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Physiological Adjustments to Fluctuating Thermal Environments: An Ecological and Evolutionary Perspective

Raymond B. Huey
Department of Zoology NJ-15
University of Washington
Seattle, Washington 98195

Albert F. Bennett
School of Biological Sciences
University of California
Irvine, California 92717

I. INTRODUCTION

The physical environment of organisms is not constant but changes through time and space. Major environmental changes cause stress, damage, or even death to organisms, but even minor changes may hamper the physiological capacities of organisms to grow, reproduce, or interact socially. Not surprisingly, organisms have evolved a variety of adjustments (e.g., behavior, acclimation, heat stress response) that help buffer the physiological impact of environmental change. These adjustments, which can have a profound effect on evolutionary fitness, can be studied from diverse perspectives. For example, what molecular and physiological bases underlie a given adjustment? What ecological and physiological circumstances favor its evolution and use?

In this paper, we adopt an ecological and evolutionary framework that has been developed to study general adjustments that organisms use to

Stress Proteins in Biology and Medicine.

buffer natural environmental variation (Levins 1968; Slobodkin and Rapoport 1974; Hochachka and Somero 1984). We apply this framework in an analysis of adjustments specifically associated with heat stress. We begin with a brief discussion of the general effects of temperature on organisms. Then, for each class of adjustment to stress, we evaluate the ecological circumstances favoring its use, describe some organismal examples from the comparative literature, and also evaluate its relevance to studies of induced aspects of the heat stress response. Our primary goal is to encourage an expanded dialogue between biologists studying the heat stress response and those interested in ecological and evolutionary physiology. As we will argue, both groups have much to contribute to each other.

That organisms are sometimes exposed to potentially stressful conditions is well illustrated with canyon lizards (*Sceloporus merriami*) living in Big Bend National Park in southern Texas (Grant and Dunham 1988). Big Bend can be very hot in midsummer, and canyon lizards die if their body temperature reaches 40–42°C. Throughout most of the day, a lizard that emerged from the relative coolness of its crevice would quickly overheat and die. Indeed, equilibrium body temperatures of these lizards exceed their upper lethal temperatures on more than 90% of the available ground surface (Fig. 1). For canyon lizards in summer, heat stress is a daily risk.

Heat stress can even occur in seemingly cold environments. For example, humans usually think of alpine areas as being cold. However, on cloudless days at 4000 meters in the Rocky Mountains, radiation loads can be intense: *Colias* butterflies suffer heat stress if the cooling winds die for more than a few minutes (Kingsolver and Watt 1983)! Heat stress can be a problem even for organisms not restricted to deserts or hot springs. Stress depends not only on local meteorological conditions, but also on the behavior, morphology, and physiology of a particular organism (Bartholomew 1958, 1964).

II. THERMAL EFFECTS AND ORGANISMAL RESPONSES

Body temperature has a profound effect on biological processes, particularly on rates of those processes. Even changes on the order of only 1°C may cause the rates of biological reactions to change by nearly 10% (Hochachka and Somero 1984; Prosser 1986; Cossins and Bowler 1987). Larger fluctuations can disrupt metabolic pathways and may lead to rapid death. Regulation of temperature or adaptation to temperature change is therefore crucial to all organisms.

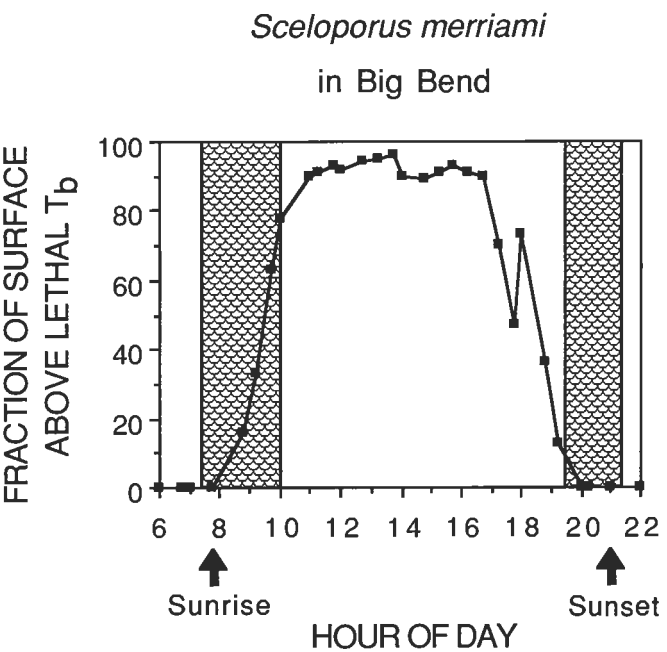

Figure 1 On a clear summer day in Big Bend National Park, Texas, equilibrium body temperatures for the canyon lizard (*Sceloporus merriami*) would exceed the lizard's lethal temperature on most of the ground surface. Lizards restrict their activity on the ground surface to early and late in the day ("scaly" regions). (Original data courtesy of B.W. Grant and A.E. Dunham.)

The range of body temperatures within which an ectothermic organism can live is often called its "tolerance zone." Within that zone, an organism's physiological capacities increase with body temperature up to a maximal value (the "optimal" body temperature) and then rapidly decrease as the organism approaches the highest temperatures it can survive (Fig. 2). The thermal sensitivity of many physiological processes shows this general pattern (Fig. 3).

