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The role of stress proteins in responses of a montane willow leaf beetle to environmental temperature variation

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The heat shock response is a critical mechanism by which organisms buffer effects of variable and unpredictable environmental temperatures. Upregulation of heat shock proteins (Hsps) increases survival after exposure to stressful conditions in nature, although benefits of Hsp expression are often balanced by costs to growth and reproductive success. Hsp-assisted folding of variant polypeptides may prevent development of unfit phenotypes; thus, some differences in Hsp expression among natural populations of ectotherms may be due to interactions between enzyme variants (allozymes) and Hsps. In the Sierra willow leaf beetle *Chrysomela aeneicollis*, which lives in highly variable thermal habitats at the southern edge of their range in the Eastern Sierra Nevada, California, allele frequencies at the enzyme locus *phosphoglucose isomerase* (PGI) vary across a climatic latitudinal gradient. PGI allozymes differ in kinetic properties, and expression of a 70 kDa Hsp differs between populations, along elevation gradients, and among PGI genotypes. Differences in Hsp70 expression among PGI genotypes correspond to differences in thermal tolerance and traits important for reproductive success, such as running speed, survival and fecundity. Thus, differential Hsp expression among genotypes may allow functionally important genetic variation to persist, allowing populations to respond effectively to environmental change.

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1. Introduction

Many organisms live in variable thermal environments, which pose substantial challenges to survival and reproduction. In response to environmental temperature variation, organisms must adapt, disperse to more favourable localities, or face extinction. Understanding mechanisms by which animals respond to environmental variation has taken on new urgency, due to increasing effects of climate change on natural systems. There are now many documented examples of shifts in species' ranges, gene frequencies, changes in mating and migratory behaviour, and local extinctions of critical species in response to climate change (Inouye *et al* 2000; Hill *et al* 2002; Rank and Dahlhoff 2002; Walther *et al* 2002; Barnosky *et al* 2003; Parmesan and Yohe 2003; Balanya *et al* 2006; Harley *et al* 2006). While the mechanisms underlying these responses to changes in natural systems are complex, effects of temperature on the physiology of ectothermic animals has been implicated as a pervasive cause (Clarke 2003; Bradshaw *et al* 2004).

Temperature affects nearly all biological processes, including the structure of proteins and biological membranes and rates of biochemical and physiological reactions (Hazel 1995; Somero 1995; Willmer *et al* 2004). Free-living ectotherms are particularly susceptible to detrimental effects of environmental temperature variation, as their body temperature is determined to a large extent by environmental temperature (Angilletta *et al* 2002; Helmuth 1999; Helmuth 2002). Effective physiological responses to temperature are especially important for ectotherms with highly variable

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body temperatures, such as sessile invertebrates living in the marine rocky intertidal region and terrestrial insects. Many of these species live close to their thermal tolerance limits, such that small changes in environmental conditions may lead to large changes in species distribution and abundance (Sagarin *et al* 1999; Tomanek and Somero 1999; Chown 2001; Denny *et al* 2006). An important unresolved question is whether these species, which are most vulnerable to environmental change, possess sufficient genetic variation to evolve in response to it (Cavicchi *et al* 1995; Hoffmann *et al* 2003; Duffy and Stachowicz 2006; Hill *et al* 2006; Jump *et al* 2006).

Current research investigating effects of temperature on species distribution, abundance, survival and fitness characters have demonstrated that the heat shock response is a critical avenue by which animals tolerate variable environmental temperatures (Feder and Hofmann 1999; Sorensen *et al* 2003; Dahlhoff 2004; Sorensen and Loeschcke 2004; Hofmann 2005; Tomanek 2005). To understand the importance of heat shock protein (Hsp) expression for animals in nature, it is necessary to describe how an animal experiences environmental temperature, measure production of stress-inducible Hsps in response to environmentally relevant temperature variation, and assess the probable physiological, ecological or fitness consequences for Hsp expression in natural populations of ectotherms. Here we first briefly discuss the importance of accurate determination of body temperatures for animals in their natural habitat and describe several classic studies that demonstrate the importance of the heat shock response for animals in nature. We then describe recent results from the Sierra willow beetle *Chrysomela aeneicollis*, in which differences in Hsp expression are related to genetic variation in a metabolic enzyme locus, *phosphoglucose isomerase* (PGI), leading to differences in thermal tolerance, locomotory performance, survival and fecundity among PGI genotypes.

2. How do we determine the body temperature of ectotherms?

Many studies of ectothermic organisms measure environmental temperature (T_E) as an index of animal body temperature (T_B), since metabolic heat production is negligible and most heat used to do metabolic work comes from the environment. It is critical to accurately predict T_B, as body temperature, not environmental temperature, drives changes in physiological process and affects structure of biochemical molecules. The relationship between T_E and T_B depends on a number of factors (Helmuth 1999; Helmuth 2002; Helmuth *et al* 2005). First, it is strongly influenced by the amount of heat gained and maintained from the environment. This includes visible and infrared radiation from, and to, the sky and the ground, conduction to and from the ground or substratum, heat convected from the animal to the surrounding air, and evaporative water loss (Willmer *et al* 2004). Second, the color and shape of an organism will determine the rate and degree of heat transfer from environment to organism, such that two organisms of different shape or color in an otherwise identical habitat may have very different T_Bs (Forsman *et al* 2002; Hazel 2002; Fitzhenry *et al* 2004). Third, the size of an organism will affect both the rate at which it gains and loses heat and its ultimate thermal capacity. Small animals heat and cool faster, due to higher surface area to volume ratios, and large animals tend to heat to higher temperatures than small animals exposed to the same conditions (Helmuth 2002; Gilchrist and Huey 2003; Kingsolver *et al* 2004; Willmer *et al* 2004). For many animals, and especially for many species of insects, life stages differ in size, shape or color, so that the thermal life of adults may be quite distinct from that of its eggs or larvae, even if they are living in the exact same location (Brown 1956; de Jong *et al* 1996; Kingsolver and Huey 1998; Kingsolver and Wiernasz 1991; Nielsen and Watt 1998; Ellers and Boggs 2004; Price 2006). Fourth, the devices used to measure temperature, such as computerized temperature loggers that have gained prominence of late, are often a different size and shape than the animals they are trying to mimic, sometimes resulting in large errors in estimating T_B from T_E (Helmuth 1999; Helmuth and Hofmann 2001; Fitzhenry *et al* 2004). Finally, many animals have the capacity to regulate body temperature behaviorally, and thus may avoid environmental extremes recorded using stationary loggers (Kingsolver and Huey 1998; Kingsolver *et al* 2004; Willmer *et al* 2004). Therefore, to predict the relationship between T_E and T_B, one must measure T_B directly, or closely match thermal properties of environmental measuring devices with the thermal properties of the organism(s) of interest (Kingsolver 1979; Huey 1991; Helmuth 1999).

