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A framework for understanding gene expression plasticity and its influence on stress tolerance

Hanny E. Rivera[1] [iD] | Hannah E. Aichelman[1] [iD] | James E. Fifer[1] [iD] | Nicola G. Kriefall[1] [iD] | Daniel M. Wuitchik[1] [iD] | Sara J. S. Wuitchik[1,2] [iD] | Sarah W. Davies[1] [iD]

[1]Department of Biology, Boston University, Boston, MA, USA

[2]FAS Informatics, Harvard University, Cambridge, MA, USA

Correspondence
Hanny E. Rivera, Hannah E.Aichelman, & Sarah W.Davies, Department of Biology, Boston University, Boston, MA, USA.
Emails: hrivera@bu.edu (H. E. R.)

haich@bu.edu (H. E. A.)

daviessw@bu.edu (S. W. D.)

Funding information
Boston University

Abstract

Phenotypic plasticity can serve as a stepping stone towards adaptation. Recently, studies have shown that gene expression contributes to emergent stress responses such as thermal tolerance, with tolerant and susceptible populations showing distinct transcriptional profiles. However, given the dynamic nature of gene expression, interpreting transcriptomic results in a way that elucidates the functional connection between gene expression and the observed stress response is challenging. Here, we present a conceptual framework to guide interpretation of gene expression reaction norms in the context of stress tolerance. We consider the evolutionary and adaptive potential of gene expression reaction norms and discuss the influence of sampling timing, transcriptomic resilience, as well as complexities related to life history when interpreting gene expression dynamics and how these patterns relate to host tolerance. We highlight corals as a case study to demonstrate the value of this framework for non-model systems. As species face rapidly changing environmental conditions, modulating gene expression can serve as a mechanistic link from genetic and cellular processes to the physiological responses that allow organisms to thrive under novel conditions. Interpreting how or whether a species can employ gene expression plasticity to ensure short-term survival will be critical for understanding the global impacts of climate change across diverse taxa.

KEYWORDS
adaptation, environmental stress, gene expression, plasticity, resilience, transcriptomics

1 | INTRODUCTION

Phenotypic plasticity – the manifestation of different physiology, morphology, behaviour, or other phenotypes under divergent environments – can allow organisms to adjust to temporal (e.g., diurnal or seasonal) and spatial (e.g., new habitats, disturbances) changes (Baythavong, 2011; Bonamour et al., 2019; Hadfield, 2016; Kelly, 2019). Theoretical interactions between environmental variability, phenotypic plasticity, and adaptation have been well described (e.g., Chevin & Hoffmann, 2017; Chevin & Lande, 2015;

Chevin et al., 2010; Waddington, 1959; West-Eberhard, 2003). Whether plasticity serves to facilitate or hinder adaptation is largely dependent on the timescale of environmental change and the strength of selection (Ghalambor et al., 2007; Gibert et al., 2019; Via & Lande, 1985). The "plasticity-first" hypothesis posits selection acts on the range of phenotypic plasticity present in the population in order to evolve towards better adapted phenotypes (Levis & Pfennig, 2016). Plasticity, therefore, may allow species to persist until genetic adaptation can "catch up" with current rates of anthropogenically-induced environmental change (Seebacher

Hanny E. Rivera, Hannah E. Aichelman are co-first authors.

et al., 2015; Visser, 2008). In particular, how plasticity may facilitate survival in organisms exposed to stress-inducing conditions is increasingly important as climate change causes environmental changes that may push species beyond their current physiological limits.

Transcriptomics has enabled investigations into how gene expression patterns vary across experimental and natural conditions. Gene expression connects genotype to cellular and organismal physiology and to potentially adaptive phenotypes; therefore, plasticity in gene expression can serve as a functional link in responding to changing environments both within and across generational timescales (De Nadal et al., 2011; Schlichting & Smith, 2002). Gene expression plasticity can be examined on a gene-by-gene basis, by collapsing genes into coregulated networks (via analyses such as WGCNA - Langfelder & Horvath, 2008), or by considering transcriptome-wide shifts in expression patterns (e.g., Kenkel & Matz, 2016).

If one is interested in understanding how the gain or loss of plasticity in a trait evolved, knowledge of the trait's ancestral range of plasticity would be ideal (reviewed by Kelly, 2019). Yet, expression of most genes is inherently dynamic (with the exception of some "housekeeping" genes) and therefore plastic, as their expression can be influenced by internal (e.g., genetic, physiological, and developmental) and external (e.g., environmental) factors. Because of this, we would expect most genes to maintain some degree of plasticity (inducible expression); however, the magnitude and direction of this plasticity can still be explored. Comparing gene expression patterns between populations and across environmental gradients can provide insights into the adaptive aspects of gene expression plasticity. For example, research has shown that northern and southern populations of killifish (*Fundulus heteroclitus*) had different upper thermal limits that were associated with differential expression of several heat shock-associated genes (Fangue et al., 2006). The southern, more thermally tolerant population, showed stronger induction (plasticity) of heat shock genes, suggesting gene expression plasticity of some key genes may be involved in thermal tolerance and contribute to biogeographical patterns (Fangue et al., 2006). With certain organisms, such as sticklebacks, the evolution of plasticity can be more readily studied as the ancestral state can be inferred by comparing marine (ancestral) and freshwater (derived) populations. Morris et al. (2014) assessed gene expression plasticity of marine and freshwater three-spine stickleback (*Gasterosteus aculeatus*) raised under extreme temperatures and found that both populations showed differential expression of similar genes; however, there was greater plasticity in freshwater populations, suggesting this plasticity was favoured under novel environments.

Decoupling the many factors that influence expression and determining how these impact organismal phenotypes remains challenging. In the context of environmental stress, transcriptomics is well poised to reveal how gene expression influences critical emergent phenotypes such as heat tolerance or drought resistance. Therefore, in this study we: (i) address the interpretation of gene expression as a plastic trait in a reaction norm framework, by comparing hypothetically stress tolerant and stress sensitive populations across conditions, and (ii) discuss the importance of timing and life history considerations in capturing gene expression responses. We concentrate on how gene expression influences tolerance, which we define as survival or maintained organismal functioning under stress; however, this framework is not limited to stress tolerance. While we acknowledge the importance of the potential influence of microbial partners on host transcriptomic responses and ultimately tolerance; however, this subject is too broad to comprehensively cover here and is deserving of its own review. We refer readers to Barfield et al. (2018), Kremer et al. (2013), Liu et al. (2020) and Richards et al. (2019) for further insights on this subject. This framework is broadly applicable to investigating gene expression plasticity in any organism, but it will be particularly useful for non-model systems, where a dearth of genetic resources (i.e., full genomes, chromosome maps, or the ability to generate gene knockouts) limits the discovery of direct genotype to phenotype links.

While we recognize and discuss the implications of the fact that gene expression, such as many phenotypic traits, is a function-valued trait (often in a multivariate state), we focus on a reaction norm framework, in which expression is measured at only a few discrete instances, as this remains the predominant experimental design for gene expression studies (e.g., comparing healthy and diseased tissues or individuals across two different conditions). We also leverage reef-building corals as a case study for non-model organisms because their ecological importance and susceptibility to climate change (Hughes et al., 2018) has led to a plethora of transcriptomic studies of stress responses (Box 1; Figure 1; Parkinson et al., 2019; Thomas et al., 2018) that provide tangible examples from which we design our conceptual framework. Nevertheless, the framework described herein can be widely used to interpret gene expression plasticity in any species.

