American Genetic Association
The Journal of Heredity
www.jhered.oxfordjournals.org

American Genetic Association

Journal of Heredity, 2016, 71–81
doi:10.1093/jhered/esv073
Symposium Article
Advance Access publication October 30, 2015

OXFORD

Symposium Article

Plastic and Evolved Responses to Global Change: What Can We Learn from Comparative Transcriptomics?

Melissa B. DeBiasse and Morgan W. Kelly

From the Department of Biological Sciences, Louisiana State University, 202 Life Sciences Building, Baton Rouge, LA 70803, USA (DeBiasse and Kelly).

Address correspondence to Morgan W. Kelly at the address above, or e-mail: morgankelly@lsu.edu.

Received February 18, 2015; First decision March 30 2015; Accepted August 6, 2015.

Corresponding Editor: Robin Waples

Downloaded from http://jhered.oxfordjournals.org/ at Louisiana State University on April 4, 2016

Abstract

Physiological plasticity and adaptive evolution may facilitate persistence in a changing environment. As a result, there is an interest in understanding species' capacities for plastic and evolved responses, and the mechanisms by which these responses occur. Transcriptome sequencing has become a powerful tool for addressing these questions, providing insight into otherwise unobserved effects of changing conditions on organismal physiology and variation in these effects among individuals and populations. Here, we review recent studies using comparative transcriptomics to understand plastic and evolutionary responses to changing environments. We focus on 2 areas where transcriptomics has played an important role: first, in understanding the genetic basis for local adaptation to current gradients as a proxy for future adaptation, and second, in understanding organismal responses to multiple stressors. We find most studies examining multiple stressors have tested the effects of each stressor individually; the few studies testing multiple stressors simultaneously have found synergistic effects on gene expression that would not have been predicted from single stressor studies. We discuss the importance of robust experimental design to allow for a more sophisticated characterization of transcriptomic responses and conclude by offering recommendations for future research, including integrating genomics with transcriptomics, testing gene regulatory networks, and comparing the equivalence of transcription to translation and the effects of environmental stress on the proteome.

Subject areas: Molecular adaptation and selection
Key words: adaptation, gene expression, global change, plasticity, RNA-seq, transcriptomics

Both evolution and phenotypic plasticity can facilitate the persistence of vulnerable species and populations during periods of environmental change (Bell and Gonzalez 2009; Chevin et al. 2010). As a result, there has been rapid growth in the past decade in research seeking to understand both plastic and evolved responses to global change stressors (Hoffmann and Sgrò 2011). Simultaneously, the advent of next generation sequencing has made genomic resources, especially transcriptomics (RNA-Seq), available to nonmodel organisms (Wang et al. 2009; Alvarez et al. 2015), allowing new ways to understand organismal responses to the environment.

The transcriptome is the entire set of RNA transcripts produced by a cell, tissue, or organism, and can change depending on developmental stage or environment. Comparative transcriptomics seeks to understand variation in the transcriptome across a variety of axes:

© The American Genetic Association. 2015. All rights reserved. For permissions, please e-mail: journals.permissions@oup.com

American Genetic Association
The Journal of Heredity
www.jhered.oxfordjournals.org

among individuals, populations, and species, in response to various sources of environmental variation, and over time. Changes in the abiotic environment are ultimately translated into population-level effects through their consequences for organismal physiology and fitness. Therefore, the utility of comparative transcriptomics stems from its potential to elucidate the linkage between the organism and its environment, opening the "black box" of organismal physiology.

In this review, we will highlight the ways that transcriptomics can be used to understand both plastic and evolutionary responses to changing environments, focusing on ectothermic organisms. We will focus on 2 areas where we feel transcriptomics plays a particularly important role: first, in understanding the genetic basis for adaptation to current environmental gradients, and second, in understanding the physiology of organismal responses to multiple stressors and how responding to multiple stressors simultaneously might constrain both plastic and evolved responses to changing environments. We will discuss the importance of a well-planned experimental design and conclude with some thoughts on future research priorities for the application of transcriptomics to understanding biological responses to changing environments.

Evolution, Plasticity, and the Transcriptome

Evolution and phenotypic plasticity are 2 possible organismal responses to changing environments. These processes are not mutually exclusive, but do occur on different timescales: evolution is the change in the genetic makeup of a population over one or more generations as a result of differential reproductive success or survival among genotypes. By contrast, phenotypic plasticity is a change in phenotype within a single organism (or a single genotype) within a generation in response to the environment. It is important to distinguish between these 2 responses in the context of global change studies for several reasons. Plastic responses are elicited within a single generation, but unlike evolution, are not expected to produce additional change over successive generations beyond the range achieved in the initial response. As a result, the buffer provided by plasticity is more immediate, but also potentially more limited (although plasticity itself can evolve, Via and Lande 1985). Plastic responses are also available to all individuals in a population, whereas evolution, by definition, results from the differential success of some genotypes over others. As a result, the costs of the 2 responses are different—the costs of evolutionary change are demographic (some individuals die, or do not reproduce) (Haldane 1957), whereas the costs of plastic responses are experienced at the level of organismal physiology (the energetic costs of producing a particular set of structures or molecules) (DeWitt et al. 1998).

Transcriptomic data can provide insight into both plastic and evolutionary responses to changing environments. Because the transcriptome is itself a phenotype, it is produced by the joint effects of the organismal genotype (G, the product of evolution), environment (E, plasticity), and the genotype by environment interaction (G × E, genetic variation in plasticity) (Rockman 2008; Levine et al. 2011; Zhou et al. 2012). Comparative transcriptomics can provide insight into any or all of these contributions to species' responses to environmental change, showing how much of the response is driven by effects of the environment (E), versus variation among individuals in their response to the environment (G × E). Many transcriptomic studies have focused on the environmental component of the transcriptomic phenotype, comparing the transcriptomes of individuals or populations in control versus experimental treatments. Fewer studies have used a common garden approach where the environmental component is controlled to test the contribution of genotypes or genotype by environment interactions to transcriptomic phenotypes. Such common garden experiments are a useful way to distinguish between plastic versus evolved transcriptomic responses. For example, Pespeni et al. (2013) maintained *Strongylocentrotus purpuratus* sea urchins collected from a northern and southern population in a common garden for 3 years, but still found differences in gene expression between the 2 populations, indicating evolved transcriptomic differences. In contrast, transcriptomic differences observed between *Zonotrichia capensis* rufous-collared sparrows sampled at low and high altitudes were no longer significant after the birds had been held in a common garden, suggesting these differences were driven solely by plasticity, and not by evolved differences between the 2 populations (Cheviron et al. 2008).

A complication of interpreting transcriptomic responses is that they are largely "upstream" of traits that will affect fitness. Because transcriptomic data are highly multivariate, it is often difficult to draw causal linkages between components of the transcriptome and those aspects of the organismal phenotype more directly tied to fitness. Nevertheless, the fine-grained nature of transcriptomic data also provides substantial advantages. Differences among populations in the transcriptomic responses to environmental stressors might provide evidence for adaptive differences not previously observed through measurement of macroscopic response variables. Similarly, nonadditive transcriptomic responses to multiple stressors might provide evidence of previously unobserved physiological trade-offs.

Leveraging Environmental Gradients to Test the Genomic Basis of Adaptation

Evolutionary change depends on genetic variation. One way to understand whether a species is likely to adapt to environmental change over time is to measure evolved differences along current environmental gradients in space. Transcriptomic studies comparing locally adapted populations can "substitute space for time" (Blois et al. 2013), measuring both the potential for adaptation and the physiological mechanisms by which adaptation to that environmental variable occurs. Locally adapted populations might also provide the raw material for future adaptation through gene flow from more tolerant to less tolerant populations. Therefore, studies of local adaptation will help inform efforts aimed at conserving the potential for evolutionary responses to future change (Aitken and Whitlock 2013; van Oppen et al. 2015). In this section, we review recent studies (see Table 1) that leverage a natural environmental gradient to test questions about plastic and evolved transcriptomic responses in locally adapted populations.