3. The heat shock response in nature

The heat shock response is critical for organisms living in a variable environment. Accumulation of denatured or partially unfolded cellular proteins due to exposure to temperature extremes or other physiological stress results in the preferential upregulation of heat-shock proteins (Hsps), which re-fold these proteins into their native, functional state or target them for degradation (Lindquist 1986, Parsell and Lindquist 1993). Upregulation of Hsps may enhance survival after stress exposure by rescuing critical metabolic enzymes from destruction, and Hsps are clearly a key component in the acquisition of thermotolerance (Krebs and Bettencourt 1999; Bettencourt *et al* 2002; Feder *et al* 2002; Garbuz *et al* 2003; Sorensen *et al* 2003). However, stress-inducible Hsp expression consumes a large amount of cellular energy,

and competes with regular metabolic processes; thus, Hsp expression may impose a fitness cost on individuals that regularly experience environmental stress (Krebs and Feder 1997; Loeschcke *et al* 1997; Krebs and Feder 1998; Krebs and Holbrook 2001; Robertson 2004). In addition, Hsp-assisted folding of mutant polypeptides may buffer organisms from developmental abnormalities resulting from exposure to environmental stress (Rutherford and Lindquist 1998; Roberts and Feder 1999; Rutherford 2003). Hsps may therefore be critical for buffering the consequences of genetic variation in an unpredictable habitat.

Differential expression of heat shock proteins may limit the distribution and abundance of ectotherms along steep thermal gradients in nature (Roberts *et al* 1997; Dahlhoff *et al* 2001; Dahlhoff 2004; Hofmann 2004; Hofmann 2005; Sorte and Hofmann 2005). For example, snails in the genus *Tegula* live in wave swept rocky intertidal habitats and experience a wide range of temperatures throughout the day with the ebb and flow of tides, which varies depending on the tidal and latitudinal distribution of each species (Watanabe 1984). *Tegula funebralis* is found in the mid-intertidal zone and experiences higher and more variable body temperatures (determined by thermally-matched snail model loggers) than its low intertidal sister species *T. brunnea* (Tomanek and Somero 1999). Thermal tolerance is greater in *T. funebralis* than *T. brunnea*, and this directly corresponds to the onset of expression of stress-inducible isoforms of Hsp70, the peak of Hsp70 expression, and the subsequent shutdown of Hsp70 expression, which for both species is at temperatures just below the lethal thermal limit, LT_{50}. Furthermore, acclimation to common garden conditions does not eliminate species difference in Hsp induction profiles (Tomanek and Somero 1999, 2002; Tomanek 2002, 2005). An important finding of these studies is that the temperature at which Hsp70 expression was upregulated for each species corresponded to temperatures that it routinely experiences in nature, whereas the lethal thermal limit (where most cellular processes, including Hsp70 synthesis, shuts down) was higher than temperatures normally experienced in nature. Thus, while the heat shock response is correlated to thermal tolerance, it is a much more sensitive and ecologically-relevant indicator of sub-lethal thermal stress, and is thus important in setting limits to distribution of species or populations along environmental temperature gradients.

In many habitats like the rocky intertidal, animals may experience a higher degree of thermal heterogeneity in T_B over small spatial or temporal scales, due to the processes discussed earlier, than over large distances (Helmuth and Hofmann 2001; Helmuth *et al* 2002; Gilman *et al* 2006; Helmuth *et al* 2006; Sagarin and Somero 2006). This heterogeneity in T_B over short spatial scales may lead to heterogeneity in the heat shock response. For example, in recent studies of the rocky intertidal mussel *Mytilus californianus*, Helmuth (2002) used automated temperature loggers that mimicked the shape, color, size and thermal properties of mussels to demonstrate that mussel T_B is more dependent on body size, location along the shore, and aspect of substratum than it was on air or water temperature. As a consequence, Hsp expression patterns for mussels within a mussel bed were complex. Mussels on the flat, horizontal surface of the rock were on average 7ºC warmer than those on vertical slopes less than 50 cm away, and Hsp70 expression was doubled for these horizontal mussels (Helmuth and Hofmann 2001). In contrast, a recent study of Hsp70 expression levels in mussels and in the dogwhelk *Nucella ostrina*, both species measured along their entire geographic range (Vancouver to Baja), were not higher in the southern, presumably warmer habitats than in the north (Sagarin and Somero 2006). Instead, peak values of Hsp70 expression were observed in northern Oregon, where intertidal animals are exposed to air in the afternoon during the warm summer months, and just south of Point Conception, California, where there is a large increase in sea surface temperature and decrease in wave exposure (and thus cooling splash). These examples illustrate the importance of rigorously tracking the response of an organism's T_B to changes in T_E, in part via the heat shock response. Such studies will be critical, given that that a predicted consequence of rapid climate change is that environmental unpredictability and fluctuation will increase, leaving more species vulnerable to stress and extinction (Easterling *et al* 2000; Parmesan and Yohe 2003; Sorensen and Loeschcke 2004; Hofmann 2005; Gilman *et al* 2006; Harley *et al* 2006, Helmuth *et al* 2006).

4. A model organism for studying effects of genetic variation on the heat shock response

The examples described above provide understanding of mechanisms by which heat shock protein expression operates in nature to buffer sometimes complex and unpredictable environmental temperature variation. Additional insights can be gained by using an organism that possesses genetic variation closely associated with the heat shock response. We have made substantial progress in this area in our studies of the Sierra willow leaf beetle *Chrysomela aeneicollis* (Schaeffer), an excellent model organism to study the relationship between thermal exposure, stress protein expression, and natural genetic variation in traits related to temperature adaptation. This beetle is abundant in cool, moist habitats at high latitudes in western North America (Brown 1956), but the range of *C. aeneicollis* extends into the Sierra Nevada mountains of Eastern California, where it occurs at high elevations (2375–3550 m). In the Sierra Nevada, *C. aeneicollis* is found in isolated sub-populations separated by permanent ice and snow at high elevations

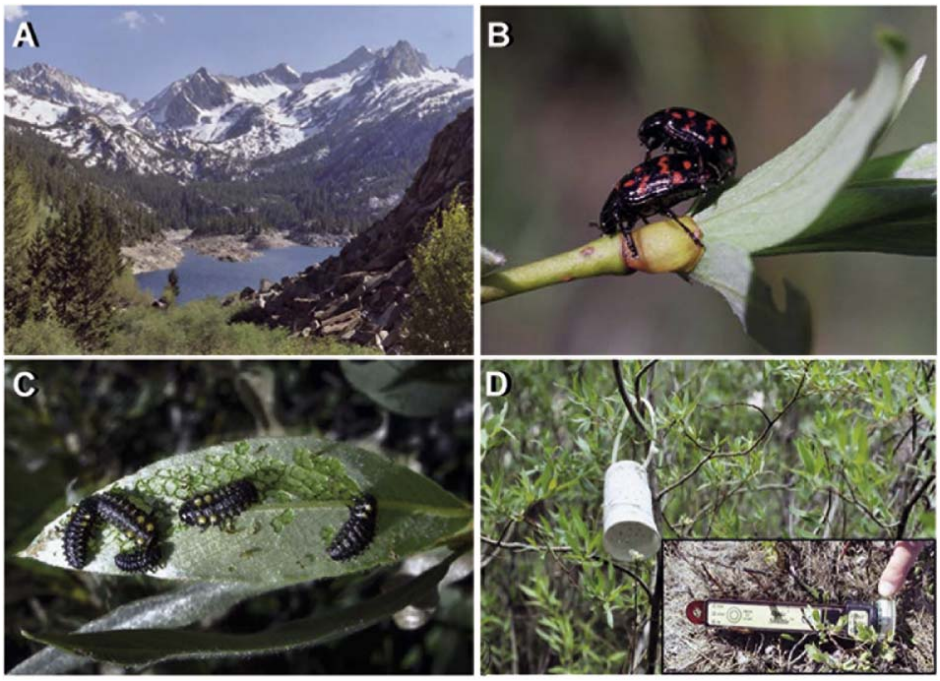

Figure 1. The willow leaf beetle *Chrysomela aeneicollis*. California populations of these beetles live in isolated drainages at high elevations in the Eastern Sierra Nevada **(A)**. Adults emerge after diapause in early June to mate and lay eggs on willow host plants **(B)**. Females lay several clutches of eggs, which hatch into larvae after a few weeks **(C)**. These larvae mature, pupate and develop into new adults before the snows return in September. Measures of environmental temperatures **(D)** with data loggers that closely match beetle body temperature (inset) demonstrate that adults and larvae experience extreme thermal variation during summer (Figure 3). Photographs: **A, B,** Nathan Rank; **C,** Sonja Otto; **D,** Elizabeth Dahlhoff; Nathan Rank (inset).

