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REVIEW

Comparative genomics in ecological physiology: toward a more nuanced understanding of acclimation and adaptation

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Summary

Organisms that live in variable environments must adjust their physiology to compensate for environmental change. Modern functional genomics technologies offer global top-down discovery-based tools for identifying and exploring the mechanistic basis by which organisms respond physiologically to a detected change in the environment. Given that populations and species from different niches may exhibit different acclimation abilities, comparative genomic approaches may offer more nuanced understanding of acclimation responses, and provide insight into the mechanistic and genomic basis of variable acclimation. The physiological genomics literature is large and growing, as is the comparative evolutionary genomics literature. Yet, expansion of physiological genomics experiments to exploit taxonomic variation remains relatively undeveloped. Here, recent advances in the emerging field of comparative physiological genomics are considered, including examples of plants, bees and fish, and opportunities for further development are outlined particularly in the context of climate change research. Elements of robust experimental design are discussed with emphasis on the phylogenetic comparative approach. Understanding how acclimation ability is partitioned among populations and species in nature, and knowledge of the relevant genes and mechanisms, will be important for characterizing and predicting the ecological and evolutionary consequences of human-accelerated environmental change.

Key words: ecological genomics, ecology, environmental genomics, evolution, mechanistic ecology, physiological genomics.

Introduction

Genome structure and functions facilitate organism–ecosystem interactions in physiological time, but are also shaped by these interactions across evolutionary time. Traditionally, genomics research has focused on many genes but few phenotypes (inbred strains of model organisms) in artificial settings (laboratories), and ecology research has focused on many phenotypes but few genes. The emerging field of ecological genomics offers to bridge this gap between phenotype-poor but gene-rich genomics and phenotype-rich but gene-poor ecology (Van Straalen and Roelofs, 2006) by deploying holistic exploratory molecular tools for investigation of whole genomes of organisms with compelling natural histories. Importantly, genomics is more than just high-throughput genetics, because genomes behave as integrated systems exhibiting complex behavior (Zhu et al., 2008). This marriage between genomics and ecology contributes to genomics by, for example, advancing the discovery of gene function in natural contexts, and advances ecology by discovering mechanisms whereby organisms integrate cues from, respond to, and are ultimately shaped by, their environment.

The success of this marriage will be accelerated by integrating the holistic systems-level hypothesis-independent research paradigm of genomics with the comparative approaches that have been a mainstay of comparative and ecological physiology since before August Krogh (the Nobel-prize-winning physiologist). Functional genomics approaches are now commonly applied to discover the genomic basis of organism–environment interactions (Aubin-Horth and Renn, 2009), though rarely within a comparative framework (Hodgins-Davis and Townsend, 2009). Comparative experimental designs facilitate discovery of genomic elements, regulatory systems and physiological processes that are conserved across taxa, thereby indicating their core importance [e.g. for aging (McCarroll et al., 2004)], and those that diverge across taxa, implicating relaxation of selective constraint or diversifying selection [e.g. for dormancy response (Ragland et al., 2010)]. Importantly, carefully crafted comparative designs can provide evidence to distinguish variation that is adaptive (and therefore relevant for promoting success in specific habitats) from variation that arose from neutral evolutionary processes.

The goal of this review is to highlight how research programs using comparative genomics in ecological physiology (CGEP) can offer more nuanced understanding of the mechanisms that enable physiological acclimation and evolutionary adaptation. By comparing closely related taxa (clones, ecotypes, populations or species) that occupy, and presumably have evolved in, different ecosystems, the goal of CGEP is to discover which genes and genomic programs are functionally important for establishing phenotypes that are suited to exploiting particular niches. First, the rationale for such a research program is briefly summarized, followed by practical, experimental design and statistical considerations. A few case studies are highlighted that offer examples of successful applications of this research program. Finally I draw attention to additional systems that are poised for exploitation using CGEP, with particular attention paid to models for climate change research. I conclude with a description of how CGEP will accelerate development of a mechanistic approach to ecology.

Genomics of acclimation and adaptation

Shifts in environmental conditions require compensatory change from resident species. Compensation for environments that change within physiological timescales can be facilitated by a flexible or plastic phenotype, which can draw on the functional genome, for example by alteration of transcription or translation. The degree to which an organism can alter its phenotype, partly governed by functional genomic mechanisms, will contribute to delimiting the range of environmental conditions to which it can acclimate. Accordingly, exploratory functional genomic approaches can contribute to systems-level mechanistic models for helping define a species' fundamental niche and its biogeographical distribution. Environmental change that emerges across generations can also be accommodated by plasticity, or alternatively may drive structural change of genomes by adaptive and demographic evolutionary processes that sort allele frequencies within populations across generations.