2 | GENE EXPRESSION PLASTICITY: A REACTION NORM FRAMEWORK

Plasticity is often measured experimentally by characterizing a trait under two or more conditions. Individuals or populations can differ in their gene expression under either condition; and the resulting gene expression "plasticity" can thus be represented by the slope of the reaction norm across conditions. The framework developed here quantifies gene expression at two levels: a baseline, no stress condition, and a peak stress condition. To link gene expression patterns to a stress tolerance phenotype, we consider two populations: one that is stress tolerant (as measured by sustained functioning in another trait such as metabolic rate, growth, survival, etc), and a second that is sensitive to the stressor (shows reduced functioning). A range of reaction norms can emerge from contrasting gene expression between these hypothetical populations (Figure 2, Figure S1). Interpreting how reaction norms in Figure 2 may facilitate or hinder a population's ability to tolerate

FIGURE 1 Corals serve as prime case study for gene expression plasticity. Warming causes healthy coral to lose their obligate endosymbionts in a process known as bleaching, leaving corals starved and white. (a) Adjacent corals can differ in bleaching tolerances. Differential bleaching in Mo'orea in 2019. The bottom, white coral is clearly bleached, while others in the figure (middle, and left) appear healthy. Image credit: Kelly Speare and Dr Marie Strader. (b) Number of publications containing topic codes "corals" and "gene expression" on the Web of Science search engine for each year from 1995 to 2019

(a)

(b)
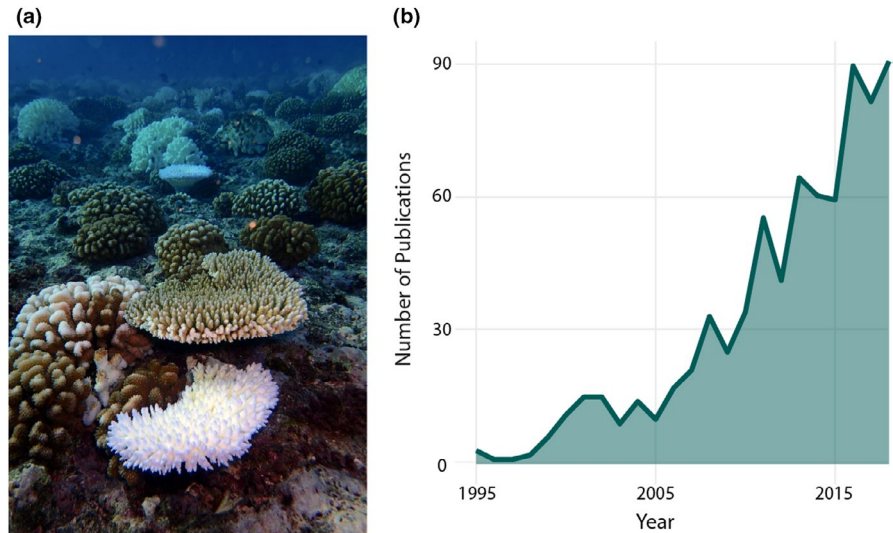
a particular stressor will depend on gene function, how expression varies with time, other regulatory mechanisms or influences (e.g., co-expression of transcription factors or inhibitors, priming, microbial influences, etc), and feedback between the phenotypic state and gene expression. We first discuss how the function of gene(s) involved in facilitating or hindering stress tolerance may lead to specific reaction norms, and later turn to influences of timing and life history.

In discussing function, we focus on the influence of two distinct expression patterns and their interactions: differing baselines and differing plasticity (i.e., reaction norm slope). Our framework is intended to generate hypotheses about which reaction norm might be expected if a gene (or network of genes) is contributing to tolerance. The presence of a specific pattern should not be taken as evidence of a particular function, especially given that gene expression is influenced by a multitude of factors simultaneously. Researchers should carefully consider the potential function of genes (if annotated) and the role they may play for the phenotype in question, as well as other conditions that could be driving expression before making functional conclusions and instead treat these interpretations as hypothesis generating. Lastly, we use the singular term "gene" throughout for simplicity, but this framework also holds for coregulated gene networks or modules (multiple genes that follow similar expression patterns).

2.1 | IF HIGHER BASELINES FACILITATE STRESS TOLERANCE

Higher constitutive expression (Figure 2a–c) can promote stress tolerance by maintaining homeostasis and cellular integrity under stress. For instance, heat shock proteins (HSPs – chaperones that prevent protein denaturation at high temperatures) are often up-regulated in response to increased temperatures across a variety of taxa including turtles, corals, nematodes, fruit flies, and fish (Bentley et al., 2017; Fangue et al., 2006; Kenkel et al., 2011; McColl et al.,

1996; Sikkink et al., 2014). Higher baseline expression of HSPs could, therefore, confer protection from frequent heat stress by providing a pre-emptive response. If a gene is important for mounting a protective stress response, then having more transcripts encoding for that protein at hand can facilitate a faster and more efficient response. This may also reduce the number of cellular resources diverted under stress allowing for the maintenance of other key cellular processes. Higher expression in the absence of stressors, however, may represent a trade-off between upfront costs and the ability to mount a rapid response.

This trade-off may be beneficial if organisms live in highly variable environments, especially if the time scale of environmental variability is shorter than the time scale of the physiological response (Barshis et al., 2013; Reed et al., 2010; Schlichting & Wund, 2014). In particular, the pattern depicted in Figure 2a has been termed "front-loading," and is hypothesized to be an adaptive form of gene expression plasticity in precisely such circumstances (Box 1; Barshis et al., 2013). Similar frontloading patterns have also been observed in intertidal sculpins (Todgham et al., 2005), yeast (Berry & Gasch, 2008), land snails (Mizrahi et al., 2012), and desert ants (Willot et al., 2017).

Figure 2b–c may represent genes where higher expression offers protection, but which may be more expensive to maintain at high expression levels, such that some degree of plasticity is still favorable. For example, expression of transcription factors that activate particular pathways could be detrimental to maintain at high levels when those pathways are unnecessary or costly. A rapid and strong induction of a transcription factor needed to prompt a protective stress response would therefore be desirable. This has been elegantly demonstrated for the DREB1A transcription factor in *Arabidopsis thaliana*, which induces expression of stress response genes that promote drought and salt tolerance (Kasuga et al., 1999). Constitutive expression of DREB1A, due to a mutated promoter region, increased stress tolerance but was also detrimental to plant growth. In contrast, the native, stress-inducible promoter had little impact on overall plant growth and showed stronger benefits for drought and salt tolerance (Kasuga et al., 1999). As another example,

BOX 1   Gene expression patterns and stress tolerance in reef-building corals

Case 1. Frontloading

One of the best characterized systems linking gene expression to stress tolerance in corals used two reefs in American Samoa: one with highly variable daily temperatures (HV) and another that is moderately variable (MV). Barshis et al. (2013) subjected HV and MV corals to an experimental heat stress and tracked changes in gene expression and endosymbiotic algal cell density. They found that HV corals bleached less (bleaching is a coral heat stress response in which endosymbiotic algae are lost), and coupled with their stress tolerance, some genes in HV corals showed a "frontloading" pattern - higher baseline expression but reduced expression plasticity (Figure 2a). These genes included heat shock protein (HSP70) and tumor necrosis factor (TNF), which promote protective immune responses (Pfeffer, 2003). Similarly, in another system of inshore and offshore reefs in the Florida Keys, more tolerant inshore corals exhibited higher baseline expression of several genes, and reduced plasticity in a subset of those genes (Kenkel et al., 2013).