(figure 1A), brush and desert scrub at low elevation. Adults (figure 1B) emerge from diapause at snowmelt (typically early June), to feed, mate, and lay eggs on willows along streams and in bogs. During this time, beetles sit and feed, or run throughout the foliage, males in search of mates and females for choice oviposition sites; however, flight is rare. Larvae (figure 1C) are found on the same host plants as the adults, and develop during the warmest months of summer (July and August). Adults from the new generation feed for several weeks before entering diapause in September (Smiley and Rank 1986; Rank 1994). Populations often reach high densities (Rank 1992a), resulting in complete defoliation of their host plants.

Since 1998, we have recorded environmental temperatures in three Eastern Sierra Nevada drainages (figure 2A): Rock Creek (RC), Bishop Creek (BC), and Big Pine Creek (BPC). Temperature dataloggers (figure 1D) were deployed at 6-20 sites along elevation gradients in each drainage that bracketed beetle distribution. Temperatures measured using loggers placed in white vented plastic cups in willow foliage 1.5 m above the soil surface closely matched beetle body temperatures (T_B) measured using fine wire thermocouple. Logger air temperatures (T_L) and beetle T_B's are 4–5°C higher than air temperatures (T_A) during the day. At night, both T_L and T_B are indistinguishable from T_A (Dahlhoff and Rank 2000; Rank and Dahlhoff 2002; McMillan *et al* 2005). We have recorded temperatures using these loggers since 1998.

Analysis of this nearly decade-long temperature record yields some striking patterns. First, there is a strong geographic pattern in thermal variation that persists through time. Average summertime logger (and thus beetle body) temperature is cooler in the northern most drainage (RC) than in the central drainage BC, which is in turn cooler than BPC (figure 2B). Typically, RC has the lowest lows and the lowest highs, and BPC the warmest lows and the highest highs (Dahlhoff and Rank 2000; Rank and Dahlhoff 2002; McMillan *et al* 2005). In all drainages, elevation is negatively related to mean daily temperature (–3.0°C per 1000 m increase in elevation). Not surprisingly, expression of a 70 kD stress-inducible heat shock protein varies among these drainages and elevations for beetles in nature (Dahlhoff and Rank 2000; McMillan *et al* 2005). Beetle T_B (not shown) and Hsp 70 expression level declines with increasing elevation (figure 2C) and Hsp70 expression is highest in the warmest drainage, BPC [(figure 2D; modified from Dahlhoff and Rank (2000)]. Within a site, beetles experience wide fluctuations in T_B during summer, from –5ºC (or lower) on

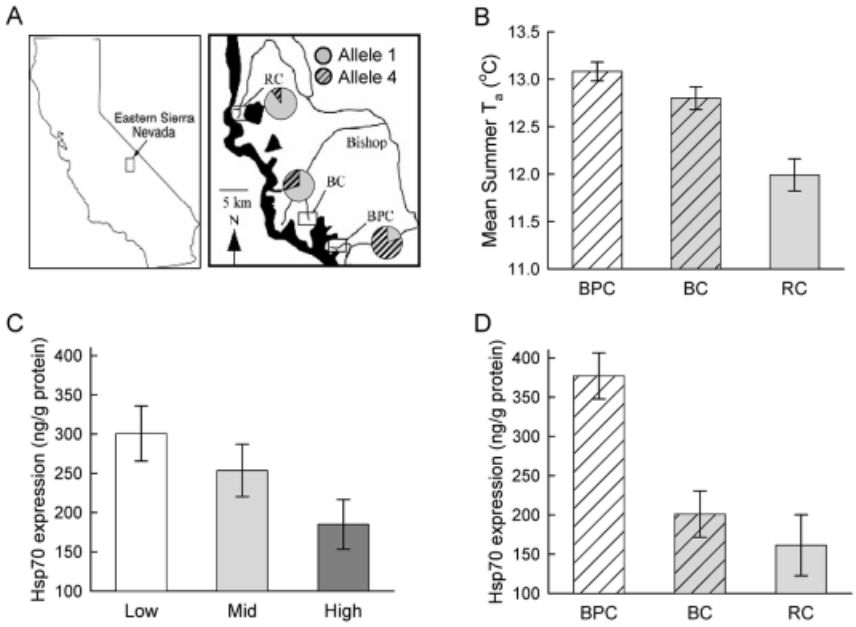

Figure 2. Genotype by environment interaction for heat shock protein (Hsp) expression in nature. (**A**) Genetic variation at the glycolyic enzyme locus *phosphoglucose isomerase* in Rock Creek (RC), Bishop Creek (BC) and Big Pine Creek (BPC); (**B**) Differences in environmental temperature between drainages. Data shown are least squares means (±SE) of average daily summertime air temperatures for 2000-2005, gathered using thermally-matched loggers deployed at five elevations in each drainage. (**C**) Hsp70 expression varies along a natural elevation gradient. Data shown are least squares means (±SE) of Hsp70 for *N*=12 beetles at low (2,750 m), mid (3,080 m) and high (3,270 m) elevation from sites in BPC, BC and RC. D: Differences in Hsp70 expression among drainages. Beetles were collected in early afternoon (1300-1400 h) and flash-frozen on dry ice immediately to preserve natural Hsp70 expression levels. Data shown are least squares means (±SE) of thorax muscle levels of a stress-inducible isoform of Hsp70, determined by Western blot analysis, for N= 36 beetles per drainage; Data modified from Dahlhoff and Rank (2000).

cold nights to over 35ºC during warm days in some lower elevation localities. An example of a trace of temperatures typically experienced by beetles over a summer at a mid-elevation site in BC is shown in figure 3. The consequences of this high degree of thermal heterogeneity on genetic variants in beetle populations and the heat shock response are discussed in detail below. Regional warming observed since 1998 has coincided with local extinction of populations at low elevations, especially in the warmest drainage BPC. In years prior to disappearance, Hsp70 expression levels of beetles living at low elevations in BPC were the highest measured in this species. Thus, environmental variability results in differential heat shock protein expression in native populations of Sierra willow beetles, as it does for other small ectotherms. In addition, high levels of Hsp expression may have negative consequences to fitness when environmental conditions become more stressful due to climate change.

