Key issues in achieving an integrative perspective on stress

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An integrative perspective on molecular mechanisms of stress resistance requires understanding of these mechanisms not just in vitro or in the model organism in the research laboratory – but in the healthy or diseased human in society, in the cultivated plant or animal in agricultural production, and in populations and species in natural communities and ecosystems. Such understanding involves careful attention to the context in which the organism normally undergoes stress, and appreciation that biological phenomena occur at diverse levels of organization (from molecule to ecosystem). Surprisingly, three issues fundamental to achieving an integrative perspective are presently unresolved: (i) Is variation in lower-level traits (nucleotide sequences, genes, gene products) seldom, commonly, or always consequential for stress resistance? (ii) Does environmental stress reduce or enhance genetic variation, which is the raw material of evolution? (iii) Is the present distribution of organisms along natural gradients of stress largely the result of organisms living where they can, or is adaptive evolution generally sufficient to overcome stress? Effective collaboration among disciplinary specialists and meta-analysis may be helpful in resolving these issues.

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"Never elaborate a baroque excrescence on top of existing but shaky ideas" (Stearns 1987)

1. Wanted: A synthetic theory of stress proteins and stress responses

Subhash Lakhotia has stated: "There is a strong need for understanding the roles of stress proteins and stress responses from an integrative biological perspective." What form might such understanding take?

Life scientists using the toolkits of molecular biology and genomics have now made considerable progress in understanding the molecular mechanisms that promote resistance to extremes of many environmental factors, including other organisms. This progress is often based in the research laboratory, and often uses the paradigm of experimental biology: If a candidate gene, gene product, or other substance is abolished, in excess, in limited quantity, mislocalized, absent when normally present, or present when normally absent, is this necessary and/or sufficient for a change? Does genetic complementation or exogenous provision of the gene or substance rescue this change? The sought-after change is often *in vitro* or in the cell, but sometimes in tissues, organs, or entire organisms – and commonly these organisms are the classical model organisms of laboratory experimental biology. The understanding we seek is simply the extension of this paradigm to non-model organisms (including ourselves) living in natural communities in natural environments (Feder and Mitchell-Olds 2003) – where "simply" is clearly an oxymoron. That is, we would like to be able to infer from an individual's genome (and its other "omes") its propensity for ecological success and evolutionary longevity/diversification, particularly under environmental stress (Feder and Mitchell-Olds 2003). We would like to know what genes, proteins, or other substances are necessary and/or sufficient for variation, both positive and negative, in these properties. With such understanding we could, for example, better understand the likely biological impact of global climate

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change and anthropogenic practices and perhaps mitigate these impacts, or improve agricultural productivity and treatment or avoidance of human disease.

Thus, the underlying paradigm is essentially linear thinking:

Δ in mechanism $\rightarrow \Delta$ in health or ecology or evolution, particularly under stress (1)

Embedded in this thinking are several caveats: (i) *Appropriate context*: Experimental conditions in the laboratory are often simple, varying one or a small number of environmental stresses at a time. The rationale for this practice is clear; it allows for the comparison of results from different laboratories and enables straightforward interpretation of outcomes (Kohler 2002). Nonetheless, such conditions are commonly poor proxies for environmental conditions outside the laboratory, which are dynamic and combinatorial (Bartholomew 1987). "Does it work in nature, in the patient, or in agriculture?" is the ultimate ground-truth. (ii) *Appropriate level*: Life is hierarchical (figure 1), and, as George Bartholomew has stated: "Any biological phenomenon finds it mechanisms at lower levels of organization and its significance at higher levels of organization" (Bartholomew 1964). A change at one level of organization, however, need not correspond to a change at another, and for that reason must be verified at levels appropriate to the question being asked. These caveats, while often ignored for expediency's sake, are at least familiar to most life scientists.

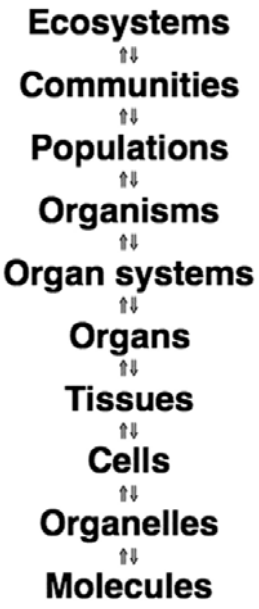

Figure 1. Levels of biological organization. As Bartholomew (1964) has stated, every biological phenomenon finds its mechanism at the levels below it and its significance at the levels above it.

