Grand Challenges in Comparative Physiology: Integration Across Disciplines and Across Levels of Biological Organization

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Introduction

Schwenk et al. (2009) provided an overview of five major challenges in organismal biology: (1) understanding the organism's role in organism–environment linkages; (2) utilizing the functional diversity of organisms; (3) integrating living and physical systems analysis; (4) understanding how genomes produce organisms; and (5) understanding how organisms walk the tightrope between stability and change. Subsequent "Grand Challenges" papers have expanded on these topics from different viewpoints, including ecomechanics (Denny and Helmuth 2009), endocrinology (Denver et al. 2009), development of additional model organisms (Satterlie et al. 2009), and development of theoretical and financial resources (Halanych and Goertzen 2009). This is the sixth paper in the "Grand Challenges" series, which offers the view from comparative physiology.

In this article, we expand upon three major challenges facing comparative physiology in the 21st century: vertical integration of physiological processes across organizational levels within organisms, horizontal integration of physiological processes across organisms within ecosystems, and temporal integration of physiological processes during evolutionary change. "Integration" is a key. It defines the scope of the challenges and must be considered in any solution. Reductive and inductive approaches both have been used with great success in biology. The reductive approach employs a simplified system to study a complex process. There is no question that such an approach has yielded a greater understanding of the molecular mechanisms of cellular processes. The inductive approach depends on observation to develop universal principles. Charles Darwin, after all, used this approach to develop the theory of natural selection. All too often these approaches are viewed as mutually exclusive, when, in fact, they are complementary and are used, to varying extents, by most biologists working today. Yet, we have fallen short of full integration across disciplines and levels of biological organization. A major impediment for further advancement has been the limitations in tools and resources. However, recent technological advances (e.g., systems biology) give us an opportunity to combine reductive and inductive approaches to study emergent properties (Boogerd et al. 2007) and now allow us to entertain

the notion that such a goal is possible, and perhaps even achievable, within the next decade.

Organismal biology in general, and comparative physiology specifically, is central to integration across disciplines. Others have promoted limited efforts for vertical integration. "Macrophysiology" integrates ecology with physiological ecology (Gaston et al. 2009). "Functional genomics" integrates gene regulation with physiology (Dow 2007). "Ecological genomics" applies molecular techniques to the study of ecology (Ungerer et al. 2008; Pennisi 2009; Stillman and Tagmount 2009). We argue that there is a need for integration from genes to ecosystems across time and space, in order to understand and predict the effects of change in the Earth's climate, pollution, habitat change, invasive species, and overexploitation (Chown and Gaston 2008).

Further, we discuss the three "integration" challenges in more detail and then offer some guidance for the development of infrastructure, tools, training, and shared resources that are essential for addressing these challenges. Included are initiatives to develop model organisms that integrate vertically across all levels of biological organization and address the social, political, and economic issues that are fundamental to our ability to successfully meet those challenges.

Vertical integration of physiological processes across organizational levels within organisms

Comparative physiologists study organisms at multiple levels of biological organization, including the behavior and metabolism of the whole organism, isolated organs, the tissues of which organs are made, cells that comprise the tissues, cellular organelles (e.g., mitochondria), and components of organelles, such as proteins and membranes. In the past decade, *cis*-, *trans*-, and epigenetic regulation of the genome, as indexed by changes in the transcriptome and proteome, have also become phenotypes of interest. Roles of regulatory RNAs (e.g., endogenous miRNA and exogenous siRNA) in control of gene expression are just starting to be understood and represent a potentially huge source of phenotypic variability (Wu and Belasco 2008). Studies at each of these organizational levels require particular expertise and laboratory resources, and these are often customized for the organisms being studied.

Krogh's Principle (Krogh 1929; Krebs 1975), that "for such a large number of problems there will be some animal of choice, or a few such animals, on which it can be most conveniently studied" has been of central importance for organismal biologists and biomedical researchers alike (Satterlie et al. 2009). Organismal biologists use Krogh's Principle to justify the study of a wide diversity of organisms that possess the appropriate combination of phenotype, ecology, and evolutionary history for addressing specific questions of physiological adaptation to a wide range of environmental conditions. In contrast, biomedical biologists use Krogh's Principle to justify a model organism-based approach, in which all fundamental questions about how organisms work can be addressed in a relatively small subset of species that are readily cultured under laboratory conditions, have a range of easily examined phenotypes, and, in some cases, possess intrinsic high mutation rates that generate a wide range of phenotypic variation.