5. Adaptive variation at a glycolytic enzyme locus

One of the unique aspects of the Sierra willow leaf beetle is that natural selection to temperature appears to act on the glycolytic enzyme locus *phosphoglucose isomerase* (PGI). Allele frequency variation at PGI across the biogrographic temperature gradient described above is much greater than for other polymorphic loci (Dahlhoff and Rank 2000, Rank 1992a, Rank and Dahlhoff 2002). Allele 1 (PGI-1) predominates in populations living in the northern drainage RC, and allele 4 (PGI-4) predominates in the southern drainage BPC (figure 2A). PGI allele 1 and 4 frequencies are intermediate in BC. Southern Sierra populations are also distinct from other populations in western North America. The PGI-1 allele predominates in Montana, Colorado, and further north in Sierra Nevada (Mount Dana, near Yosemite Park), whereas PGI-4 is at near fixation in the most southern location we have found these beetles (near Taboose Pass in King's Canyon National Park) (Fearnley 2003).

Annual and seasonal variation in climatic conditions causes shifts in beetle distribution, abundance and allele frequency variation at PGI, but not other polymorphic loci (Fearnley 2003, Rank and Dahlhoff 2002). We sampled allele frequency variation at five allozyme loci in populations in all three drainages in 1988 and 1996. During that time, the frequency of PGI-1 increased by 11% in BC, while PGI-4 decreased. These directional shifts did not occur at other polymorphic enzyme loci, suggesting that changes at PGI

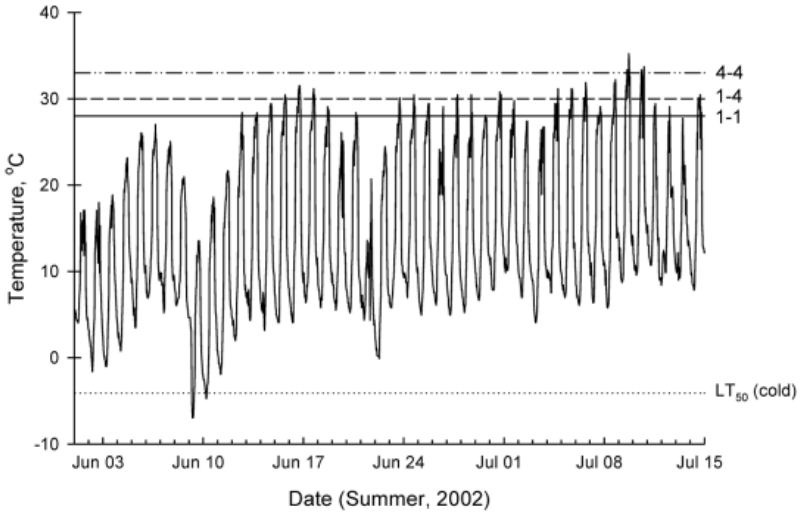

Figure 3. Environmental temperature variation routinely experienced in nature results in differential expression of Hsp70 among PGI genotypes at elevated temperatures, and occasional mortality after nighttime freezing. Temperature data shown were measured using a thermally-matched logger deployed at mid-elevation (3,130 m) in Bishop Creek, where both PGI alleles 1 and 4 are common, during summer 2002. Lines indicate temperatures of up-regulation of stress-inducible Hsp70 for PGI 1-1, 1-4 and 4-4 adult beetles, and LT_{50} cold, the temperature at which 50% of adults recently emerged from diapause die from cold exposure. Hsp70 expression and cold tolerance data modified from Rank and Dahlhoff (2002) and Neargarder *et al* (2003).

resulted from natural selection favoring the PGI-1 allele. In 1996, Eastern Sierra populations had recently re-colonized lower elevations after widespread declines during a dry period in late 1980's. The increased frequency of the PGI-1 allele may have resulted from cooler, wetter conditions that occurred during summers of 1995–96 before the second sampling period (Rank and Dahlhoff 2002). We have also observed rapid shifts in PGI allele frequency over short time scales. In a single summer (2001), PGI-1 increased in frequency by 5.6% early in the season (over-wintered adults to larvae), but decreased by 5.9% later in summer (larvae to new adults). The magnitude of the increase was related to daily maximum air temperature. Genotypic differences in fecundity or adult survival may have caused the initial increase in PGI-1 frequency, while differential larval or pupal survival may have caused its decrease. These data suggest that the relative fitness of PGI genotypes depends on life stage (Fearnley 2003). A possible outcome of such changes in relative fitness would be a net fitness advantage for heterozygotes (Mitton 1997), which could result in balancing selection and promote long-term maintenance of the PGI polymorphism.

6. The importance of genetic variation at PGI for the heat shock response

One of the most surprising findings of this work has been that Hsp70 induction temperature and total expression level varies among PGI genotypes (Dahlhoff and Rank 2000; Rank and Dahlhoff 2002; Neargarder *et al* 2003; McMillan *et al* 2005; Rank *et al* 2007). Differences in induction temperature among PGI genotypes occur at environmentally relevant temperatures (figure 3). PGI 1-1 individuals start to upregulate Hsp70 at 28ºC, a temperature routinely experienced while beetles are mating and larvae are developing, whereas PGI 4-4 individuals upregulate Hsp expression at temperatures rarely experienced in that habitat (33ºC). PGI 1-4 heterozygotes are intermediate. While we have not observed direct mortality due to elevated temperatures in nature, our own recent work (discussed below), along with other studies of ectotherms, have demonstrated that over-expression of Hsps may reduce performance or reproductive success (Patton and Krebs 2001; Roberts *et al* 2003; Sorensen and Loeschcke 2004; Folk and Gilchrist 2005). Differences in Hsp70 expression among PGI genotypes may be important for cold tolerance as well, as Hsps have been demonstrated to enhance cold hardiness in other insects (Chown 2001; Kelty and Lee 1999; Yiangou *et al* 1997; Yocum 2001). Though it may seem counterintuitive that a montane insect suffers cold mortality in summer, we have observed this phenomenon on several occasions (Rank 1994; McMillan *et al* 2005). Larval mortality in nature is related to minimum nighttime temperature, and larval survival is significantly lower in the coldest drainage RC than in BC or BPC. Survival after cold exposure differs among PGI genotypes (1-1>1-4>4-4) in adults and larvae (Rank and Dahlhoff 2002; Neargarder *et al* 2003). Because beetles experience exposure to cold

each night after several hours of exposure to extreme high temperatures, upregulation of Hsps during the day (which differs among PGI genotypes) may afford protection from cold in some individuals the following night. Thus, cold, like heat, is probably a significant selective force in these populations.

7. Adaptive significance of the PGI polymorphism to temperature adaptation in beetles

We have used this intriguing PGI polymorphism to study the consequences of naturally occurring genetic variation in a species adapted to physically challenging environments (figure 4). Allozyme loci provide excellent models to study this problem at multiple levels of biological organization. Mitton (1997) and Feder and Watt (1993) advocated a multi-tier approach to determining the adaptive significance of allozyme loci which includes investigations of (i) functional and biochemical properties of allozymes, (ii) physiological and performance consequences of differences in functional properties; (iii) the link between physiology, performance and components of reproductive success and (iv) a probable mechanism by which the polymorphism is maintained by selection in the population or interest. In this final section of the paper, we briefly describe our application of this approach to demonstrate a probable mechanism by which the PGI polymorphism is maintained in these habitats, and the relevance of the heat shock response at each level of organization.