Temperature

Increasing temperature is the most obvious consequence of global change. As a result, many studies have compared the response of populations distributed along temperature gradients to ambient and elevated temperature treatments using performance and transcriptomic data (Table 1). In 3 of the 8 studies reviewed here, differential gene expression between heat stressed and control treatments was greater in the thermally tolerant population compared to the thermally sensitive population (whitefly, Mahadav et al. 2009; seagrass, Franssen et al. 2014; trout, Narum and Campbell 2015). However, in the 5 other studies, the thermally *sensitive* population had greater

Downloaded from http://jhered.oxfordjournals.org/ at Louisiana State University on April 4, 2016

American Genetic Association
The Journal of Heredity
www.jhered.oxfordjournals.org

Table 1. List of recent studies using transcriptomic data to examine the response of individuals and populations to environmental stress

Taxon	Variable	Populations compared	Citation
Corals (*Acropora hyacinthus*)	Temperature	Highly and moderately variable reefs	Barshis et al. (2013)
Corals (*Porites astreoides*)	Temperature	Offshore and inshore reefs	Kenkel et al. (2013)
Snails (*Chlorostoma funebralis*)	Temperature	North and south populations	Gleason and Burton (2015)
Seagrasses (*Zostera marina* and *Nanozostera noltii*)	Temperature	Subtidal and intertidal, north and south populations	Franssen et al. (2014)
Redband trout (*Oncorhynchus mykiss gairdneri*)	Temperature	Desert and montane populations	Narum and Campbell (2015)
Copepods (*Tigriopus californicus*)	Temperature	North and south populations	Schoville et al. (2012)
Whiteflies (*Bemisia tabaci*)	Temperature	B and Q biotypes	Mahadav et al. (2009)
Killifish (*Fundulus heteroclitus*)	Temperature	North and south	Dayan et al. (2015)
Sea urchins (*Strongylocentrotus purpuratus*)	Ocean acidification	Low and high pH	Evans et al. (2013)
Sea urchins (*Strongylocentrotus purpuratus*)	Ocean acidification	Low and high pH	Pespeni et al. (2013)
Coccolithophores (*Emiliania huxleyi*)	Ocean acidification	Experimental evolution	Lohbeck et al. (2014)

differential gene expression between heated and control treatments (copepod, Schoville et al. 2012; coral, Barshis et al. 2013; snail, Gleason and Burton 2015). This suggests that large changes in gene expression could indicate an adaptive response, or alternatively, a large transcriptomic response could signal greater levels of stress. Therefore, inter and intraspecific comparisons of stress responses should be interpreted with caution in the absence of additional fitness or performance data. Moving forward, future studies comparing differential expression among sets of orthologous loci across taxa as opposed to simply the number of differentially expressed genes will be useful in determining whether transcriptomic changes under stressful conditions represent a panic response or an adaptive, coordinated response in targeted pathways.

Despite their previously demonstrated importance in heat tolerance (Feder and Hofmann 1999), heat shock protein (*hsp*) genes were not universally upregulated in response to heat stress. For example, although there was high differential expression between heat exposed northern and southern populations of the seagrass *Nanozostera noltii*, *hsp* genes were not upregulated (Franssen et al. 2014). Barshis et al. (2013) found that of the 78 genes that responded identically to heat treatments in the sensitive and tolerant *Acropora hyacinthus* coral populations, typical stress response genes including those for *hsps*, molecular chaperones, and antioxidants were absent. These data paint a portrait of organismal responses to heat stress that is more nuanced than a simple upregulation of heat shock proteins. Importantly, this view is made possible by whole transcriptome sequencing, which lacks the ascertainment bias inherent to qPCR-based studies of specific candidate genes.

Many studies observed significant differential gene expression among populations in the control treatments (Mahadav et al. 2009; Barshis et al. 2013; Kenkel et al. 2013; Franssen et al. 2014; Narum and Campbell 2015; Gleason and Burton 2015). Some of these found that populations varied in constitutive (i.e. baseline) levels of gene expression for a given gene or set of genes under control or low stress conditions and that some of those same genes were also involved in the plastic response to heat stress. For example, Barshis et al. (2013) found that 60 genes upregulated under temperature stress in the heat-sensitive corals had a reduced response in the heat-tolerant corals. These same genes also had higher baseline expression in the heat-tolerant compared to sensitive corals under ambient conditions, a phenomenon referred to by the authors as "frontloading." Metabolic genes in heat-tolerant *Porites astreoides* from inshore locations had high baseline expression, consistent with frontloading (Kenkel et al. 2013). Gleason and Burton (2015) found molecular chaperones and antioxidant genes had higher baseline expression in southern versus northern control *Chlorostoma funebralis* snail populations but were not differentially expressed in southern populations under heat stress. Finally, in *Zostera marina* seagrass, heat responsive genes were more highly expressed in the southern population than the northern population in both control treatment (i.e., higher baseline expression) and heat-stressed treatments. Higher baseline expression of key stress response genes noted by these studies might represent an evolved response to a frequently encountered stress that allows tolerant populations to initiate a reduced, and hence more efficient, change in gene expression. Frontloading is also interesting from a theoretical standpoint, as it provides a link between plastic and evolutionary processes: evolutionary adaptation to a gradient in environmental stress in these cases appears to have occurred through baseline upregulation of the plastic response to that stressor. At this point however, the number of studies demonstrating front-loading are relatively small. More data are needed to understand whether it represents a general mechanism for adaptation to environmental stress.

One of the challenges when making comparisons between populations along a gradient is distinguishing between differences driven by adaptation to the gradient and differences that are due to drift (Khaitovich et al. 2004) or other sources of selection that co-vary with the variable of interest (Harrison et al. 2012; 2014). Dayan et al. (2015) present one possible solution to this problem, using a phylogenetic comparison to differentiate between neutral and adaptive variation in gene expression in the killifish *Fundulus heteroclitus* and its sister species *F. grandis* acclimated to different temperatures. Under neutrality, northern and southern populations of *F. heteroclitus* should be more similar to each other than either is to *F. grandis*;

Downloaded from http://jhered.oxfordjournals.org/ at Louisiana State University on April 4, 2016

American Genetic Association
The Journal of Heredity
www.jhered.oxfordjournals.org

however, natural selection may drive non-neutral patterns of gene expression correlated with environmental conditions, not species boundaries. The authors used a 2-way ANOVA to compare the mean gene expression of a cold-adapted northern *F. heteroclitus* population to the pooled mean of warm-adapted southern *F. heteroclitus* and *F. grandis* populations to find divergent patterns of expression resulting from natural selection. When intraspecific variation exceeded interspecific variation in a direction that correlated with temperature, the pattern of expression was considered adaptive. Although developmental plasticity and maternal effects could not be ruled out in this study, there were non-neutral, likely heritable, differences in gene expression among *Fundulus* populations adapted to warm and cold temperatures.

Ocean Acidification

Most global change transcriptomic studies have focused on the effects of temperature, but in marine systems, ocean acidification (OA) (through the absorption of atmospheric CO_2) is another important consequence of CO_2 emissions (Doney et al. 2009). OA causes shifts in seawater chemistry, alterations in biogeochemical nutrient cycles, and especially impacts organisms that use calcium carbonate to form their skeletons and shells (Orr et al. 2005). Episodic upwelling can bring cold, naturally acidified water to the surface (Feely et al. 2008), so populations in regions of regular upwelling could become adapted to local water chemistry conditions (De Wit and Palumbi 2013). Many studies have examined plastic responses to OA in marine taxa, testing whether the capacity to tolerate present day pH variation might confer resistance to future OA (Evans and Hofmann 2012; Kelly and Hofmann 2013). Far fewer have compared these responses among multiple populations from regions with distinct pH regimes to test for local adaptation to low pH (Table 1).

Studies focusing on coccolithophores and sea urchins point to the potential for adaptation to future pH conditions. Lohbeck et al. (2014) found that although the short-term response to OA in the coccolithophore *Emiliania huxleyi* was a downregulation of candidate genes involved in growth and calcification, the evolved response showed a restoration of gene expression and an upregulation in genes involved in cytosolic pH regulation. Pespeni et al. (2013) observed higher expression in genes involved in growth and biomineralization in southern versus northern *S. purpuratus* sea urchins. These results matched data showing southern urchins had faster spine regeneration rates, pointing to evolved, and potentially adaptive, differences among urchin populations in traits related to the response to OA.

In contrast to Pespeni et al. (2013), the results from Evans et al. (2013) did not strongly support the potential to persist under future OA conditions in *S. purpuratus*. Evans et al. (2013) found transcriptomic differences between developmental stages in the urchins with early stage larvae having a greater response to increased OA compared to later stage larvae. Although the early stage larvae had a large transcriptional response to the treatment corresponding to pH levels experienced during present day upwelling, neither larval stage responded to increased OA levels predicted for the end of the century, suggesting the ability to cope with present day variation in pH does not imply persistence under future conditions. However, because Pespeni et al. (2013) and Evans et al. (2013) tested the response to OA in different life stages, adults versus larvae, respectively, it is difficult to draw any firm conclusions about the potential for *S. purpuratus* as a species to adapt to OA based on these results. Ideally, future studies to predict species' persistence under climate change would incorporate data from multiple developmental phases (see Future Directions section).

Evolutionary Trade-Offs in the Face of Multiple Stressors

Most species will experience climate warming simultaneously with other changes (Etterson and Shaw 2001; Duputié et al. 2012; Chevin 2013). As a result, the magnitude and direction of both plastic and evolutionary responses to climate change will ultimately depend on trade-offs, which occur when a relationship between 2 traits prevents them from being simultaneously optimized. Trade-offs occur within a single organism when limited energy budgets mean that allocation to one function (e.g., stress tolerance) leads to diminished allocation to other functions, such as growth or reproduction. Evolutionary trade-offs occur because genetic variation and natural selection are usually multivariate, and evolution depends on the degree to which genetic variation is aligned with the "multivariate direction of selection" (Blows and Hoffmann 2005). For example, a population might possess genetic variation for performance at both high and low temperatures, but if there is a negative genetic correlation between these 2 traits, it might not be possible to evolve substantially increased performance at both high and low temperatures simultaneously (Berger et al. 2014).

