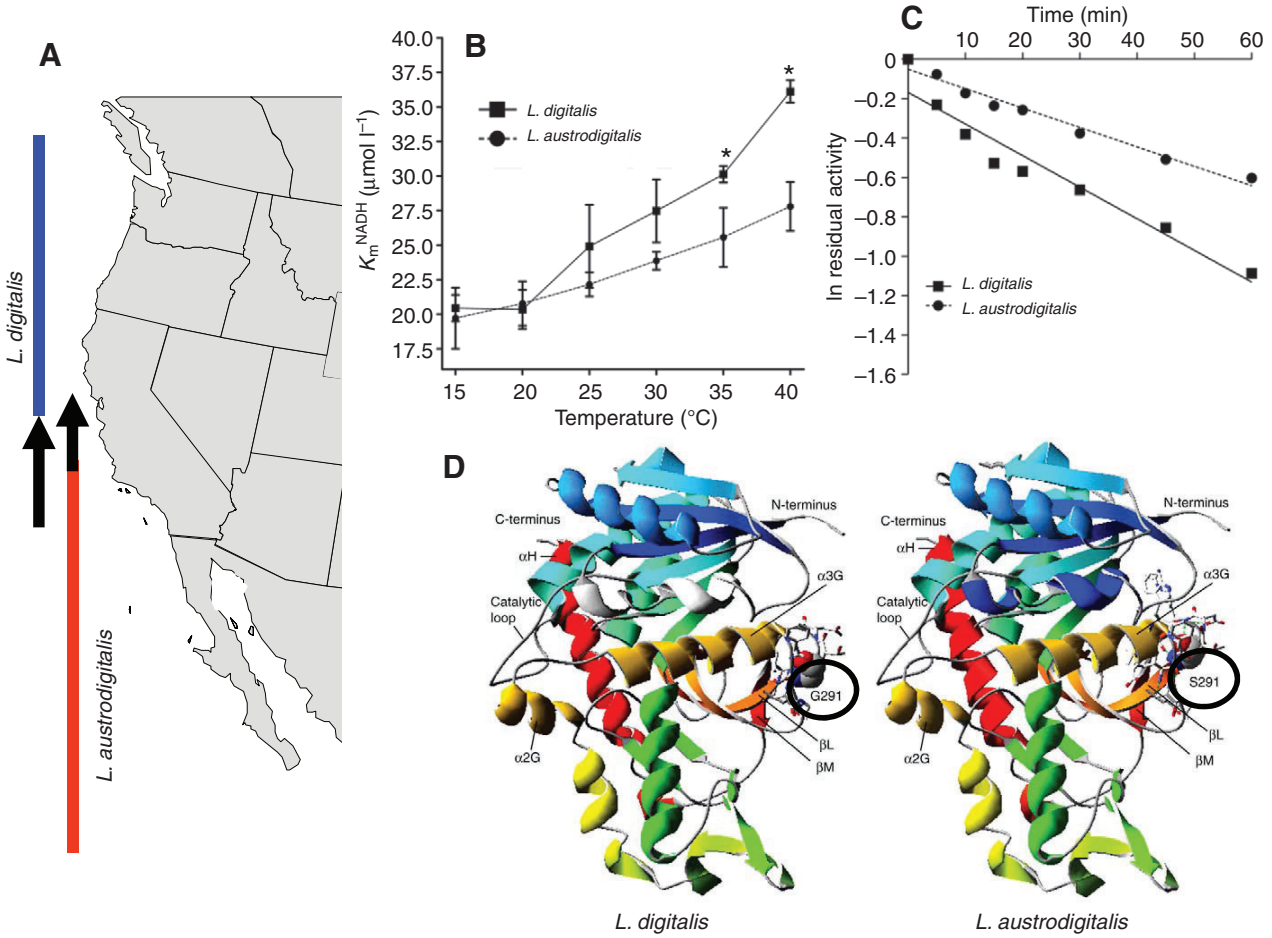

Fig. 4. Biogeography and characteristics of cMDHs of *Lottia digitalis* and *L. austrodigitalis*. (A) Biogeographic distributions of the two congeners in 1998. Black arrows show contraction of the southern range for *L. digitalis* and expansion of the northern range for *L. austrodigitalis* between 1978 and 1998 (Crummett and Eernisse, 2007). (B) Effect of measurement temperature on the K_m of NADH for cytosolic malate dehydrogenase (cMDH). (C) Loss of activity during heating at 42°C for cMDH. (D) Three-dimensional structures and the single amino acid replacement (site 291) for cMDHs of *L. digitalis* and *L. austrodigitalis* [B–D from Dong and Somero (Dong and Somero, 2009)].

many 'targets' needs to be 'hit' to achieve adaptation, then adaptive evolution can be achieved by a number of different substitutions. If so, evolution will be relatively rapid, compared with a scenario in which changes in only one or very few specific sites in the protein sequence can lead to adaptation. The minor number of sequence changes required for temperature adaptation and the likelihood that many sites in the protein are able to support adaptive change has implications for rates of evolution, i.e. for the issue of whether adaptive evolution of proteins can 'keep up with' the pace of climate change. Although a general, quantitative evaluation of evolutionary rates in this context is not possible, in species with short generation times, high reproductive outputs and substantial genetic polymorphism, adaptive protein evolution might be able to occur at a rate needed to counteract the effects of a warming climate.

