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Pleiotropy, plasticity, and the evolution of plant abiotic stress tolerance

David L. Des Marais and Thomas E. Juenger

Section of Integrative Biology, University of Texas at Austin, Austin, Texas

Address for correspondence: Thomas E. Juenger, Section of Integrative Biology, University of Texas at Austin, 1 University Station, C0930, Austin, TX 78712. tjuenger@austin.utexas.edu

Progress in understanding the mechanisms of adaptive plant abiotic stress response has historically come from two separate fields. Molecular biologists employ mutagenic screens, experimental manipulations, and controlled stress treatment to identify genes that, when perturbed, have fairly large effects on phenotype. By contrast, quantitative and evolutionary geneticists generally study naturally occurring variants to inform multigenic models of trait architecture in an effort to predict, for example, the evolutionary response to selection. We discuss five emerging themes from the molecular study of osmotic stress response: the multigenic nature of adaptive response, the modular organization of response to specific cues, the pleiotropic effects of key signaling proteins, the integration of many environmental signals, and the abundant cross-talk between signaling pathways. We argue that these concepts can be incorporated into existing models of trait evolution and provide examples of what may constitute the molecular basis of plasticity and evolvability of abiotic stress response. We conclude by considering future directions in the study of the functional molecular evolution of abiotic stress response that may facilitate new discoveries in molecular biology, evolutionary studies, and plant breeding.

Keywords: abiotic stress; *Arabidopsis*; constraint; drought; physiology; pleiotropy; tolerance

Introduction

Plants live in an ever-changing and often unpredictable environment. As sessile organisms, plants must cope with whatever perturbations afflict their particular microhabitat. In the most general sense, we can think of plants as employing three strategies to cope with habitat heterogeneity. First, plants can evolve a specialist strategy to maximize fitness under a narrow range of environmental conditions. Depending on the extent of such evolutionary change and its fitness impact, specialization might lead either to endemic species or to locally adapted populations. Second, a generalist species can maximize fitness by producing a "general purpose" phenotype that survives in several environments. At a local scale in a heterogeneous environment, a generalist would potentially experience lower fitness than a specialist taxon. On the other hand, generalists may perform better across a range of environments, buffer populations across environmental extremes, and possibly aid in dispersal to novel environments. A third possible strategy is an adaptive plastic response wherein a genotype produces the appropriate phenotype for a particular environmental condition. This strategy might include plastic growth and development leading to environment-specific altered stature, branching, or specific functional traits related to the specific environmental perturbation (e.g., stomatal closing in response to drought).

A number of organismal and environmental features may influence the origination or stability of these three strategies. The temporal frequency and spatial arrangement of "stressful" environments across the geographical range of a species will create the opportunity for selection that can drive adaptive evolution and the occurrence of specialists versus generalists.[1,2] The evolution of a phenotypically plastic response strategy will depend strongly on any cost of that strategy which might arise from

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misperception of the cue or the deleterious effects of genetic machinery needed for such a strategy.[3,4] Finally, the scale of environmental heterogeneity—expressed as the distance between locally adapting populations—will shape the role of gene flow that may counteract the effects of local adaptation in response to natural selection.

To what extent is adaptation to diverse abiotic stresses limited by genetics, including the degree and architecture of genetic variability or functional trade-offs in traits? To what extent is adaptation limited by contrasting selection pressures across heterogeneous or stressful environments? How important is the spatial or temporal pattern of environmental variability in determining the evolutionary outcomes (e.g., the evolution of generalist, specialist, or plastic strategies)? Many of these questions are broad and encompass analysis at several levels of organization—from the level of genomes to genetic variability within and among populations, and across ecological and evolutionary timescales. Not surprisingly, much of the progress in answering these questions has come from conceptual and theoretical models of evolution. Molecular biologists have also tackled the field of abiotic stress tolerance but from a reductionist perspective. These studies have discovered a vast diversity of mechanisms that relate to plant performance in particular environmental contexts. To what extent can these disparate endeavors inform each other? How might evolutionary theory help to interpret and synthesize the molecular work? Similarly, how can insights from molecular biology lead to more realistically parameterized models of trait evolution?