Comparing transcriptomic data from multiple studies points to possible trade-offs for taxa facing multiple climate change stressors. In corals, which will be exposed to the combined effects of increased temperature and acidification in the coming decades, temperature stress caused a downregulation of calcification genes (Barshis et al. 2013; Kenkel et al. 2013) while acidification led to upregulation in genes controlling internal calcium carbonate chemistry (Moya et al. 2012). Taken together, these results suggest that corals may be constrained in their ability to tolerate both of these stressors simultaneously. Transcriptomic data can also illuminate the relative importance of co-occurring stressors. In Eastern oysters (*Crassostrea virginica*) from natural populations exposed to a range of environmental variables (temperature, pH, salinity, dissolved oxygen, and pollutants), temperature and pH had the largest effect and influenced gene expression in a nonlinear fashion (Chapman et al. 2011). Similarly, the C4 grass *Andropogon gerardii* mounted a greater transcriptional response to heat stress than to desiccation stress, but results also suggest it will respond differently to the combined effects of global warming and reduced water availability than to either stressor individually (Travers et al. 2010).

Some transcriptomic studies of stress tolerance have revealed unexpected tradeoffs. *Drosophila melanogaster* selectively bred for heat tolerance performed better than control flies at high and low but not intermediate temperature conditions, as did flies selectively bred for cold tolerance, suggesting a trade-off: better performance at extreme temperatures at the expense of performance at intermediate ones (Kristensen et al. 2007). In contrast, salt tolerant *Daphnia pulex* do not seem to pay a cost for their broader tolerance, maintaining high fitness at nonstressful salinities. However, many of the genes involved in salinity adaptation are also involved in the plastic morphological response to predators, suggesting that the costs of generalization might be experienced as trade-offs on other environmental axes (Latta et al. 2012).

Although temperature and pH elicited different transcriptomic responses in the studies mentioned above, some environmental stressors could lead to overlapping patterns of gene expression. For example, preliminary data from our lab indicate overlap in

Downloaded from http://jhered.oxfordjournals.org/ at Louisiana State University on April 4, 2016

American Genetic Association
The Journal of Heredity
www.jhered.oxfordjournals.org

the transcriptomic response to heat shock and high salinity stress in *Tigriopus californicus* copepods, suggesting that the plastic response elicited by one stressor might confer resistance to the other. Evolutionary responses in 2 different traits can also be positively correlated. For example, larvae of adult Sydney rock oysters, *Saccostrea glomerata*, selectively bred for rapid growth and disease resistance were more resilient to OA than wild-type oysters (Parker et al. 2011).

The few studies that have explicitly tested for transcriptomic effects of 2 or more climate change stressors acting simultaneously have revealed complex effects on gene expression that would not have been predicted from single stressor treatments. Genes differentially expressed in *Mytilus galloprovincialis* mussels exposed to heat stress alone, nickel stress alone, or to heat and nickel stress simultaneously fell in distinct functional categories (Mohamed et al. 2014). In the symbiotic sea anemone *Anemonia viridis*, exposure to ultraviolet and thermal stress elicited changes in expression for 24 genes whose expression was unaffected by either of the single stressor treatments (Moya et al. 2012). When Vidal-Dupiol et al. (2014) exposed *Pocillopora damicornis* coral fragments to increased temperature in the presence and absence of the bacteria *Vibrio coralliilyticus*, they observed upregulation of heat shock proteins and some immune genes, but downregulation in other immune genes, suggesting thermal stress alters coral immune response in complicated ways. For *S. purpuratus* sea urchin larvae exposed to both elevated OA and temperature, one group of transcripts, dominated by metabolic genes, showed a strong downregulation in the 2-stressor treatment but were unaffected by either of the stressors individually, suggesting that some stressors might magnify each other's effects (Padilla-Gamiño et al. 2013). All of these results highlight the need for multistressors studies, as the synergistic effects they revealed would not have been predicted from single stressor treatments.

Interpreting Transcriptomic Data: The Importance of Experimental Design and Functional Testing

Transcriptomic data are becoming easier and cheaper to collect, but without close attention to robust experimental design and statistical analyses, data will lack power to answer questions of interest. Here, we discuss important considerations for data collection and analyses and examine how functional testing can help determine whether transcriptomic responses are adaptive.

Experimental Design

Regardless of whether data are collected by RNA-Seq or microarrays, researchers should adhere to the 3 fundamental aspects of experimental design: replication, randomization, and blocking (Auer and Doerge 2010). For example, a robust experimental design for a hypothetical study to test for intraspecific transcriptomic variation in response to environmental stress would include many individuals (replication) from different populations (blocking) assigned to different experimental treatments (randomization). Although some studies have found technical variation in RNA-Seq studies to be low (i.e., read count differences not attributed to biological differences, Bainbridge et al. 2006; Marioni et al. 2008; Mortazavi et al. 2008; Hashimoto et al. 2009), others find it to be a significant problem (McIntyre et al. 2011; Gilad and Mizrahi-Man 2015). Technical variation can result from biases in library preparation (i.e., PCR amplification and reverse transcription artifacts) and/or sequencing (i.e., lane and base calling errors). Multiplexing barcoded samples and sequencing libraries from the same experimental treatment across multiple lanes will ensure that biological and technical variation can be partitioned (e.g., see Figure 4 in Auer and Doerge 2010).

Biological replication is also required for robust experimental design because it allows the estimation of variability within a treatment group (Granados-Cifuentes et al. 2013; Seneca and Palumbi 2015). Although some questions will require deep sequencing (i.e., differential expression of exons, alternative splicing), empirical evidence suggests biological replication is generally more important than sequencing depth to estimate differential gene expression (Liu et al. 2014; Rapaport et al. 2013). For example, Liu et al. (2014) found adding sequencing depth beyond 10 million reads per library did not improve the power to find differentially expressed genes in the human genome while adding biological replicates increased power significantly regardless of sequencing depth. However, the appropriate number of biological replicates and sequencing depth will vary depending on the study system, transcriptome size, genomic resources available, and question of interest, and thus should be determined on a case-by-case basis.

Another important consideration for studies comparing multiple populations is how to distinguish between neutral and adaptive differentiation. Although we have robust null models of nucleotide substitution (Kimura 1983), models of gene expression evolution are less well developed and often fail to account for the complexity and noise associated with transcriptomic data (Harrison et al. 2012). Furthermore, models of transcriptomic change are often not sufficiently sophisticated to determine whether changes in gene expression are due to neutral divergence among populations or species (i.e., genetic drift) or the result of adaptive processes driven by natural selection. The development of appropriate null models of gene expression changes is essential to further our understanding of transcriptome evolution (Khaitovich et al. 2004; Harrison et al. 2012; 2014).

Researchers must also decide what is the optimal number of time points to sample and when to sample them. Many comparative transcriptomic studies collect expression data at a single point, capturing only a brief snapshot in time. Ideally, projects that sample multiple time points will provide a more complete view of changes in gene expression in response to an environmental stressor and how organisms acclimate to the stressor initially and over the long term. For example, many studies sampling multiple time points have found that despite an immediate, and in some cases extreme, transcriptomic response to a stressor, gene expression returns to baseline levels over time, a phenomenon called transcriptional resilience (Franssen et al. 2011). Sampling at 7 time points over 48 h, Telonis-Scott et al. (2014) found different isoforms of the *stv* gene were expressed at different times in heat stressed *D. melanogaster* individuals. Brennan et al. (2015) found that *F. heteroclitus* killifish challenged with salinity stress had significant changes in physiology (blood chemistry) and gene expression at the 6-h mark with a subsequent return to baseline conditions based on 5 samples collected over the next 14 days. In *Acropora millepora* coral juveniles, exposure to acute (3 days) OA conditions caused high levels of differential expression, but after a prolonged (9-day) exposure, expression of most genes returned to the control level (Moya et al. 2015). There was also little overlap in the genes responding to the acute and prolonged exposures. In temperature-stressed *Acropora hyacinthus* corals, over a quarter of the transcriptome was differentially regulated after a one hour exposure, but at the 15-h time point, the early responding genes had

Downloaded from http://jhered.oxfordjournals.org/ at Louisiana State University on April 4, 2016

American Genetic Association
The Journal of Heredity
www.jhered.oxfordjournals.org

returned to control expression levels and a different set of genes was successively regulated (Seneca and Palumbi 2015). Had these experiments terminated after a single time point, the authors would likely have come to very different conclusions about the stress response of their focal taxa.

In the same spirit as increasing the number of time points sampled, quantifying organismal responses over a *gradient* of environmental variation (as opposed to 2 levels of a single condition) might reveal thresholds at which important physiological and transcriptomic transitions take place. For example, *F. heteroclitus* killifish span an osmotic gradient from fresh to saltwater and are able to remodel their gill epithelia as they transition between environments (Whitehead et al. 2011). Experiments spanning this salinity threshold have elucidated the precise salinity that triggers this remodeling and the genomic basis for this physiological transition (Whitehead et al. 2011, 2013; Brennan et al. 2015). In a study of geographic variation in heat tolerance, Osovitz and Hofmann (2005) exposed *S. purpuratus* sea urchins to 7 °C temperatures over a gradient ranging from 10 to 36 °C and found the incubation temperature that induced maximum *hsp70* expression differed between Oregon (23.8 °C) and California (26.8 °C) populations.