Case 2. Dampening and transcriptome resilience

Reduced plasticity (regardless of baseline expression) has also been associated with stress tolerance in corals, and is termed "dampening" (Figures 2a,d,g). Corals with dampened expression of metabolic and ribosomal processing genes under stress showed higher thermal tolerance (Bay & Palumbi, 2017). Transcriptomic resilience of genes associated with apoptosis and heat-shock protein gene ontology terms were also correlated with increased thermal tolerance (Seneca & Palumbi, 2015; Figure 3). Additionally, Thomas et al. (2019) found that tolerant coral species returned to baseline gene expression levels more quickly after heat stress.

Case 3. Whole transcriptome plasticity

An organism's ability to shift the profile of its transcriptome when subjected to new conditions is likely to correlate with its ability for overall physiological plasticity (while frontloading or dampening may be beneficial for specific genes). Indeed, in a reciprocal transplant experiment in the Florida Keys, Kenkel and Matz (2016) found that corals who were able to shift their global transcriptomic patterns to better match those of corals native to the transplant environment showed higher survival and faster growth than those who failed to adequately shift their gene expression, even when the transplant environment was of lower quality than their home environment. These results underscore the role of gene expression in determining physiological plasticity of emergent traits.

the stress-activated protein kinase (SAPK) pathway in yeast helps maintain RNA translation in the face of oxidative stress; however, maintaining its activation under ambient conditions leads to overproduction of unnecessary proteins (Dunand-Sauthier et al. 2005). As such, being able to strongly induce this pathway when required (such as in Figure 2c,f,i) is probably more effective than assimilating plasticity into a constitutively higher expression level with lower plasticity (such as Figure 2a), which would risk detrimental effects.

Similarly, the Spx gene in *Bacillus* and *Staphylococcus* controls a variety of critical cellular pathways, such that a low expression level renders cells hypersensitive to a variety of stressors (Pamp et al., 2006). Higher expression levels at both baseline and stress conditions of Spx or similarly functioning genes could allow for greater stress tolerance (Figure 2b,c). Higher plasticity in the tolerant population (Figure 2c) may also arise because these genes are in the initial stages of genetic accommodation, where increased plasticity is favoured (Box 2; Kelly, 2019). However, under continued environmental selection over the course of generations, genetic assimilation may occur, leading to decreased plasticity in the tolerant population and resulting in the expression pattern in Figure 2a. Both Figure 2a,c may also represent patterns that emerge as a result of G × E interactions wherein tolerant and sensitive genotypes exhibit divergent responses in new environments. Such patterns may facilitate the assimilation of gene expression or drive selection for particular reaction norms.

2.2 | If higher baselines hinder stress tolerance

Genes following Figure 2g–i show lower baseline expression in the tolerant population, suggesting that these genes may drive detrimental physiological responses to stress, or that overexpression can be energetically costly and hinder survival. For genes that control cell apoptosis, reduce growth, alter metabolic processes, or trigger dysbiosis (in the case of corals or other symbiotic organisms), tolerant populations would benefit from lower constitutive expression. In white shrimp, high levels off caspase-3 and cathepsin-B under nitrite stress coincide with increased levels of cell apoptosis (Guo et al., 2013). Similarly, in human cells, accumulation of acetylated kinase HIPK2 serves as a threshold inducer of apoptosis under reactive oxygen species (de la Vega et al., 2012). When considering genes that serve similar purposes, populations or individuals with lower baseline expression may benefit from never reaching levels that trigger apoptosis or other cellular stress pathways, especially if the plasticity of response is also reduced (as in Figure 2g). Such a pattern would be particularly favourable for genes that induce a threshold response, meaning that once expression reaches a certain level, specific pathways are activated.

For the pattern shown in Figure 2i, the tolerant population has reduced baseline expression but higher plasticity, with expression levels of these genes increasing post-stress. This may represent genes similar to those in Figure 2c, in which there is a detriment (or trade off) to higher expression. However, in this case the cost

FIGURE 2 Reaction norm framework for upregulated genes. Possible reaction norms for an upregulated gene (or coregulated gene networks) in a stress tolerant and sensitive population. The function of genes that show different patterns may either confer resistance or promote a stress response and detrimental physiological effects. For a downregulation version see Figure S1
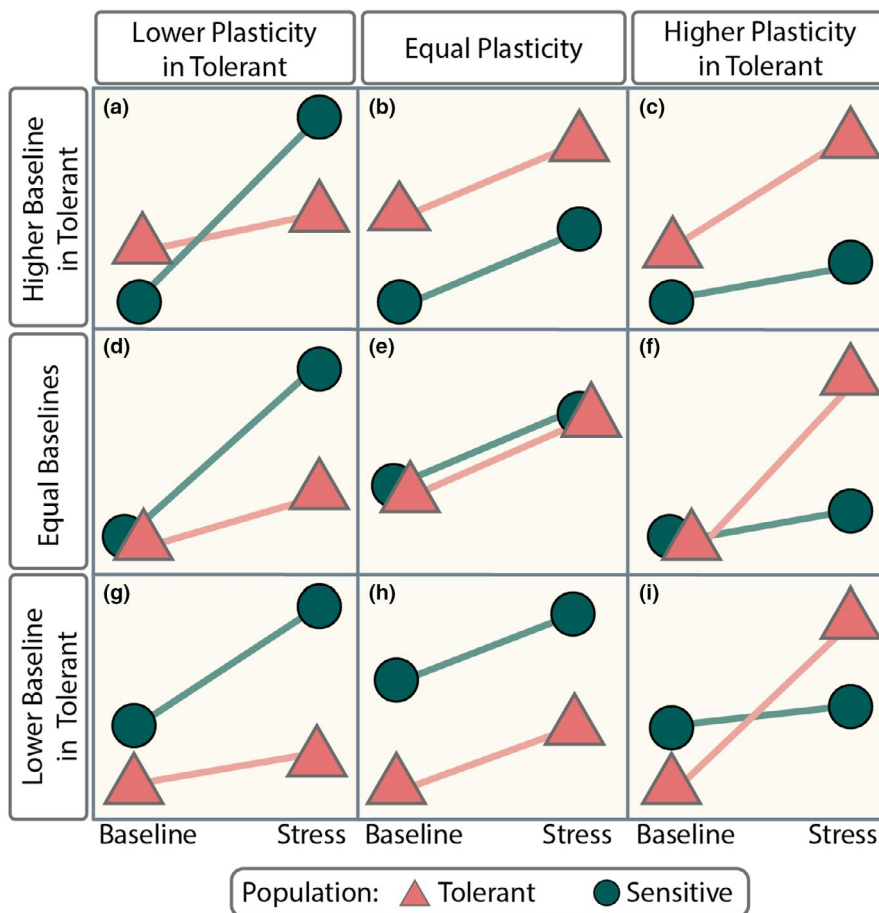

may be much higher, such that baseline expression is also reduced in tolerant populations, perhaps for reasons unrelated to the immediate stress response. For example, in pied flycatchers expression of certain HSPs (namely HSP60 but not HSP70) were associated with reduced immune response, and females with higher HSP60 levels had lower antibody levels following exposure to killed bacteria in an immune challenge (Morales et al., 2006). In such a case, having lower baseline expression while being able to activate the response when necessary would result in increased tolerance (as in Figure 2i). A similar pattern has been observed in *Drosophila*, whose reproductive output decreased in inbred lines that evolved higher HSP70 expression and increased thermal tolerance (Bettencourt et al., 1999), and in pea leaf miners (*Liriomyza huidobrensis*), whose fecundity decreased under heat stress concurrent with higher expression of HSP70 and HSP20 (Huang et al., 2007). If genes following Figure 2i are contributing to tolerance, their function could be to regulate other energetically costly processes, thereby allowing reallocation of resources towards stress responses or maintenance of homeostasis. For instance, in mammals, the AMP-activated protein kinase (AMPK) system is induced under stress to shut down energy intensive cellular processes (Alexander & Walker, 2011; Jeon et al., 2012). This and other genes with similar functions would be most beneficial to stress tolerance if they are inactive under baseline conditions, but can be quickly upregulated under stress.