Our aim in this article is to review the current state of our understanding of the genetic basis of plant physiological response to abiotic stresses—particularly those dealing with osmotic relations—and to consider the evolutionary mechanisms that shape the ongoing evolution of such traits. In particular, we will argue that many of the emerging themes from the molecular literature are readily understood in the conceptual framework of environment-dependent phenotypic plasticity. In this context, we will discuss the evolutionary processes that might impact adaptive physiological response to abiotic stress. We conclude by considering the empirical priorities for the field of functional evolutionary genetics in light of recent developments in molecular biology and quantitative genetics.

Arabidopsis as a tool for evolutionary studies

Over the past decade, many insights in plant biology have come from detailed studies of the model angiosperm, *Arabidopsis thaliana* (Brassicaceae). *Arabidopsis* is a weedy, predominantly selfing, annual whose natural distribution in Eurasia encompasses a wide range of habitats from subtropical to subarctic climates, and from sea level to over 2,000 m. The abundant natural nucleotide variation found in *Arabidopsis*[5] has been exploited by geneticists toward two general ends. First, workers interested in the functional characterization of genes can use natural accessions as a large mutant pool to determine the function of uncharacterized genes through direct assays on functional variants or through QTL and association mapping approaches. Like any functional characterization of a gene, QTL mapping can be a time-consuming process, but some success stories have been reported (reviewed in Refs. 6–9). Second, the study of natural variation itself, its distributions, origins and maintenance as well as the effects of selection on the origin of adaptations, has received considerable attention (reviewed in Refs. 6–8,10). Because of the excellent coverage from other recent reviews, we will only highlight a few emerging themes regarding functional genetic variation in *Arabidopsis*. For example, QTL studies frequently detect many loci contributing to functional differences between parental lines, suggesting that standard quantitative genetic models may be reasonably employed in evolutionary studies in *Arabidopsis*. These QTL studies have uncovered considerable transgressive segregation, usually as a result of a mixture of alleles of opposing phenotypic effects in the parental accessions. There is also some evidence of the important role played by epistatic (nonadditive) interactions, especially in flowering time and fitness related traits (e.g., Refs. 11–17). Finally, QTL with broadly pleiotropic effects have been uncovered in a number of studies (see van Zanten *et al.*[18] as an example).

Plant-abiotic interactions

Plants are constantly challenged by their abiotic environment. Even at a small geographic scale, considerable variation can be found in water availability, temperature and soil characteristics such as salinity, micronutrient content, and toxic metal abundance.

Of these, osmotic stress is likely the environmental factor that most strongly limits the natural distribution of plant species.[19–21] From an agronomic standpoint, osmotic stress commonly reduces crop yields to less than half of potential yields.[22,23] Osmotic stress encompasses stress imposed by water deficit, excess salt, and mild cold, which are often studied collectively because they impose similar challenge to plant cells. Water is essential for metabolism, transport and, for herbaceous plants like *Arabidopsis*, for generating the turgor that allows an upright habit. Plants rely on an open transport system to move water and solutes between tissues, and also to provide ready access to atmospheric CO_2 for photosynthesis. However, the open pores necessary for such adaptations causes plants to lose most of the water that they transport from the soil. Variation in water availability is caused not only by geographic variation in rainfall and temperature but also by subtle differences in soil characteristics, which result in variation in the water potential of the substrate that roots attempt to exploit. Salt stress is closely tied to soil water potential because salt concentration at the root surface increases with decreasing water and the resulting gradient in solute concentration makes it more difficult for roots to extract water passively from the soil. At the cellular level, water deficit inherently leads to increase in solute concentration and, hence, to the possibly damaging effects of high salt concentration. Cold imposes a similar set of stresses on plants, beyond its effect on the rate of cellular metabolism. Moderate cold temperatures decrease cell membrane fluidity and thereby interfere with diffusion of water and solutes into and out of cells. Extremely cold temperatures can result in direct damage to cells and cell compartments through decreases in membrane integrity.