For a long while, organismal biologists studied a broad array of organisms but lacked the ability to develop molecule–organism integration as the biomedical research community has done for its relatively small set of study organisms. Recent advances in high-throughput approaches to genomics and proteomics have started to blur what constitutes a model organism (Crawford 2001; Gracey 2007; Dalziel et al. 2009). Generation of genome sequence for any study organism is now possible and will likely continue to become both less expensive and more straightforward to do so in the future. For organismal biologists interested in understanding physiological diversity across space and time (Gaston et al. 2009), there is great promise for application of genomics and proteomics to develop extremely high-resolution assays to compare transcriptome (Gracey et al. 2008; Stillman and Tagmount 2009), proteome (Dowd et al. 2010; Tomanek and Zuzow 2010), and/or epigenome (Jablonka and Raz 2009) "fingerprints" of physiological "state." These assays may reveal very fine-scale differences among individuals and/or populations across ecologically relevant scales, but for elucidation of physiological mechanisms affected by those differences, these genomic–proteomic approaches yield only hypotheses about which genes may be involved in physiological processes.

To directly test hypotheses resulting from "-omics" studies of nonmodel organisms, we must turn to both classical methods in protein biochemistry and cellular physiology to determine what specific gene products do, as well as novel methods in reverse genetics (e.g., RNA interference) to determine what changes in phenotype occur when those genes are not expressed (Dow 2007). Such studies require substantial resources to build necessary personnel and research infrastructure specific to study organisms, as reverse genetic methods are often taxon-specific. Such infrastructure is already present for the small number of model organisms used by the biomedical research community, yet the challenges of translating a transcriptome profile into an integrated physiological response are still great. For example, Dow (2007) estimated that the 300,000 researcher-years spent conducting studies of the model arthropod *Drosophila melanogaster* have resulted in functional understanding of about 20% of the known genes, and those genes are, for the most part, associated with developmental phenotypes for which clearly indexed assays exist. As it is likely that many gene products will function the same way across all organisms, we can reasonably predict pathways and cellular roles of known genes for non-model organisms. However, Dow (2007) suggests that a third of the genes from any genome are sufficiently novel that their function cannot be predicted without further empirical experimentation.

Schwenk et al. (2009) suggested that an important grand challenge to organismal biologists is to integrate across vertical levels, from the genome to the organism in what has been termed GCOB #4 by Halanych and Goertzen (2009). The *D. melanogaster* research community is likely large enough to have a chance of functionally characterizing all the genes in the fly genome. Is this task achievable for organismal biologists working on a nonmodel organism for which a small research community exists? Dow (2007) argued that comparative physiologists must rely on model organisms in which to test functional hypotheses, because application of reverse genetics is only currently available in a small set of organisms. But what if the phenotypic variation we study is not present in an organism tractable to reverse genetics? A drawback of the reverse-genetics approach is that it tests the phenotypic function of single genes, whereas complex phenotypes (e.g., metabolic rate, thermal tolerance) are certain to be polygenic. How will we know if we are assessing the appropriate functional aspect of those genes if the changes we induce are taken out of context of the cellular network upon which selection has acted? These are issues worth considering before testing functional hypotheses resulting from nonmodel organisms in a model organism system.

Much promise and hope among comparative physiologists is that computational *in silico* reverse genetics may be valuable in assessing predicted phenotypic change. Assuming that an adequate amount of information regarding the functioning of gene products can be determined, at least part of our ability to predict emergent properties integrating across genes to organisms will rely on computational solutions, including quantitative systems biology.

Quantitative systems biology is a theoretical approach for integration of phenotypic responses across vertical levels of integration. In a quantitative systems biology approach to understanding emergent properties of cells, researchers are using the types of "omics" data that are increasingly easier and less expensive to generate (e.g., genome, transcriptome, proteome, and metabolome) and using quantitative models to understand the linkages across those vertical levels by which changes in the environment are transduced from one "ome" to another. In doing so, researchers can develop "predictive models" for how biological systems respond to changes in the environment. Systems biology aims to understand emergent properties of organismal function from interaction networks of subcellular characteristics, such as the interrelatedness of genes, proteins, and biochemical pathways. From a theoretical standpoint, systems approaches to organismal biology are like complex interaction networks of species within an ecosystem, except for that a systems approach includes the nested hierarchy of the central dogma of biology.