Wild populations contain individuals across all life stages, and we may expect different transcriptomic and phenotypic responses to environmental stress in larvae versus adults. If adults easily tolerate a stress but larvae are severely affected, conclusions drawn based on studies of adults will underestimate the impact of the stressor on the species as a whole (Byrne 2011, 2012). On the other hand, one life stage might appear to tolerate a stress but at the expense of future fitness. For example, several studies have documented phenotypic (Sunday et al. 2011; Kelly et al. 2013) and gene expression differences (O'Donnell et al. 2009, 2010; Padilla-Gamiño et al. 2013) in *S. purpuratus* sea urchin larvae reared under ambient and low pH treatments and these effects might be compounded by prior adult exposure to OA (Dupont et al. 2012). Although some studies have independently tested the transcriptomic response of environmental stress in different life stages of a given taxon (Dubansky et al. (2013) and Pilcher et al. (2014), embryonic and adult *F. heteroclitus* killifish, respectively), the most robust inferences will be drawn from studies that follow individuals over development. Although challenging, studies that test the response of an organism across life stages or test the subsequent fitness effects of environmental stress will better inform predictions about how the species will respond and persist in the face of global change (Kristensen et al. 2015).

Data Analysis

Early transcriptomic studies used microarrays and serial analysis of gene expression (SAGE) but the field is shifting to RNA-Seq (Malone and Oliver 2011; McGettigan 2013) performed on a variety of sequencing platforms (Oshlack et al. 2010; Li et al. 2014). Because RNA-Seq is a relatively recent approach the field is still in the process of finding the most effective methods for data analyses. Recent RNA-Seq analyses have relied heavily on approaches using Poison or negative binomial distributions to model gene count data and test differential gene expression among treatments (Rapaport et al. 2013). However, a method originally developed for microarray data (limma, Smyth 2005), has recently been adapted for RNA-Seq data and has been shown to lower type I error rates (calling a gene significantly differentially expressed when it is not) because it does not depend on fitting a negative binomial distribution to the data (limma-voom, Law et al. 2014).

Are Plastic Responses Adaptive?

A robust experimental and statistical design will allow for the confident identification of differentially expressed genes, but the researcher is still left to interpret her results. The difficulty in interpreting transcriptomic responses to environmental stress gets to the heart of a deeper question in evolutionary ecology: To what extent are plastic organismal responses adaptive (Chan et al. 2012)? It has been known for some time that plasticity is not always adaptive (Grether 2005). For example, maladaptive plasticity can occur in extreme environments through loss of homeostasis or because that environment had not previously been experienced in the species' evolutionary history (Ghalambor et al. 2007). Plasticity can also be maladaptive in unpredictable environments (Reed et al. 2010) and might fail to evolve in habitats that are demographic sinks (Holt and Gaines 1992). In the case of transcriptomic responses to stress, changes in gene expression could confer resilience to that stressor, or they could be part of a generalized stress response that does nothing to increase organismal tolerance.

Comparing transcriptomic responses to environmental stress between locally adapted populations (as summarized above) will allow researchers to begin to tease out the portion of the plastic response that is adaptive. However, this approach still faces 2 challenges inherent in most transcriptomic studies of nonmodel organisms. First, most transcripts in *de novo* assemblies remain unannotated, limiting the ability to make connections between gene expression and organismal function (Pavey et al. 2012). Fortunately, gene ontologies are species-neutral so annotations derived from work in model systems can be used to inform work in nonmodel systems (Primmer et al. 2013). However, not all species of ecological interest have a closely related model, and as much as 3 quarters of a *de novo* assembly might fail to make a blast match to an annotated sequence from another organism (Meyer et al. 2015). Function can also be inferred using methods such as InterProScan (Jones et al. 2014), which searches nucleotide or protein sequences against the InterPro consortium, a collection of databases containing predictive protein models. One drawback to this approach is that the algorithms are computationally intensive and parallelization is often needed to analyze a large number of sequences in a practical time frame (Jones et al. 2014). Second, data generated from population comparisons are entirely correlative—it is impossible to tell from transcriptomic data alone whether expression of a particular transcript actually increases tolerance of a particular stress, and comparisons between natural populations typically do not allow investigators to separate the effects of multiple sources of selection, drift, and population history (Whitehead et al. 2013). Clearly, an important next step for the field is to move past correlational studies by testing functional hypotheses that link adaptive phenotypes to genotypes.

Transcriptome sequencing studies allow for the identification of candidate genes and regions that might be responsible for adaptive physiological phenotypes but few studies have explicitly tested the connection between gene expression and phenotype (Alvarez et al. 2015). Experimental evolution and reverse genetic techniques are 2 approaches that identify and test candidate loci predicted to influence phenotype and fitness. For species that can be bred in the lab, experimental evolution, selection experiments, and/or quantitative trait loci (QTL) mapping allow researchers to target the genetic basis of adaptation to specific stressors (Becks et al. 2012; Norman et al. 2014). For example, Dhar et al. (2011) evolved *Saccharomyces cerevisiae* under constant salt stress in the lab and used both transcriptomics and whole genome sequencing to discover expression and

Downloaded from http://jhered.oxfordjournals.org/ at Louisiana State University on April 4, 2016

American Genetic Association
The Journal of Heredity
www.jhered.oxfordjournals.org

sequence changes associated with increased fitness under salt stress. However, one important caveat to evolve and re-sequence studies is that performance of selectively bred lines in the lab might not accurately predict performance under field conditions (Kristensen et al. 2007).

While traditional forward genetics seeks to determine the genetic basis for a phenotypic trait, reverse genetics aims to determine or confirm the phenotype that results from variation at a given genetic locus of interest. Reverse genetic techniques such as RNA interference (RNAi) (Hannon 2002) and CRISPR interference (CRISPRi) (Qi et al. 2013) suppress the translation of a putatively adaptive candidate gene whose expression is hypothesized to confer an adaptive advantage. Once function of the gene is knocked out, researchers can test how organismal phenotype and fitness change in response to environmental stress. CRISPRi has been used in taxa including bacteria, plants, fish, and mammals (Bikard et al. 2013; Hwang et al. 2013; Jiang et al. 2013; Wang et al. 2013) and RNAi has been used successfully to quantify the role of putative candidate genes in determining phenotype in a wide range of taxa including cnidarians, sponges, flatworms, rotifers, tardigrades, gastropods, arachnids, and crustaceans (Barreto et al. 2015 and references therein). In RNAi, double stranded RNA (dsRNA) molecules with gene specific sequences bind to, and degrade, endogenous mRNAs of a target gene before they can be translated, thereby suppressing expression of the gene (Fire et al. 1998; Hannon 2002). Barreto et al. (2015) developed RNAi for *T. californicus,* targeting the heat-shock beta 1 (*hspb1*) gene, which was differentially expressed in thermally sensitive and tolerant *T. californicus* populations during extreme heat stress (Schoville et al. 2012) and is likely an important locus for adaptation to temperature. *Hspb1* dsRNA was introduced using electroporation and successfully knocked down expression of the heat shock gene. Copepods treated with *hspb1* dsRNA had lower survivorship under high temperature stress relative to control copepods, demonstrating a clear link between *hspb1* expression and thermal tolerance.

Future Directions

As researchers continue to use transcriptomics to understand plastic and evolutionary responses to environmental change, we see important future directions that will allow increasingly robust interpretations of transcriptomic data.

Integrating Genomic and Phenomic Techniques and Data with Transcriptomics

QTL analysis is a statistical method that utilizes genomic and phenotypic data to map phenotypic traits to their underlying genomic regions in an attempt to explain the genetic basis of variation in complex traits (Doerge 2002). By viewing gene expression as a heritable, quantitative phenotypic trait for which there is large within and among population and species variation, expression QTL (eQTL) analyses borrow genomic linkage and mapping tools to identify the genomic regions responsible for regulating gene expression and polymorphisms within those regions (Gilad et al. 2008; Montgomery and Dermitzakis 2011). Although eQTL can be highly context dependent and difficult to compare across studies (Box 3 in Gilad et al. 2008), eQTL mapping has the potential to provide substantial insight into the evolution of gene regulation and expression. For example, Gagnaire et al. (2013) simultaneously mapped phenotypic quantitative trait loci (pQTL) and eQTL in ecologically divergent limnetic dwarf and normal benthic *Coregonus clupeaformis* lake whitefish. They found multiple groups of eQTL were clustered in hot spots at the extreme edges of 14 linkage groups and overlap between pQTL and eQTL on 15 linkage groups. The eQTL hotspots suggest the presence of master regulatory loci controlling expression in lake whitefish (Gagnaire et al. 2013). Rohlfs and Nielsen (2015) have also developed a new method that treats gene expression as a quantitative trait. Their Expression Variance and Evolution model parameterizes the ratio of population to evolutionary expression variance. This allows for tests for lineage-specific shifts in expression and a phylogenetic ANOVA that detects genes with increased or decreased ratios of expression divergence to within-population diversity, analogous to the HKA test for nucleotide data.