The genomic elements that facilitate plasticity in physiological time and the genomic targets of adaptive evolutionary processes may often overlap. For example, genes regulated during acclimation in killifish are more likely to show patterns of adaptive population divergence than genes that are not associated with acclimation (Whitehead et al., 2012; Whitehead et al., 2011), and genes harboring signatures of natural selection in marine and freshwater stickleback fish are enriched for genes that are functionally involved in physiological regulation of osmotic homeostasis (Shimada et al., 2011). Indeed, variation in gene expression is extensive within populations and can serve as crucial substrate for evolutionary change (Crawford and Oleksiak, 2007; Rees et al., 2011; Townsend et al., 2003; Whitehead and Crawford, 2006b).

The comparative approach in ecological physiology research

Physiology research, in the simplest sense, seeks to understand how complex biological systems work. Comparative approaches, where physiologies of more than one taxon are compared, have a long tradition in physiology research and offer insight into fundamental physiological mechanisms that would not have been possible from examination of only a single species. At the most fundamental level, biologists make comparisons because we want to understand the nature of biodiversity, and we tend to be particularly interested in two components of biodiversity: (1) the traits or mechanisms that are universal and unite related functions across many diverse taxa, and (2) the traits or mechanisms that are uniquely evolved to enable novel life histories. These, of course, are two sides of the same coin. For example, as highlighted by Somero, comparative studies led to the discovery that the select group of molecules that serve as intracellular organic osmolytes across diverse taxa do so by virtue of unique physical properties that stabilize protein structure and function (Somero, 2000). This is one side of the coin. An example of the other side of the coin is lineage-specific adoption of urea as an osmolyte, which tends to destabilize proteins, thereby providing an exception to the rule, but this led to the discovery of parallel lineage-specific use of methylammonium compounds to counteract the protein-destabilizing effects of urea. That is, the comparative approach enabled discovery of biological universals as well as lineage-specific novelties.

Different comparative designs are more or less appropriate depending on which side of the coin (universal *versus* lineage-specific phenomena) one is most interested in. If the focus is on identifying mechanisms that underlie universal traits, then ideally one should include the broadest sample of taxa that share that trait. In contrast, if the focus is on mechanisms that underlie novel lineage-specific and presumably adaptive traits, then more nuanced and strategic comparative designs are useful. The goal of these studies is often to determine, among those traits that vary between taxa, those that are adaptive and therefore crucially important for life in a particular habitat. The development of explicit evolutionary and statistical models has greatly facilitated rigorous exploration of alternative evolutionary explanations for trait differences among related species [formally called the comparative method in evolutionary biology (Harvey and Pagel, 1991)]. A core component of these models is the use of phylogenies to reconstruct the historical divergence of the trait (Felsenstein, 1985). Phylogenies provide a framework to test how many times a particular trait evolved; they help answer whether traits are shared because they have repeatedly evolved to support a shared way of life (indicative of adaptive relevance), or alternatively, because those species share recent common ancestry (indicative of phylogenetic baggage).

The advent of modern genomics enabled deployment of massively parallel tools that offer systems-level insights into how the environment interacts with, and ultimately shapes, the genome, and mechanistic insights into how genome regulation and variation are linked to phenotypic plasticity and phenotypic evolution. Indeed, the expression of genes is one of the first steps along the path linking genotype to higher-level phenotypes such as morphology or physiology. Genome biology has been comparative since its inception; among the earliest questions was, "which genes are common across all species (and represent core biological processes) and which are species-specific (implying niche-specific relevance)?" In contrast, functional genomics has tended to either examine genome regulation in response to environmental perturbation with no comparative component (e.g. within a single species, inbred strain, or cell line) or compare genome expression across species but in a static environment (no environmental manipulation). Environmental manipulation enables discovery of genes and pathways functionally involved in phenotypic plasticity. Inclusion of species contrasts of course advances understanding of the universality, lineage specificity or niche specificity of these responses to the environment. Table 1 summarizes the types of research questions, methodologies and data that are associated with comparative physiology, physiological genomics and CGEP research programs. Experimental designs that would facilitate discovery of taxon-by-environment interactions have not been commonly exploited. Yet, given the exponentially increasing accessibility of genomic-scale tools for application in non-traditional model systems, and the legacy of discoveries derived from comparative approaches in physiology and evolutionary biology, comparative physiological genomics is likely to accelerate the pace of integration in 21st century comparative physiology (Mykles et al., 2010).