My principal point is: Despite the attractiveness and simplicity of the linear paradigm (1), and even if the foregoing caveats are appropriately reflected in our scientific practices, we are a long way from establishing the underlying assumptions that would enable it to be applied, and the paradigm itself may be fundamentally inapplicable. In large part, this is because we have not yet reconciled several fundamental contradictions in our understanding of life under stress.

2. Hierarchical and systems thinking: Point, counterpoint and synthesis

2.1 *Point*

Experimental and natural variations in the mechanisms that underlie stress resistance have clear, consistent, and predictable consequences for ecological and evolutionary attributes. One might logically expect the following to be true: species that have robust mechanisms for stress tolerance (e.g. effective molecular chaperones in appropriate concentrations in appropriate cells) are able to exploit more stressful environments or persist longer in them than species that do not have such robust mechanisms. Or *vice versa*: species with greater stress tolerance exhibit more robust mechanisms than species with lesser stress intolerance. Experimental diminution of such mechanisms (e.g. genetic knock-outs, inhibitors) reduces ecological success, health, and/or evolutionary persistence. Genetic variation (e.g. polymorphisms) at critical points in nucleotide sequences are consequential for stress resistance. Indeed, evidence for such findings is considerable. The literature of comparative physiology and biochemistry is replete with cases in which stress tolerance is correlated with specific mechanisms for stress tolerance (Hochachka and Somero 2002; Watt and Dean 2000). For most molecular chaperones, genetic evidence (and corresponding work *in vitro*) firmly establishes the role of these chaperones in stress tolerance (Feder and Hofmann 1999). Medical genetics (Jorde 2003) and its counterpart in agriculture are essentially a massive catalogue of instances in which segregating polymorphisms give rise to disease.

2.2 *Counterpoint*

Experimental and natural variations in the mechanisms that underlie stress resistance do not have clear, consistent, and predictable consequences for ecological and evolutionary attributes. Surprisingly, the experimental deletion of the vast majority of genes in the vast majority of genomes in which this has been attempted has no detectable phenotypes (Feder and Walser 2005). That is, the majority of genes and

the products they encode presently seem inconsequential. Recently, Wagner concluded that this phenomenon has counterparts at every level of biological organization: from RNAs to ecosystems, components can be varied with no detectable impact on function (Wagner 2005). One possible explanation for such findings emerges from the ongoing explosion in the analysis of biological networks. Biological networks (e.g. interactions among all genes in the genome, all proteins in the proteome, intracellular signalling networks, food webs in ecosystems) are complex, scale-free, and modular (Barabâasi 2003; Carroll 2005; Pereira-Leal and Teichmann 2005 Csermely 2006; Pereira-Leal *et al* 2006). Just as the internet (a non-biological but complex, scale-free, and modular network) can function when any given server or node is removed, so can biological networks. Another finding is that components in any system are highly redundant.

2.3 *Synthesis*

Four points are relevant. First, network thinking predicts that any node in a network should be consequential in proportion to its connectedness, and evidence for this prediction is growing. As biological networks are described in increasing detail, a possible outcome is that hubs (i.e. highly connected nodes) are consequential if deleted or varied whereas less-connected elements are not. That is, any network organization will give rise to a predictable spectrum of synthetic phenotypes. Second, the description of phenotypes is contingent upon experimental conditions. A knock-out or polymorphism may have no phenotype under permissive condition, and might have a phenotype only under a highly circumscribed combination of stresses (e.g. only at a specific temperature and humidity and salinity, and only in a specific season and at a specific life cycle stage, and only when these stresses have specific kinetics of onset) (Feder and Walser 2005). This is the biological version of "the curse of infinite dimensionality" (Bellman 1961). Pragmatically, the combinatorial manipulation of each nucleotide and possible stress may be impossible. Third, true redundancy may itself be adaptive in the context of stress (providing its cost is not exorbitant) if it forestalls catastrophic failure under stress or facilitates repair. Finally, redundancy present at any moment may represent an inevitable but transient balance between ongoing processes that generate it (e.g. gene duplication) and other processes that remove it (degeneration via natural selection, diversification/complementation) (Lynch and Conery 2000); if the latter processes are less powerful or act more slowly than the former, redundancy will persist even if it is not presently advantageous. Importantly, such transient redundancy, particularly at the level of genes, can be an important source of raw material for evolutionary change: one redundant gene can take on new functions if another is present to perform the pre-existing functions (Lynch and Conery 2000).