Organisms cope with the profound effects of temperature on physiological capacities in two general ways. One solution involves regulation of body temperature (i.e., thermoregulation), which enables an organism to gain some control over its physiological capacities as well as to avoid extreme temperatures. The complementary solution involves the reorganization of an organism's thermal sensitivity: This solution includes both short-term (e.g., acclimation or acclimatization) and long-term (evolutionary adaptation) processes.

Many animals go to great lengths to regulate body temperatures. Mammals and birds ("endotherms"), for example, expend major portions

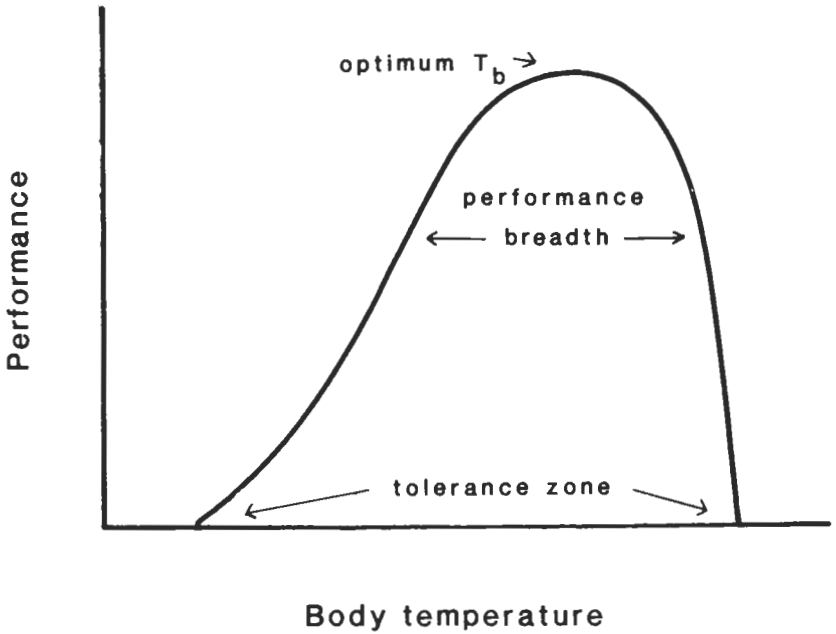

Figure 2 Effect of body temperature on the physiological performance capacity of a hypothetical ectotherm. (Modified, with permission, from Huey and Stevenson 1979.)

of their total energy budgets to stabilize body temperature metabolically (Cossins and Bowler 1987). Many arthropods, fish, and reptiles ("ectotherms") behaviorally regulate body temperature (Cowles and Bogert 1944; Casey 1981) at levels near optimal for physiological functioning (Fig. 3).

Under certain circumstances, the thermoregulatory abilities—metabolic or behavioral—of organisms are inadequate or inappropriate, but animals can still buffer environmental fluctuations by adjusting the thermal sensitivity of their physiological capacities. Comparative physiologists (Prosser 1986) often distinguish between adjustments that affect survival at extreme temperatures ("resistance" adaptations) and those that affect physiological capacities at non-extreme body temperatures ("capacity" adaptations). Included among these capacity and resistance adaptations are acclimation responses, developmental effects, and evolutionary adaptations.

III. TIME SCALES AND SEVERITY OF FLUCTUATIONS

To understand the types of adjustments to environmental fluctuations, one must consider the time scales both of the fluctuation and of the ad-

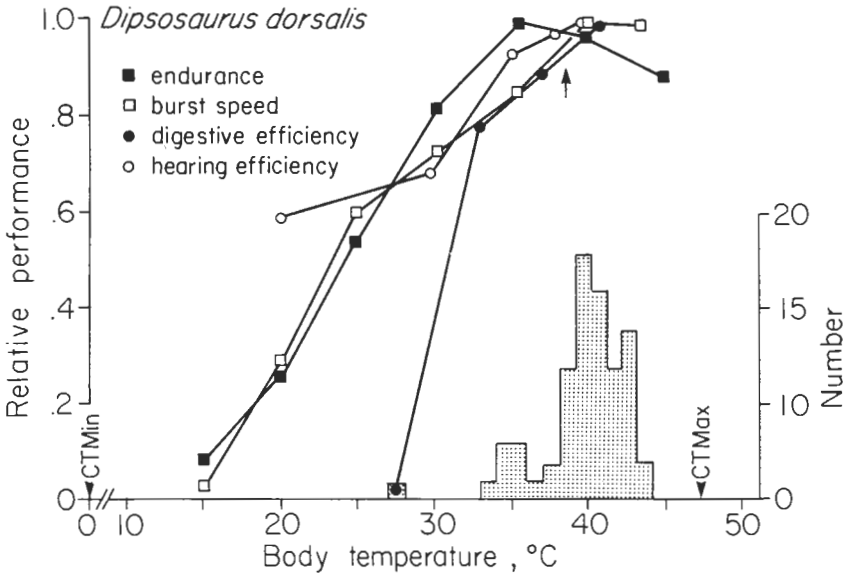

Figure 3 Sensitivity of several physiological functions to body temperature in a lizard, the desert iguana *Dipsosaurus dorsalis*. The body temperatures of lizards in nature (histogram) or in a laboratory thermal gradient (up arrow) enable the lizards to perform at or near maximal physiological capacities. Critical thermal limits (loss of righting response) are also shown (down arrow). Original sources of data in Huey (1982). (Modified, with permission, from Huey 1982.)

justment (Levins 1968; Slobodkin and Rapoport 1974; Hochachka and Somero 1984). An adjustment that takes a week to activate is clearly inappropriate if the stress itself is likely to last only for an hour or two (Levins 1968; Hochachka and Somero 1984). Thus, the response should be matched in time with the environmental fluctuation. Moreover, the magnitude of the response should be matched to the potential severity of the stress: Mobilizing a major physiological response may prove maladaptive if the stress is relatively minor (Palumbi 1984).