Plant physiologists recognize three general strategies for dealing with osmotic stress.[24] First, plants may adjust their rates of water uptake and loss in response to environmental water deficit under a strategy of dehydration avoidance. This is often achieved through physiological regulation of root function or rate of water loss through transpiration. Dehydration avoidance is a conservative strategy whereby plants "save" water in the soil and maintain good internal water status but at the cost of current photosynthesis and growth. Second, plants may display dehydration tolerance, which allows them to survive periods of internal water deficit in a dry environment. Many plants have evolved spectacular strategies to withstand and recover from extended periods of internal water deficit (e.g., so-called resurrection plants such as *Anastatica hierochuntica* and several species in the genus *Selagninella*) and, while *Arabidopsis* is not strictly dehydration tolerant in this sense, it does employ many strategies that reduce cellular damage from short term dehydration stress. Third, plants can escape stress entirely by accelerating their progression through the life cycle and flowering either before stress is anticipated to arrive, or by flowering in direct response to stress. Many arid-land grain crops have been improved through breeding programs that result in crop escape from seasonal drought.[25]

Dehydration avoidance

For many plants, dehydration avoidance is achieved largely by stomatal regulation,[26,27] which minimizes water loss and can be a rapid and effective strategy but may result in reduced CO_2 uptake and growth.[28,29] This trade-off is a fundamental constraint on land-plant form and physiology. The efficiency with which plants fix CO_2 relative to their rate of water loss is called water-use efficiency (WUE) and when high, WUE can mitigate the trade-off between CO_2 uptake and water loss.

Variation in WUE among *Arabidopsis* accessions has been reported in several studies.[30–32] In evolutionary and ecological studies WUE is most often measured as the ratio of ^{13}C to ^{12}C in leaf tissue as compared to a universal standard. This ratio has been reported to range from −22% to −34% for terrestrial C3 plant species across many different plant sizes, habits and geographical distributions.[33] Carbon isotope ratios from *Arabidopsis* accessions grown in a common garden cover a significant portion of this range, spanning −26.7% to −32.4% (Fig. 1; A. Kenney unpublished results). Clear geographic patterns in this variation have not been identified, beyond the expected finding that some accessions from relatively wet locales have lower WUE than accessions originating in drier habitats.[34] Broad surveys of accessions have also reported a strong positive correlation between the drought avoidance trait WUE and the drought escape trait flowering time.[30] Genetic studies in three mapping populations, Landserg *erecta* × Columbia,[35] L*er* × Cape Verdi Islands,[32,35] and Tsushima × Kashmir[34] have identified multiple genetic loci affecting

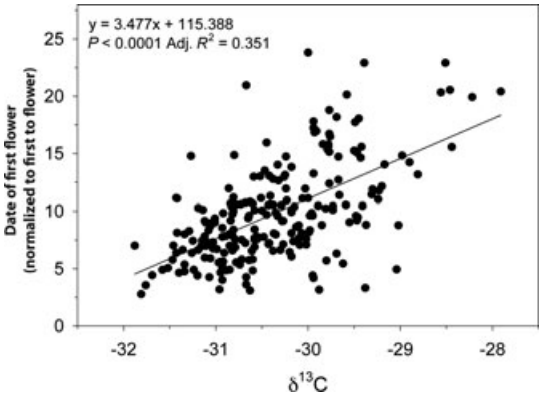

Figure 1. **The genetic correlation between date of first flowering and WUE (measured using carbon isotope discrimination) among a panel of spring annual accessions of *Arabidopsis*. Each point represents the genotypic value for a particular accession in well-watered conditions.**

variation in WUE. Juenger *et al.*[32] identified five genomic regions affecting variation in WUE, and two of these appear to be tightly linked to regions controlling variation in flowering time. Moreover, these QTL harbor alleles of differing effect, such that progeny show more extreme WUE phenotypes than either of the parents.

Dehydration tolerance

Mechanisms for dehydration tolerance can also be quite diverse and include accumulation of osmolytes and changes in metabolism. By one simple measure—average reduction in total leaf area despite greater than average loss of relative water content under soil water deficit—some accessions of *Arabidopsis* show evidence of dehydration tolerance.[36] Natural variation in vegetative salt stress was reported by Quesada *et al.*[37] for a small RIL population descended from Col and L*er* parents. Both parents contained alleles that increase and decrease salt tolerance, demonstrating the complex genetic basis of this trait and the possibility of considerable genetic variation between populations in the salt tolerance of vegetative tissue.