3. Environmental stress as a driver of evolution: Point, counterpoint and synthesis

3.1 *Point*

By exposing populations to distinctive environmental regimes, often at range limits where population sizes are small, environmental stress may favour novel genetic variants that would be eliminated in less stressful regimes. Typically the range and combinations of stresses that individuals of all species, including our own, undergo is not uniform; some individuals are more stressed than others. For abiotic stresses, the intensity is often thought to vary across a species' geographic or altitudinal range, with abiotic stress at the margins or limits being more intense than elsewhere. A growing number of systematic studies are corroborating this expected pattern of stress intensity, although stresses may interact to yield exceptional patterns (Helmuth *et al* 2002; Hofmann 2005). Extremes of stress, then, are hypothesized to be distinctive sources of evolutionary novelty. The major tenets of this hypothesis are (Hoffmann and Parsons 1997): (i) Environmental extremes influence the expression of phenotypic and genetic variation. (ii) Extreme conditions are periods of intense natural selection. (iii) Selection under extremes can produce characteristics that are not normally favored. (iv) Extreme conditions can influence population size.

Clearly each tenet *can be* correct, as Hoffmann and Parsons (1997) amply document. Of particular note are several associated evolutionary mechanisms that may act primarily in relation to stress. The first is molecular chaperones acting as "evolutionary capacitors" (Rutherford and Lindquist 1998). These chaperones may normally suppress extreme phenotypes, but reveal these phenotypes under the influence of stress and expose the encoding genes to selection. A second is mobile genetic elements, which under stress can rearrange genomes (Kazazian 2004). Another is accidents in recombination, segregation, and reproductive isolation resulting in segmental or whole-genome variation (Feder 2006). These may interact. For example, molecular chaperone-encoding genes are especially susceptible to the insertion of mobile genetic elements in their proximal promoter regions (Walser *et al* 2006). Whatever the source of the variation, it is the raw material of evolutionary change. In principle, however, any given change ought to be difficult to establish in a species with many populations, as exchange of alleles with many non-marginal populations ought to swamp a rare novel allele. Here the fourth point of Hoffmann and Parsons (1997) is especially crucial, as is the potential for extreme stress to isolate marginal populations and inhibit gene flow throughout

a species' range. In isolated and small populations, chance and natural selection may interact to favour the proliferation and fixation of novel alleles or combinations thereof. When stress abates and gene exchange resumes, the marginal populations can serve as a more potent source of variation (*see* also Peck and Welch 2004). Alternatively, if reproductive isolating mechanisms have arisen in the novel or marginal population, it may then not exchange genes with less-marginal populations but go forward as an independent species. By this logic, extreme environments ought to be potent generators and incubators of biological diversity.