Combining high throughput phenotyping (HTP) with genomic and transcriptomic methods has the potential to revolutionize trait discovery, allowing tests of hypotheses about the effect of genotype and environment on phenotype, and improving phenotypic predictions based on genotypic information (Li et al. 2014). HTP has been implemented in lab and field-based studies on plants to noninvasively collect time series data on growth, development, canopy temperature, and chlorophyll fluorescence as a proxy for photosynthetic function, and could be amenable to other systems (Furbank and Tester 2011; Zhang et al. 2012; Brown et al. 2014). For example, using a time-lapse imaging HTP approach, Zhang et al. (2012) found major genetic components for 3 growth-related traits in *Arabidopsis thaliana*. Topp et al. (2013) combined high throughput 3D imaging and phenotyping with a multivariate QTL analysis to reveal the genetic basis of root architecture in *Oryza sativa* rice, identifying 89 QTL at 13 hotspots for 25 phenotypic traits. Interestingly, some QTL for uncorrelated phenotypic traits were found clustered together while other phenotypic traits with almost complete correspondence had QTL that colocalized at only a few loci.

Testing Population Genomics Questions with Transcriptome Sequence Data

A handful of studies have tested population genomic questions using single nucleotide polymorphism (SNP) genotype data extracted from transcriptome sequences. Tepolt and Palumbi (2015) found neutral and non-neutral processes drove population structure in the invaded and native ranges, respectively, of the European green crab, *Carcinus maenas*, based on 10 000+ SNP loci. Bay and Palumbi (2014) genotyped 23 *Acropora hyacinthus* coral individuals from populations known to differ in transcriptomic thermal stress response (Barshis et al. 2013) at 15 000+ SNPs and identified 114 highly divergent SNPs as candidates for environmental selection, providing further support for genetic differentiation among sites. Despite no signal of population differentiation among *Haliotis rufescens* abalone sampled from 3 sites distinct in temperature, hypoxia, and water chemistry, De Wit and Palumbi (2013) found almost 700 SNPs extracted from transcriptomic sequences were F_{ST} outliers, suggesting genes containing these SNPs might be candidate loci for local adaptation. De Wit et al. (2015) promote collecting SNPs from transcriptomes as a reduced representation technique that retains functional information, but potential weaknesses exist. For example, allele specific expression, where one allele is more highly expressed than the other, will skew genotype frequency estimates, particularly for pooled samples. Testing for allele specific expression is possible but not trivial. One option is to collect supplementary genomic sequence data for heterozygote individuals and compare the distribution of the 2 alleles to the distribution observed in the RNA-Seq data. Another option is to test for allele specific expression across the transcriptome and at

Downloaded from http://jhered.oxfordjournals.org/ at Louisiana State University on April 4, 2016

American Genetic Association

The Journal of Heredity

www.jhered.oxfordjournals.org

specific loci using statistical models, such as the Bayesian framework developed by Skelly et al. (2011). Although debate exists on the prevalence of allele specific expression (Lemay et al. 2013; Konczal et al. 2014), researchers mining SNPs from transcriptome sequences should quantify allele specific expression in their data before estimating allele frequencies (Degner et al. 2009; Skelly et al. 2011). Another important consideration is whether a single experimental design or data set can reliably provide the data required to test hypotheses about population genomics *and* differential gene expression. Undoubtedly, the full potential of transcriptome sequences will be further revealed as RNAseq data accumulate.

Using Transcriptomic Data to Identify Gene Regulatory Networks

Examining sets of interacting genes in regulatory networks can provide insight into whether upstream changes in gene expression lead to downstream effects on organismal fitness. Additionally, model-based methods testing coexpression of genes in regulatory networks can differentiate between changes in expression due to selection and those that evolved due to drift and therefore lack functional consequences (Khaitovich et al. 2004; Oldham et al. 2006). For example, Filteau et al. (2013) used regulatory network analyses to find possible targets of natural selection in ecologically divergent dwarf and normal *C. cupleaformis* whitefish by identifying modules of coexpressed genes related to phenotypic variation in reproduction, growth, morphology, and behavior. In contrast, Runcie et al. (2012) found that temperature-induced expression changes in upstream genes in a *S. purpuratus* sea urchin regulatory network did not influence expression of the downstream genes they regulate, suggesting the downstream genes were buffered against the effects of environmental stress and hence shielded from natural selection. Incorporating methods to reconstruct and test gene regulatory networks will improve our understanding of how genes interact with each other, how their expression is regulated, and their functional importance to organismal fitness (Cline et al. 2007; Shojaie et al. 2014).

Incorporating the Translatome into Transcriptomic Studies

An important assumption in transcriptomics is that all mRNA molecules transcribed are subsequently translated into proteins and that changes in transcription correspond closely to changes in translation. Although some studies have shown a close link between mRNA abundance and protein abundance (Newman et al. 2006), growing evidence suggests differences at the mRNA level do not always exist at the protein level (Diz et al. 2012; Khan et al. 2013) and the equivalence of transcription and translation is likely to be system (Ueda et al. 2012) and/or stressor (Halbeisen and Gerber 2009; Yángüez et al. 2013) dependent. Furthermore, mRNA might be targeted for degradation before translation by other noncoding RNAs, which are increasingly recognized as playing an important role in gene regulation (Morris and Mattick 2014).

Recent studies that have extracted and sequenced premature mRNA and mature, ribosome-bound mRNA (total RNA-seq versus polyribosomal RNA-seq) have suggested that the complexity in plant and animal transcriptomes is due in part to the inclusion of spurious isoforms and incompletely spliced pre-mature mRNAs that do not contribute to functional proteins. For example, Zhang et al. (2015) found decreased complexity and diversity in the *Arabidopsis* translatome compared to the transcriptome. A promising future direction for the field of transcriptomics is to explore in more detail how environmental stress influences transcription and translation and how these 2 biological processes relate to each other and to plasticity and adaptation.

Conclusions

Transcriptome sequencing is a powerful tool for testing questions about how individuals and populations will respond to climate change over ecological and evolutionary timescales. We reviewed recent studies measuring the transcriptomic response of locally adapted populations to environmental stress and studies testing organismal responses to multiple climate change stressors. Transcriptomic studies comparing populations that are locally adapted in space might illuminate the mechanisms by which adaptation to that environmental variable might occur through time. One mechanism highlighted in several studies provides a possible linkage between plastic and evolved responses to environmental stress: the permanent upregulation of the plastic stress response as a part of the evolved response to that stressor in locally adapted populations. More data are needed, however, to understand whether this represents a general mechanism for stress adaptation. We find that the few studies testing multiple stressors simultaneously have found synergistic effects on gene expression that would not have been predicted from single stressor studies, highlighting the need for more studies testing organismal responses to multiple stressors. The interpretation of transcriptomic data in nonmodel organisms is limited by difficulties in separating adaptive from neutral differences among populations, incomplete annotations of de novo transcriptomes, and the correlative nature of transcriptomic data in general. Future studies employing functional testing and combining transcriptomic and genomic data will help determine the evolutionary significance of variation in transcriptomic responses environmental stress among populations and species, providing important insights into plastic and evolved responses to global change.

Funding

Louisiana State University Department of Biological Sciences.

Acknowledgments

We thank Robin Waples, Maheshi Dassanayake, Karine Posbic, and 3 anonymous reviewers, whose constructive comments greatly improved the manuscript.

References

Aitken SN, Whitlock MC. 2013. Assisted gene flow to facilitate local adaptation to climate change. *Annu Rev Ecol Evol Syst*. 44:367–388.

Alvarez M, Schrey AW, Richards CL. 2015. Ten years of transcriptomics in wild populations: what have we learned about their ecology and evolution? *Mol Ecol*. 24:710–725.

Auer PL, Doerge RW. 2010. Statistical design and analysis of RNA sequencing data. *Genetics*. 185:405–416.

Bainbridge MN, Warren RL, Hirst M, Romanuik T, Zeng T, Go A, Delaney A, Griffith M, Hickenbotham M, Magrini V, *et al.* 2006. Analysis of the prostate cancer cell line LNCaP transcriptome using a sequencing-by-synthesis approach. *BMC Genomics*. 7:246.

Barreto FS, Schoville SD, Burton RS. 2015. Reverse genetics in the tide pool: knock-down of target gene expression via RNA interference in the copepod *Tigriopus californicus*. *Mol Ecol Resour*. 15: 868–879.

Downloaded from http://jhered.oxfordjournals.org/ at Louisiana State University on April 4, 2016

American Genetic Association
The Journal of Heredity
www.jhered.oxfordjournals.org

Barshis DJ, Ladner JT, Oliver TA, Seneca FO, Traylor-Knowles N, Palumbi SR. 2013. Genomic basis for coral resilience to climate change. *Proc Natl Acad Sci USA*. 110:1387–1392.

Bay R, Palumbi SR. 2014. Multilocus adaptation associated with heat resistance in reef-building corals. *Curr Biol*. 24:R1166–R1168.

Becks L, Ellner SP, Jones LE, Hairston NG Jr. 2012. The functional genomics of an eco-evolutionary feedback loop: linking gene expression, trait evolution, and community dynamics. *Ecol Lett*. 15:492–501.