Genetic lesions – DNA decay – may limit abilities to adapt to climate change

Loss of protein-coding genes

A more challenging problem in adapting to climate change arises for species that have lost genetic information required for life at increasing temperatures as a consequence of long evolutionary periods under highly stable conditions of low temperature. This type of genomic loss is termed 'DNA decay'; it has been observed in a variety of contexts, in addition to evolution under stable temperatures, where lack of purifying selection leads to losses from the genetic 'tool kit' (Harrison and Gerstein, 2002; Hoffmann and Willi, 2008).

Three types of lesions in DNA may influence how well an ectotherm will be able to respond to rising temperatures. First, when protein-coding genes are partially or wholly lost from the genome, the proteome of the organism is correspondingly reduced in complexity. This process is unlikely to be reversible if an entire protein-coding gene or a major fraction thereof is lost through mutation. Second, mutations that disrupt open reading frames in protein-encoding DNA (pseudogene generation) cause loss of the corresponding proteins from the organism's proteome. These mutations, if limited in extent, may be reversible. Third, lesions in gene regulatory regions, whether through loss of DNA or mutations in sequence, that eliminate the capacity to regulate gene expression in response to thermal stress could truncate the ability of organisms to adequately modify the phenotype during acclimatization. In addition to these lesions, failure to translate a mRNA that is transcribed successfully can lead to shortfalls in the proteome.

Lesions of these four types may be prevalent in cold-adapted stenotherms of the Southern Ocean, which have evolved for millions of years in highly stable cold and well-oxygenated waters (Eastman,

1993). Loss of protein-coding genes was initially suggested several decades ago when Ruud (Ruud, 1954) demonstrated that icefishes of the family Channichthyidae lacked erythrocytes. Subsequent studies have shown that a single mutational event in the Channichthyidae lineage caused the complete loss of the gene encoding the β-globin subunit of hemoglobin plus a portion of the 5′ region of the α-globin gene (Cocca et al., 1997). All members of the Channichthyidae so examined were found to contain the gene for myoglobin, but expression of myoglobin protein has been lost at least three times, by two different molecular mechanisms: disruption of the reading frame or failure of the myoglobin message transcribed from the gene to be translated into protein (Sidell et al., 1997). Loss of these two oxygen transport proteins is thought to be closely related to several of the unusual anatomical and physiological properties of icefishes, including major increases in the size of their hearts and gills, and in and blood volume (Montgomery and Clements, 2000; Sidell and O'Brien, 2006). How these unique characteristics of the Channichthyidae will play out in the context of global warming is not clear, but it seems likely that warmer waters with lower dissolved oxygen could pose significant challenges for these 'white-blooded' fish.

Loss of gene regulatory abilities

The consequences of 'DNA decay' in species adapting to stable temperatures may include reduced ability to modify the phenotype when changes in environmental conditions are encountered. The genetic differences between stenothermal and eurythermal species that account for their different abilities to acclimate to temperature remain largely unknown. However, because adaptive modification of the phenotype during acclimation (or acclimatization) is certain to involve large-scale alterations in gene expression, one of the fundamental distinctions between stenotherms and eurytherms may lie in their relative capacities to modify transcriptional processes in the face of thermal stress. With the advent of new molecular tools like cDNA and oligonucleotide DNA microarrays ('gene chips') for examining environmentally induced changes in the transcriptome (the population of mRNA present in cells), insights into the role played by plasticity in transcriptional processes are beginning to be achieved in a variety of species, including Antarctic marine ectotherms (Buckley and Somero, 2009).

One of the genetic lesions that has been discovered in Antarctic fishes and invertebrates is the loss of the heat-shock response (HSR) (Hofmann et al., 2000; Clark and Peck, 2009), a process commonly regarded, at least in textbooks, as being 'ubiquitous' among species. Through their activities as protein chaperones, heat-shock proteins play critical roles in reducing and repairing the damage to cellular proteins that arises from several forms of physical and chemical stress, notably high temperatures. Although many chaperones are synthesized constitutively in cells, heat stress almost invariably causes an up-regulation of heat-shock protein synthesis. The initial discovery that this response is missing in the Antarctic notothenioid fish *Trematomus bernacchii* (Hofmann et al., 2000) has been followed by discovery of losses of the HSR in a variety of other Antarctic marine ectotherms (for a review, see Clark et al., 2009). Although the basic molecular lesions underlying these losses of the HSR largely remain to be discovered, the significance of losing the HSR seems apparent. Most obviously, the species lacking the HSR will have reduced abilities to minimize the damage to their protein pool that is caused by elevated temperatures. This lack of ability to cope with heat may be one reason for the extreme stenothermality of Antarctic marine ectotherms (Somero and DeVries, 1967; Peck et al., 2004; Podrabsky and Somero, 2006).