3.2 *Counterpoint*

By reducing population size and purging populations of genetic variation, environmental stress may limit evolution. Given that genetic and phenotypic variation is the raw material of evolutionary change, its lack may impede the generation of biological diversity – and stress can readily purge a population of alleles that might be permissible under more benign conditions. Although "pre-genomic", Bradshaw (1991) provides an especially cogent overview of how lack of genetic variation, which he terms "genostasis", may limit evolution in stressful environments. A 2005 review co-authored by the Hoffmann of Hoffmann and Parsons (1997) reaches a similar conclusion (Blows and Hoffmann 2005). As Bradshaw also states, strong selection (such as that due to extreme stress) can exhaust the genetic variation in a population (*see* also Frankham 2005 and Jump and Penuelas 2005). Due to prevailing stress, marginal populations have a high propensity for extinction and, when extinct, obviously can no longer contribute to a species' or community's diversity. Indeed, in an excellent review of both point and counterpoint, (Jump and Penuelas 2005) single out habitat fragmentation at range margins as a key factor leading to the loss of genetic variation. Moreover, even if a marginal population develops incipient adaptation, gene flow from larger, more central populations may well overwhelm the genetic uniqueness of the marginal population (Alleaume-Benharira *et al* 2006; Kirkpatrick and Barton 1997; Lenormand 2002). An entirely different point is that many of the key assumptions of the opposing view can be challenged. Populations in the center of a species' range may well experience more favourable abiotic conditions than populations at the range's margin. To the extent this is true for both, any given population and the other members of its biological community, biotic stress may be greater for central populations than for marginal populations due to increased intra- and inter-specific competition, predation, etc. at the centers of ranges. If conditions favor increased gene flow among central populations and between central and peripheral populations, as plausibly they should, central populations may sample far more genetic variation than peripheral populations (Alleaume-Benharira *et al* 2006). Indeed, Ackerly (2003) has suggested that these phenomena interact, with selection for attributes that favour success in central populations typically overwhelming selection in marginal populations when these populations exchange genes (*see* also Kirkpatrick and Barton 1997; Lenormand 2002; Alleaume-Benharira *et al* 2006).

3.3 *Synthesis*

Clearly, stress sometimes plays a key role in the genesis of biological novelty, but sometimes does not. We can imagine some of the variables that contribute to this dichotomy: pre-existing genetic variation, rate at which new variation originates within a population, severity of the stress(es) in both magnitude and rate of change, effective population size and population structure, gene flow among populations of a species, and structure of the biological communities in which both central and marginal populations reside, among others. Evolution under stress may well require conditions in which the value of each relevant variable must be not too weak, not too strong, but just right. Defining such values might well benefit from a meta-analysis of individual species' patterns. At this point, however, whether stress plays a distinctive role in evolution is unresolved.

4. Sufficiency of evolution to overcome environmental stress: Point, counterpoint, and synthesis

4.1 *Point*

As indicated by numerous studies of adaptive convergence, modelling, and documentation of evolution in real time, natural selection typically is competent to enable organisms to withstand stress (Endler 1986; Elena and Lenski 2003; Davis *et al* 2005; Jump and Penuelas 2005). As noted, the literature of comparative physiology and biochemistry is replete with cases in which populations or species tolerate remarkable extremes of stress, and specific human populations exhibit distinctive capacities to function in the face of stress. Much other literature reviews accounts of adaptation to climate change (Chen and Kang 2005; Davis *et al* 2005; Jump and Penuelas 2005; Bradshaw and Holzapfel 2006). It is difficult to imagine how such capacities may have arisen except through evolutionary processes such as adaptation or exaptation – no matter how repeatedly distributional patterns suggest that such processes are insufficient for the origin of stress tolerance. Indeed, empirical studies of selection, both in nature and in the laboratory, have repeatedly demonstrated that evolution is remarkably effective and rapid in changing stress-relevant traits (Endler 1986).

4.2 *Counterpoint*

As indicated by widespread covariation of traits with environmental stress regimes and the extensive climatic zonation of species distributions, species mainly "live where they can" and natural selection typically is either too weak or too slow to allow species to adapt to changing stress regimes. Alternatively, natural selection "reinforces" a species' traits in individuals that disperse to environments they find favourable – a process termed stabilizing or purifying selection – whereas individuals dispersing to unfavourable environments simply fail to reproduce (Ackerly 2003). Well before their field had a name, ecologists had recognized that every species has a limited range – a suite of microclimates and competing species within which it could flourish but outside of which it could not. Some selected classical statements of this recognition include Merriam's (1898) "life zones" and Grime's (1977) CSR classification of plant species. Thus when, for example, climate changes, species either migrate to maintain their pre-existing climatic conditions and/or undergo extinction in their pre-existing range, but do not adapt in place as the environment changes. This process is "ecological sorting" (Weiher and Keddy 1995; Ackerly 2003). Similarly, agriculturalists may switch crops or stock as conditions change to maintain productivity, which would be unnecessary if crops or stocks could adapt to changing agricultural conditions. Recent interest in anthropogenic climate change has spawned hundreds of studies with findings consistent with ecological sorting (Parmesan 2006). Parmesan and Yohe (2003), for example, summarized data for nearly 1600 species, of which 59% had undergone change in phenology and/or distribution during the past two centuries. Among the most convincing evidence for ecological sorting is phylogenetic constraint (Figure 3). If natural selection were typically all-powerful, then the traits of phylogenetically related species should vary according to