Sensitive and tolerant populations can also exhibit equal plasticity in response to stress, while differing in baseline expression (Figure 2b,h). In Figure 2b,h, both populations have the same underlying capacity to modulate expression in response to stress (i.e., same reaction norm slope); however, their phenotypic outcomes may differ. Higher baseline expression of those particular genes or gene networks could be protective (Figure 2b) or detrimental (Figure 2h) under stress. Lastly, both populations could have the same baseline and plastic response (Figure 2e), suggesting that these networks play no major role in contributing to stress tolerance differences between populations and may instead represent a universal response to environmental change or time (e.g., circadian or developmental genes).

2.3 | If higher baselines facilitate stress tolerance

Elevated plasticity may hinder (Figure 2d) or facilitate (Figure 2f) stress tolerance. However, given the comparable baselines in both populations, it is perhaps more likely that these genes/networks simply represent stress networks that are activated in the sensitive population but not in the tolerant population (Figure 2d), and conversely protective genes/networks that the tolerant population is capable of activating to higher levels than the sensitive population (Figure 2f).
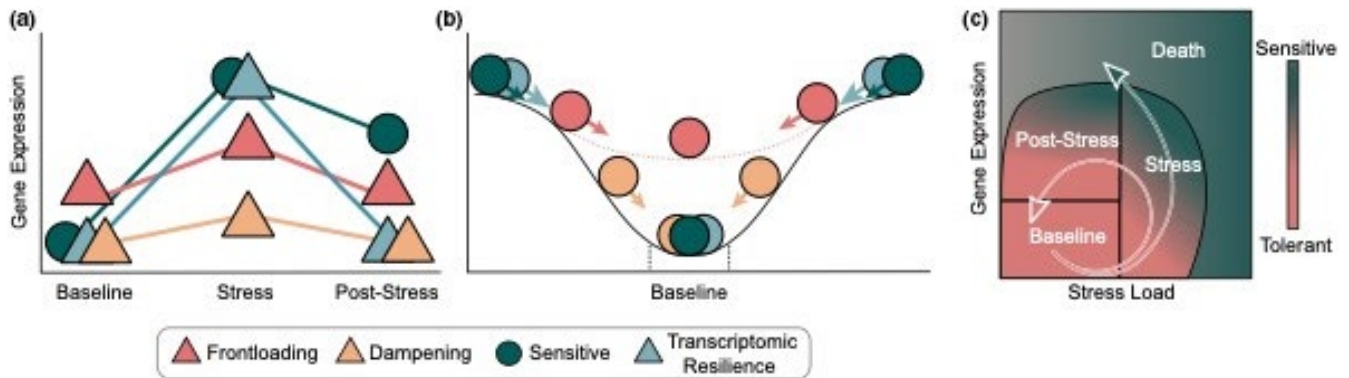
FIGURE 3 Transcriptomic resilience and timing. (a) Gene expression reaction norms of four strategies during recovery after a stressor. We use triangles again for patterns that may confer tolerance and circles for patterns associated with stress sensitivity. While all triangle paths show a return to baseline (resilience) the pink (frontloading) and yellow (dampening) are also depicting differences in baseline and plasticity and are therefore labelled differently. (b) Adapted from the rolling ball analogy commonly used for ecological resilience and depicted in Hodgson et al. (2015). Each ball represents a gene showing a color-matched expression pattern in (a). Landscapes represent expression possibilities during a stress event. In the absence of stress, the ball will settle in a trough, representing baseline expression levels. Elasticity (rate of return to the baseline) is represented by the size of the arrow (i.e., larger arrows have faster rates of return). Pink dotted line is the expression landscape for the frontloaded ball. (c) Using Torres et al. (2016) loops through disease space as an alternative framework of an organism's path through stress response and recovery. The colour gradient represents the resulting phenotype for a given path through stress and recovery space, though *x*- and *y*-axis can denote any two parameters that are correlated but with a time lag

Alternatively, differences in response time and sampling may generate such patterns and these considerations are discussed in detail below. To correlate the influence of gene expression plasticity on overall stress tolerance, differences in gene expression under baseline conditions are illuminating for interpreting the potential role of particular genes and networks in facilitating or hindering stress tolerance. Plasticity on its own should also be considered, but such an analysis may be better suited for examining global shifts in overall transcriptome patterns such as those discussed in Box 1, Case 3 (e.g., Kenkel & Matz, 2016).

3 | The evolution of reaction norms

The framework of gene expression reaction norms presented here could be easily generated by studying two populations or individuals. We have chosen to present our framework in this manner as this is often how gene expression studies are conducted, especially in non-model systems. However, there is much to be gained by also considering these reaction norms in an evolutionary context; for instance, by comparing species with known phylogenetic relationships or incorporating population genetics and demographic analyses into the interpretations of reaction norm differences between groups. In sticklebacks, the ancestral and derived state of plasticity can be inferred because marine lineages of stickleback are ancestral to freshwater derived populations (Lescak et al., 2015). Similar analyses may be possible for invasive species or range expansion events, where the ancestral population can be easily identified (for such considerations see Schneider & Meyer, 2017). For a fantastic example of how a framework similar to ours can be useful we refer the reader to Campbell-Staton et al. (2020), which examined different reaction

norms of gene expression of *Anolis* lizards that had independently colonized urban environments when exposed to forest and urban environment, and found that genes with particular reaction norms were associated with heat tolerance and could be adaptive for urban living.

A large body of literature addresses the evolution of reaction norms in phenotypically plastic traits (e.g., Angilletta et al., 2003; Day & Rowe, 2002; Gomulkiewicz & Kirkpatrick, 1992). This literature includes differences in generalist versus specialist reaction norms (e.g., Gilchrist, 1995), trade-offs where a reaction norm gains fitness in one subset of conditions but loses fitness in another (e.g., Powers & Schulte, 1998), and overall shifts in the position of a reaction norm such that fitness is higher across the entire range of conditions (e.g., Yamahira & Conover, 2002). Understanding the evolution of gene expression reaction norms can be challenging, as they are influenced by many internal and external inputs (as discussed here).