Drought escape

While the functional basis of flowering in direct response to drought stress is largely unknown, many studies have documented the genetic variation of flowering in response to other environmental cues.[38–40] A correlation between genetically determined flowering time and latitudinal variation in day length (which can serve as a biological proxy for temperature) was long predicted and first demonstrated in *Arabidopsis* by Stinchcombe *et al.*[39] It has also been postulated that climates with summer droughts might select for earlier flowering time and, while this has not yet been shown in *Arabidopsis*, a recent study in *Brassica* demonstrated a direct role for early summer drought on flowering time.[41] Much of the variation in flowering time between Arabidopsis accessions can be explained by segregating null alleles at the FRIGIDA and FLOWERING LOCUS C loci (see below). Natural variation in genes involved in light detection and regulation of FRI and FLC also contribute to functional differences in water use and patterns of whole-plant gas exchange, emphasizing the complex genetic nature of this trait.[30]

Molecular mechanisms of osmotic stress response

The three general strategies plants employ to prevent damage from osmotic stress should not be thought of as either on or off at a moment in time: plants likely maximize their fitness when they express a phenotypic response scaled to the type and severity of osmotic stress they encounter. Elucidating the mechanisms, genes, and pathways that sense and control these responses has been of great interest to molecular biologists interested in improving crop yield in marginal or unpredictable environments[42] and also due to the attractiveness of osmotic stress response mechanisms as model systems for understanding intracellular signaling.[43] In this section, we describe several emerging themes from the integrated molecular study of abiotic stress response in *Arabidopsis*. For a more complete treatment of these themes please consult several excellent recent reviews (e.g., Refs. 42–55).

Multigenic nature of abiotic stress response

Drought and cold stress affect the expression of hundreds if not thousands of genes in *Arabidopsis*.[52,56–58] Some of these genes are engaged in mechanisms to prevent direct water loss to the atmosphere through open stomates. Many more dehydration-responsive genes signal and code cellular processes involved in dehydration tolerance. While most attention has focused on genes that are upregulated during stress, many genes are downregulated as well. Stress alters many aspects of plant physiology, including

the slowing or cessation of growth, and so these downregulated genes may reflect changes in normal metabolism.[57] One complication in the interpretation of large lists of stress-responsive genes is identifying which genes reflect an adaptive response to the stress and which represent "unintended" transcriptional changes that may be involved in senescence or malfunctioning regulation.

Genes involved in dehydration tolerance are part of a large and interconnected signaling network stimulated by cold, water deficit and high salinity conditions. Specificity in the response to these diverse signals seems to occur largely at the level of transcriptional and posttranslational modification. Early work on drought-responsive genes in *Arabidopsis* identified a consensus DNA regulatory element that is activated during dry conditions.[59] These Dehydration Responsive Elements (DRE)—which are highly similar to elements called C-Repeat (CRT) found associated with cold-responsive genes[60]—are found in the proximal promoters of 531 *Arabidopsis* genes[61] (searching with a less conservative motif identifies DRE core motifs in 5,547 genes[62]). A second important component of dehydration-tolerance signaling is the Abscisic acid (ABA)-Responsive Element (ABRE[63]) promoter motifs. ABREs are widely distributed throughout the genome and associated with genes coding for diverse functions (the core motif is found in the promoter regions of 1,256 genes). In addition to activating genes involved in abiotic stress response, ABREs are found in the promoters of genes known to be involved in response to pathogen stress,[64] suggesting a possible point of cross-talk between abiotic and biotic stress signaling networks.

The target genes containing DREs and ABREs include signaling intermediates as well as genes that likely directly confer dehydration tolerance; for simplicity, we will refer to these latter genes collectively as effector genes. Considerable physiological work in multiple plant systems has shown that osmotic stress induces accumulation of sugars (especially sucrose, raffinose, and trehalose), sugar alcohols (such as mannitol), amino acids (particularly proline), and amines (reviewed in Ref. 45). While genes involved in synthesis of these compounds can be considered candidate effector genes, it is important to note that in very few cases has a functional link been made between osmotic stress-induced upregulation of a gene product with an increase in a particular solute and a concomitant increase in dehydration tolerance (but see Refs. 65,66). An additional class of putative effector genes are those coding for proteins that directly stabilize cellular components or degrade components damaged by osmotic stress such as the late embryogenesis abundant proteins (LEA[67]), heat shock proteins (HSP[68]), and various proteinase inhibitors. The overwhelming majority of genes characterized to date as effector genes are simply those found to be upregulated during experimentally induced cold, water and salt stress, or those whose regulation is dramatically affected by knockout or overexpression lines of key signaling proteins. If the genes identified in such assays are reflective of the number and kinds of genes involved expressed in nature, then we may conclude that the adaptive response to osmotic stress involves a large fraction of the *Arabidopsis* genome.