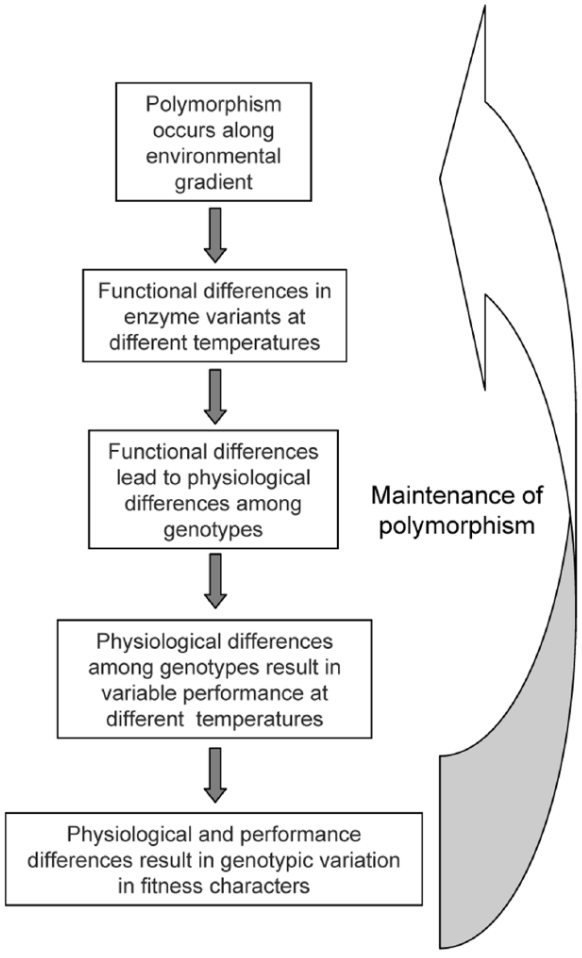

Figure 4. Willow beetles as a model for studying the adaptive significance of genetic variation in natural populations. Variation in Hsp expression along environmental temperature gradients and among *phosphoglucose isomerase* genotypes results in differential thermal tolerance among genotypes. Differences in Hsp expression are also linked to effects of temperature on running speed among genotypes, and to fitness characters like larval growth, survival and female fecundity. These interactions may ultimately cause the persistence of the PGI polymorphism in these populations.

7.1 *Functional differences among PGI allozymes.*

Our studies of PGI kinetics in *C. aeneicollis* have shown that there are small differences among PGI allozymes in the Michaelis-Menten binding constant, (K_m) and enzyme thermal stability (4-4 > 1-4 > 1-1). The K_m (which varies inversely with binding affinity) for the PGI allozyme common in RC (PGI 1-1) is greater at all measurement temperatures than PGI 4-4, the allozyme common in BPC (PGI 4-4; Dahlhoff and Rank 2000). PGI 1-4 heterozygotes showed intermediate K_m, especially at high measurement temperature. In addition, catalytic efficiency, indexed by V_{max}/K_m, is higher for 1-1 than 4-4 allozymes at moderate temperature (E P Dahlhoff, unpublished data). These data suggest that PGI allele 4 (a slow migrating allele) is more thermostable, and thus less efficient at moderate temperatures, than allele 1 (a fast-migrating allele). These results are consistent with other functional studies of PGI allozymes in ectotherms (Watt 1977, 1983 Hoffmann 1981a, 1981b). Homologous charge substitutions across multiple taxa could be responsible for the observation that PGI 'fast' alleles tend to make thermolabile allozymes, 'slow' alleles thermostable ones (Riddoch 1993; Wheat *et al* 2006).

Variation in thermostability among PGI alleles could be one mechanism driving differential expression of Hsps among PGI genotypes. After moderate heat stress (in the laboratory or in nature), individuals possessing the thermolabile PGI 1-1 allozyme have higher Hsp70 expression levels in thorax tissue than those possessing the more thermostable 4-4 allozyme. This may be in response to higher cellular levels of partially unfolded PGI, the concentration of which will depend on PGI genotype (1-1 > 1-4 > 4-4). While PGI is one of many cytosolic proteins that Hsp70 will chaperone, PGI is typically one of the most abundant glycolytic enzymes in the cell (Maughan *et al* 2005). In addition, recent studies of

the role of the heat shock response in targeting cancer cells for destruction by the mammalian immune system have demonstrated that Hsps act as "chaperokinins" to facilitate the presentation of tumor antigens to immune system cells (Asea 2005). One of the most common antigen sequences found on tumor cells is homologous with PGI (Yanagawa *et al* 2004; Funasaka *et al* 2005). While we have no evidence that there is a preferential response of Hsp70 to PGI over other glycolytic enzymes in willow beetles, these data are suggestive of a more general causal connection between Hsp70 and PGI.

7.2 *Differences in thermal tolerance among PGI genotypes*

Functional differences among PGI allozymes suggest that onset of a physiological response to elevated temperature should occur at lower temperatures for 1-1 individuals than for 1-4 or 4-4 individuals. Results for the physiological traits of Hsp70 expression and thermal tolerance are consistent with this prediction (Neargarder *et al* 2003, Rank and Dahlhoff 2002). We quantified thermal tolerance using critical thermal maximum (CT_{max}), the temperature at which an individual exposed to slowly increasing temperatures loses neuromuscular control, and LT_{50}, the temperature at which 50% of beetles did not survive a 4 hr exposure. Mean CT_{max} was 39.2°C for adults and LT_{50} for heat ranged from 35.7 to 39.2°C. For exposure to cold, LT_{50} ranged from –6.3 to –6.6°C. Larvae were less tolerant of thermal extremes than adults. Previous exposure to acutely cold or warm temperatures enhanced beetles' ability to survive subsequent exposure to extreme temperatures. Most importantly, CT_{max} values and survival after exposure to LT_{50} (heat and cold) were consistently related to PGI genotype for adults and larvae (1-1 > 1-4 > 4-4). Beetles with greatest tolerance of extreme heat or cold generally expressed higher levels of Hsp70, results consistent with other studies of thermotolerance in ectotherms (Feder *et al* 1996; Krebs 1999; Tomanek and Somero 1999). In addition, field data suggest that upregulation of Hsp70 after exposure to daytime high temperatures may increase tolerance of subsequent nighttime cold (McMillan *et al* 2005; Rank *et al* 2007).

7.3 *Differences in physiology and performance among PGI genotypes*

To investigate mechanisms by which physiological differences among PGI genotypes affect performance characters, especially after exposure to repeated thermal stress, we measured larval growth, metabolic rate, and adult and larval running speed. In all experiments, we collected beetles from a population where all three genotypes were relatively abundant, thus starting with naturally bred and reared individuals whose phenotype is shaped by interactions between genotype and natural environment. We found that differences among PGI genotypes existed for all three characters, but that the rank order of genotypes depended on treatment temperature (McMillan *et al* 2005; Rank *et al* 2007). For example, PGI 4-4 larvae grew faster than other genotypes when repeatedly exposed to a moderately elevated temperature (27°C), but not at lower temperatures. PGI genotypes also differed in effects of temperature on running speed. Adults possessing the PGI 1 allele ran faster than PGI 4-4 homozygotes in nature and after one exposure to extreme temperature, but repeated exposure to extreme temperature in adults, or a single extreme exposure for larvae, resulted in greater running speed in 4-4 homozygotes. In these beetles, Hsp70 expression depended on recent thermal history and on PGI genotype. However, the ranking of each PGI genotype with respect to Hsp70 expression depended on whether the animal had been exposed to a single stress or to repeated bouts of extreme temperature. Thus, phenotypic expression of genetic differences at PGI depended on recent environmental conditions experienced by the beetle, suggesting phenotypic plasticity in performance characters and the stress response.