One avenue is to consider the evolution of eQTLs. Work on eQTLs combines gene expression analyses with genome wide association studies to uncover polymorphisms, some examples being polymorphisms that may contribute to differential expression of genes involved in human diseases (reviewed in Barbosa et al., 2013; Cookson et al., 2009; Gilad et al., 2008) and those underlying functional traits in *Arabidopsis* (Keurentjes et al., 2007; Lovell et al., 2015; Lowry et al., 2013). For example, a single nucleotide polymorphism within the HLA gene (an antigen) had additive effects on expression level, and individuals with high expression showed lower HIV viral load (Fellay et al., 2007). In plants, similar analyses have identified expression variants linked to drought response and leaf senescence (Wehner et al., 2016). However, for gene expression patterns to evolve, there must be some degree of heritability of expression levels. Indeed, studies have found gene expression can be highly

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BOX 2 Glossary

Function-valued trait

A trait whose characteristics may be described as a function of another variable. For example, height can be described as a function of age (through development), or gene expression of an immune response gene could be described as a function of viral load. In other words, a trait with a continuous reaction norm (Gomulkiewicz et al., 2018).

Genetic accommodation

An adaptive (increasing fitness) shift in the plasticity of a trait, encompassing both increased and decreased plasticity (Ehrenreich & Pfennig, 2016).

Canalization

A subset of genetic accommodation, where a trait that was originally responsive to environmental cues (plastic) loses that plasticity and the trait becomes less variable (Ehrenreich & Pfennig, 2016).

Genetic assimilation

An extreme case of genetic accommodation, wherein all trait plasticity is lost and the trait is now constant across environments (Ehrenreich & Pfennig, 2016).

Genetic compensation

A subset of genetic accommodation, where increased plasticity is favoured. Can present as counter-gradient variation when populations from different environments are brought into common garden (Grether, 2005).

Priming

When previous exposure to particular environmental conditions provides protection against future conditions of a similar or more severe magnitude.

Frontloading

A specific gene expression reaction norm (Figure 2a,i) with higher baseline expression and reduced plasticity under new conditions. Usually used in association with more tolerant populations showing this pattern for genes involved in stress response (Figure 2a; Barshis et al., 2013). If advantageous, frontloading can represent genetic assimilation of expression levels for specific genes, assuming the ancestral state exhibited higher plasticity.

Dampening

A more general case in which the gene expression response appears muted (lower plasticity) under different conditions. Usually discussed in association with more tolerant populations showing this pattern for genes involved in stress response (Figure 2a,d,g) (Bay & Palumbi, 2015).

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heritable in species ranging from yeast (Brem & Kruglyak, 2005; Brem et al., 2002), *Drosophila* (Jin et al., 2001; Nourmohammad et al., 2017), corals (Davies, 2014; Meyer et al., 2011), butterflies (Kvist et al., 2013), turtles (Tedeschi et al., 2016), maize (Schadt et al., 2003), to human cell lines (Monks et al., 2004). This heritable variation in gene expression can serve as raw material for the evolution of reaction norms (Whitehead & Crawford, 2006). The framework we present does not require consideration of the evolution of the reaction norm, but additional insights are likely to arise from such considerations and are strongly encouraged.

4 | CAVEATS TO THE REACTION NORM FRAMEWORK

Many studies quantify gene expression across discrete time points or conditions, in a linear reaction norm context, which is the framework we have used here. However, it is important to acknowledge that gene expression may be better studied as a function-valued trait, where expression level depends on an independent factor (i.e., time, developmental stage, environmental condition, intensity of a stressor, etc), and the gene expression profile becomes a curve that

varies as a function of that factor. As a recognizable analogue, thermal performance is often considered a function-valued trait, with performance of a particular trait (such as time to righting in reptiles) described as a function of temperature. Considering gene expression as a function-valued trait can have several benefits, including higher statistical power to detect genes that contribute to particular phenotypes, and easier detection of genotype-by-environment (G × E) interactions that influence gene expression (Hodgins-Davis & Townsend, 2009). This is especially true in the analysis of expression quantitative trait loci (eQTLs), which are associated with variation in mean expression (G) or the magnitude of gene expression plasticity (G × E; e.g., Li et al., 2006). Re-considering gene expression data as a function-valued trait can also facilitate the identification of genes responsible for transitions between life stages (e.g., metamorphosis) or differentiation of different cell types, as was done across cell-phase stages in yeast (Leng & Müller, 2006). However, sampling in a way that fully characterizes gene expression as a function-valued trait is not trivial. One study that developed methods to analyse gene expression as a function-valued trait sampled *Arapidopsis thaliana* across 24 timepoints (Stegle et al., 2010). Even with decreasing sequencing costs, sampling at high frequencies is often not feasible depending on the organism, budget, question of interest, or due to the often-destructive sampling that occurs in order to obtain organismal RNA.

It is also important to acknowledge that not all population differences in gene expression are adaptive, and we encourage researchers to consider the additional factors that could be contributing to observed differences in gene expression, for example the effects of drift in small populations, linkage to other genes under selection, or the influence of other environmental conditions that are not measured (e.g., toxins). Here, we discuss gene expression plasticity in the context of a "tolerant" and a "sensitive" population, and, similar to many published studies, this leaves us with a sample size of one for these population comparisons. A recent study by Koch and Guillaume (2020) provides an excellent framework for how to address these concerns that involves measuring both fitness and gene expression at the level of the individual. Therefore, these authors were able to estimate selection pressures on gene expression across environments, and further explore the intensity of selection on plasticity in gene expression (Koch & Guillaume, 2020). Whether gene expression plasticity is adaptive or maladaptive can also be explored using experimental evolution at the population level, as was shown by Ghalambor et al. (2015), who studied gene expression divergence in guppies introduced to a low predation environment from an ancestral high predation environment. A small subset of genes showed gene expression patterns that matched a historically derived low predation population, but of these only a small subset (11) showed adaptive gene expression plasticity in the ancestral high predation population, with most others showing maladaptive plasticity. These results suggest that the direction of gene expression plasticity should be interpreted with caution when comparing contemporaneous groups as the effect of evolution over several generations may reveal that plasticity may actually be maladaptive.

5 | TIMING IS EVERYTHING

Above, we present a framework that illustrates gene expression reaction norms and how they may relate to stress tolerance across populations. However, gene expression is dynamic and can change drastically, even on the scale of hours (e.g., Andrew et al., 2015; Tarrant et al., 2019; Wuitchik et al., 2019; reviewed by López-Maury et al., 2008). Interpretation of transcriptomic patterns relies not only on understanding potential gene functions, but also on careful consideration of sampling timing. Given that sampling time points, organismal life history, additional stressors, feedback cycles, and epigenetic or transgenerational effects may all interact to influence expression patterns, careful experimental design is critical for interpreting gene expression plasticity.

5.1 | Transcriptomic resilience: what happens after the acute stress response

While the gene expression response during stress can be critical for survival of an organism, its ability to recover, or show resilience, can also be a strong indicator of overall tolerance. In the context of function-valued traits, one can consider gene expression as a function of time before, during, and after stress. In transcriptomics, the concept of resilience signifies a return to baseline (prestress) expression levels and indicates how patterns stabilize after the removal of a stressor (Figure 3). Borrowing from the field of ecosystem resilience (Grimm et al., 1996; Hodgson et al., 2015; Holling, 1973; Pimm, 1984), we can also consider the elasticity, or the rate at which this return to baseline occurs (Figure 3b). The influence of transcriptomic resilience on stress tolerance was first considered in seagrasses, where stress tolerant populations exhibited more elastic gene expression patterns (Franssen et al., 2011), a pattern that has also been observed in corals (Seneca & Palumbi, 2015; Box 1). Transcriptomic resilience may be beneficial when stress-induced changes in gene expression incur significant costs. For example, continued overexpression of heat shock proteins can divert energy away from metabolism, growth and reproduction (Tomanek, 2010). Thus, a rapid return to homeostasis (i.e., transcriptomic resilience) could limit this expense (De Nadal et al., 2011), while still providing the benefit of increased expression during stress.