Abiotic stress response is modular

Particular abiotic stresses, or levels of stress, induce specific responses. The molecular processes that effect such specific responses are often described as stress "modules." The complex expression patterns of DRE/CRT-containing genes are driven by a small family of transcription factors that, while fairly conserved in their protein function, seem to confer considerable diversity of action through their own unique patterns of transcriptional activity. In screens for proteins that interact with the DRE/CRT DNA motifs, Liu *et al.*[69] and Stockinger *et al.*[70] isolated and characterized the DREB1 (DRE-Binding Protein) and CBF (CRT-Binding Factor) genes, respectively; further analysis revealed that the two groups identified the same genes. CBF1/DREB1b, CBF2/DREB1c, and CBF3/DREB1a are arranged in tandem and act as transcriptional regulators of DRE/CRT-containing genes. Transcripts from all three of these genes accumulate within 15 min of exposure to 4° C[71] and their transcriptional targets collectively constitute the "CBF Regulon."[46] Additional DRE/CRT-activating transcription factors have been identified under drought and/or high salinity conditions and named DREB1d/CBF4, DREB2, DREB1e/DDF2 and DREB1f/DDF1 (reviewed by Ref. 47). The DNA-binding domains of the DREB/CBF/DDF proteins are highly similar and, not surprisingly, all of these genes appear to interact with the same DRE/CTR motifs. However,

not all genes containing DRE/CRT are direct targets of all DREB/CBF transcription factors. The precise determinants of DRE/CRT binding specificity remain unclear, but functional specificity may arise from the sequences surrounding the conserved DRE/CRT element, which might recruit particular family members. Alternatively, distinct *cis*-elements in a single promoter region may recruit multiple transcription factors to induce full transcriptional activity.[54] The DREB-family of transcription factors has as many as 56 members, the majority of which remain uncharacterized.[72]

ABA—a phytohormone commonly known for its role in inhibiting seed germination—is also a key player in abiotic stress signaling. ABA is generally believed not to be involved in cold-stress response[69] and, accordingly, most evidence suggests that expression of CBF1,2,3/DREB1a,b,c are unresponsive to ABA. The DREB2 proteins, whose transcription respond most strongly to drought and salinity, also appear to act via ABA-independent pathways (Refs. 69,73; and see Fig. 2). However, the dehydration-responsive CBF4/DREB1d gene is responsive to ABA-treatment and therefore represents an ABA-dependent pathway for activation of DRE/CBF-containing genes.[74]

ABREs are bound by the AREB/ABF (Abscisic acid Response Element Binding factors) family of transcription factors,[75–78] which are expressed in aerial organs and roots in response to drought and