Horizontal integration of physiological processes across organisms within ecosystems

With rapid and unprecedented global change in the Earth's climate (GCEC), organisms experience a more unpredictable and extreme environment (IPCC, 2007). Although temperature is known to have ubiquitous effects on rates of physiological processes and the integrity of macromolecular structures and thus is the main abiotic factor to which we pay attention (Hochachka and Somero 2002), it is by far not the only one of importance for predicting the effects of change in the Earth's climate on the physiologies of organisms. With the oceans buffering the terrestrial increase in temperatures by absorbing much of the carbon dioxide and the heat itself, their chemistry is changing rapidly, greatly affecting the physiologies of marine organisms (Pörtner et al. 2005). Despite the fact that the extent of change in oceanic and coastal pH, as well as their natural variation, is still debated, acidification of the world's oceans through increasing levels of carbon dioxide dissolving and forming carbonic acid has been identified as one of the main effects of GCEC, with potentially broad consequences for the ability of organisms to build their calcium-based shells, exoskeletons, and reefs (Riebesell et al. 2000; De'ath et al. 2009). Warm air can hold more water and this leads to changes in precipitation. However, the change is not just "more precipitation" but more intense and less predictable patterns of precipitation, which leads to heavy winter run-offs into rivers and along the coasts, posing a challenge to stenohaline and moderately euryhaline marine organisms (Richmond et al. 2007). On land, this can lead to long periods of drought, interrupted by episodes of heavy and unpredictable precipitation, which affect the availability of food for terrestrial organisms from temperate to tropical regions (Malhi and Wright 2004). A thorough understanding of the basic physiological responses to these changes and its evolutionary variation in "emerging" or "new" model organisms from various habitats and a set of organisms from diverse phylogenetic groups is needed to provide a basis for predicting the effects of GCEC (Pörtner 2010; Somero 2010; Tomanek 2010). This work will present a direct contribution to our need to predict and adapt to the organismal consequences of GCEC.

What is missing from this view is the importance of biological interactions for predicting the biological effects of GCEC (Segal 2010; Tabachnick 2010). The closer the interaction, e.g., pathogen or symbiont versus predator, the more important it is to consider the "thermal sensitivities" of the interacting organisms to predict the effect of temperature (or other abiotic changes) on their association. This will become especially important for our society if we evaluate the interplay between phytophagous insects or pollinators and our crop plants (Dukes et al. 2009). Other examples have shown that longer reproductive seasons can allow insect pests to have devastating effects on forests. The spread of avian malaria affects a number of bird assemblages in tropical regions already, and we know little about the characteristics and variation of the immune responses of natural bird populations to pathogens (Segal 2010). Amphibians have made

headlines due to their recent declines, which have been linked to the effects of warmer temperatures on their immune system and one of their major pathogens, among a number of other factors (Hayes et al. 2010). The same is the case for *Perkinsus*, a pathogen of oysters that seems to spread possibly due to increasing temperatures, eutrophication of coastal waters, or a combination of both (Ford and Smolowitz 2007).

Corals and their symbiotic algae (*Symbiodinium*) are greatly affected by temperature extremes and provide an excellent example for the challenge we face when predicting the biological effects of GCEC (Mydlarz et al. 2010). Bleaching, or the expulsion of *Symbiodinium*, occurs when temperatures reach a certain threshold, depriving the coral of a major food resource and leading to death. However, the algal population is heterogeneous and thus some genetic strains survive and re-colonize the coral polyp, leading to the recovery of coral reefs. How a warmer and more acidic ocean will change the balance between greater or lesser survival rates of corals through impacts on both the symbiont and the host is an area of active study (Anthony et al. 2008; De'ath et al. 2009; Barshis et al. 2010). Furthermore, eutrophication may also add another stress to the symbiosis. Developing a better understanding of the physiological effects of multiple co-stressors on biological interactions, often not just one, is what comparative physiologists have to deliver in order to accurately predict the biological effects of GCEC.