Fortunately for organisms, both the magnitude and duration of environmental fluctuations are sometimes predictable. For example, organisms can use environmental cues (e.g., photoperiod) to anticipate general environmental shifts associated with daily and seasonal cycles in temperature.

The various adjustments animals use to deal with the environmental fluctuations have various lag times (Hochachka and Somero 1984; Prosser 1986): Some are activated instantaneously, but others take days or even weeks. To some extent, variation in response time of different physiological adjustments is probably adaptive. For example, fast-acting

responses can be activated quickly to cope with brief, but still dangerous perturbations (Palumbi 1984). However, major physiological adjustments such as acclimatization, which may be metabolically expensive, are activated only by sustained environmental fluctuations, such as those associated with weather fronts or especially with seasons. Thus, differences in response times among adjustments effectively enable an organism to deal with variation in the likely persistence and seriousness of a given environmental fluctuation.

IV. RESPONSES TO SHORT-TERM FLUCTUATIONS

Short-term thermal fluctuations, those on the order of a minute or a few hours, may not even be noticed by large endotherms or even ectotherms (e.g., Komodo dragons, Galapagos tortoises), whose body size is an effective thermal buffer (Spotila et al. 1973; Stevenson 1985). However, short-term fluctuations can be very stressful to small organisms (e.g., copepods, fruitflies, butterflies, and lizards), because their body temperatures equilibrate quickly with environmental temperatures. Adjustments that deal effectively with short-term fluctuations must respond quickly, otherwise the fluctuation might have done its damage by the time the compensatory adjustment is activated. Several behavioral and physiological adjustments meet this criterion of a fast response time (Table 1).

Simple behavioral adjustments can be effective in helping animals avoid short-term stress. For example, *Sceloporus merriami* in Big Bend avoid stress during the summer by restricting above-ground activity to very early and very late in the day, the only times when much of the ground surface area is relatively cool (Fig. 1). During the heat of the day, the lizards retreat into crevices and caves (Grant and Dunham 1988). Ground squirrels (*Xerus inauris*) use their fluffy tails as a parasol on hot days in the Kalahari desert of Africa but must still shuttle to cool burrows at midday (Bennett et al. 1984). Behavior is often the most effective way of avoiding stress in a dangerously hot locality (Bartholomew 1958, 1964; Stevenson 1986).

Animals may supplement behavioral adjustments with fast-acting ones involving physiology and morphology. The first four of these responses (Table 1) can quickly change rates of heating and cooling or can change the equilibrium temperature of an animal (Turner and Tracy 1983; Stevenson 1986), but they do not directly alter the way that temperature influences physiological performance. In contrast, the last three adjustments do modify the effect of temperature on physiology. Because these last two adjustments are of special relevance to the theme of this book, we discuss them in the next section.

Table 1 Behavioral and physiological adjustments to short-term temperature fluctuations

Behavioral adjustments	regulate time of activity—avoid times of extreme conditions
	shuttling—regulate time in sun versus shade
	postural changes—change surface area exposed to heat sources or sinks
Physiological and morphological adjustments	circulatory adjustments—vasoconstriction vasodilation
	evaporative cooling—panting
	color change—alter reflectivity
	thermogenesis
	tolerance of hypothermia and of hyperthermia
	immediate compensation
	heat hardening
	heat shock response

For elaboration on behavioral adjustments, see Stevenson (1986); on physiological adjustments, see Bartholomew (1982) and Cossins and Bowler (1987).

V. HEAT HARDENING AND THE HEAT STRESS RESPONSE

"Heat hardening," a transient increase in the heat tolerance of an organism, is stimulated by briefly preexposing the animal to a near-lethal temperature (Hutchison and Maness 1979). This is an organismal-level phenomenon, and its mechanistic bases are unclear. The "heat stress response," which can also be triggered by brief exposure to high temperature (or by other environmental stimuli, Lindquist 1986), is also associated with an increase in heat tolerance. However, the heat stress response is a cellular-level phenomenon that specifically involves the induced synthesis of some proteins and often the repression of synthesis of others (Ashburner and Bonner 1979). It appears to have repair and protective functions (Li and Werb 1982; Pelham 1986; Schlesinger 1986; Landry et al. 1987; Riabowol et al. 1988; Chapters 1, 4, 10, 12, 14). Both heat hardening and the heat stress response have very fast response times, sometimes as fast as 15–30 minutes (Burns et al. 1986; Easton et al. 1987); both are transient phenomena. Moreover, both phenomena are activated by exposure to high body temperature (although the heat stress response is activated by many factors). In effect, these induced responses appear to reflect last-ditch, go-for-broke responses to short but dangerous environmental conditions. However, some salamanders in nature voluntarily expose themselves to temperatures high enough to induce hsp70 mRNA (Easton et al. 1988), suggesting that the adaptive bases of the heat stress response may be complex.