7.4 *Fecundity differs among PGI genotypes*

We compared female PGI genotypes with respect to fecundity in the laboratory and field (Bruce 2005). In the laboratory under mild conditions (20ºC), PGI 1-1 females produced more eggs than PGI 4-4 females. However, at elevated temperatures (32ºC), PGI 1-1 female fecundity declined, while number of eggs laid by PGI 4-4 females increased. After several weeks' exposure to routine stress, Hsp70 expression levels tended to be higher in 4-4 than 1-1 genotypes, suggesting that PGI genotypes vary in protection for protein synthesis critical for egg production. At field sites in RC, PGI 1-1 individuals produced more eggs than PGI 4-4 individuals. However, in BPC, 4-4 individuals produced more eggs than 1-1 individuals, corresponding to the naturally occurring distribution of PGI alleles (figure 2A, map). These data are especially powerful since beetles used in field and laboratory experiments were from a single source population in BC where PGI allele frequencies are intermediate. Differences in fecundity, like differences in locomotor performance and thermal tolerance, may be responsible for the observed geographic divergence in PGI allele frequency.

8. Conclusions

In this paper, we have described environmental and phenotypic factors that influence expression of stress

proteins that buffer organisms from environmental change, and described model systems where the relationship between environment and stress protein expression have been well characterized. We have also shown that a model organism that possesses genetic variation needed to evolve in response to environmental change can be used to develop a more profound understanding of the adaptive significance of the heat shock response in nature. We hope that these studies provide new momentum to further research on the potential for organisms to evolve in response to environmental change, and that they also illustrate the limits of adaptive evolution to compensate for human-caused global warming.

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References

Angilletta M J, Niewiarowski P H and Navas C A 2002 The evolution of thermal physiology in ectotherms; *Thermal Biol.* **27** 249–268

Asea A 2005 Stress proteins and initiation of immune response: Chaperokine activity of Hsp72; *Exercise Immunol. Rev.* **11** 34–45

Balanya J, Oller J M, Huey R B, Gilchrist G W and Serra L 2006 Global genetic change tracks global climate warming in *Drosophila subobscura*; *Science* **313** 1773–1775

Barnosky A D, Hadly E A and Bell C J 2003 Mammalian response to global warming on varied temporal scales; *J. Mammal.* **84** 354–368

Bettencourt B R, Kim I, Hoffmann A A and Feder M E 2002 Response to natural and laboratory selection at the *Drosophila* hsp70 genes; *Evolution* **56** 1796–1801

Bradshaw W E, Zani P A and Holzapfel C M 2004 Adaptation to temperate climates; *Evolution* **58** 1748–1762

Brown W J 1956 The New World species of *Chrysomela* L. (Coleoptera: Chrysomelidae); *Can. Entomol.* **88** 1–54

Bruce D A 2005 *Effects of PGI genotype and temperature on fecundity, mating success and running speed of a Sierra willow leaf beetle;* Masters Thesis, Department of Biology, Sonoma State University, Rohnert Park. C A, USA

Cavicchi S, Guerra D, Latorre V and Huey R B 1995 Chromosomal Analysis of Heat-Shock Tolerance in *Drosophila melanogaste*r Evolving at Different Temperatures in the Laboratory; *Evolution* **49** 676–684

Chown S L 2001 Physiological variation in insects: hierarchical levels and implications; *J. Insect Physiol.* **47** 649–660

Clarke A 2003 Costs and consequences of evolutionary temperature adaptation; *Trends in Ecol. and Evol.* **18** 573–581

Dahlhoff E P 2004 Biochemical indicators of stress and metabolism: Applications for marine ecological studies; *Annu. Rev. Physiol.* **66** 183–207

Dahlhoff E P, Buckley B A and Menge B A 2001 Physiology of the rocky intertidal predator *Nucella ostrina* along an environmental stress gradient; *Ecology* **82** 2816–2829

Dahlhoff E P and Rank N E 2000 Functional and physiological consequences of genetic variation at phosphoglucose isomerase: heat shock protein expression is related to enzyme genotype in a montane beetle; *Proc. Natl. Acad. Sci. USA* **97** 10056–10061

de Jong P W, Gussekloo S W S and Brakefield P M 1996 Differences in thermal balance, body temperature and activity between non-melanic and melanic two-spot ladybird beetles (*Adalia bipunctata*) under controlled conditions; *J. Exp. Biol.* **199** 2655–2666

Denny M W, Miller L P and Harley C D G 2006 Thermal stress on intertidal limpets: long-term hindcasts and lethal limits; *J. Exp. Biol.* **209** 2420–2431

Duffy J E and Stachowicz J J 2006 Why biodiversity is important to oceanography: potential roles of genetic, species, and trophic diversity in pelagic ecosystem processes; *Mar. Ecol. Prog. Series* **311** 179–189

Easterling D R, Meehl G A, Parmesan C, Changnon S A, Karl T R and Mearns L O 2000 Climate extremes: observations, modeling, and impacts; *Science* **289** 2068–2074

Ellers J and Boggs C L 2004 Functional ecological implications of intraspecific differences in wing melanization in *Colias* butterflies; *Biol. J. Linn. Soc.* **82** 79–87

Fearnley S L 2003 *Adaptation at an enzyme locus in Chrysomela aeneicollis: situating the PGI polymorphism in a functional and historical context*; Masters Thesis, Department of Biology, Sonoma State University, Rohnert Park. C A, USA

Feder M, Bedford T, Albright D and Michalak P 2002 Evolvability of Hsp70 expression under artificial selection for inducible thermotolerance in independent populations of *Drosophila melanogaster*; *Physiol. Biochem. Zool.* **75** 325–334

Feder M E, Cartano N V, Milos L, Krebs R A and Lindquist S L 1996 Effect of engineering Hsp70 copy number on Hsp70 expression and tolerance of ecologically relevant heat shock in larvae and pupae of *Drosophila melanogaster*; *J. Exp. Biol.* **199** 1837–1844

Feder M E and Hofmann G E 1999 Heat-shock proteins, molecular chaperones, and the stress response: Evolutionary and ecological physiology; *Annu. Rev. Physiol.* **61** 243–282

Feder M E and Watt W B 1993 Functional biology of adaptation; in *Genes in ecology* (eds) R J Berry, T J Crawford and G M Hewitt (Oxford: Blackwell) pp 365–392

Fitzhenry T, Halpin P M and Helmuth B 2004 Testing the effects of wave exposure, site, and behavior on intertidal mussel body temperatures: applications and limits of temperature logger design; *Mar. Biol.* **145** 339–349

Folk D G and Gilchrist G W 2005 Heat-shock response and locomotory performance in *Drosophila* populations selected for divergent knockdown temperatures; *Integr. Comp. Biol.* **45** 955–1103 doi: 10.1093/icb/45.6.955

Forsman A, Ringblom K, Civantos E and Ahnesjo J 2002 Coevolution of color pattern and thermoregulatory behavior in polymorphic pygmy grasshoppers *Tetrix undulata*; *Evolution* **56** 349–360

Funasaka T, Yanagawa T, Hogan V and Raz A 2005 Regulation of phosphoglucose isomerase/autocrine motility factor expression by hypoxia; *FASEB J.* **19** 1422–1430