In corals and other organisms for which symbiosis plays an important role in physiological responses to the environment, studies are needed that investigate the physiologies both of host and symbiont, as well as different genetic strains of symbionts, no trivial task given the dependent nature of the organisms' relationship. To add to the difficulty of this task, we now understand that corals are a community of more than just the cnidarian host and the *Symbiodinium* alga; the coral holobiont also includes specific types of microbes that live on the surface and inside the skeleton of corals (Thurber et al. 2009). Microbes are likely important modulators of the biology of complex animals or animal interactions in ways that organismal biologists are only beginning to appreciate, such as understanding the causative agents in coral disease (Thurber et al. 2009; Sunagawa et al. 2010). Many of the microbes living around, on, and inside organisms we study are not able to be cultured, but direct sequencing of phylogenetically informative loci allows estimates both of diversity and abundance to be made (Sogin et al. 2006). Organisms, at least humans, are a community comprised of more microbial cells than animal cells and high-throughput sequencing is being used to characterize variation in microfloral diversity and abundance within and between individuals (Costello et al. 2009). Through this research, we are learning that organisms may be more microbial cells than animal cells (at least this has been shown in humans) and that while we once thought that microbial symbionts were monocultures (e.g., gut microflora) we are now learning that there is a great diversity of microbiota present. What is the impact of our ability to characterize the microbial world on how we study the organisms so thoroughly inundated with those bacteria?

Although clearly a major priority for funding agencies, support for predicting the effects of GCEC has to be balanced with other challenges concerning a wider set of fundamental research questions. For example, an integrated approach, using several ''omic''-platforms, must be used to obtain a system-level understanding of how organisms respond to the environment. This poses a tremendous challenge, as one laboratory alone cannot possess the expertise to conduct transcriptomic, proteomic, and metabolomic analyses. Outsourcing to genome and proteome centers has its limits. The maintenance of those facilities is costly, which requires them to focus on high-throughput and, in the case of proteomic and metabolomic studies, a limited set of model organisms. It also deprives students from being part of discovery and the generation of new questions, such as the power of mass spectrometry to analyze posttranslational modifications and their importance for cellular signal processes (Marks et al. 2009). Thus, scientific consortia have to develop to support collaborating laboratories, often specializing in one technique, to work together and exchange students to pursue a systems analysis. Importantly, the analysis of the data that emerge from these projects requires computational tools that are not always available for emerging model organisms, due to a lack of inter-relational databases that integrate the results of the different platforms. There is also a risk in applying gene ontologies, which are based on only a few major model organisms, to analyze datasets investigating the response of emerging model organisms to a novel stress, such as acidification. An advantage of systems biology techniques is to discover new hypotheses, which include identifying novel protein functions when an organism is exposed to different challenges.

The emphasis on systems technologies can easily make a comparative physiology student wonder how they will be able to do research at smaller universities that lack the support for such projects. And how can one involve undergraduate students in systems biology research? First, the technology is becoming cheaper and more accessible to everyone, sometimes through collaborations. Second, the systems biology platforms, although crucial in many aspects, are only a tool for generating new hypotheses that require careful verification, using standard physiological techniques, sometimes as simple and yet powerful as enzyme assays. Funding agencies will have to balance the need to push forward new technologies, with their applications to new questions, and the verification of new hypotheses that are generated by these technologies with smaller scale, more targeted approaches to advance both discovery-driven and hypothesis-driven science. Innovation in comparative physiology comes in different flavors that range from high-throughput sequencing platforms, application of mass spectrometry, analysis and integration of data,

and targeted verification of hypotheses to social changes in the scientific community as a whole.