It is unlikely that the entire transcriptome will show resilience. Proteins have varying half-lives in cells and gene expression dynamics should reflect the lifespan of proteins they encode (as an aside, there is often disagreement between mRNA and protein levels, but we direct the reader to Dar et al. (2016), Greenbaum et al. (2003), and Pascal et al. (2008) for in-depth considerations). Furthermore, some genes may be able to return to baseline conditions more quickly. For example, despite finding evidence for transcriptomic resilience, Seneca and Palumbi (2015) still found many genes to be differentially expressed in both tolerant and sensitive coral populations relative to unexposed controls at 20 h post-heat stress. While this study did not measure gene expression again at a later point to track

FIGURE 4 Timing and sampling of a stress response. (a) Hypothetical gene expression profile of a tolerant (pink) and sensitive (green) population that share identical amplitude and plasticity in the overall response to stress yet differ in the onset of the response. This could result in the sensitive population being considered more plastic if sampled during time points 2 and 5 (b) or less plastic if sampled during time points 1 and 3 (c)
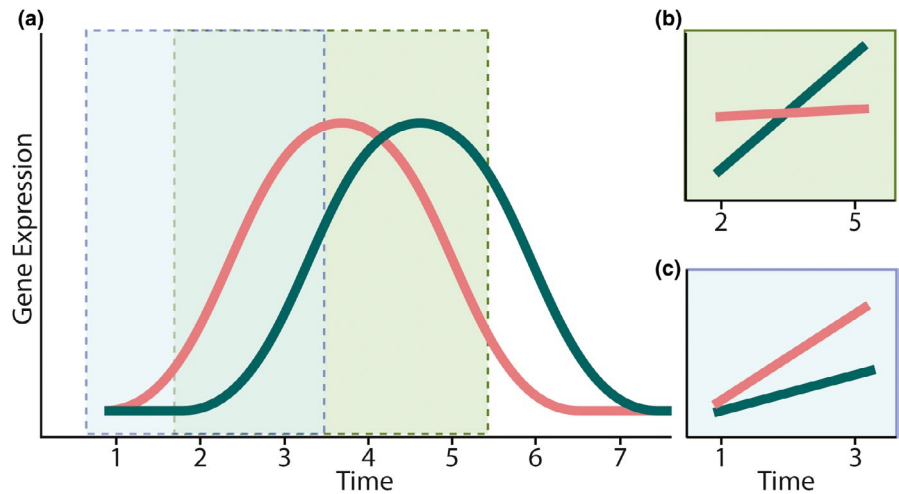
longer-term recovery, other studies found that certain genes can show lingering effects and remain differentially expressed months after stress events, long after physiological signs of stress subside (Pinzón et al., 2015; Thomas et al., 2019; Thomas & Palumbi, 2017).

While transcriptomic resilience can differ from acute patterns represented in Figure 2, they may overlap. For instance, transcriptional dampening (lower plasticity) can result from a combination of frontloading and/or transcriptomic resilience, depending on sampling timing (e.g., if there is high elasticity). The association between transcriptomic resilience and stress tolerance provides two mechanisms by which dampening (reduced plasticity) could confer tolerance: (i) via reduced expression during stress, and (ii) via higher elasticity. The benefits of frontloading may also be intertwined with transcriptomic resilience, as frontloading might confer tolerance to stress partially due to faster recovery of baseline levels (as expression levels do not have to shift as far to return to baseline). Indeed, some genes identified as frontloaded in corals also show transcriptomic resilience (Barshis et al., 2013; Seneca & Palumbi, 2015).

Transcriptional profiles can also be impacted by internal cellular stress response mechanisms, which further complicate our interpretation of expression patterns and their relationship with resilience. For example, in bacteria, microRNAs (miRNAs) and small interfering RNAs (siRNAs) such as IsR can delay activation of stress response proteins as well as facilitate their return to baseline, thereby preventing or postponing the build-up of stress proteins during short-term stress events, which could result in a signature of transcriptome resilience (Legewie et al., 2008). In eukaryotes, mRNAs that contain internal ribosome entry site sequences in their 5′ untranslated region can proceed to direct and rapid translation. This has been shown to occur for some proteins necessary under stress conditions (Holcik & Sonenberg, 2005). While the latter system would not directly impact initial production rates of mRNA (transcription), RNA-sequencing may not detect increased expression because these transcripts are more quickly translated into proteins (and therefore not measured by RNA capture).

These potentially confounding interactions highlight the benefit of considering gene expression and the resultant phenotype as a function-valued trait, where one can account for at least some

of the interactions between samples. One elegant example of this approach is Torres et al. (2016), which tracked gene expression of markers for various cell types along with parasite load, and red blood cell (RBC) counts in mice infected with *Plasmodium chabaudi* and analysed how these parameters co-varied through time. They were interested in parameters that had a hysteretic relationship, that is, where there is a time lag between some inducer (e.g., parasite load) and a product (RBCs). The authors found that mapping two parameters with a hysteretic relationship provided much higher predictive power on health outcome than looking at one parameter alone. For example, if mice had both low mature RBCs and low RBC precursors (measured via gene expression), they were likely to die from the infection; however, examining these parameters on their own provided little predictive power (Torres et al., 2016). In addition, the trajectory that individuals took across the two-parameter space predicted the ability of the organism to recover from the stress, with smaller loops (quicker return to baseline) predicting positive outcomes (Figure 3c). Mapping stress and recovery in a multidimensional parameter space could thus assist with identifying inflection points at which higher gene expression shifts from being beneficial to harmful. This approach goes beyond thinking only in terms of consequences of frontloading, dampening or transcriptomic resilience, which by themselves may not necessarily depict the entire picture of stress and recovery.

5.2 | Dynamics of gene expression; sampling versus response timing

Given the diversity of processes that can influence gene expression, timing of sampling must be carefully considered to be able to relate differences in gene expression with stress tolerance. Poor experimental design can result in confounding effects that can lead to erroneous conclusions. Consider, for example, a hypothetical gene expression profile for a tolerant (pink) and a sensitive (green) individual or population (Figure 4a). The magnitude and direction of the responses are identical, except that the tolerant population responds more quickly than the sensitive population. Several of the

reaction norms depicted in Figure 2 could be derived from these two curves depending on when sampling occurs (Figure 4b–c). It should be noted that such a lag can also occur in reverse (tolerant lagging behind sensitive), and this example serves simply as one hypothetical outcome of many possible scenarios to highlight the complexity of interpreting gene expression patterns when few time points along a continuum are explored.

Sampling at only two time points can make it difficult to determine whether differences between populations indicate variation in gene expression plasticity or simply a mismatch in response time. For example, the frontloading pattern described in Box 1 is characterized by higher baseline gene expression in tolerant populations coupled with reduced plasticity (Figure 2a). Sampling at the edges of the green shaded region in Figure 4a would yield a frontloading-like reaction norm in the tolerant population (Figure 4b), while sampling within the blue shaded region would point to the tolerant population exhibiting higher expression plasticity of the same genes (Figure 4c).