Bell G, Gonzalez A. 2009. Evolutionary rescue can prevent extinction following environmental change. *Ecol Lett*. 12:942–948.

Berger D, Walters RJ, Blanckenhorn WU. 2014. Experimental evolution for generalists and specialists reveals multivariate genetic constraints on thermal reaction norms. *J Evol Biol*. 27:1975–1989.

Bikard D, Jiang W, Samai P, Hochschild A, Zhang F, Marraffini LA. 2013. Programmable repression and activation of bacterial gene expression using an engineered CRISPR-Cas system. *Nucleic Acids Res*. 41:7429–7437.

Blois JL, Williams JW, Fitzpatrick MC, Jackson ST, Ferrier S. 2013. Space can substitute for time in predicting climate-change effects on biodiversity. *Proc Natl Acad Sci USA*. 110:9374–9379.

Blows MW, Hoffmann A. 2005. A reassessment of genetic limits to evolutionary change. *Ecology*. 86:1371–1384.

Brennan RS, Galvez F, Whitehead A. 2015. Reciprocal osmotic challenges reveal mechanisms of divergence in phenotypic plasticity in the killifish. *J Exp Biol*. 218:1212–1222.

Brown TB, Cheng R, Sirault XR, Rungrat T, Murray KD, Trtilek M, Furbank RT, Badger M, Pogson BJ, Borevitz JO. 2014. TraitCapture: genomic and environment modelling of plant phenomic data. *Curr Opin Plant Biol*. 18:73–79.

Byrne M. 2011. Impact of ocean warming and ocean acidification on marine invertebrate life history stages : vulnerabilities and potential for persistence in a changing ocean. In: Gibson R, Atkinson R, Gordon J, Simth I, Hughes D, editors. *Oceanography and marine biology: an annual review*. Boca Raton (FL): Taylor & Francis. p. 1–42.

Byrne M. 2012. Global change ecotoxicology: Identification of early life history bottlenecks in marine invertebrates, variable species responses and variable experimental approaches. *Mar Environ Res*. 76:3–15.

Chan Z, Bigelow PJ, Loescher W, Grumet R. 2012. Comparison of salt stress resistance genes in transgenic Arabidopsis thaliana indicates that extent of transcriptomic change may not predict secondary phenotypic or fitness effects. *Plant Biotechnol J*. 10:284–300.

Chapman RW, Mancia A, Beal M, Veloso A, Rathburn C, Blair A, Holland AF, Warr GW, Didinato G, Sokolova IM, *et al.* 2011. The transcriptomic responses of the eastern oyster, *Crassostrea virginica*, to environmental conditions. *Mol Ecol*. 20:1431–1449.

Chevin LM. 2013. Genetic constraints on adaptation to a changing environment. *Evolution*. 67:708–721.

Chevin LM, Lande R, Mace GM. 2010. Adaptation, plasticity, and extinction in a changing environment: Towards a predictive theory. *PLoS Biol*. 8:1–8.

Cheviron ZA, Whitehead A, Brumfield RT. 2008. Transcriptomic variation and plasticity in rufous-collared sparrows (*Zonotrichia capensis*) along an altitudinal gradient. *Mol Ecol*. 17:4556–4569.

Cline MS, Smoot M, Cerami E, Kuchinsky A, Landys N, Workman C, Christmas R, Avila-Campilo I, Creech M, Gross B, *et al.* 2007. Integration of biological networks and gene expression data using Cytoscape. *Nat Protoc*. 2:2366–2382.

Dayan DI, Crawford DL, Oleksiak MF. Forthcoming 2015. Phenotypic plasticity in gene expression contributes to divergence of locally-adapted populations of *Fundulus heteroclitus*. *Mol Ecol*. 24:3345–3359.

Degner JF, Marioni JC, Pai AA, Pickrell JK, Nkadori E, Gilad Y, Pritchard JK. 2009. Effect of read-mapping biases on detecting allele-specific expression from RNA-sequencing data. *Bioinformatics*. 25:3207–3212.

De Wit P, Palumbi SR. 2013. Transcriptome-wide polymorphisms of red abalone (*Haliotis rufescens*) reveal patterns of gene flow and local adaptation. *Mol Ecol*. 22:2884–2897.

De Wit P, Pespeni MH, Palumbi SR. 2015. SNP genotyping and population genomics from expressed sequences - current advances and future possibilities. *Mol Ecol*. 24:2310–2323.

DeWitt TJ, Sih A, Wilson DS. 1998. Costs and limits of phenotypic plasticity. *Trends Ecol Evol*. 13:77–81.

Dhar R, Sägesser R, Weikert C, Yuan J, Wagner A. 2011. Adaptation of *Saccharomyces cerevisiae* to saline stress through laboratory evolution. *J Evol Biol*. 24:1135–1153.

Diz AP, Martínez-Fernández M, Rolán-Alvarez E. 2012. Proteomics in evolutionary ecology: linking the genotype with the phenotype. *Mol Ecol*. 21:1060–1080.

Doerge RW. 2002. Mapping and analysis of quantitative trait loci in experimental populations. *Nat Rev Genet*. 3:43–52.

Doney SC, Fabry VJ, Feely RA, Kleypas JA. 2009. Ocean acidification: the other CO2 problem. *Annu Rev Mar Sci*. 1:169–192.

Dubansky B, Whitehead A, Miller JT, Rice CD, Galvez F. 2013. Multitissue molecular, genomic, and developmental effects of the Deepwater Horizon oil spill on resident Gulf killifish (*Fundulus grandis*). *Environ Sci Technol*. 47:5074–5082.

Dupont S, Dorey N, Stumpp M, Melzner F, Thorndyke M. 2012. Long-term and trans-life-cycle effects of exposure to ocean acidification in the green sea urchin *Strongylocentrotus droebachiensis*. *Mar Biol*. 160:1835–1843.

Duputié A, Massol F, Chuine I, Kirkpatrick M, Ronce O. 2012. How do genetic correlations affect species range shifts in a changing environment? *Ecol Lett*. 15:251–259.

Etterson JR, Shaw RG. 2001. Constraint to adaptive evolution in response to global warming. *Science*. 294:151–154.

Evans TG, Hofmann GE. 2012. Defining the limits of physiological plasticity: how gene expression can assess and predict the consequences of ocean change. *Philos Trans R Soc Lond B Biol Sci*. 367:1733–1745.

Feder ME, Hofmann GE. 1999. Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. *Annu Rev Physiol*. 61:243–282.

Feely RA, Sabine CL, Hernandez-Ayon JM, Ianson D, Hales B. 2008. Evidence for upwelling of corrosive "acidified" water onto the continental shelf. *Science*. 320:1490–1492.

Filteau M, Pavey SA, St-Cyr J, Bernatchez L. 2013. Gene coexpression networks reveal key drivers of phenotypic divergence in lake whitefish. *Mol Biol Evol*. 30:1384–1396.

Fire A, Xu S, Montgomery M, Kostas S, Driver S, Mello C. 1998. Potent and specific genetic interfernece by double-stranded RNA in *C. elegans*. *Nature*. 319:806–811.

Franssen SU, Gu J, Bergmann N, Winters G, Klostermeier UC, Rosenstiel P, Bornberg-Bauer E, Reusch TBH. 2011. Transcriptomic resilience to global warming in the seagrass *Zostera marina*, a marine foundation species. *Proc Natl Acad Sci USA*. 108:19276–19281.

Franssen SU, Gu J, Winters G, Huylmans AK, Wienpahl I, Sparwel M, Cover JA, Oslen JL, Reusch TB, Bornberg-Bauer E. 2014. Genome-wide transcriptomic responses of the seagrasses *Zostera marina* and *Nanozostera noltii* under a simulated heatwave confirm functional types. *Mar Genomics*. 15:65–73.

Furbank RT, Tester M. 2011. Phenomics–technologies to relieve the phenotyping bottleneck. *Trends Plant Sci*. 16:635–644.

Gagnaire PA, Normandeau E, Pavey SA, Bernatchez L. 2013. Mapping phenotypic, expression and transmission ratio distortion QTL using RAD markers in the Lake Whitefish (*Coregonus clupeaformis*). *Mol Ecol*. 22:3036–3048.

Ghalambor CK, McKay JK, Carroll SP, Reznick DN. 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Funct Ecol*. 21:394–407.

Gilad Y, Mizrahi-Man O. 2015. A reanalysis of mouse ENCODE comparative gene expression data. *F1000Res*. 121:1–32.

Gilad Y, Rifkin SA, Pritchard JK. 2008. Revealing the architecture of gene regulation: the promise of eQTL studies. *Trends Genet*. 24:408–415.

Gleason LU, Burton RS. 2015. RNA-seq reveals regional differences in transcriptome response to heat stress in the marine snail *Chlorostoma funebralis*. *Mol Ecol*. 24:610–627.

Granados-Cifuentes C, Bellantuono AJ, Ridgway T, Hoegh-Guldberg O, Rodriguez-Lanetty M. 2013. High natural gene expression variation in

Downloaded from http://jhered.oxfordjournals.org/ at Louisiana State University on April 4, 2016

American Genetic Association

The Journal of Heredity

www.jhered.oxfordjournals.org

the reef-building coral *Acropora millepora*: potential for acclimative and adaptive plasticity. *BMC Genomics*. 14:228.