Temporal integration of physiological processes during evolutionary change

In 1973, the geneticist and evolutionary biologist Theodosius Dobzhansky boldly claimed, ''Nothing in biology makes sense except in the light of evolution'' (Dobzhansky 1973). Yet, the degree to which comparative physiology has embraced evolutionary biology as a unifying theory that drives research remains a largely unfulfilled goal. To be fair, much progress has been made to incorporate evolutionary theory into studies of physiology (Garland and Carter, 1994; Feder and Hofmann, 1999; Zera and Harshman 2001), but evolutionary physiology remains a relatively small subdiscipline. Contemporary evolutionary physiology largely traces its origin to the landmark book edited by Feder et al. (1987) entitled ''New Directions in Ecological Physiology'', which brought physiologists and evolutionary biologists together with the goal of encouraging direction and growth in the field. Since then, substantial progress has been made in (1) the incorporation of phylogenetic relationships and the development of associated statistical tools in comparative studies (Garland et al. 2005), (2) the incorporation of evolutionary biology in the study of human physiology and the rise of Darwinian medicine (Williams and Neese 1991; Hales et al. 1992, 2001; Cordain et al. 1998), and (3) the use of laboratory experiments on the selection of physiological traits (Bennett and Lenski 1999; Gibbs 1999; Bennett 2003; Garland 2003). Nevertheless, the ''language'' and theories developed within evolutionary biology to explain adaptive evolution are largely absent from studies seeking to understand the physiological basis of adaptation to different environments. Such a separation is unfortunate, as the goals of comparative physiologists and evolutionary biologists are broadly overlapping, with each field informing the other (Garland and Carter 1994; Bradley and Zamer 1999). Here, we suggest that the time is approaching when the techniques used by comparative physiologists and evolutionary biologists are converging, and, as such, there is a growing need for conceptual unification around topics that span the disciplines. Below, we highlight two areas in which comparative physiology and evolutionary biology would benefit from this type of unification. First, we focus on the idea of phenotypic plasticity as a unifying and guiding framework for both disciplines. Second, we discuss the importance of trade-offs and relating physiological variation to fitness.

Phenotypic plasticity is the capacity for a given genotype to produce different phenotypes in response to different environments. Said differently, plasticity is the reprogramming of the genome in response to the external and internal environment (Aubin-Horth and Renn 2009). Thus, all phenotypic traits, whether they are physiological, behavioral, morphological, or some component of the transcriptome or proteome, can be studied from the perspective of phenotypic plasticity if changes in the environment alter expression. Evolutionary biologists have long been interested in the phenomenon of phenotypic plasticity, in part because of the centrality of the environment in shaping the phenotype. Indeed, a central problem in quantitative, ecological, and evolutionary genetics is to partition the phenotypic variation observed in populations into its genetic and environmental components to better understand the expected response to selection (Falconer and MacKay, 1996; Roff, 1997; Lynch and Walsh 1998; Connor and Hartl 2004). A large body of theory has been developed to explain the selective pressures that favor the evolution of a plastic genotype over a canalized, or nonplastic one (Schlichting and Pigliucci, 1998) and what role this plasticity might play in evolutionary adaptation (Ghalambor et al. 2007). The traits of greatest interest to evolutionary biologists are those that are most closely related to fitness, which are usually continuous, complex, and determined by many gene loci whose expression is sensitive to the environment; this also describes most physiological traits. Evolutionary biologists use specific terms and language when describing the genetic and environmental inputs that determine phenotypes. For example, the visual representation of a plastic trait as a line or function that describes how the value of the trait changes as a function of the environment is called a *reaction norm*. Thus, nonplastic traits exhibit flat reaction norms, whereas plastic traits exhibit reaction norms that have a particular slope or curvature that, in turn, may be reversible or fixed during development. Variation among individual genotypes in their reaction norms to the same environmental conditions is called *genotype by environment interaction*; or a measure of how much plasticity varies within a population. The connections of these terms to comparative physiology are apparent. Many, if not most, physiological traits are plastic, as their expression is dependent on the environment. How these physiological traits specifically change as a function of temperature, pH, salinity, or availabilitiy of oxygen are the reaction norms for the trait. While some physiologists explicitly use the language of evolutionary biology by referring to reaction norms for physiological traits (Angilletta et al. 2002; Cossins et al. 2006; Kingsolver and Huey 2008), most do not, thus restricting effective discourse between disciplines. Even fewer physiological studies of animals attempt to quantify the amount of variation among individuals within a population (Zamer et al. 1999; Whitehead and Crawford, 2006), and instead remain focused on studying specific pathways under controlled environmental and genetic backgrounds. Yet, as has been repeatedly pointed out, this variation provides the raw material for evolution to occur (Whitehead and Crawford 2006). Why are these concepts important beyond the small population of evolutionary physiologists? Technological advances now allow physiologists and evolutionary biologists to move beyond phenotypes and explicitly examine the genetic basis of phenotypes. Microarrays, quantitative PCR, and high-throughput sequencing are causing a convergence in the

experimental methods, datasets, and statistical tools throughout the biological sciences. Thus, the conceptual connection between the work of comparative physiologists studying how populations or species differ in the way that temperature alters the number of copies of a particular transcript, and the evolutionary biologist interested in the way that selection acts on reaction norms is tantalizingly close. How this convergence in approach advances the goals of comparative physiology is a grand challenge that we feel will come from understanding what maintains physiological variation within populations and how this variation is related to fitness.