Experimental design considerations for comparative ecophysiological genomics research

Outlined here are some conceptual and practical issues to be considered in designing robust comparative physiological genomics experiments. These include choice of experimental design for manipulating the environment, defining the comparative framework (choice of species and their evolutionary relationships), choice of data collection platform (microarrays *versus* RNA sequencing), statistical analysis, and data interpretation.

Environmental manipulation

In so far as the goal of CGEP is to compare and contrast responses of several taxa to the environment (i.e. to compare the 'norms of

Table 1. Types of research questions, methodologies and data that are associated with comparative physiology, physiological genomics and comparative genomics in ecophysiology research programs

	Research questions	Typical methodologies	Types of data generated
Comparative physiology	What traits or mechanisms are universal and unite related functions across many diverse taxa? What traits or mechanisms are uniquely evolved to enable novel life histories?	Experimental manipulation of environmental variables; compare physiological response function and associated mechanisms among taxa.	Phylogeny, phenotypic characterization (e.g. biochemistry, physiology, behavior)
Physiological genomics	What are the genomic elements or regulatory programs that facilitate physiological response to environmental change?	Experimental manipulation of environmental variables; characterize genomic response function.	Genome response function
Comparative genomics in ecophysiology (CGEP)	What are the genomic elements that are conserved across taxa and that unite shared functional traits? What is the genomic basis for adaptive divergence in physiological compensatory abilities among taxa?	Following environmental challenge, compare genomic response function among taxa within a phylogenetic comparative framework. Most powerful when genome response is linked to higher-order (physiological) response functions.	Phylogeny, phenotypic characterization, genome response function

reaction'), the environment must be manipulated with species in a 'common garden' (Schlichting and Pigliucci, 1998). This is often achieved by raising organisms within a controlled laboratory setting and challenging them with carefully controlled environmental manipulation, such as changes in temperature, salinity, oxygen, pollutants or symbionts. These experiments offer careful control over environmental factors of interest, but induced genome responses may differ from those expressed under natural conditions. An alternative design is field transplant experiments, where organisms collected from different environments may be transplanted to alternate habitats, and differences in genome expression measured across environments. These experiments are often practically difficult and offer less control over environmental variables, many of which may covary, but they are perhaps more ecologically realistic (e.g. Cheviron et al., 2008).

Comparative framework

If the comparative study seeks to investigate the adaptive mechanisms underlying lineage-specific trait divergence, this requires comparison of more than two species and requires a robust estimate of shared ancestry (phylogeny). Comparisons between two species are useful for distinguishing traits that are under strong constraint from those that are more evolutionarily labile, but not for distinguishing variation that is adaptive *versus* neutral. Trait variance between any given pair of species may be governed by an interaction between neutral genetic drift and natural selection. The influence of neutral drift is scaled by time since shared ancestry. That is, one would expect two species that share a recent common ancestor to share more similarities than would more distantly related species because of neutral genetic drift alone. If comparing just two species, one cannot determine whether the trait divergence