Garbuz D, Evgenev M, Feder M E and Zatsepina O 2003 Evolution of thermotolerance and the heat-shock response: evidence from inter/intraspecific comparison and interspecific hybridization in the *virilis* species group of *Drosophila*. I. Thermal phenotype; *J. Exp. Biol.* **206** 2399–2408

Gilchrist G W and Huey R B 2003 Is plasticity an adaptation? Body size as a function of temperature within and among parallel clines in *Drosophila subobscura; Integr. Comp. Biol.* **43** 841

Gilman S E, Wethey D S and Helmuth B 2006 Variation in the sensitivity of organismal body temperature to climate change over local and geographic scales; *Proc. Natl. Acad. Sci. USA* **103** 9560–9565

Harley C D G, Hughes A R, Hultgren K M, Miner B G, Sorte C J B, Thornber C S, Rodriguez L F, Tomanek L and Williams S L 2006 The impacts of climate change in coastal marine systems; *Ecol. Lett.* **9** 228–241

Hazel J R 1995 Thermal adaptation in biological membranes: Is homeoviscous adaptation the explanation?; *Annu. Rev. Physiol.* **57** 19–42

Hazel W N 2002 The environmental and genetic control of seasonal polyphenism in larval color and its adaptive significance in a swallowtail butterfly; *Evolution* **56** 342–348

Helmuth B 1999 Thermal biology of rocky intertidal mussels: Quantifying body temperatures using climatological data; *Ecology* **80** 15–34

Helmuth B 2002 How do we measure the environment? Linking intertidal thermal physiology and ecology through biophysics; *Integr. Comp. Biol.* **42** 837–845

Helmuth B, Broitman B R, Blanchette C A, Gilman S, Halpin P, Harley C D G, O'Donnell M J, Hofmann G E, Menge B A and Strickland D 2006 Mosaic patterns of thermal stress in the rocky intertidal zone: Implications for climate change; *Ecol. Monogr.* **76** 461–479

Helmuth B, Harley C D G, Halpin P M, O'Donnell M, Hofmann G E and Blanchette C A 2002 Climate change and latitudinal patterns of intertidal thermal stress; *Science* **298** 1015–1017

Helmuth B, Kingsolver J G and Carrington E 2005 Biophysics, physiological ecology, and climate change: Does mechanism matter?; *Annu. Rev. Physiol.* **67** 177–201

Helmuth B S T and Hofmann G E 2001 Microhabitats, thermal heterogeneity, and patterns of physiological stress in the rocky intertidal zone; *Biol. Bull* **201** 374–384

Hill J K, Hughes C L, Dytham C and Searle J B 2006 Genetic diversity in butterflies: interactive effects of habitat fragmentation and climate-driven range expansion; *Biol. Lett.* **2** 152–154

Hill J K, Thomas C D, Fox R, Telfer M G, Willis S G, Asher J and Huntley B 2002 Responses of butterflies to twentieth century climate warming: implications for future ranges; *Proc. R. Soc. London Series B Biol. Sci.* **269** 2163–2171

Hoffmann A A, Hallas R J, Dean J A and Schiffer M 2003 Low potential for climatic stress adaptation in a rainforest *Drosophila* species; *Science* **301** 100–102

Hoffmann R J 1981a Evolutionary genetics of *Metridium senile*. I. Kinetic differences in phosphoglucose isomerase E.C. 5.3.1.9 allozymes; *Biochem. Genet.* **19** 129–144

Hoffmann R J 1981b. Evolutionary genetics of *Metridium senile*. II. Geographic patterns of allozyme variation; *Biochem. Genet.* **19** 145–154

Hofmann G E 2005 Patterns of Hsp gene expression in ectothermic marine organisms on small to large biogeographic scales; *Integr. Comp. Biol.* **45** 247–255

Huey R B 1991 Physiological Consequences of Habitat Selection; *Am. Nat.* **137** S91–S115

Inouye D W, Barr B, Armitage K B and Inouye B D 2000 Climate change is affecting altitudinal migrants and hibernating species; *Proc. Nat. Acad. Sci. USA* **97** 1630–1633

Jump A S, Hunt J M, Martinez-Izquierdo J A and Penuelas J 2006 Natural selection and climate change: temperature-linked spatial and temporal trends in gene frequency in *Fagus sylvatica*; *Mol. Ecol.* **15** 3469–3480

Kelty J D and Lee R E 1999 Induction of rapid cold hardening by cooling at ecologically relevant rates in *Drosophila melanogaster*; *J. Insect Physiol.* **45** 719–726

Kingsolver J G 1979 Thermal and Hydric Aspects of Environmental Heterogeneity in the Pitcher Plant Mosquito; *Ecol. Monogr.* **49** 357–376

Kingsolver J G and Huey R B 1998 Evolutionary analyses of morphological and physiological plasticity in thermally variable environments; *Am. Zool.* **38** 545–560

Kingsolver J G, Izem R and Ragland G J 2004 Plasticity of size and growth in fluctuating thermal environments: Comparing reaction norms and performance curves; *Integr. Comp. Biol.* **44** 450–460

Kingsolver J G and Wiernasz D C 1991 Seasonal Polyphenism in Wing-Melanin Pattern and Thermoregulatory Adaptation in *Pieris* Butterflies; *Am. Nat.* **137** 816–830

Krebs R A 1999 A comparison of Hsp70 expression and thermotolerance in adults and larvae of three *Drosophila* species; *Cell Stress Chaperones* **4** 243–249

Krebs R A and Bettencourt B R 1999 Evolution of thermotolerance and variation in the heat shock protein, Hsp70; *Am. Zool.* **39** 910–919

Krebs R A and Feder M E 1997 Deleterious consequences of Hsp70 overexpression in *Drosophila melanogaster* larvae; *Cell Stress Chaperones* **2** 60–71

Krebs R A and Feder M E 1998 Hsp70 and larval thermotolerance in *Drosophila melanogaster*: how much is enough and when is more too much?; *J. Insect Physiol.* **44** 1091–1101

Krebs R A and Holbrook S H 2001 Reduced enzyme activity following Hsp70 overexpression in *Drosophila melanogaster*; *Biochem. Genet.* **39** 73–82

Lindquist S 1986 The heat-shock response; *Annu. Rev. Biochem.* **55** 1151–1191

Loeschcke V, Krebs R A, Dahlgaard J and Michalak P 1997 High-temperature stress and the evolution of thermal resistance in *Drosophila*; (ed.) Bijlsma R L, in *Experientia Supplementum (Basel); Environmental stress, adaptation and evolution*, (Basel) pp 175–190

Maughan D W, Henkin J A and Vigoreaux J O 2005 Concentrations of glycolytic enzymes and other cytosolic proteins in the diffusible fraction of a vertebrate muscle proteome; *Mol. Cell. Proteomics* **4** 1541–1549

McMillan D M, Fearnley S L, Rank N E and Dahlhoff E P 2005 Natural temperature variation affects larval survival, development and Hsp70 expression in a leaf beetle; *Funct. Ecol.* **19** 844–852

Mitton J B 1997 *Selection in natural populations* (New York: Oxford University Press)

Neargarder G, Dahlhoff E P and Rank N E 2003 Variation in thermal tolerance is linked to phosphoglucose isomerase genotype in a montane leaf beetle; *Funct. Ecol.* **17** 213–221

Nielsen M G and Watt W B 1998 Behavioural fitness component effects of the alba polymorphism of *Colias* (Lepidoptera, Pieridae): resource and time budget analysis; *Funct. Ecol.* **12** 149–158