A fundamental assumption of integrative biology is that organisms are made up of complex interacting systems, such that physiological traits represent the integration of numerous biochemical, morphological, and behavioral traits. This perspective implies that adaptive changes in physiological pathways and systems will often involve trade-offs between different interacting components (Pörtner et al. 2006). Evolutionary biologists share this viewpoint, but tend to emphasize integration at the genetic level in the form of genetic correlations (e.g., antagonistic pleiotropy) and the direct and correlated responses to selection (Arnold 1983; Lande and Arnold 1983). Arnold (1987) referred to this as the ''physiology to gene approach,'' where variation in physiology is related back to the action of multiple interacting genes and traits, with the goal of understanding how evolutionary changes in physiology will affect the evolution of other traits. Ultimately, comparative physiologists and evolutionary biologists agree that how selection acts on physiological traits is determined by the fitness costs and benefits of changing suites of interacting traits (Ghalambor et al. 2003; Dalziel et al. 2009). In contrast, candidate gene and molecular approaches to the study of physiological traits tend to examine specific pathways or networks in isolation of the other components of the phenotype. While such molecular approaches have been extremely successful in the discovery of the mechanistic ways in which organisms respond to the environment, they often require looking at such pathways in isolation of the whole organism and the environments in which they occur. Arnold (1987) referred to this as the ''gene to physiology approach'' because it starts with variation at a specific gene locus and elucidates the pathway from the gene to the physiological phenotype. The grand challenge facing comparative physiologists is how to incorporate both approaches to improve our understanding of mechanism and to relate physiological variation to fitness under field conditions that expose organisms to diverse selective pressures (Dalziel et al. 2009). How can such a challenge be overcome? We suggest it will require comparative physiologists to become more comparative and collaborative in their research programs. Below, we expand on these ideas.

While much attention has previously been given to defining what comparative physiology is, most practitioners would agree that it involves the study of diverse physiological systems, in diverse organisms, adapted to diverse environments. To date, most of the taxonomic diversity studied by comparative physiologists has focused on interspecific comparisons and has relied heavily on laboratory-based measurements. But comparative biology may also encompass comparisons between individual genotypes occupying the same environment, and comparisons between populations occupying different environments. Such comparisons are integral to incorporating gene-to-physiology and physiology-to-gene approaches for several reasons. First, a comparison of individuals within populations is the starting point for describing the amount of standing variation in physiological systems (Zamer et al. 1999; Whitehead and Crawford 2006; Crawford and Oleksiak 2007). For example, recent work on birds has shown how variation within and between populations in the *Clock* gene is related to physiological and behavioral differences related to fitness (Liedvogel et al. 2009). Similarly, genetic variation of the metabolic enzyme phosphoglucose isomerase has been linked to variation in flight metabolic rates and dispersal rates in butterflies (Haag et al. 2005). Crawford and Oleksiak (2007) reported substantial differences between genetic lines in the pathways that explain substrate-specific metabolism. That different genotypes are able to accomplish the same performance in different ways, has troubling implications for the generality of conclusions drawn from a limited number of genotypes (Crawford and Oleksiak 2007). Collectively, these results point towards a future when there will be a greater appreciation for genetic diversity and the processes that maintain it. Second, while there have been repeated calls in the past for taking laboratory-based studies of physiological pathways to the field (Arnold 1983; Dalziel et al. 2009), transcriptomic and proteomic techniques now allow for quantifying variation among individuals and populations under natural field conditions. Such studies are critical not only to better understand the diversity of physiological strategies used by organisms under the heterogeneous conditions in the field, but also are critical to linking physiological traits to individual fitness. Field studies based on laboratory research enable comparative physiologists to test hypotheses in a context where the trade-offs associated with expression of a physiological trait are exposed. Controlled laboratory environments by definition shield individuals from these types of fitness trade-offs (Reznick and Ghalambor 2005). For example, laboratory-based research has shown that the genetic and physiological pathways responsible for resistance to insecticides comes about through the affects of many alleles of small effect distributed among various genetic lines (McKenzie and Batterham, 1994). However, under field conditions, resistance evolves through the substitution of single genes of large effect that arise as rare mutations that then spread through migration (McKenzie and Batterham 1994; Lenormand et al. 1998). These results suggest that the use of genetic lines established from a small number of individuals does not do a good job of predicting how resistance evolves in nature. Furthermore, the benefits of resistance to insecticides are highly context-specific and potentially costly to individual fitness.