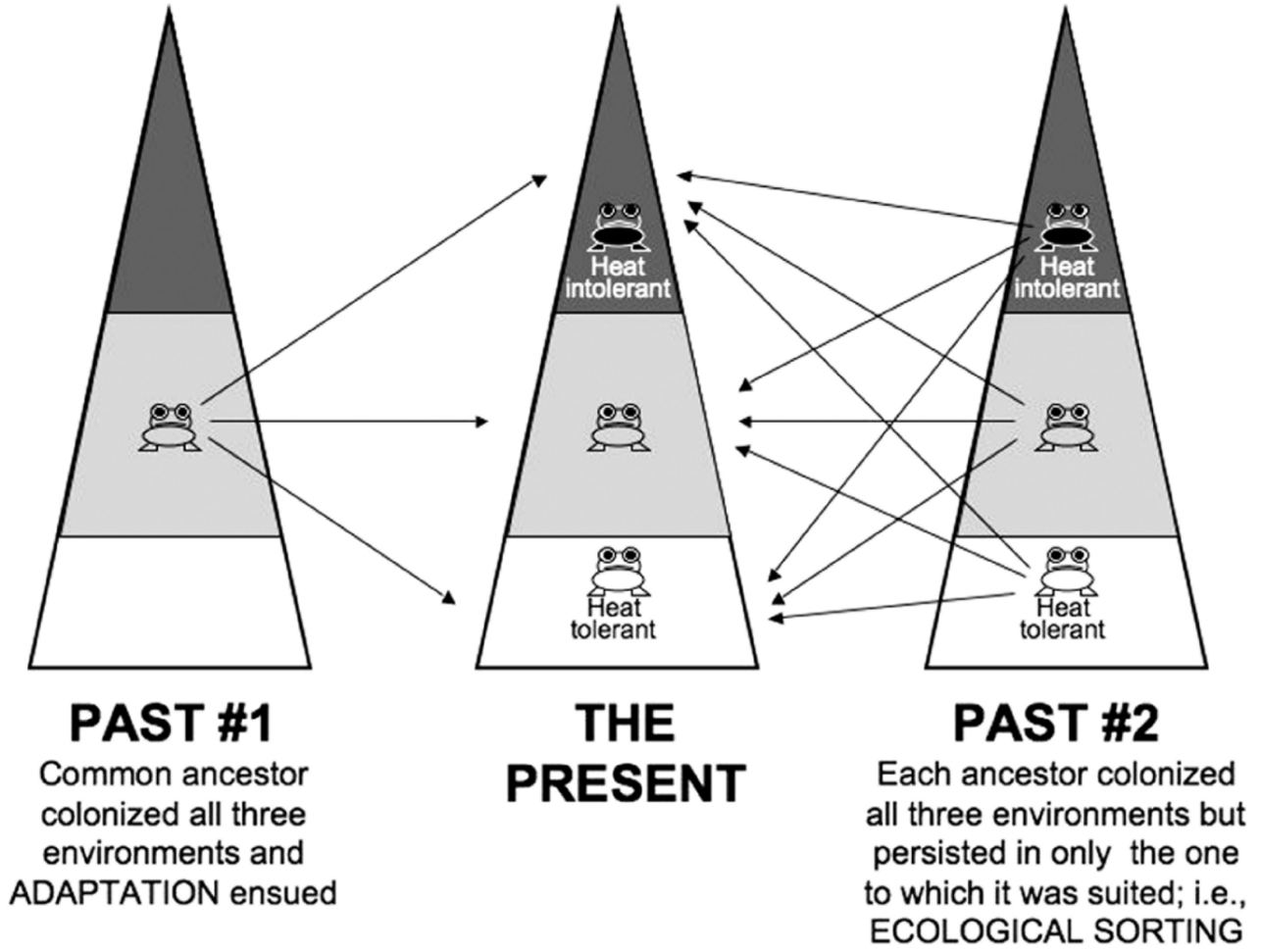

Figure 2. Two diametrically opposite processes may yield species' and populations' distributions that are correlated with their stress tolerance (center). In the first, ancestors colonize diverse environments and adapt to those environments through natural selection and other evolutionary processes. In the second, ecological sorting, species simply live where they can and tolerances are static.