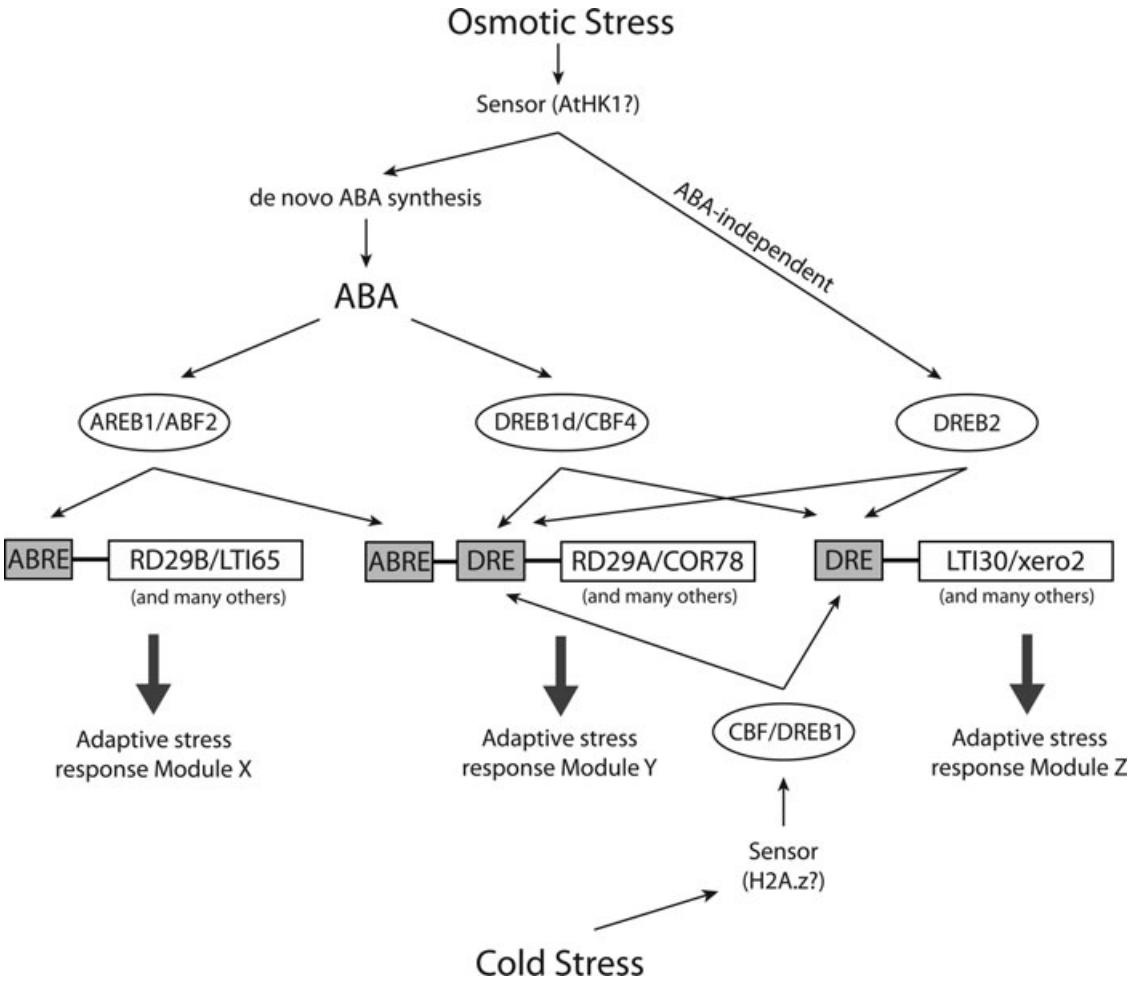

Figure 2. **Modular nature of AREB and DREB signaling in response to osmotic and cold stress. Grey boxes represent promoter motifs, white boxes are genes, and circles are proteins. There are many additional pathways not shown here, and arrows indicate flow of information and do not necessarily represent a functionally validated direct interaction.**

high salinity conditions but, notably, are generally not transcribed in seeds.[76,78] While little is known about in vivo functions of most of the AREB/ABF proteins (beyond their roles as transcriptional regulators), some patterns have emerged from expression studies, from transgenic lines overexpressing AREB/ABFs, and from the few functional knockouts that are available. Like the DREB/CBF transcription factors, the AREB/ABFs seem to be under differential transcriptional control and may participate in diverse signaling networks.[76,78] AREB1 expression is highly responsive to ABA treatment in all vegetative tissues and also responds to glucose treatment whereas AREB2/ABF3 has more spatially restricted expression and its mutants have no glucose-related phenotype.[77] All studied AREB proteins require phosphorylation for full activity.[79] Lines overexpressing AREB/ABF genes generally exhibit enhanced tolerance to drought and high salinity conditions whereas lines with multiple AREB/ABF genes knocked-out seem to be hypersensitive to drought and high salinity.[75–77,79] These phenotypes are believed to be the result of AREB/ABF-mediated transcriptional activation of ABRE-containing target genes including several late embryogenesis abundant (LEA) genes as well as regulatory genes.[75] In addition, there may be a direct correlation between the number of ABREs in a gene's promoter and its level of inducibility by AREB/ABF proteins.[75] Differential inducibility of the AREB/ABF proteins may therefore act in a combinatorial manner with complex promoter structures to permit considerable specificity of cellular response under various stresses.