Studies of heat hardening seem to have developed largely independently from those of the heat stress response. Attention to heat hardening has come largely from organismal biologists (Hutchison and Maness 1979). An example of heat hardening in a salamander is shown in Figure 4. Easton and his co-workers (1987) have shown that the temperature necessary to induce heat spasms can be increased transiently by briefly exposing a salamander to a temperature 2°C below that which normally induces spasms. Interestingly, heat hardening can also be induced by exposing the salamander for 1 hour to temperatures as low as 5°C below the spasm temperature (P.S. Rutledge, pers. comm.). It is tempting to speculate that the heat shock response is the primary physiological mechanism behind heat hardening. However, several studies show that the acquisition of organismal heat tolerance can be uncoupled from hsp synthesis (Burns et al. 1986; Easton et al. 1987; Hightower and Renfro 1988). Thus, heat hardening and the heat stress response may sometimes be mechanistically unrelated.

To an evolutionary physiologist, two aspects of the heat stress response are striking. First, the omnipresence and remarkable conservatism of many heat stress proteins (at least the hsp70 family, Bienz 1984; Lindquist 1986) implies either (1) that stress is a common and universal experience of organisms or (2) that the proteins also play a fundamental role in normal physiological responses (Chapters 10, 12, 14). Otherwise, protein sequences would have long ago diverged by genetic drift. In any

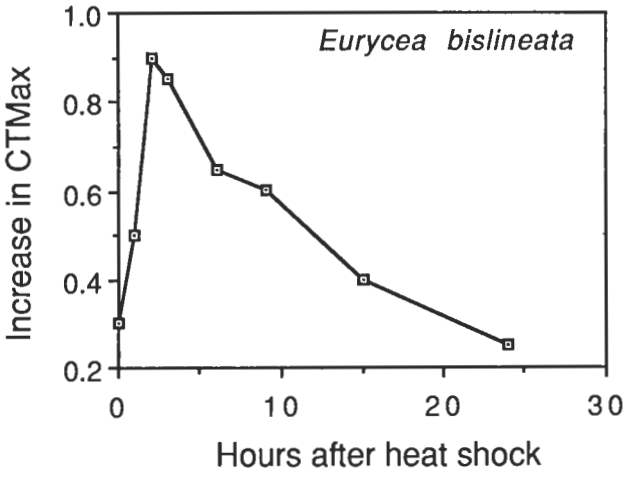

Figure 4 Heat hardening (i.e., increase in critical thermal maximum, CTM, measured as onset of spasms) in the salamander *Eurycea bislineata* following a 1-hr exposure to a temperature 2°C below an individual's (previously established) CTM. (Modified, with permission, from Easton et al. 1987.)

case, the conservatism in protein sequence strongly implies the response arose very early in evolution. Although the evolutionary importance of stress has long been recognized (Haldane 1932; Kingsolver and Watt 1983; Parsons 1983, 1987), the omnipresence of the heat stress response encourages organismal and evolutionary physiologists to pay closer attention to the role of stress in physiological adaptation. Second, the observation that some heat stress proteins are not routinely expressed is interesting, given the possible thermoprotective function they serve (Chapters 1, 4, 12, 14). A priori, one might indeed expect that organisms would maintain heat stress proteins as a "prophylactic" against stress. The nonconstitutive nature of some heat stress proteins might reflect either the excessive metabolic costs of maintaining the proteins (see Levins 1968, p. 11) or perhaps some detrimental effects on normal physiological functioning.

Nevertheless, heat shock proteins are present in some animals even in the apparent absence of a heat stress. For example, some heat stress proteins are seemingly constitutive in *Fundulus heteroclitus* (M. Koban, pers. comm.), a fish living in salt marshes and estuaries where temperatures frequently become very hot. Koban and his co-workers speculate that these proteins may provide the fish with "anticipatory" protection against sudden heat shock.

VI. RESPONSES TO SEASONAL FLUCTUATIONS

Some environmental fluctuations take place over a week or so (a weather front) or months (seasonal changes). For fluctuations of such length, the types of short-term adjustments discussed in the previous section would be inappropriate or perhaps inadequate. Accordingly, organisms can supplement these short-term adjustments with others that reversibly alter the animal's sensitivity to temperature. Such physiological reorganizations are called acclimation (in controlled laboratory environments) or acclimatization (in nature) responses.

The thermal sensitivities of many physiological processes can be radically shifted during acclimation, thereby compensating for the effects of temperature. For example, during chronic exposure to low temperature, many ectotherms increase metabolic rates, in effect thereby compensating for the depressing effects of cold on metabolism (Precht et al. 1973; Prosser 1986). Thus, when measured at common body temperatures, processing rates of cold-acclimated individuals are often greater than those of warm-acclimated ones. (Not all acclimation responses are "compensatory," and the presumed significance of these alternative responses is discussed in Tsuji [1988].) Acclimation is normally reversible if the

animal is returned to its original body temperature, but only after a period of reacclimation. The mechanisms of capacity acclimation are highly varied, including synthesis of different isozymes, differential utilization of metabolic pathways, altered rates of protein synthesis and degradation, and changes in membrane composition (Hochachka and Somero 1984; Prosser 1986; Cossins and Bowler 1987).

Resistance adaptations (e.g., critical thermal limits) also respond to acclimation, perhaps even more frequently than do capacity adaptations such as metabolic rate. A particularly striking example is seen in seasonal shifts in critical temperatures (Fig. 5) of the crayfish (*Orconectes rusticus*), studied by Layne et al. (1987). Critical temperatures in crayfish in nature change dramatically, roughly paralleling seasonal changes in stream and probably body temperatures (Fig. 5).