their current environment. By contrast, if natural selection were typically weak, then phylogenetically related species in different environments should resemble one another. Ackerly, Prinzing, and their colleagues (e.g., Prinzing *et al* 2001; Ackerly 2003; Prinzing 2005), have studied numerous plant species in North American and Europe, respectively, and found considerable support for the latter pattern. Additionally, Prinzing has carefully documented how species resort themselves into new microhabitats as climate changes. Indeed, Parmesan (2006, p. 656) concludes: "... there is little theoretical or experimental support to suggest that climate warming will cause absolute climatic tolerances of a species to evolve sufficiently to allow it to conserve its geographic distribution in the face of climate change and thereby inhabit previously unsuitable climatic regimes." Climate change is not so unique a stress that Parmesan's conclusion should not be broadly pertinent to stress in general.

4.3 *Synthesis*

Ackerly (2003) has hypothesized that ecological sorting should predominate in some circumstances, and adaptation in others. In brief, in multi-species biological communities, competition constrains species' niches; evolving novel stress tolerances, and hence a new niche, would be problematic if another species already occupies that niche. Hence, in many cases the path of least resistance in the face of a changing stress regime may be for a species to track or migrate with the geographic zone of favorable climatic conditions. By contrast, when close competitors are not present (e.g., on islands or in zones of extreme stress), the way is clear for species to evolve novel stress tolerances and directional natural selection should be sufficient for such evolution. Of course, if a species cannot track or migrate with the geographic zone of favourable climatic conditions, it will either adapt or disappear. Ackerly's hypothesis is an attractive one because it seems amenable to testing. The circumstances in which adaptation and ecological sorting predominate should be related to pre-existing species diversity in a community, any given species' capacity for dispersal, etc. Of great interest would be a systematic survey of flora and fauna in which adaptation and ecological sorting would be characterized according to such variables.

Interestingly, Bradshaw and Holzapfel (2006) concluded that most instances of climatic adaptation concern alterations