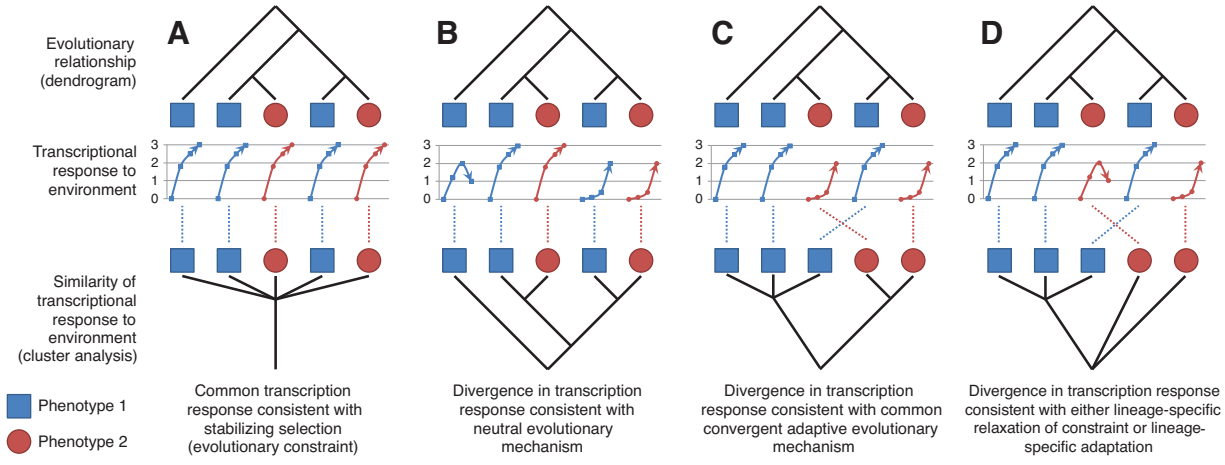

Fig. 1. Four different scenarios of similarity or divergence of genomic response to environmental challenge across taxa. The dendrograms at the top indicate the phylogenetic relationship uniting five taxa that vary in phenotype (blue squares and red circles indicate the phenotypic identity of each taxon). The line graphs in the middle show the trajectory of gene expression response to environmental challenge for each taxon. The dendrograms at the bottom represent the results of cluster analysis uniting taxa with the greatest similarity of gene expression response to environmental challenge. Panels A–D each indicate different scenarios of how gene expression response to the environment might vary across taxa, with the evolutionary inference summarized at the bottom of each panel.

observed is different from that expected by time since shared ancestry, especially because selective constraint is gene- or trait-specific, or which character state is derived or ancestral (trait polarity). However, if three or more species are compared, the neutral genetic distance uniting all pairs of species can be used as a matrix of expected covariances, where species united by the shortest genetic distances (most recent shared ancestry) would be expected to share greatest similarity for any given trait (physiology or gene expression) (Felsenstein, 1985), and trait polarity can be determined. Against this neutral framework of expected covariances, one may test whether observed trait variance among taxa matches the phylogenetic (neutral) expectation, or rejects it in favor of the alternative (presumably adaptive) hypothesis.

The phylogenetic comparative approach can be used to test which genes (or other traits) contribute to adaptive phenotypic divergence among taxa (e.g. Whitehead and Crawford, 2006a). Examples are illustrated in Fig. 1. Consider a group of five species that each express one of two physiological or morphological phenotypes (represented by the blue boxes and the red circles). The blue species may be freshwater fishes that exhibit low salt tolerance, and the red species may be fish that occupy marine habitats and have high salt tolerance. The dendrogram in the top panel is an estimate of their evolutionary history (phylogeny), indicating that high salt tolerance has evolved twice independently. One may conclude that high salt tolerance is a derived adaptive state in the marine lineages, and seek to identify genomic programs relevant for regulating osmotic acclimation and adaptation through common-garden salinity challenge experiments. The middle panel (line graph) represents the expression response of four different genes (scenarios A–D) to salinity challenge, each indicating a different comparative response. The bottom dendrogram is the result of a cluster analysis that unites species with similar gene expression responses to environmental challenge (clustering of similar norms of reaction).

Many genes may not respond transcriptionally to environmental challenge, although of those that do, one may test whether the response function across species is consistent with neutral expectations (Fig. 1, scenarios A and B) or rejects neutral expectations (Fig. 1, scenarios C and D). Transcriptional response to the environment may be identical across all species (Fig. 1, scenario A). This pattern may be indicative of genes under strong evolutionary constraint, say for a universally shared core signaling or stress response. In scenario B (Fig. 1), patterns of transcriptional response for these genes do differ among species, but in a pattern that mirrors recency since shared ancestry and therefore does not reject the neutral expectation. Although these genes (Fig. 1, scenario B) may be relevant to the adaptive salt tolerance, their pattern of divergence does not reject the null (neutral) hypothesis. Genes with patterns of transcriptional response matching scenario C (Fig. 1) are most similar among species sharing similar phenotypes, consistent with a convergent genomic mechanism supporting the repeatedly evolved salt-tolerant phenotypes. Genes showing this comparative pattern of transcriptional response clearly reject the neutral expectation and are strong candidates for genes supporting adaptive physiological convergence. It is important to note that repeated evolution of similar traits in common environments (convergent evolution) may not always indicate adaptation, for example because of architectural constraint (Gould and Lewontin, 1979) or exaptation (Gould and Vrba, 1982). However, distinguishing adaptations from exaptations may not be important if seeking to establish the current adaptive value of a trait in a given environment (Hochachka and Somero, 2002). Genes included in scenario D (Fig. 1) show unique transcriptional responses in each of the derived salt-tolerant lineages. This pattern also rejects the neutral expectation, but indicates either relaxation of selective constraint in the two derived lineages (for example because of decreased population size accelerating neutral drift), or independent lineage-specific mechanistic solutions to a common adaptive challenge. Statistical approaches for identifying conserved or diverged transcriptional responses are discussed below. These examples illustrate how a carefully considered comparative design enables not only discovery of common and divergent genome expression responses, but enables one to assign the lineage-specific environmental response patterns for each gene to alternative evolutionary hypotheses (neutral *versus* adaptive), thereby accelerating the pace of discovery of genes and pathways that are crucially important for facilitating success in particular habitats.