Does the heat stress response show similar seasonal shifts? If so, the threshold temperature for protein induction (or the magnitude of the response) should be lower in winter than in summer. Although such seasonal shifts have not yet been examined for natural populations, several studies have compared the heat stress response in laboratory experiments where acclimation temperature was varied. For example, the threshold temperature for the heat stress response of hepatocytes of channel catfish (*Ictalurus punctatus*) is seemingly unaffected by acclimation temperature (however, the temperature steps were 2.5°C, which may obscure a subtle effect of acclimation on threshold temperatures), but the temperature for maximum synthesis is higher for warm-acclimated fish (Koban et al.

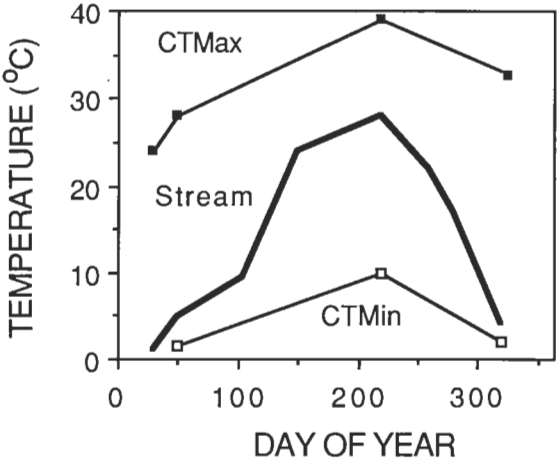

Figure 5 Critical thermal limits of the crayfish *Orconectes rusticus* and water temperatures at different seasons. Note the parallel shifts in critical limits and in water temperatures. (Redrawn, with permission, from Layne et al. 1987.)

1987). In unpublished studies, Koban and co-workers (pers. comm.) find that the heat stress response of the killifish (*Fundulus heteroclitus*) is also sensitive to acclimation temperature, but the patterns are complex. Interestingly, Bradley et al. (1988) found that copepods raised at low temperature (4°C or 15°C) induced somewhat different proteins following heat shock than did copepods raised at higher temperatures (20°C). These studies imply that some aspects of the dynamics of the heat stress response are affected by the prior thermal history of an organism. If so, acclimation protocols might prove to be a useful experimental tool for molecular biologists interested in manipulating the heat stress response.

Further studies of the effects of acclimation on the heat stress response of whole organisms would clearly be of interest, especially of temperate-zone organisms, which often experience dramatic seasonal shifts in body temperature (Fig. 5; Tsuji 1988). However, a caveat is warranted: Experiments designed to stimulate acclimation may need to vary photoperiod, not just temperature (Tsuji 1988). As Levins (1968, p. 13) noted, the type of environmental information (e.g., photoperiodic cues) an animal uses to anticipate environmental fluctuations may be different from the factor (e.g., temperature) for which the organism's physiology is actually compensating.

Although most acclimation responses are reversible, one special class—sometimes called "developmental switching"—results in phenotypic modifications that are fixed throughout life (Maynard Smith 1958; Levins 1968). Thus, the physiological or morphological phenotype of an organism is determined by the conditions experienced during development. For example, butterflies raised at low ambient temperatures have darker wings than those raised at higher ambient temperatures, and this switch is presumably adaptive by enhancing the effectiveness of basking at low ambient temperatures (Watt 1969). Similarly, exposure of newly emerged *Drosophila* females to high temperature (30.5°C for 5–12 days) has a profound effect on life span at 20°C, possibly by causing a permanent reduction in metabolic rate (Maynard Smith 1958).

Developmental switching is rarely studied in thermal biology, and we know of no studies examining whether the temperature threshold or the magnitude for the heat stress response depends on developmental experiences. In theory (Levins 1968), developmental switching should be restricted to species for which the projected environmental conditions last at least as long as the life span of the organism. Otherwise, the organism would have an inappropriate phenotype for part or much of its life. Accordingly, developmental switching should be found in animals (e.g., many microorganisms, insects) that go through several nonoverlapping generations during the course of a year. However, developmental

switching could be favored in long-lived organisms that are distributed along sharp thermal gradients, such as up a mountain.

VII. RESPONSES TO LONG-TERM FLUCTUATIONS

As environmental temperatures change or as organisms colonize areas with different thermal conditions, selection may favor the evolution—not just acclimation—of altered thermal sensitivity of physiological processes (Levins 1968; Slobodkin and Rapoport 1974; Parsons 1983; Bradley and Ketzner 1982; Huey and Kingsolver 1989). Specifically, selection should favor evolutionary changes in thermal sensitivity that improve the match between the body temperatures experienced by the animals and those optimizing physiological and ecological performance (Huey and Bennett 1987; Lynch and Gabriel 1987). For example, because a lineage invading a hot desert will experience higher body temperatures, selection should favor the evolution of higher optimal temperatures and critical thermal limits. Do such evolutionary changes occur? We examine both interspecific and intraspecific patterns.

Most of the data germane to this topic are based on interspecific studies comparing species living in areas with different thermal regimes, and these data frequently document differences in both capacity and resistance adaptations (Hochachka and Somero 1984; Prosser 1986). Among different species of fish, for example, those from polar regions have substantially higher metabolic rates than do those from temperate waters when measured at common body temperatures; likewise, metabolic rates of temperate fish exceed those of tropical fish, a situation reminiscent of warm and cold temperature acclimation (Brett and Groves 1979). Resistance adaptations also vary dramatically with latitude. Specifically, upper and lower lethal (or critical) temperatures are typically high in the tropics and lower in temperate and especially polar zones (e.g., fish, Fig. 6; frogs [John-Alder et al. 1988], lizards [van Berkum 1988]). Remarkably, arctic fish die of heat stress at temperatures that would cause tropical fish to die of cold stress (Fig. 6). Resistance adaptations often vary with habitat (Prosser 1986). For example, bacteria show exceptional variation in heat resistance, and "thermophilic" bacteria in particular are heat tolerant (Henle 1987).