The complex interaction between signaling pathways is often manifested at the level of *cis*-regulatory control of effector genes, and cellular decisions about which module(s) to activate may be *cis*-regulatory in nature (Fig. 2). (For an excellent review of the structure, function and evolution of *cis*-regulatory motifs, see. Ref. 80) As discussed earlier, drought-stress signaling in *Arabidopsis* is achieved through both ABA-dependent and ABA-independent pathways. The existence of these two separate pathways (and these are only two of many others currently known) may reflect the action of distinct genetic components during sensing, signaling and cellular response. The integration of multiple pathways may also facilitate a greater diversity of responses, or perhaps the action of parallel pathways may serve to amplify a single environmental signal. Genetic work in multiple plant systems has shown that a single ABRE is insufficient to induce gene expression in response to ABA treatment. ABRE-induced gene expression requires an additional "coupling" DNA element, which may take the form of a second ABRE or one of several related elements. RD29a/COR78 is an LEA-class gene of unknown function that is strongly upregulated by drought, cold, salt and ABA treatment.[59] The proximal promoter of RD29a has one ABRE and two DREs, which interact synergistically: in reporter gene assays with dissected RD29a promoter constructs, the single ABRE is insufficient to drive expression during ABA treatment, but its pairing with one or more the DREs confers high expression.[81] In contrast, a promoter construct containing just the two DREs was sufficient to induce transcription under salt, cold and drought stress. Moreover, coincident binding of both AREB (via the ABRE) and DREB (via the DREs) proteins to the wild-type RD29a promoter confers higher expression than binding of a single class of transcription factor.[81] It seems likely, therefore, that expression of genes like RD29a can be controlled at a low level by the ABA-independent stress signaling pathway, and also at a higher level through integration of both ABA-dependent and ABA-independent pathways. Taken together, a staggering number of distinct response modules may be realized through the combination of these DREBs, AREBs, and additional transcription factors in combination with their corresponding DNA motifs in the promoters of target genes.

Pleiotropy of key signaling proteins

As just discussed, the stimulus-specific response of individual modules depends on their activation by key regulatory and signal integration proteins. Because the function of such regulators influences the expression and activity of many downstream genes we might expect that mutations in central regulators exhibit considerable pleiotropy. This expectation has not been fully borne out by empirical studies. However, we currently have a very incomplete picture of the regulatory hierarchy of abiotic stress signaling and much of our understanding of such signaling comes from experiments involving small subnetworks; accurate estimation of the pleiotropic effects of key signalers is still several years away.

PHOT1 is a light sensor with a particularly complex role in *Arabidopsis* plant-water relations. Expressed in above-ground tissues, PHOT1 positively regulates stomatal opening in response to blue light,[82] influences chloroplast movements, and plays an important role in stem phototropism. PHOT1 is also expressed in roots where it seems to aid in negative phototropism, causing roots to travel further in the soil per unit root growth as compared to PHOT1-deficient plants.[83] Knockout mutations in PHOT1 also lead to lower biomass accumulation under dry conditions and lower WUE,[83] suggesting the many traits potentially affected by mutations in this key environmental sensing protein.

Mitogen-activated protein kinases (MAPKs) are upstream regulators of many aspects of plant cell signaling. MAPK cascades typically involve three components: MAPK kinase kinases (MPKKKs), which phosphorylate MAPK kinases (MPKKs), which phosphorylate MAPKs, which phosphorylate diverse proteins. The *Arabidopsis* genome contains 20 MAPKs, 10 MPKKs, and more than 60 MPKKKs,[84] which are deployed in seemingly limitless combinations to effect a diversity of signaling cues during development and stress response. MPK4 and MPK6 have received considerable attention for their role in abiotic stress signaling.[85] Posttranslational activation of these two kinases is stimulated by cold, low humidity, salt, wounding, reactive oxygen species, and touch.[86,87] Loss of function of MPK6 leads to a host of deleterious phenotypes throughout the plant, including improper spacing of stomates during leaf development, hypersensitivity to ozone (likely due to elevated intracellular reactive oxygen species, ROS) and reduced resistance to some fungal pathogens.[88–90] Given the large number of phenotypes potentially affected by MPK6, it seems likely that any particular response-specific activity may be determined through cell context dependency, or by its activation by upstream partners in MAP kinase signaling.[91]