Data collection platforms

For multi-taxon genome expression comparisons, two general data collection platforms are available: hybridization-based tools (microarrays) and quantitative RNA sequencing, each of which has advantages and disadvantages, although these are rapidly changing. For microarrays, options are to use microarrays designed for each experimental species where challenges include extensive development effort and identification of orthologous genes, or to hybridize multi-species RNA to a platform designed from a single species (heterologous hybridization). Heterologous hybridizations have been the most commonly exploited solution, although one must be careful to control biases and artifacts caused by decrease in hybridization efficiency with increasing genetic distance from the platform species. Clearly, hybridization bias will be minimized for closely related taxa (populations or ecotypes of the same species) because beyond a certain genetic distance hybridization efficiency and specificity will be sufficiently compromised to preclude meaningful analysis. Other recent excellent reviews summarize many of the important considerations (Bar-Or et al., 2007; Renn et al., 2004). In addition, downstream statistical analysis can filter the potential biases associated with heterologous hybridization. For example, if taxon Y is hybridized to a microarray platform with probes designed from taxon X, then inference of lower transcription for any given gene when comparing X with Y is confounded by potential decrease in hybridization efficiency because of accumulated substitutions. However, in comparative physiological genomics experiments often the factor of interest is not necessarily absolute differences in expression between species (where taxon is the main effect), but rather differences in their response to the environment (significant taxon-by-environment interaction). In this case, although one cannot clearly interpret the cause of differences in the positions of reaction norms, the slopes of those reaction norms are interpretable as conserved or taxon-specific responses to the environment.

Although microarrays are the more mature technology in terms of development, deployment and data analysis, massively parallel RNA sequencing is rapidly replacing microarrays for many applications (Wang et al., 2009). RNA sequencing avoids biases associated with heterologous hybridization, offers greater dynamic range of detection, and delivers more nuanced data (e.g. detection of splice variants). However, development of analytical and statistical tools is not mature, costs per sample remain higher than microarrays, and the most effective use requires a reference genome sequence, which is unavailable for many species, although these current drawbacks are likely to be corrected soon with the present

exponential increase in adoption and decrease in parallel-sequencing costs.

Statistical framework

The statistical design usually used to compare norms of reaction across species is ANOVA. In this test, overall variance in expression for each gene is partitioned between an environmental main effect (the norm of reaction) and a taxon main effect (variation in expression level across taxa). Most importantly, ANOVA provides an explicit statistical test for a taxon-dependent response to the environment: significant taxon-by-environment interaction (Sokal and Rohlf, 2001). Unfortunately, the literature is littered with statistical designs that purport to have identified population-specific transcriptional responses to the environment by just scanning for non-overlapping sets of environmentally responsive genes from multiple taxon-specific *t*-tests or one-way ANOVAs; this is statistically indefensible. Clustering methods (multi-dimensional scaling, principal components analysis) are also widely used in exploring patterns of genome expression divergence. Most applications of these tools are not statistically robust for defining genes variably expressed by environment or taxon. Rather, they should be reserved for exploring patterns of correlation and divergence with the subsets of genes identified as population- or environment-responsive by statistically robust methods. In addition to ANOVA, spline-fitting analyses can statistically model and distinguish common and taxon-specific responses to the environment, and can be particularly appropriate for genome responses that may be complex and non-linear, such as dose responses or time-course responses (Storey et al., 2005).