Have induction temperatures for the heat stress response similarly evolved to match environmental thermal regimes? Unfortunately, few data are available for heat stress responses of whole organisms, which may be different from those of cell cultures. Nevertheless, insects (e.g., desert locusts vs. *Drosophila*, Whyard et al. 1986) or fish (killifish or

Talapia vs. rainbow trout; Kothary and Candido 1982; Chen et al. 1988; M. Koban et al., pers. comm.) living in warm environments typically synthesize heat stress proteins at higher temperatures than do species living in cooler environments. Interestingly, initial studies of the Antarctic fish *Notothenia rossii,* which can tolerate temperatures no warmer than 12°C, suggest that hsp70 gene (incubated spleen) may be maximally expressed at 8°C (Maresca et al. 1988). Overall, these limited comparative data suggest that the evolution of the heat stress response may well be involved with the evolution of habitat selection and of geographic distribution (Parsons 1987; Spotila et al. 1989).

The interpretation of available comparative data on the heat stress response is, however, weakened by two considerations. First, most studies have looked at responses of cultured cells rather than of whole organisms: Organismal responses are more relevant both ecologically and evolutionarily (Bartholomew 1958, 1964; Huey and Stevenson 1979). Second, most studies involve comparisons of distantly related taxa rather than of closely related ones. Although desert locusts and *Drosophila* (or killifish, arctic fish, and rainbow trout) differ in their heat stress responses, these species have long had separate evolutionary histories. Consequently, any observed differences in their heat stress responses might merely reflect those separate histories and thus need not have anything to do with adaptation to specific thermal environments

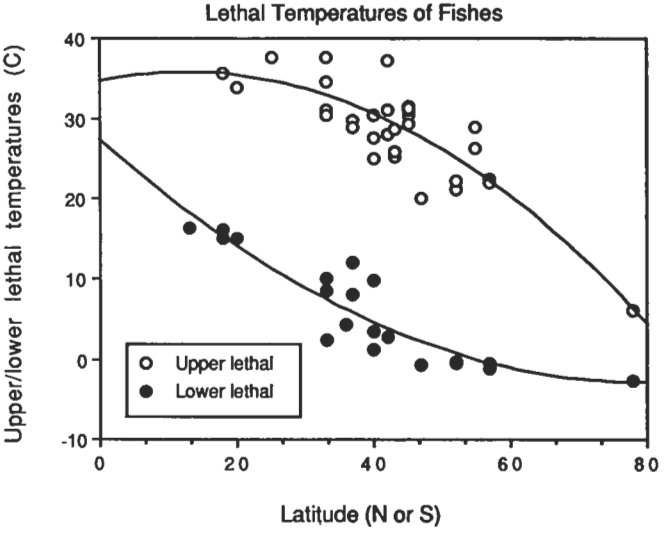

Figure 6 Upper and lower lethal temperatures for fishes (post-embryonic states only) from different latitudes. Curves fitted by polynomial regression. (Redrawn, with permission, from Brett 1970.)

that modern species currently occupy (Hochachka and Somero 1984; Huey and Bennett 1986; Huey 1987). This is the classic ambiguity plaguing any comparison of "apples and oranges."

One solution to the apples and oranges problem inolves studying geographic variation within rather than between species (Powers 1987). Very often heat tolerance of a population is directly related to the ambient temperature of the environment in which it lives (Hertz et al. 1983; Hochachka and Somero 1984; Prosser 1986; but see Coyne et al. 1983). However, geographic variation in the heat stress response has thus far been examined only in killifish, *Fundulus heteroclitus* (M. Koban, pers. comm.). Distributed along the eastern coast of the United States, this fish is subjected to an extraordinarily sharp thermal gradient (1°C per degree of latitude, Powers 1987). Different populations have different threshold temperatures for heat stress proteins, but the actual divergence between *Fundulus* from Maine and Georgia is minor. Curiously, northern populations initiate synthesis at a temperature slightly higher than do southern populations (M. Koban, pers. comm.).

An alternative solution to historical complications in comparative studies involves studying closely related species and then interpreting observed patterns with explicit reference to the phylogenetic relations and history of those species (Lauder 1981; Huey and Bennett 1986, 1987; Huey 1987). We recently developed an evolutionary approach for studying interspecific patterns of capacity and resistance adaptations, including the analysis of heat tolerance, with a method that uses phylogenetic information (Huey and Bennett 1987). We studied the thermal dependence of sprint speed and the critical thermal limits (temperatures at which the righting response is lost) of several species of scincid lizards from Australia that live either in temperate coastal regions or in hot interior deserts. These lizards differ strikingly in both optimal temperature for sprinting and in their critical thermal maxima, the upper temperature at which righting response is lost. Using a minimum-evolution or "parsimony" algorithm, we estimated the probable preferred temperature, critical thermal limits, and habitat associations of the hypothetical ancestors of this group (Fig. 7). This method allowed us to examine the probable *changes* in optimal temperatures and in heat tolerance that occurred when a given lineage invaded a new habitat.