Grether GF. 2005. Environmental change, phenotypic plasticity, and genetic compensation. *Am Nat*. 166:E115–E123.

Halbeisen RE, Gerber AP. 2009. Stress-dependent coordination of transcriptome and translatome in yeast. *PLoS Biol*. 7:1–15.

Haldane JBS. 1957. The cost of natural selection. *J Genet*. 55:511–514.

Hannon G. 2002. RNA interference. *Nature*. 418:244–251.

Harrison PW, Wright AE, Mank JE. 2012. The evolution of gene expression and the transcriptome-phenotype relationship. *Semin Cell Dev Biol*. 23:222–229.

Harrisson KA, Pavlova A, Telonis-Scott M, Sunnucks P. 2014. Using genomics to characterize evolutionary potential for conservation of wild populations. *Evol Appl*. 7:1008–1025.

Hashimoto SI, Qu W, Ahsan B, Ogoshi K, Sasaki A, Nakatani Y, Lee Y, Ogawa M, Ametani A, Suzuki Y, *et al.* 2009. High-resolution analysis of the 5'-end transcriptome using a next generation DNA sequencer. *PLoS One*. 4:1–10.

Hoffmann AA, Sgrò CM. 2011. Climate change and evolutionary adaptation. *Nature*. 470:479–485.

Holt R, Gaines M. 1992. Analysis of adaptation in heterogeneous landscapes: implications for the evolution of furidamental niches. *Evol Ecol*. 6:433–447.

Hwang WY, Fu Y, Reyon D, Maeder ML, Tsai SQ, Sander JD, Peterson RT, Yeh JR, Joung JK. 2013. Efficient genome editing in zebrafish using a CRISPR-Cas system. *Nat Biotechnol*. 31:227–229.

Jiang W, Zhou H, Bi H, Fromm M, Yang B, Weeks DP. 2013. Demonstration of CRISPR/Cas9/sgRNA-mediated targeted gene modification in Arabidopsis, tobacco, sorghum and rice. *Nucleic Acids Res*. 41:e188.

Jones P, Binns D, Chang HY, Fraser M, Li W, McAnulla C, McWilliam H, Maslem J, Mitchell A, Nuka G, *et al.* 2014. InterProScan 5: genome-scale protein function classification. *Bioinformatics*. 30:1236–1240.

Kelly MW, Hofmann GE. 2013. Adaptation and the physiology of ocean acidification. *Funct Ecol*. 27:980–990.

Kelly MW, Padilla-Gamiño JL, Hofmann GE. 2013. Natural variation and the capacity to adapt to ocean acidification in the keystone sea urchin *Strongylocentrotus purpuratus*. *Glob Chang Biol*. 19:2536–2546.

Kenkel CD, Meyer E, Matz MV. 2013. Gene expression under chronic heat stress in populations of the mustard hill coral (Porites astreoides) from different thermal environments. *Mol Ecol*. 22:4322–4334.

Khaitovich P, Weiss G, Lachmann M, Hellmann I, Enard W, Muetzel B, Wirkner U, Ansorge W, Pääbo S. 2004. A neutral model of transcriptome evolution. *PLoS Biol*. 2:0682–0689.

Khan Z, Ford MJ, Cusanovich DA, Mitrano A, Pritchard JK, Gilad Y. 2013. Primate transcript and protein expression levels evolve under compensatory selection pressures. *Science*. 342:1100–1104.

Kimura M. 1983. *The neutral theory of molecular evolution*. New York (NY): Cambridge University Press.

Konczal M, Koteja P, Stuglik MT, Radwan J, Babik W. 2014. Accuracy of allele frequency estimation using pooled RNA-Seq. *Mol Ecol Resour*. 14:381–392.

Kristensen TN, Loeschcke V, Hoffmann AA. 2007. Can artificially selected phenotypes influence a component of field fitness? Thermal selection and fly performance under thermal extremes. *Proc Biol Sci*. 274:771–778.

Kristensen TN, Overgaard J, Lassen J, Hoffmann AA, Sgrò C. 2015. Low evolutionary potential for egg-to-adult viability in *Drosophila melanogaster* at high temperatures. *Evolution*. 69:803–814.

Latta LC, Weider LJ, Colbourne JK, Pfrender ME. 2012. The evolution of salinity tolerance in Daphnia: a functional genomics approach. *Ecol Lett*. 15:794–802.

Law CW, Chen Y, Shi W, Smyth GK. 2014. voom: Precision weights unlock linear model analysis tools for RNA-seq read counts. *Genome Biol*. 15:R29.

Lemay MA, Donnelly DJ, Russello MA. 2013. Transcriptome-wide comparison of sequence variation in divergent ecotypes of kokanee salmon. *BMC Genomics*. 14:1–11.

Levine MT, Eckert ML, Begun DJ. 2011. Whole-genome expression plasticity across tropical and temperate *Drosophila melanogaster* populations from Eastern Australia. *Mol Biol Evol*. 28:249–256.

Li Y, Cheng R, Spokas KA, Palmer AA, Borevitz JO. 2014. Genetic variation for life history sensitivity to seasonal warming in Arabidopsis thaliana. *Genetics*. 196:569–577.

Li S, Tighe SW, Nicolet CM, Grove D, Levy S, Farmerie W, *et al.* 2014. Multi-platform assessment of transcriptome profiling using RNA-seq in the ABRF next-generation sequencing study. *Nat Biotechnol*. 32:915–925.

Liu Y, Zhou J, White KP. 2014. RNA-seq differential expression studies: more sequence or more replication? *Bioinformatics*. 30:301–304.

Lohbeck KT, Riebesell U, Reusch TB. 2014. Gene expression changes in the coccolithophore *Emiliania huxleyi* after 500 generations of selection to ocean acidification. *Proc R Soc B*. 281: 20140003.

Mahadav A, Kontsedalov S, Czosnek H, Ghanim M. 2009. Thermotolerance and gene expression following heat stress in the whitefly *Bemisia tabaci* B and Q biotypes. *Insect Biochem Mol Biol*. 39:668–676.

Malone JH, Oliver B. 2011. Microarrays, deep sequencing and the true measure of the transcriptome. *BMC Biol*. 9:34.

Marioni JC, Mason CE, Mane SM, Stephens M, Gilad Y. 2008. RNA-seq: an assessment of technical reproducibility and comparison with gene expression arrays. *Genome Res*. 18:1509–1517.

McGettigan PA. 2013. Transcriptomics in the RNA-seq era. *Curr Opin Chem Biol*. 17:4–11.

McIntyre LM, Lopiano KK, Morse AM, Amin V, Oberg AL, Young LJ, Nuzhdin SV. 2011. RNA-seq: technical variability and sampling. *BMC Genomics*. 12:1–13.

Meyer B, Martini P, Biscontin A, De Pittà C, Romualdi C, Teschke M, Frickenhaus S, Harms L, Freier U, Jarman S, *et al.* Forthcoming 2015. Pyrosequencing and de novo assembly of Antarctic krill (*Euphausia superba*) transcriptome to study the adaptability of krill to climate induced environmental changes. *Mol Ecol Resour*.

Mohamed B, Hajer A, Susanna S, Caterina O, Flavio M, Hamadi B, Aldo V. 2014. Transcriptomic responses to heat stress and nickel in the mussel *Mytilus galloprovincialis*. *Aquat Toxicol*. 148:104–112.

Montgomery SB, Dermitzakis ET. 2011. From expression QTLs to personalized transcriptomics. *Nat Rev Genet*. 12:277–282.

Morris KV, Mattick JS. 2014. The rise of regulatory RNA. *Nat Rev Genet*. 15:423–437.

Mortazavi A, Williams BA, McCue K, Schaeffer L, Wold B. 2008. Mapping and quantifying mammalian transcriptomes by RNA-Seq. *Nat Methods*. 5:621–628.

Moya A, Ganot P, Furla P, Sabourault C. 2012. The transcriptomic response to thermal stress is immediate, transient and potentiated by ultraviolet radiation in the sea anemone *Anemonia viridis*. *Mol Ecol*. 21:1158–1174.

Moya A, Huisman L, Forêt S, Gattuso JP, Hayward DC, Ball EE, *et al.* 2015. Rapid acclimation of juvenile corals to CO2 -mediated acidification by upregulation of heat shock protein and Bcl-2 genes. *Mol Ecol*. 24:438–452.

Narum SR, Campbell NR. 2015. Transcriptomic response to heat stress among ecologically divergent populations of redband trout. *BMC Genomics*. 16:103.

Newman JR, Ghaemmaghami S, Ihmels J, Breslow DK, Noble M, DeRisi JL, Weissman JS. 2006. Single-cell proteomic analysis of *S. cerevisiae* reveals the architecture of biological noise. *Nature*. 441:840–846.

Norman JD, Ferguson MM, Danzmann RG. 2014. Transcriptomics of salinity tolerance capacity in Arctic charr (*Salvelinus alpinus*): a comparison of gene expression profiles between divergent QTL genotypes. *Physiol Genomics*. 46:123–137.