Case studies

Social insects

Eusocial insect models have been efficiently exploited to uncover the genomic mechanisms governing social behavior, and these studies illustrate clever application of 'Krogh's principle', which posits that for any question in biology, nature offers an ideal study system (Jorgensen, 2001; Krogh, 1929). To facilitate cross-taxon comparisons, some studies have used heterologous hybridization (Sen Sarma et al., 2007) whereas others have developed species-specific tools that efficiently exploited genomic information from related species (Toth et al., 2007). Comparative studies indicate that brain genome regulation associated with behavioral maturation is largely conserved across species of bees (Sen Sarma et al., 2007), and much of the subtle species-specific divergence is accounted for by genetic distance, indicating strong evolutionary constraint on genomic mechanisms governing behavioral maturation. Between wasps and bees, which are separated by ~100 million years of evolution, genome regulation associated with provisioning and foraging behaviors is largely conserved, again emphasizing strong evolutionary constraint (Toth et al., 2010). In contrast, genome regulation associated with reproductive behavior tends to be lineage specific in wasp compared with bees, likely reflecting accelerated divergence in reproductive strategies between these clades (Toth et al., 2010). Further comparative studies identified genes associated with aggression behavior, where genes induced with environmentally provoked aggressive behavior within European honeybees were largely the same genes as those that were differentially expressed between brains of European honeybees and the more aggressive (derived) African honeybees (Alaux et al., 2009), thereby providing evidence for evolution by genetic assimilation of plastic responses to the environment (West-Eberhard, 2005).

Killifish

Killifish from the genus *Fundulus* are emerging as models in ecological genomics (Burnett et al., 2007) partly because individuals of some species are highly plastic, in so far as their physiologies are highly flexible to accommodate large changes in environmental conditions such as salinity, temperature or hypoxia. Yet, closely related species vary in physiological flexibility (Griffith, 1974; Whitehead, 2010), thereby offering a comparative system to study not only the genomic mechanisms that facilitate plasticity, but also to discover the genomic elements that have evolved to underpin expansion or contraction of physiological plasticity. Along natural salinity gradients, *F. heteroclitus* population divergence in physiological plasticity rejects neutral expectations, and patterns of divergence in genome regulation in response to osmotic challenge was assigned to neutral or adaptive models (Whitehead et al., 2011). Interestingly, of the genes that varied in expression between populations, those that also varied in response to salinity stress were more likely to show patterns of adaptive population divergence than genes that were not environmentally responsive (Whitehead et al., 2011). This offered greater scope for inference about the ecological importance of particular genes and physiological pathways associated with osmotic niche partitioning.

In contrast to extensive physiological plasticity, some populations of *F. heteroclitus* have evolved dramatic adaptation to localized stressors such as pollution that is heritable, fixed and not inducible (not plastic) (Nacci et al., 2010). Pollution tolerance has evolved several times independently, offering the opportunity to study whether common or unique molecular mechanisms underlie repeatedly evolved adaptive phenotypes. In a common-garden comparative pollutant-challenge experiment (Whitehead et al., 2012), three tolerant populations were each paired with nearby reference (pollution-sensitive) populations with which they shared most recent ancestry. Using genes that were not transcriptionally responsive to pollutant challenge but that did differ between populations, genome expression of geographic neighbors was most similar, which was consistent with the neutral expectation. However, population differences in genes that were transcriptionally responsive to pollutant challenge clearly grouped tolerant populations as highly similar but dramatically distinct from each of their sensitive neighbors, rejecting the neutral expectation, and revealing a common evolved mechanism for repeated adaptive pollution tolerance. Pollutant challenge was crucial in revealing evolved mechanisms between populations, because few genome expression differences exist between populations in the absence of pollution stress (Bozinovic and Oleksiak, 2010). Genes transcriptionally responsive to an ecologically relevant environmental perturbation (salinity in the first example or pollution in the second example) were most informative for revealing mechanisms of adaptive population divergence.