A coastal distribution is thought to be ancestral in these Australian skinks (Greer 1980). However, two genera *(Eremiascincus* and *Ctenotus)* that we studied appear to have invaded the hot interior deserts of Australia (Fig. 7). Paralleling this change in habitat are increases in the critical thermal maxima (Fig. 7) and optimal sprint temperature (not shown) of both genera, presumably reflecting selection for higher heat tolerance in

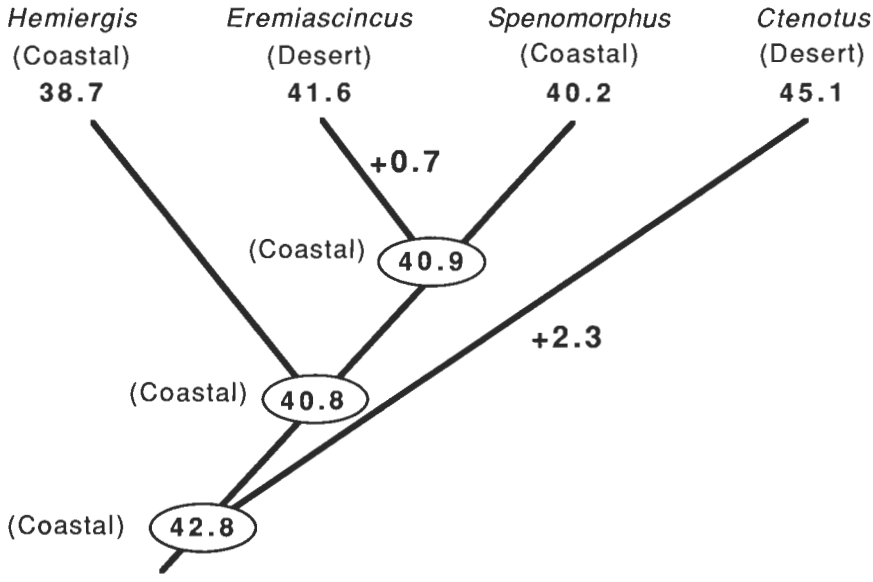

Figure 7 A partial phylogeny of some Australian lizards (Scincidae), showing critical thermal maxima (loss of righting response) and current habitat associations (desert vs. coastal) for the different genera and for their presumed ancestors. (Intrageneric variation in critical thermal maxima is minor in these lizards.) The values of hypothetical ancestors are estimated using an algorithm that minimizes the evolutionary change in the tree (see text). Note that the invasion of the Australian desert by two genera *(Eremiascincus, Ctenotus)* is associated with an apparent increased heat tolerance (critical thermal maximum). From data in Huey and Bennett (1987).

deserts. Whether the heat stress response has also evolved in the Australian skinks is currently unknown.

Drosophila would be an ideal genus for a phylogenetic analysis of the heat stress response: Good phylogenies are available for many species groups, species occur in diverse thermal habitats, species can differ dramatically in heat tolerance (Levins 1969; David et al. 1983; Parsons 1983), and the techniques for studying the heat stress response of *Drosophila* are well developed. Preliminary evidence (Table 2; S. Lindquist, pers. comm.) suggests that larvae of two desert species (*D. mojavensis* and *D. arizonensis)* have a higher temperature for maximal hsp70 synthesis than do larvae of *D. melanogaster*.

Phylogenetic techniques may also provide molecular biologists with a new comparative tool for testing functional hypotheses generated by conventional molecular techniques. Given both a phylogeny and protein sequences for a series of related species, one can use parsimony algorithms

Table 2 Heat stress responses of two desert species (*mojavensis* and *arizonensis*) of *Drosophila* are shifted to high temperature (°C) relative to those of *D. melanogaster*

Species	Habitat	Temperature at maximum hsp70 synthesis	Temperature at shutdown of all protein synthesis
melanogaster	widespread	35–37°C	37°C
mojavensis	desert	39°C	41°C
arizonensis	desert	41°C	43°C

Preliminary data, courtesy of S. Lindquist (pers. comm.). Larvae were raised at 25°C and acutely exposed to a graded series of temperatures.

to reconstruct ancestral protein sequences and then attempt to associate specific evolutionary changes in protein sequence with specific evolutionary changes in function. Indeed, this general approach has been used to trace the evolution of hemoglobin (Goodman et al. 1975; Goodman 1976), and it could well be used with the heat stress response.

Phylogenetic reconstructions are not without limitations. Historical reconstructions vary somewhat depending on the parsimony algorithm that is used, and parsimony itself can sometimes produce misleading inferences concerning history (Felsenstein 1983). Moreover, historical reconstructions are not amenable to experimental validation (Huey and Bennett 1986). Nevertheless, we argue that phylogenetic and historical considerations must be incorporated into comparative analyses. Otherwise, one is forced to accept the false assumption that organismal phenotypes are adapted only to contemporary conditions and thus bear no vestiges of history (Huey 1987).

VIII. SELECTION EXPERIMENTS

Evolutionary biologists sometimes use selection experiments to explore the dynamics of evolutionary change in a given trait. Selection experiments include two general classes (Rose and Graves 1989): (1) selective breeding of individuals with specified phenotypes ("artificial selection," often used to improve crop yields), or (2) raising outbred lines for several generations under specified environmental conditions ("natural selection" in the laboratory). Both types of studies provide information on the evolutionary lability of traits as well as of the degree to which their evolution might be constrained by genetic correlations with other traits (Falconer 1981; Arnold 1987). Selection experiments hold considerable potential as a tool for comparative or molecular physiologists (Hill 1980; Service 1987; Huey and Kingsolver 1989; Rose and Graves 1989).