O'Donnell MJ, Hammond LM, Hofmann GE. 2009. Predicted impact of ocean acidification on a marine invertebrate: Elevated CO2 alters response to thermal stress in sea urchin larvae. *Mar Biol*. 156:439–446.

O'Donnell M, Todgham A, Sewell M, Hammond L, Ruggiero K, Fangue N, Zippay ML, Hofmann GE. 2010. Ocean acidification alters skeletogenesis and gene expression in larval sea urchins. *Mar Ecol Prog Ser*. 398:157–171.

Oldham MC, Horvath S, Geschwind DH. 2006. Conservation and evolution of gene coexpression networks in human and chimpanzee brains. *Proc Natl Acad Sci USA*. 103:17973–17978.

Downloaded from http://jhered.oxfordjournals.org/ at Louisiana State University on April 4, 2016

American Genetic Association
The Journal of Heredity
www.jhered.oxfordjournals.org

van Oppen MJ, Oliver JK, Putnam HM, Gates RD. 2015. Building coral reef resilience through assisted evolution. *Proc Natl Acad Sci USA*. 112:2307–2313.

Orr JC, Fabry VJ, Aumont O, Bopp L, Doney SC, Feely RA, Gnanadesikan A, Gruber N, Ishida A, Joos F, *et al.* 2005. Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature*. 437:681–686.

Oshlack A, Robinson MD, Young MD. 2010. From RNA-seq reads to differential expression results. *Genome Biol*. 11:220.

Osovitz CJ, Hofmann GE. 2005. Thermal history-dependent expression of the hsp70 gene in purple sea urchins: biogeographic patterns and the effect of temperature acclimation. *J Exp Mar Bio Ecol*. 327:134–143.

Padilla-Gamiño JL, Kelly MW, Evans TG, Hofmann GE. 2013. Temperature and CO(2) additively regulate physiology, morphology and genomic responses of larval sea urchins, *Strongylocentrotus purpuratus*. *Proc Biol Sci*. 280:20130155.

Parker LM, Ross PM, Raftos D, Thompson ET, O'Connor WA. 2011. The proteomic response of larvae of the Sydney rock oyster, *Saccostrea glomerata* to elevated *p*CO2. *Aust Zool*. 35:1011–1023.

Pavey SA, Bernatchez L, Aubin-Horth N, Landry CR. 2012. What is needed for next-generation ecological and evolutionary genomics? *Trends Ecol Evol*. 27:673–678.

Pespeni MH, Barney BT, Palumbi SR. 2013. Differences in the regulation of growth and biomineralization genes revealed through long-term common-garden acclimation and experimental genomics in the purple sea urchin. *Evolution*. 67:1901–1914.

Pilcher W, Miles S, Tang S, Mayer G, Whitehead A. 2014. Genomic and genotoxic responses to controlled weathered-oil exposures confirm and extend field studies on impacts of the Deepwater Horizon oil spill on native killifish. *PLoS One*. 9:e106351.

Primmer CR, Papakostas S, Leder EH, Davis MJ, Ragan MA. 2013. Annotated genes and nonannotated genomes: cross-species use of gene ontology in ecology and evolution research. *Mol Ecol*. 22:3216–3241.

Qi LS, Larson MH, Gilbert LA, Doudna JA, Weissman JS, Arkin AP, Lim WA. 2013. Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. *Cell*. 152:1173–1183.

Rapaport F, Khanin R, Liang Y, Pirun M, Krek A, Zumbo P, Mason CE, Socci ND, Betel D. 2013. Comprehensive evaluation of differential gene expression analysis methods for RNA-seq data. *Genome Biol*. 14:R95.

Reed TE, Waples RS, Schindler DE, Hard JJ, Kinnison MT. 2010. Phenotypic plasticity and population viability: the importance of environmental predictability. *Proc Biol Sci*. 277:3391–3400.

Rockman MV. 2008. Reverse engineering the genotype -phenotype map with natural genetic variation. *Nature*. 456:738–744.

Rohlfs R V, Nielsen R. 2015. Phylogenetic ANOVA : The Expression Variance and Evolution (EVE) model for quantitative trait evolution. *Syst Biol*. 64:695–708.

Runcie DE, Garfield DA, Babbitt CC, Wygoda JA, Mukherjee S, Wray GA. 2012. Genetics of gene expression responses to temperature stress in a sea urchin gene network. *Mol Ecol*. 21:4547–4562.

Schoville SD, Barreto FS, Moy GW, Wolff A, Burton RS. 2012. Investigating the molecular basis of local adaptation to thermal stress: population differences in gene expression across the transcriptome of the copepod *Tigriopus californicus*. *BMC Evol Biol*. 12:170.

Seneca FO, Palumbi SR. 2015. The role of transcriptome resilience in resistance of corals to bleaching. *Mol Ecol*. 24:1467–1484.

Shojaie A, Jauhiainen A, Kallitsis M, Michailidis G. 2014. Inferring regulatory networks by combining perturbation screens and steady state gene expression profiles. *PLoS One*. 9:e82393.

Skelly DA, Johansson M, Madeoy J, Wakefield J, Akey JM. 2011. A powerful and flexible statistical framework for testing hypotheses of allele-specific gene expression from RNA-seq data. *Genome Res*. 21:1728–1737.

Smyth G. 2005. Limma: linear models for microarray data. In: *Bioinformatics and computational biology solutions using R and Bioconductor*. New York (NY): Springer. p. 397–420.

Sunday JM, Crim RN, Harley CD, Hart MW. 2011. Quantifying rates of evolutionary adaptation in response to ocean acidification. *PLoS One*. 6:e22881.

Telonis-Scott M, Clemson AS, Johnson TK, Sgrò CM. 2014. Spatial analysis of gene regulation reveals new insights into the molecular basis of upper thermal limits. *Mol Ecol*. 23:6135–6151.

Tepolt CK, Palumbi SR. 2015. Transcriptome sequencing reveals both neutral and adaptive genome dynamics in a marine invader. *Mol Ecol*. 24:4145–4158.

Topp CN, Iyer-Pascuzzi AS, Anderson JT, Lee CR, Zurek PR, Symonova O, Zheng Y, Bucksch A, Mileyko Y, Galkovskyi T, *et al.* 2013. 3D phenotyping and quantitative trait locus mapping identify core regions of the rice genome controlling root architecture. *Proc Natl Acad Sci USA*. 110:E1695–E1704.

Travers SE, Tang Z, Caragea D, Garrett KA, Hulbert SH, Leach JE, Bai J, Saleh A, Knapp AK, Fay PA, *et al.* 2010. Variation in gene expression of *Andropogon gerardii* in response to altered environmental conditions associated with climate change. *J Ecol*. 98:374–383.

Ueda K, Matsuura H, Yamaguchi M, Demura T, Kato K. 2012. Genome-wide analyses of changes in translation state caused by elevated temperature in *Oryza sativa*. *Plant Cell Physiol*. 53:1481–1491.

Via S, Lande R. 1985. Evolution of phenotypic plasticity. *Evolution*. 39:505–522.

Vidal-Dupiol J, Dheilly NM, Rondon R, Grunau C, Cosseau C, Smith KM, Frietag M, Adjeroud M, Mitta G. 2014. Thermal stress triggers broad *Pocillopora damicornis* transcriptomic remodeling, while *Vibrio coralliilyticus* infection induces a more targeted immuno-suppression response. *PLoS One*. 9:e107672.

Wang Z, Gerstein M, Snyder M. 2009. RNA-Seq: a revolutionary tool for transcriptomics. *Nat Rev Genet*. 10:57–63.

Wang H, Yang H, Shivalila CS, Dawlaty MM, Cheng AW, Zhang F, Jaenisch R. 2013. One-step generation of mice carrying mutations in multiple genes by CRISPR/cas-mediated genome engineering. *Cell*. 153:910–918.

Whitehead A, Galvez F, Zhang S, Williams LM, Oleksiak MF. 2011. Functional genomics of physiological plasticity and local adaptation in killifish. *J Hered*. 102:499–511.

Whitehead A, Zhang S, Roach JL, Galvez F. 2013. Common functional targets of adaptive micro- and macro-evolutionary divergence in killifish. *Mol Ecol*. 22:3780–3796.

Yángüez E, Castro-Sanz AB, Fernández-Bautista N, Oliveros JC, Castellano MM. 2013. Analysis of genome-wide changes in the translatome of Arabidopsis seedlings subjected to heat stress. *PLoS One*. 8:e71425.

Zhang X, Hause RJ, Borevitz JO, Coruzzi GM. 2012. Natural genetic variation for growth and development revealed by high-throughput phenotyping in *Arabidopsis thaliana*. *G3 Genes Genomes Genetics*. 2:29–34.

Zhang X, Rosen BD, Tang H, Krishnakumar V, Town CD. 2015. Polyribosomal RNA-Seq reveals the decreased complexity and diversity of the Arabidopsis translatome. *PLoS One*. 10:e0117699.

Zhou S, Campbell TG, Stone EA, Mackay TF, Anholt RR. 2012. Phenotypic plasticity of the Drosophila transcriptome. *PLoS Genet*. 8:e1002593.

Downloaded from http://jhered.oxfordjournals.org/ at Louisiana State University on April 4, 2016