It is important to note that we are not discounting the possibility that a lag in response may be a real biological signal of tolerance or sensitivity, merely that it may influence the patterns and interpretations of data obtained from minimal timepoints. Statistical approaches can improve detection of when genes become differentially expressed and provide insight into biological significance that may underlie temporal differences (Stegle et al., 2010). These considerations underscore the care that researchers should take in interpreting gene expression patterns, and stress the benefits of adopting a function-valued approach that aims to maximize sampling across a continuum of conditions or timepoints. While increased sampling may become financially prohibitive depending on the study system and questions, there are certain statistical methods that can approximate such analyses even with limited sampling (e.g., Vanhatalo et al., 2019). However, these approaches have yet to be explored in the context of gene expression. In the interim, it is critical to have strong a priori hypotheses of patterns that may be expected from reaction norms of stress responsive genes in each study species.

Another alternative is to combine gene expression with multiple physiological measures to help overcome some of these limitations. For instance, Torres et al. (2016) identified sensitive mice by measuring the correlation between parameters that were modulated out of phase with each other (lagged) to create looping maps of gene expression and disease space (which can be substituted with other stressors). The direction of the response (i.e., moving towards recovery or towards death) could be inferred by associating gene expression at only a few time points with blood cell counts (Figure 3c). Such analyses can be facilitated by correlation network methods like WGCNA, which identify coregulated networks of genes showing similar expression patterns and then correlate these gene expression values across continuous or binary measures of interest (Langfelder & Horvath, 2008).

These considerations are especially important for the type of stress response and the kind of organism or life stage being studied. Gene expression studies of larval organisms would benefit from sampling individuals in as near synchrony as possible, since developmental changes in gene expression can change on the course of several hours and have the potential to confound observed patterns. Especially in the context of temperature, studies examining developing (e.g., larval or seedling) organisms should take additional care, as temperature is well-known to affect developmental rates across a variety of taxa, from plants (i.e., Atkinson & Porter, 1996; Jacott & Boden, 2020) to ectothermic animals (i.e., Gillooly et al., 2002; Jarošík et al., 2004). Additionally, organisms may mount different gene expression changes in response to acute versus chronic stress. For instance, a meta-analyses of gene expression studies in coral found distinct gene expression signatures when corals were sampled after an acute heat stress (several hours) compared to a chronic exposure (several days to weeks) (Dixon et al., 2020). In developing zebrafish, a gene expression response to domoic acid (a neurotoxin) can be detected at 3 days post fertilization, but not seven (Panlilio et al., 2020). Therefore, care should be taken to synchronize sampling relative to the stressor across individuals and treatments, especially when considering acute responses.

Given the decreasing costs of sequencing and methods for low-cost gene expression profiling (i.e., Tag-seq; Lohman et al., 2016; Meyer et al., 2011), we recommend that studies leveraging gene expression to understand stress tolerance should aim to increase replication across sampling time points and within individuals to the degree possible. Furthermore, we stress the importance of avoiding technical biases in addition to biological replication. If the experiment is conducted across multiple time points and has a large number of samples, it is important to randomize sample processing and manage sequencing runs in order to avoid batch effects (Conesa et al., 2016; Liu & Markatou, 2016), although statistical packages exist to deal with such issues (i.e., *limma*; Smyth, 2005).

In more complex organisms, gene expression patterns are often tissue-specific (Lonsdale et al., 2013), and tissue type should, therefore, be carefully considered during experimental design. Even with simple colonial animals that can be easily fragmented to generate genetically identical pieces (such as corals), distinct patterns of gene expression are found in the growing tips and branch bases of branching corals (Hemond et al., 2014) and similarly in growing segments of plants (e.g., Knauer et al., 2019; Tian et al., 2019). Clonal organisms, however, serve as a powerful way to constrain variance in gene expression across conditions by controlling for genotype thus facilitating robust G × E analyses.

5.3 | Developmental plasticity and priming: influence of life history and environment on gene expression

Outside the time frame of a particular study, an organism's environmental and/or life history can influence gene expression patterns in ways that could mislead interpretations. Early exposure to environmental conditions can trigger specific developmental programmes that then limit the response landscape of adults, which is often

called developmental plasticity (West-Eberhard, 2003). Phenotypic plasticity, which is generally considered a reversible process, can be distinguished from developmental plasticity, which is more likely to be irreversible (Novoplansky, 2002). In developmental plasticity, pre-adult developmental changes occur after integrating information from the environment (Beldade et al., 2011; Novoplansky, 2002). While some authors use phenotypic plasticity and developmental plasticity as synonyms (West-Eberhard, 2003), here we consider developmental plasticity to be a subtype of phenotypic plasticity. Developmental plasticity is important to consider because it is difficult to control in systems where studying F2 generations is unfeasible, potentially making it difficult to disentangle how past experiences modulate gene expression between populations within the framework we provide.

During development, phenotypic variation induced by the external environment (both biotic and abiotic factors such as temperature, nutrition, and presence of predators) can be discrete (e.g., a switch that produces alternative phenotypes) or continuous (Beldade et al., 2011). It is valuable to consider gene expression in the context of developmental plasticity, as any developmental change caused by an environmental cue can result from a change in gene expression (Beldade et al., 2011) and these changes can be misinterpreted as local adaptation. Additionally, hormones often play an important role in linking the environment to developmental plasticity (Nijhout, 2003). For example, in honeybees, juvenile hormone (JH) responsive genes (such as "growth genes" in developing queens) influence caste differentiation (Barchuk et al., 2007). An iconic example of developmental plasticity is the water flea, *Daphnia*, in which the presence of predators (as detected by concentrations of fish kairomone) triggers a helmeted phenotype (mediated by epigenetic changes) in juveniles, which is retained in the next generation of progeny (Harris et al., 2012). If one were to analyse gene expression patterns of progeny during early larval stages, larvae from parents exposed to predators might show upregulation of genes responsible for helmet formation (Miyakawa et al., 2010); however, this pattern would be unrelated to the stressor being tested and instead be influenced by the organism's prior history. These sorts of examples highlight the complexities of interpreting gene expression data and the importance of considering uncontrolled variables before drawing conclusions.

Exposure to a specific stressor may lead to hardening against that stressor (also called priming, or hormesis if the stressor is a toxin). Repeated exposure, as may occur for stressors that fluctuate on predictable timescales like light or temperature, can also trigger physiological changes that better equip an individual to respond under subsequent exposure. For instance, in the pea leaf miner exposure to a 4 h heat stress led to sustained elevated expression of HSP20 and HSP70 and increased thermal tolerance under subsequent heat stress (Huang et al., 2007). For the ornate tree lizard (*Urosaurus ornatus*), exposure to sublethal temperatures resulted in a significant increase in thermal tolerance 6 h after the initial challenge. Improvement in thermal tolerance also differed among populations from different altitudes, suggesting that the impact of hardening can vary by population and the

level of plasticity induced may itself have a genetic basis (Gilbert & Miles, 2019). Physiological measurements or gene expression sampling that fails to account for short-term effects could lead to a misinterpretation of thermal tolerance dynamics and its cellular and molecular underpinnings. The subject of thermal hardening has also been studied extensively in *Drosophila* (e.g., Kelty & Lee, 2001; Malmendal et al., 2006; Sejerkilde et al., 2003), other insects (reviewed by Teets & Denlinger, 2013), as well as plants (e.g., Beck et al., 2004; Johnson-Flanagan & Singh, 1987; Vierling & Nguyen, 1992).