More broadly, lack of certain types of heat-shock proteins leads to disruption of important cellular regulatory processes, including those that control the intrinsic (mitochondria-dependent) and extrinsic (death-receptor-mediated) pathways of programmed cell death (apoptosis), where heat-shock protein 70 (Hsp 70) is of critical regulatory significance (Creagh et al., 2000; Beere, 2004). Up-regulation of Hsp 70 and certain other molecular chaperones can block apoptosis through inhibition of the caspase proteins that are pivotal in the apoptotic destruction of the cell (Beere, 2004). This regulatory reaction can be viewed as a means of providing molecular chaperones like Hsp 70 with adequate time to renature damaged proteins, before the cell makes the decision of whether or not it will 'commit suicide' through the apoptotic process. Failure to up-regulate Hsp 70 during stress thus may eliminate the opportunity for chaperone-mediated processes to repair cellular proteins and, if repair is successful, eliminate the need for apoptosis. The finding that mRNA levels for proteins associated with apoptosis and DNA damage increased following heat stress in the Antarctic notothenioid fish *T. bernacchii*, whereas mRNA for Hsp70 did not change, suggests a potential for regulatory failure of apoptosis following heat stress in this species (Buckley and Somero, 2009). Other heat-shock proteins, notably Hsp 90, are important in a variety of signal transduction processes under stressful and non-stressful conditions (Pratt, 1997). The absence of heat-induced changes in Hsp 90 synthesis (Hofmann et al., 2000; Buckley and Somero, 2009) provides further evidence that Antarctic fishes lack abilities found in less stenothermal animals to regulate cellular processes in the face of temperature change.

Although the contents of the genomes of cold-adapted stenotherms like Antarctic notothenioid fishes remain to be fully explored by sequencing programs, the absence of heat-induced transcriptional changes characteristically observed in heat-stressed eurythermal species is suggestive of a truncated acclimatory potential in notothenioids relative to eurythermal fishes (Podrabsky and Somero, 2004; Buckley et al., 2006; Buckley and Somero, 2009). Notothenioid fishes and other Antarctic stenotherms may have lost many of the genetic capacities – both protein-coding and regulatory functions – that will be needed to allow their survival in a warming world. More broadly, changes in a variety of anatomical, physiological and behavioural capacities in Antarctic notothenioids raise a more general question about the abilities of these species to cope with climate change (Montgomery and Clements, 2000; Pörtner et al., 2006).

Summary

Animal species differ widely in the degree of threat they face from global warming. Warm-adapted species, including terrestrial ectotherms native to the tropics and intertidal invertebrates occurring high along the subtidal-to-intertidal gradient, and cold-adapted stenothermal species, notably ectotherms of the Southern Ocean, appear most vulnerable to climate change. Not only are their LT_{50} values close to the current thermal maxima of their habitats but also their abilities to acclimatize to warmer temperatures are highly limited. In addition, the vulnerability of species to climate change is also linked with the rate of warming found at different latitudes. Warming rates are generally greatest at high latitudes, so Arctic and Antarctic species that currently encounter temperatures near their thermal maxima may be among the first species to experience severe stress from global warming and may have the least time available to undergo adaptive evolutionary change.

Mechanistically, limitations to aerobic performance may be an important factor in setting the thermal optima and limits for many

animals. Acute and chronic heat stress may be governed in significant measure by shortfalls in cardiac performance that lead to inadequate provision of oxygen to support aerobic respiration.

Orthologous proteins differ in thermal responses among differently adapted congeners, and these differences may contribute to changes in biogeographic patterning as temperatures increase. The discovery that temperature-adaptive changes in proteins can result from a single amino acid substitution at one of many sites within a protein that govern conformational mobility has implications for rates of protein adaptation to rising temperatures. Depending on generation time, population size, the amount of genetic variation in a population and other factors, adaptive evolution of proteins potentially may occur rapidly enough to 'keep pace with' climate change. Species possessing this potential for rapid evolution could emerge as 'winners' – survivors – in a warming world.

More challenging are the genetic lesions that appear to accompany long periods of evolution in thermally stable habitats, lesions that appear to be at least partially responsible for the extreme stenothermy found in species like ectotherms of the Southern Ocean. Such losses of protein-coding genes and gene regulatory mechanisms may in many cases be essentially irreversible, and may place cold-adapted stenothermal species in extreme vulnerability from the predicted rise in air and sea temperatures. These genetically depauperate species seem most destined to be the major 'losers' in climate change.

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