At the two loci involved in insecticide resistance (*ace-1* and *Ester*), the alleles that provide a fitness advantage in the presence of insecticide through the production of acetylcholinesterase and other esterases have negative pleiotropic effects that result in increased developmental time and reduced wing length (Chevillon et al. 1999; Bourguet et al. 2004). Thus, individuals carrying insecticide-resistance alleles in populations not exposed to pesticides are likely to be at a disadvantage and selected against, resulting in genetic variation among populations as a function of their exposures to pesticide (Chevillon et al. 1999; Bourguet et al. 2004). It is likely that most physiological pathways have similar pleiotropic effects that will only be revealed under field conditions (Reznick and Ghalambor 2005). After all, if we cannot demonstrate that the body of laboratory research conducted by comparative physiologists in the laboratory translates into meaningful adaptive patterns in nature, it calls into question the utility of our entire research programs.

Proposed initiatives

In 2009, the National Science Foundation convened a workshop to discuss the challenges for 21st century biologists. The main conclusions in the workshop report are: (1) the need for tools to acquire, archive, access, and interpret vast amounts of information; (2) developing new model organisms for forward and reverse genetics; and (3) developing an infrastructure that promotes interdisciplinary training and collaboration between people (Robinson et al. 2010). In this article, we have addressed three challenges that are essential for forward progress of comparative physiology, as well as for other disciplines in comparative biology. Further, we outline some steps that must be taken to meet those challenges.

Develop model organisms that integrate vertically across all levels of biological organization

Both physiologists and evolutionary geneticists seek to understand the mechanisms underlying organismal adaptation and evolution. However, physiologists, for the most part, do not assess the genetic basis of variation in ecologically important phenotypes (''traits'') for the organisms they study. Conversely, evolutionary geneticists do not know how variation in genetic markers mechanistically relates to ecologically important phenotypes. Since the root processes, namely changes at the genome level, are the same for both comparative physiologists and evolutionary geneticists, both disciplines can benefit from analyses of whole genomes of the organisms they study. Furthermore, evolutionary genetics will directly link to mechanistic physiology across the bridge of the genome. Until recently, the costs of producing a complete genomic sequence have been prohibitively expensive. Innovations in ''next generation'' sequencing technology have reduced costs and increased efficiencies in obtaining and cataloging genomic sequences (Metzker 2010). The estimated cost for the first complete human genomic sequence that was published in 2004 is $300M; in contrast, current estimates for a ''personal genome'' are as low as $5K (Metzker 2010). With the decline in costs, proposals have come to vastly expand the number of species for genomic sequencing. The Genome 10K Project aims to sequence the genomes of 10,000 representative vertebrate species for a comprehensive understanding of the evolution of vertebrates (Haussler et al. 2009).

Relating variation in physiological capacities and responses to population genetics requires whole-genome sequencing of thousands of individuals within and between populations. Physiological responses to environmental change are complex and probably involve hundreds of genes. Adding further complexity is that the assemblage of genes involved may change over temporal scales, and regulation by epigenetic and/or miRNA adds further complexity. Population-level genome sequencing is analogous to the National Institutes of Health (NIH) 1000 Genomes Project, which is assessing variations in the genomes of at least a thousand human individuals to identify regions of the genome associated with common human diseases (http://www.genome.gov/27528684) (Kuehn 2008) and the NIH Genes, Health, and Environmental Initiative (GEI) to discover genetic susceptibilities of humans to environmental risks (http://www.genome.gov/19518663) (Christensen and Murray 2007). Such efforts have revealed variation between individuals ranging from single base pair mutations, known as single nucleotide polymorphisms, to changes in genes' copy numbers arising from duplications or deletions of large fragments of the DNA (Christensen and Murray 2007). Genomic screens have identified mutations in coding and noncoding regions associated with diseases or developmental defects (Christensen and Murray 2007; Cauchi et al. 2008; Boles et al. 2009).