Thermal trait variation in adults can be the result of additive genetic variance (i.e., inheritance of particular allele(s) that affects the phenotype), but also encompasses the cumulative impact of the thermal history experienced throughout their lifetime (e.g., through canalization or plasticity-induced effects). Temperature-associated traits and their plasticity can be influenced by developmental exposure (e.g., Bowler & Terblanche, 2008), especially in ectothermic organisms that rely on ambient temperatures to modulate growth and metabolic rates (Beitinger et al., 2000; Ward et al., 2010). For example, in sculpins, temperature drives size- and age-class distributions within and among populations across thermally divergent environments (Metaxas & Scheibling, 1993; Wuitchik et al., 2018). Additionally, early exposure to thermal variability can result in fixation of certain gene expression patterns in intertidal sculpins such that upon sampling later in life, "baseline" expression levels in fact reflect prior history (Todgham et al., 2005). This variation integrated within the reaction norm framework may be misinterpreted as local adaptation, even though trait data from earlier life stages is unknown. Therefore, for organisms that exhibit bipartite life histories, it may be valuable to study thermal trait variation across several representative life history stages to control for environmental history (Figure 5). Considering the influence of environmental history on thermal traits along with sampling across multiple life stages may paint a clearer picture of how an organism can respond to current and future environmental conditions.

While hardening and related phenomena have been well-studied in their own right, their influence on gene expression and physiological plasticity is often overlooked. These interactions between early environmental history and subsequent performance under stress need not be a barrier to experimentation; instead, considering or explicitly controlling these variables can generate powerful experimental designs that correlate gene expression patterns to phenotypic responses to stress. In fact, contrasting populations or individuals with different life or environmental histories may lead to the discovery of genes or networks involved in developmental plasticity or canalization (see Ehrenreich & Pfennig, 2016 for a full discussion).

While not the focus of this synthesis, it is also important to highlight that epigenetic modifications can influence gene expression within and across generations (Colicchio et al., 2015; Eirin-Lopez & Putnam, 2019; Leung et al., 2016; Putnam et al., 2016), and environmental cues can induce these modifications (reviewed in Feil & Fraga, 2012). In plants, cold winter temperatures facilitate
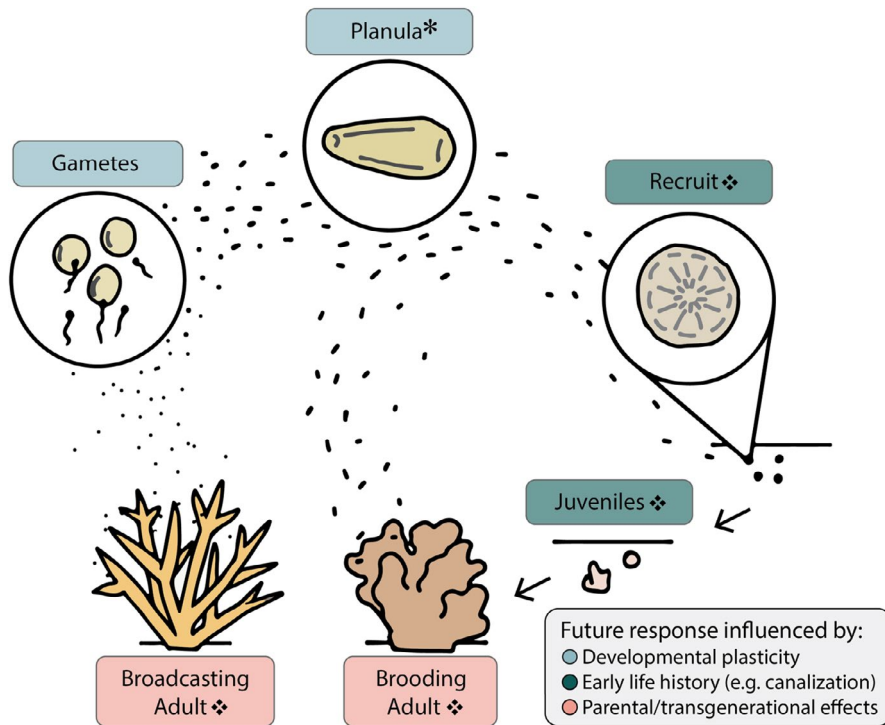
FIGURE 5 Influence of life and environmental history on future responses. For sessile organisms with bipartite life histories, such as corals, early life history and developmental plasticity are especially important processes as life stages before maturation are when the organism is motile and can be exposed to drastically different environments. When sampling adults, you are observing the cumulative effects of these prior life history stages in combination with the current adult environment. In addition, transgenerational impacts transmitted by parents can contribute to gene expression signatures of their progeny. * Algal symbionts in brooders only. ❖ Algal symbionts in both brooders and broadcasters

vernalization, the ability to flower in more temperate climates (Feil & Fraga, 2012; Kim et al., 2009). Additionally, temperature changes in *Arabidopsis thaliana* mediate histone variant enrichment and generate gene expression patterns that correlate with temperatures experienced (Kumar & Wigge, 2010). Revisiting an earlier example, DNA methylation in honeybees has also been linked to caste differences in gene expression (Elango et al., 2009). Transgenerational plasticity in the context of climate change can also impact multiple traits linked to population persistence (Shama et al., 2016; Shama & Wegner, 2014). However, epigenetic plasticity may be more likely to govern tolerance over longer timescales (within an organism's lifetime and between generations) when compared to gene expression dynamics (hours to days) under stress (Strader et al., 2020). Overall, the role of epigenetics and acclimatization in driving responses to climate change remains a budding area of research and offers many exciting possibilities for future study (Burggren, 2015; Eirin-Lopez & Putnam, 2019).

6 | CONCLUSIONS

In the fast-paced era of genomics, it is now highly feasible to use gene expression data to better understand organismal performance and generate new insights into cellular and molecular stress response mechanisms across taxa. In this synthesis, we provide a framework for interpreting gene expression patterns, and discuss many of the nuances in linking gene expression to important phenotypes such as stress tolerance. While transcriptomic studies can offer real-time insights into plastic organismal responses, we encourage researchers to carefully consider alternative mechanisms that could influence

gene expression patterns prior to, during, and beyond the scope of their experiments. This includes, but is not limited to: onset of the stress response, life history, environmental history, and microbial influences. Given the inherently dynamic nature of gene expression, sampling regimes should aim to span the time frame of organismal responses to the condition of interest in order to more carefully relate gene expression changes to plastic phenotypic outcomes. Gaining a more comprehensive understanding of how gene expression plasticity can influence stress tolerance will also elucidate its role in facilitating (or hindering) species persistence in light of rapid environmental change. The framework provided here can aid interpretation of gene expression patterns and improve experimental design by limiting confounding variables in future transcriptomic studies.

AUTHOR CONTRIBUTIONS

S.W. Davies conceived the original concept. All authors contributed to research, writing, and development of figures. H.E. Rivera and H.E. Aichelman oversaw all writing from conception to publication.

DATA AVAILABILITY STATEMENT

There are no data associated with this manuscript.

ORCID

Hanny E. Rivera https://orcid.org/0000-0003-4747-1339

Hannah E. Aichelman https://orcid.org/0000-0001-6999-5518

James E. Fifer https://orcid.org/0000-0002-4356-9024

Nicola G. Kriefall https://orcid.org/0000-0002-1930-3998

Daniel M. Wuitchik https://orcid.org/0000-0002-4090-6400

Sara J. S. Wuitchik https://orcid.org/0000-0002-1913-0396

Sarah W. Davies https://orcid.org/0000-0002-1620-2278

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.