Selection experiments have now been conducted on heat and cold resistance in flagellates (Dallinger 1887), wasps (White et al. 1970), *Drosophila* (David et al. 1983), and fish (Shah 1985). In all cases, heat and cold tolerance was altered by selection (for review, see Huey and Kingsolver 1989).

Natural and artificial selection have also been used to explore changes in heat resistance as well as in the heat stress response of *Drosophila melanogaster*. For example, Alahiotis and Stephanou (1982; see also Alahiotis 1983; Stephanou and Alahiotis 1983; Kilias and Alahiotis 1985) maintained two lines at different temperatures (14°C or 26°C), thereby subjecting them to natural selection for growth and reproduction at low versus high temperature. After 7 years, the high-temperature line had higher resistance to heat stress and also had a higher heat stress response than did the low-temperature line. Both differences seem to be maternally inherited (Stephanou and Alahiotis 1983). Interestingly, both heat sensitivity and the heat stress response apparently evolved even though the flies were kept at temperatures (14°C, 26°C) well below the supposed temperature threshold for the induction of heat stress proteins in *Drosophila*. Perhaps the heat stress response is active during chronic exposure to temperatures *well below* those required to trigger an acute response. In other words, perhaps induction of the response is sensitive not only to temperature, but also to the duration of exposure to temperature (Lindquist 1986; Easton et al. 1987; Rutledge et al. 1987a). If so, the tendency of most studies to focus exclusively on acute heat *shock* experiments may have inadvertently masked an inherent flexibility of the heat stress response.

Flies were also selectively bred for resistance or sensitivity to acute heat shock (40°C for 25 min; Alahiotis and Stephanou 1982; Stephanou and Alahiotis 1983; Kilias and Alahiotis 1985). Only 6.5% of flies from the sensitive line survived the heat shock, whereas 76.2% from the resistant line survived. Moreover, the capacity for synthesis of hsp70 following heat shock was seemingly higher for the resistant line than for the sensitive line (Stephanou et al. 1983).

Further selection studies on the heat stress response are certainly warranted. Of particular interest would be a comparison of the evolution of heat stress responses in lines subject to artificial selection for tolerance versus intolerance to high temperature (e.g., White et al. 1970; Morrison and Milkman 1978; Stephanou et al. 1983). Individual variation (Bennett 1987) in heat hardening and heat resistance is well known in diverse organisms (Bradley and Ketzner 1982; Hosgood and Parsons 1968; Rutledge et al. 1987b), suggesting that the heat stress response has the genetic potential to evolve (Bennett 1987; see also Stephanou et al. 1983; Rut-

ledge et al. 1987b). Because of the conservatism in some hsp sequences, evolutionary change might be most conspicuous at the control level. If so, selection experiments might prove a useful tool for molecular biologists interested in control mechanisms.

IX. FUTURE STUDIES ON THE EVOLUTION AND ECOLOGY OF HEAT STRESS RESPONSES

The heat stress response is one of many ways (Table 1) that organisms use to buffer environmental stresses. On the basis of its kinetics and physiological side effects, the heat stress response appears to be a "last-resort" response to brief but severe perturbations. It may convey some thermoprotection and repair, but at some probable (though undefined) cost to the organism.

To an evolutionary biologist, the heat stress response offers a largely unexplored system for comparative study. Organismal biologists have only recently begun to exploit the opportunities provided by the heat stress response and by the technology of molecular biology (Alahiotis 1983; Parsons 1987; Spotila et al. 1989). Obvious topics in need of further work are acclimation sensitivity, ontogenetic changes in heat resistance and in the heat stress response (Lindquist 1986), and patterns of interspecific and intraspecific divergence (e.g., Table 2). These comparative studies may be of interest to molecular biologists because divergence in responses among related organisms may provide useful clues as to the regulation and functions of the response itself.

To a physiological ecologist, heat stress proteins may also serve as a useful bioassay of how frequently organisms are stressed in nature (Saunders 1988; Spotila et al. 1989) and perhaps even of the type or severity of the stress (Neidhardt et al. 1984; Rutledge et al. 1987a)—issues significant to debates in population biology and in physiological evolution (Haldane 1932; Kingsolver and Watt 1983; Parsons 1987; Spotila et al. 1989). The omnipotence and apparent conservatism of the heat stress response could suggest that organisms routinely experience stress in nature (see above). However, by determining the proportion of individuals expressing heat stress proteins as a function of geography or season or habitat, one could quantify the actual frequency of stress. Work along these lines has recently begun (Easton et al. 1988).

X. CONCLUDING REMARKS

The heat stress response encompasses an area of biology where organismal and molecular biologists can learn much from each other. Molecular

techniques (e.g., hsp assays, genetic engineering) can help evolutionary physiologists measure the frequency of stress in nature as well as document the patterns of the evolution of the response. Conversely, organismal and evolutionary techniques (e.g., acclimation manipulations, phylogenetic analysis, selection experiments) provide molecular biologists with novel sources of "experimental" variation, which in turn may lead to new insights as to the mechanics and regulation of the heat stress response.

An expanded dialogue between molecular and evolutionary biologists will almost certainly be mutually beneficial. We suspect that organismal and evolutionary biologists will increasingly become involved in heat stress research now that the physiological and protective functions (if not the physiological costs) of the response at the *organismal* level are being clarified.

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