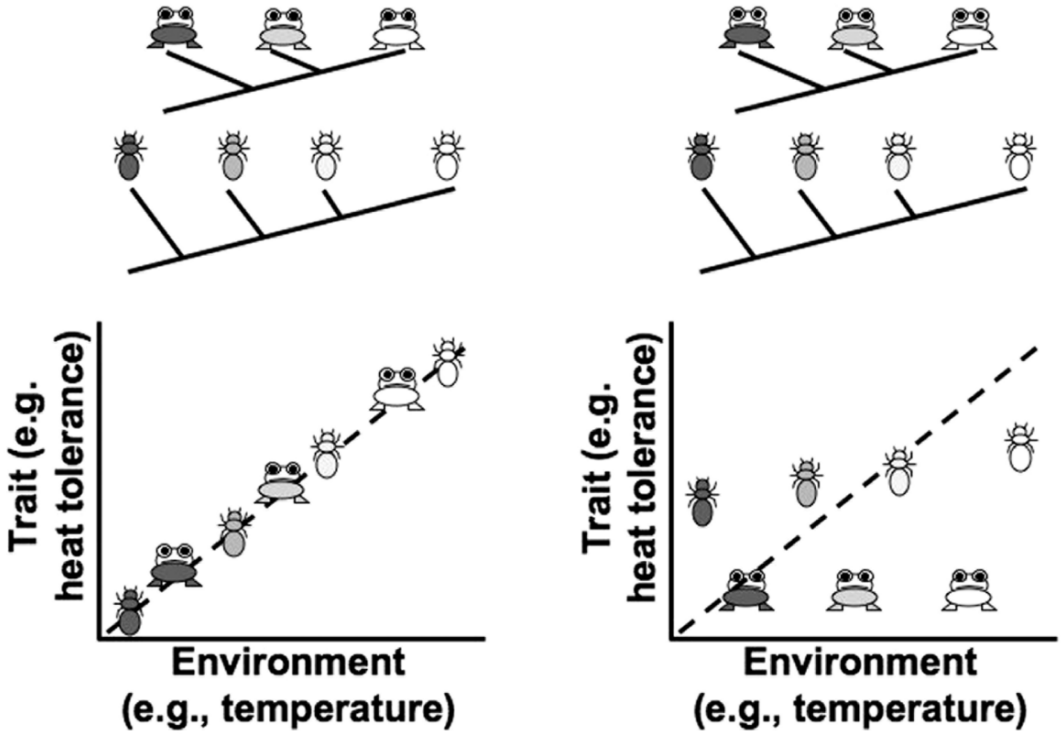

Figure 3. Phylogenetic conservatism (right) or its lack (left) may distinguish between adaptation and ecological sorting as causes of species' and populations' distributions. In each case, the cartoon on top indicates the phylogenies of two separate groups of organisms. *Left:* If evolution can readily adjust the stress tolerance of species and populations, then tolerance and environment should be correlated within phyla and differences among phyla should be minimal. *Right:* If the stress tolerance of species and populations is static or resistant to evolution, then tolerance and environment should be poorly correlated within phyla and differences among phyla should be distinct.

in biological timing, so that reproduction (for example) is shifted to the time of year when climate is most appropriate. The authors conclude (p. 1477): "We are not aware of any examples of genetic changes in animal populations toward either higher thermal optima or greater heat tolerance that are correlated with recent climate warming."

5. Conclusion

Before we can achieve an integrative perspective on stress proteins and stress responses, we need to establish its foundations. Surprisingly, at least three key fundamental elements remain controversial or unresolved. (i) One view is that the components of cells or organisms routinely give rise to stress resistance or susceptibility, whereas another is that they do not. (ii) One view is that stress is a potent generator of biological novelty, whereas another is that it generally inhibits the genesis of biological novelty. (iii) One view is that adaptation is a potent mechanism that can overcome biological stress, whereas another is that it is seldom capable of doing so such that populations and species "live where they can". Although the foregoing sections propose potential syntheses, these views are presently so diametri-cally opposite that the likely time course of resolution is unclear.

One possibility is that no resolution is possible. Outside of the laboratory, in both natural environments and human societies, is the phenomenon of "place" (Kohler 2002). That is, every individual organism or location has a unique history, undergoes a unique suite of conditions or stresses, and has a unique combination of genes, if alive. If so, truly general laws or perspectives may be elusive.

Another possibility is that resolution is possible, but that scientists have only recently organized themselves to achieve it. Especially if biological "signal" is prone to distortion by the "noise" of place, detailed sampling and powerful analysis will be necessary to detect it – and such sampling and analysis is beyond the capacity of any single scientist. While the physical sciences furnished the first proof of this concept, the biological sciences have exploited it in the sequencing of a growing number of complex genomes and the description of a growing number of transcriptomes, proteomes, metabolomes, interactomes, etc. Indeed, the International Union of Biological Sciences (IUBS, *http://www.iubs.org*) and its several themes was an early proponent of team science in biology on an international scale. Presently the major impediment to the resolution of the three fundamental controversies (above) may not be so much in data acquisition as in analysis and synthesis. Of note, therefore, may be two mechanisms funded by the U.S. National Science Foundation: the National Center for Ecological Analysis and Synthesis (NCEAS, *http://www.nceas.ucsb.edu*) in California and the National Evolutionary Synthesis Center (NESCent, *http://www.nescent.org*) in North Carolina. The key innovation of these and other centers are the "working groups", peer-reviewed assemblages of scientists that meet at these centers to analyze pre-existing data and make progress on the most fundamental issues. In an example relevant to the present discussion, one such group that assembled on "'The Ecological and Evolutionary Dynamics of Species Borders" recently published 6 consecutive papers in *Oikos* (Holt and Keitt 2005). In another context, "jamborees" for genome annotation (Ashburner 2006) function similarly. Importantly, although both NCEAS and NESCent have the word "National" in their title, working groups typically include the most relevant scientists regardless of their nationality. The ongoing controversies fundamental to an integrative perspective on stress (outlined in sections 2-4) may well yield to such an approach.

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