Parmesan C and Yohe G 2003 A globally coherent fingerprint of climate change impacts across natural systems; *Nature (London)* **421** 37–42

Parsell D A and Lindquist S 1993 The function of heat-shock proteins in stress tolerance: degradation and reactivation of damaged proteins; *Annu. Rev. Genet.* **27** 437–496

Patton Z J and Krebs R A 2001 The effect of thermal stress on the mating behavior of three *Drosophila* species; *Physiol. Biochem. Zool.* **74** 783–788

Price T D 2006 Phenotypic plasticity, sexual selection and the evolution of colour patterns; *J. Exp. Biol.* **209** 2368–2376

Rank N E, Bruce D A, McMillan D M, Barclay C and Dahlhoff E P 2007 Phosphoglucose isomerase genotype affects running speed and heat shock protein expression after exposure to extreme temperatures in a montane willow beetle; *J. Exp. Biol.* **210** 750–764

Rank N E 1992a A hierarchical analysis of genetic differentiation in a montane leaf beetle (*Chrysomela aeneicollis*); *Evolution* **46** 1097–1111

Rank N E 1992b Host plant preference based on salicylate chemistry in a willow leaf beetle (*Chrysomela aeneicollis*); *Oecologia (Berlin)* **90** 95–101

Rank N E 1994 Host plant effects on larval survival in a salicin-using leaf beetle *Chrysomela aeneicollis* (Coleoptera: Chrysomelidae); *Oecologia (Berlin)* **97** 342–353

Rank N E and Dahlhoff E P 2002 Allele frequency shifts in response to climate change and physiological consequences of allozyme variation in a montane insect; *Evolution* **56** 2278–2289

Riddoch B J 1993 The adaptive significance of electrophoretic mobility in phosphoglucose isomerase (PGI); *Biol. J. Linn. Soc.* **50** 1–17

Roberts D A, Hofmann G E and Somero G N 1997 Heat-shock protein expression in *Mytilus californianus*: Acclimatization (seasonal and tidal-height comparisons) and acclimation effects; *Biol. Bull.* **192** 309–320

Roberts S P and Feder M E 1999 Natural hyperthermia and expression of the heat shock protein Hsp70 affect developmental abnormalities in *Drosophila melanogaster*; *Oecologia* **121** 323–329

Roberts S P, Marden J H and Feder M E 2003 Dropping like flies: Environmentally induced impairment and protection of locomotor performance in adult *Drosophila melanogaster*; *Physiol. Biochem. Zool.* **76** 615–621

Robertson R M 2004 Thermal stress and neural function: adaptive mechanisms in insect model systems; *J. Thermal Biol.* **29** 351–358

Rutherford S L 2003 Between genotype and phenotype: Protein chaperones and evolvability; *Nat. Rev. Genet.* **4** 263–274

Rutherford S L and Lindquist S 1998 Hsp90 as a capacitor for morphological evolution; *Nature (London)* **396** 336–342

Sagarin R D, Barry J P, Gilman S E and Baxter C H 1999 Climate-related change in an intertidal community over short and long time scales; *Ecol. Monogr.* **69** 465–490

Sagarin R D and Somero G N 2006 Complex patterns of expression of heat-shock protein 70 across the southern biogeographical ranges of the intertidal mussel *Mytilus californianus* and snail *Nucella ostrina*; *J. Biogeogr.* **33** 622–630

Smiley J T and Rank N E 1986 Predator protection versus rapid growth in a montane leaf beetle; *Oecologia* **70** 106–112

Somero G N 1995 Proteins and temperature; *Annu. Rev. Physiol.* **57** 43–68

Sorensen J G, Kristensen T N and Loeschcke V 2003 The evolutionary and ecological role of heat shock proteins; *Ecol. Lett.* **6** 1025–1037

Sorensen J G and Loeschcke V 2004 Effects of relative emergence time on heat stress resistance traits, longevity and hsp70 expression level in *Drosophila melanogaster*; *J. Thermal Biol.* **29** 195–203

Sorte C J B and Hofmann G E 2004 Changes in latitudes, changes in aptitudes: *Nucella canaliculata* (Mollusca : Gastropoda) is more stressed at its range edge; *Mar. Ecol. Prog. Series* **274** 263–268

Sorte C J B and Hofmann G E 2005 Thermotolerance and heat-shock protein expression in Northeastern Pacific *Nucella* species with different biogeographical ranges; *Mar. Biol.* **146** 985–993

Tomanek L 2002 The heat-shock response: Its variation, regulation and ecological importance in intertidal gastropods (genus *Tegula*); *Integr. Comp. Biol.* **42** 797–807

Tomanek L 2005 Two-dimensional gel analysis of the heat-shock response in marine snails (genus *Tegula*): interspecific variation in protein expression and acclimation ability; *J. Exp. Biol.* **208** 3133–3143

Tomanek L and Somero G N 1999 Evolutionary and acclimation-induced variation in the heat-shock responses of congeneric marine snails (genus *Tegula*) from different thermal habitats: Implications for limits of thermotolerance and biogeography; *J. Exp. Biol.* **202** 2925–2936

Tomanek L and Somero G N 2002 Interspecific- and acclimation-induced variation in levels of heat-shock proteins 70 (hsp70)

and 90 (hsp90) and heat-shock transcription factor-1 (HSF1) in congeneric marine snails (genus *Tegula*): implications for regulation of hsp gene expression; *J. Exp. Biol.* **205** 677–685

Walther G R, Post E, Convey P, Menzel A, Parmesan C, Beebee T J C, Fromentin J M, Hoegh-Guldberg O and Bairlein F 2002 Ecological responses to recent climate change; *Nature (London)* **416** 389–395

Watanabe J M 1984 The Influence of Recruitment, Competition, and Benthic Predation on Spatial Distributions of 3 Species of Kelp Forest Gastropods (Trochidae, *Tegula*); *Ecology* **65** 920–936

Watt W B 1977 Adaptation at specific loci. I. Natural selection on *phosphoglucose isomerase* of *Colias* butterflies: biochemical and population aspects; *Genetics* **87** 177–194

Watt W B 1983 Adaptation at specific loci. II. Demographic and biochemical elements in the maintenance of the *Colias* pgi polymorphism; *Genetics* **103** 691–724

Wheat C W, Watt W B, Pollock D D and Schulte P M 2006 From DNA to fitness differences: Sequences and structures of adaptive variants of *Colias* phosphoglucose isomerase (PGI); *Mol. Biol. Evol.* **23** 499–512

Willmer P, Stone G and Johnston I 2004 *Environmental physiology of animals* (Oxford: Blackwell Science)

Yanagawa T, Funasaka T, Tsutsumi S, Watanabe H and Raz A 2004 Novel roles of the autocrine motility factor/phosphoglucose isomerase in tumor malignancy; *Endocrine-Related Cancer* **11** 749–759

Yiangou M, Tsapogas P, Nikolaidis N and Scouras Z G 1997 Heat shock gene expression during recovery after transient cold shock in *Drosophila auraria* (Diptera: Drosophilidae); *Cytobios* **92** 91–98

Yocum G D 2001 Differential expression of two HSP70 transcripts in response to cold shock, thermoperiod, and adult diapause in the Colorado potato beetle; *J. Insect Physiol.* **47** 1139–1145

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