Future research

CGEP can be approached from two directions: (1) exploit the extensive genomic resources that are established for traditional laboratory models to explore phenotypic variation in natural ecotypes; and (2) develop genomic resources to exploit species with well-characterized phenotypic variation among natural populations with well-characterized ecologies. Two example systems using the former strategy include the model plant *Arabidopsis* and the yeast *Saccharomyces*. Natural variation has been characterized within and among species of *Arabidopsis*, for example in flowering time, salinity tolerance, temperature tolerance, and metal accumulation

and tolerance (Lefebvre et al., 2009). Comparative genomics studies have offered insight into mechanisms of divergence in salt tolerance between *Arabidopsis* and a close relative (Taji et al., 2004), and in metal accumulation among *Arabidopsis* species (Weber et al., 2006). Moreover, microarray analysis has shown that genome expression is highly responsive to temperature stress, and for some of these temperature-responsive genes expression variation among ecotypes correlates with the latitude from which they originate (Swindell et al., 2007): a pattern suggestive of adaptive divergence. Extensive genome resources for *Arabidopsis* are enabling genome re-sequencing of populations (e.g. Turner et al., 2010), so synthesis between functional genome variation, population genomic variation and phenotypic variation is accessible for this model.

Similarly, extensive genomic resources are available for yeast species and isolates, including characterization of the genetic architecture governing variation in gene expression response to the environment (Smith and Kruglyak, 2008), and extensive phenotypic and functional genome variation that exists among *Saccharomyces* strains that is suggestive of both similarity by shared ancestry and similarity by evolution in common environments (Kvitek et al., 2008). The functional genome of *Saccharomyces cerevisiae* is highly responsive to environmental perturbation (Gasch et al., 2000), so this comparative system is well-positioned to offer insights into how genome responses to the environment are similar, converge or diverge between ecotypes occupying unique or common niches with unique or common physiologies.

The second approach to CGEP is to first choose models that are uniquely positioned to address specific biological questions, for example in invasion biology or biological responses to climate change, then borrow or develop appropriate genomics toolkits. Shallow-water or intertidal species can experience rapid periodic and stochastic fluctuations in their environment and harbor much physiological plasticity, but may be particularly at risk from climate change (Harley et al., 2006). Species may respond to climate change through movement, acclimation, or adaptation (Parmesan, 2006). Of these, movement has received much attention in the literature particularly in terms of climate envelope modeling. For the latter two responses, relevant and timely questions accessible by CGEP include: (1) what genomic systems enable physiological resilience; (2) does functional resilience vary between populations and species and, if so, what are the genomic elements that contribute to this variance; and (3) what are the costs and limits to resilience? Genomics tools have been developed for several models that are particularly appropriate for addressing these types of questions. For example, genome-scale tools have been designed for some species of coral that are thought to live near their thermal maxima (Berkelmans and Willis, 1999) and are therefore at particular risk from ocean warming (Hoegh-Guldberg, 1999). For the scleractinian coral *Montastraea faveolata*, regional variation exists in genome response to temperature challenge (Polato et al., 2010). Although the adaptive relevance of this variation remains to be determined, these data indicate geographically distributed variation in mechanisms that govern acclimation to heat stress, even in the face of extensive gene flow, that may buffer populations from changing thermal environments. One consequence of increasing CO_2 is ocean acidification, to which some marine species are sensitive (Hofmann et al., 2010). Experiments in the particularly sensitive sea urchin *Lytechinus pictus* revealed that exposure to elevated CO_2 (decreased pH) disrupts skeletal development in parallel with the regulation of genes involved in energy metabolism, biomineralization and ion regulation (O'Donnell et al., 2010). This was discovered using heterologous hybridization to microarrays from the closely related genomic model *Strongylocentrotus purpuratus*. In addition, sea urchins pre-exposed to elevated CO_2 showed a compromised response to heat shock compared with those exposed to ambient CO_2 (O'Donnell et al., 2009), highlighting the potential for synergistic impacts of climate change, and providing an opportunity to study the genomic mechanisms underlying conflict between co-occurring stressors. Biogeographic variation in heat tolerance across latitudinal clines (e.g. Osovitz and Hofmann, 2005), coupled with geographic variation in ocean pH and CO_2, may contribute to locally evolved differences in tolerance to combined stressors associated with climate change that could be predictive of future acclimation and adaptation.