We propose an expanded effort to assess whole-genome variation in organisms or groups of organisms that can serve as ''diagnostic indicators'' for particular habitats. It requires organisms (1) that are distributed over a wide geographic range and are key components of a biological community, (2) that show variation in physiological responses to abiotic factors, and (3) in which transcriptomic and proteomic tools have been developed (Dalziel et al. 2009). Having an annotated genome to model organisms would facilitate analysis of transcriptional and posttranscriptional responses. Furthermore, comparing genomic data from individuals that differ in response may identify assemblages of loci associated with a particular physiological trait. The power of this approach is that differences in both regulatory and structural domains of mRNA genes can be identified (Boles et al. 2009). This would provide the tools to assess biologically relevant variation within a population. Individuals could be screened for these loci, using high-throughput methods, to assess how a population may respond to an environmental challenge, such as hypoxia, acidification, or temperature extremes. An example of the successful application of this approach is a recent study of the genetic variation of the innate immune response to infections in *Drosophila* (Sackton et al. 2010).

Develop an infrastructure of tools, training, and resources

The required resources both in terms of data and computational capacity in order to undertake a systems approach are not trivial. Presently, approaches in systems biology are making a lot of headway in the biomedical sciences where significantly larger amounts of funding are available. The sociopolitical and economic leverage of the biomedical sciences in the quest to improve the health and quality of life for humans are able to foot the large price tag of systems biology that require massive investment to recruit and train scientists, to fund laboratories generating the necessary foundational data, and to purchase, maintain, and improve computational resources. For organismal biologists, who have often benefitted by taking the successes from the biomedical research community without having to also endure the failures, there are two foreseeable outcomes from the initiatives in systems biology presently underway. In the first outcome, advances in systems biology may result in the development of computational tools and of methods for collecting empirical data that are inexpensive, broadly applicable, and widely accepted. In that case, organismal biologists can pick up those tools and use them to address questions of interest with any study organism. In a second possible outcome, advances in systems biology may result in the development of taxon-specific approaches. For example, we may need different quantitative models for gene interaction and protein interaction networks for each model organism because those organisms, even at the cellular level, could have physiological variability that requires a unique approach. What are organismal biologists to do if quantitative analyses in systems biology must be performed for each of the diverse range of organisms we study? Let us hope that the first possible outcome occurs.

In either event, even if the well-funded biomedical research community develops a set of tools in systems biology that any organismal biologist can adopt, there will still be significant costs involved in generation of empirical data and management of those data. How will organismal biologists obtain such funding? As Halanych and Goertzen (2009) indicated, the greatest Grand Challenge in Organismal Biology (#12) may be garnering increased public support for the research that we do. Organismal biologists need to do a better job in communicating to the public that we are not solely concerned with the health and quality of life for one highly privileged species (our own), we are concerned with the health and well being of all the animals, plants, and other organisms with whom we share our planet and ultimately, on whom we depend for our sustenance. What value do governments place on understanding every organism on earth besides humans? Biomedical lobbyists for public-interest groups and the pharmaceutical/biotechnology industry can influence public perception and, as pointed out by Halanych and Goertzen (2009), the complex sociopolitical and economic issues that underlie funding decisions. There is room for the Society for Integrative and Comparative Biology and organismal biologists to better communicate to the public that what we do is important to nearly all animal life on the planet, including our own species.

Assuming that organismal biologists manage to garner the kind of financial support necessary, should organismal biologists be encouraged to undertake a systems biology approach that integrates across all of the levels of biological organization we study? How should researchers with a finite amount of resources and facilities spend their time and money in addressing questions of physiological adaptation or physiological responses to environmental change? Are there some organisms that we should be studying, such as ''new'' model organisms that now have complete genome sequences? Are some levels of biological organization (e.g., transcriptome versus proteome versus metabolome) more informative, so that we make a greater attempt to understand those levels? Answers to these questions are important aspects of future decisions about funding.

We are on the verge of an exciting era of discovery brought about by revolutionary advances in how we acquire, access, analyze, and integrate large datasets from different levels of biological organization. Biological systems are constantly adapting and evolving in response to biotic and abiotic factors. If we are to better understand the robustness of ecosystems to perturbation, we, as comparative physiologists, must break through boundaries between disciplines and build, with the support of funding agencies, research teams that can tackle the complexities intrinsic to biological systems across vertical, horizontal, and temporal scales. As scientists trained to establish ''independent'' research programs, this may be the greatest challenge of all.

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