Genomic toolkits and phylogenetic information for many other comparative systems could be quickly developed or expanded to address diverse cutting-edge questions in physiological and evolutionary ecology. For example, Antarctic notothenioid fishes have radiated to occupy extreme cold niches in the Southern Ocean (Eastman, 2005), phylogenetic information exists (Near et al., 2004) and genomic resources are in development (Detrich and Amemiya, 2010). This system offers a wonderful opportunity to uncover genomic mechanisms governing morphological evolution that is correlated with trophic specialization (Albertson et al., 2010) and physiological evolution that facilitated persistence in extreme cold but at the cost of low tolerance to warming environments (Podrabsky and Somero, 2006; Somero and Devries, 1967). A variety of sculpin occupy diverse nearshore environments, where species variation in hypoxia tolerance limits matches the environmental hypoxia niche in which they are found (Mandic et al., 2009b). Comparative studies using phylogenetic comparisons have started to offer insight into evolved physiological mechanisms of adaptive niche partitioning (Mandic et al., 2009a; Mandic et al., 2009b), which could be further facilitated with genomics-scale tools. Additional comparative systems may be powerful for advancing understanding of genomic mechanisms that facilitate invasive success of alien species. For example, when grown in a common environment extensive genome expression variation is apparent between weedy and non-weedy populations of sunflower, much of which is lineage-specific, indicating independently derived mechanisms associated with repeated evolution of invasiveness (Lai et al., 2008). Population-by-environment interaction experiments should facilitate further insight into genomic mechanisms that drive the physiological divergence that enables invasive success. Similarly, an invasive mussel has largely replaced a native congener in intertidal habitats along the Californian coast, and comparative studies using custom-designed microarrays are offering insights into genomic mechanisms that underlie species-specific differences in response to temperature challenge (Lockwood et al., 2010) and salinity challenge (Lockwood and Somero, 2011).

In this post-genomics era, the option of first choosing the most appropriate study system for a particular biological question, truly in the spirit of Krogh's principle, and then developing the requisite genomics tools (rather than existing availability of genomic resources dictating choice of study organism) is rapidly becoming more feasible. This is because of the accessibility of low-cost massively parallel sequencing to generate genome-scale resources *de novo*. Moreover, with increased availability of genome-enabled species comes greater opportunities for sharing of resources between closely related species.

Although the barrier for entry into genomics studies with non-traditional models is rapidly decreasing because of rapid technological and analytic developments, the sophisticated tools necessary for validation of gene function are still mainly restricted to traditional laboratory models. That is, gene knockout and knockdown techniques are accessible mainly for genetically tractable organisms, which currently excludes many excellent ecological and physiological models. However, studying genomic responses of diverse wild species to diverse ecologically relevant challenges will facilitate an alternate model of empirical gene function prediction (Colbourne et al., 2011). Computational analytical tools are progressing in parallel with advances in data gathering, driving progression from making biological inferences at the single gene level, through to gene lists and functional category enrichment, to network and biology pathway modeling and other sophisticated systems biology modeling (Aggarwal and Lee, 2003; Kim et al., 2010). These advances offer a more nuanced understanding of how genomes evolve and function to produce complex and plastic phenotypes.

Genomics and a mechanistic approach to ecology research

Recent efforts seek to accelerate development of a mechanistic approach to ecology, where deployment of biophysical or biomechanical models serves to extend our understanding of how organisms work as well as how organisms interact with their environment (Denny and Gaylord 2010). Genomics approaches, including those outlined in this article, can also be used to extend our mechanistic understanding of how organisms work to how organisms interact with their environment, in parallel and interactively with other approaches outlined in this issue. For example, physiological, behavioral and biomechanical response functions are the proximate contributors to defining fitness and delineating the fundamental and realized niches of populations and species (Kearney, 2012). Comparative functional genomics approaches are appropriate for accessing an additional layer of biological organization and contribute to illuminating the ultimate (genetic and evolutionary) source of these phenotypic response functions. That is, the phenotypic response function is an emergent feature of genome structure and function, both of which are directly manipulated by evolutionary phenomena. Importantly, organism-level and genome-scale data reciprocally offer increased scope for inference, where physiological data enhance interpretation of genomic data and *vice versa*. Although the task of mapping genotype to phenotype is not trivial – indeed, it represents one of the ‘grand challenges’ of 21st century biology (Rose and Oakley, 2007; Schwenk et al., 2009) – comparative functional genomics studies are a reasonable and powerful approach for discovering the genomic mechanisms that are important for facilitating ecologically relevant phenotypes and phenotypic responses to environmental change. For many, the ultimate goal is to eventually map how the meta-genome content (all the genes present across genomes) and meta-genome regulation of a biological community is predictive of community structure and function; important progress has been gained particularly in the field of environmental microbiology. This long vision will clearly require syntheses across many disciplines, including how organism-level response functions (ecomechanics) predict the outcome of individual and population interactions with the biotic and abiotic environments, and how those response functions (and variants in those response functions) are encoded, actualized and how they evolve at the genome level (ecogenomics).

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