Interpreting the pleiotropic effects of genes at downstream regulatory nodes is also challenging. As discussed above, the AREB and DREB-family proteins are believed to transactivate hundreds (potentially thousands) of genes in response to cold and drought stress. We might expect, then, that loss of function mutants in these transcription factors incur considerable costs. To the contrary, individual knockout mutations of AREB1, ABF3, DREBs and SnRK2s (kinases involved in AREB1 activation) show surprisingly small phenotypic effects beyond changes in expression of downstream genes.[75,77,92] Stronger phenotypes, including ABA insensitivity, hypersensitivity to drought stress, and overgrown rosettes, were found in a transgenic line in which AREB1 and related genes were knocked down using a broad-spectrum repression domain.[75] A line lacking functional copies of three SnRK2-family proteins also shows severely reduced survival following drought stress and ABA insensitivity.[92] The DRIP proteins negatively regulate DREB2a and while single knockouts of either DRIP proteins show modest effects, mutants carrying T-DNA insertions in both DRIP proteins show substantial upregulation of DREB2a-regulated proteins and much higher survival following dehydration stress.[93] The striking phenotypic difference between lines with single genes knocked out as compared to those lacking multiple genes suggests the existence of functional redundancy in the genome; an alternative prospect is that the single gene mutants may confer deficiencies in unsampled phenotypes. Regardless, clear difficulties remain in estimating the extent of pleiotropy of a gene from studies of severe structural mutations or from synthetic manipulation of transcription.

Appropriate stress response involves integrating multiple environmental cues

Tissue damage from osmotic stress is often unavoidable; in such cases a plant's fitness may be optimized by setting seed before environmental conditions become severe. We might imagine two general strategies to cope with drought-induced mortality. First, if the onset of drought is seasonal and thereby reasonably predictable plants can use environmental cues to trigger changes in life history to ensure they flower and set seed before seasonal drying. Second, if the onset of stress is gradual plants can rapidly accelerate the transition to flowering using cues that faithfully predict the coming stress. While this second strategy is commonly observed in the laboratory and field, the molecular basis of flowering in direct response to stress is currently unknown in *Arabidopsis*. However, the recent discovery of temperature-sensitive histones which affect transcription of flowering-time genes may suggest one mechanism for flowering in direct response to environmental stress.[94]

Arabidopsis accessions display two very different life history strategies. So-called winter annual

accessions germinate in the fall and overwinter as vegetative rosettes. These accessions require a prolonged cold treatment followed by lengthening days to stimulate the transition to flowering. Spring/summer annuals—including popular lab strains such as Columbia, Landsberg *erecta*, and Wassilewskija—germinate in the spring and do not require cold treatment to induce flowering; under long days these accessions generally flower within weeks of germination. Because the end of the growing season at a particular locale (due to seasonal drought or onset of winter) occurs at an approximately constant time of year, *Arabidopsis* uses winter cold (in the case of vernalization-dependent winter annuals) and change in daylength to set a flowering schedule. The molecular genetic basis of the transition from a vegetative to floral meristem is one of the best understood aspects of plant biology. Many excellent recent reviews on the subject are available,[48,49,53,95] so we will only briefly consider the mechanisms underlying flowering time.

Flowering time in *Arabidopsis* requires integration of at least three factors: temperature, light and the internal circadian clock (Fig. 3). The circadian clock determines transcript abundance of CONSTANS (CO), which peaks late in the daytime under long day conditions.[96] CO protein activity is, in turn, a function of environmental light.[97] The photoreceptors Phytochrome A (PhyA) and Cryptochrome 2 (CRY2) directly sense far red and blue light, respectively, and respond by stabilizing CO protein. CO protein then promotes expression of Flowering Locus T (FT). (Interestingly, CO stability can be compromised by PhyB, which responds to red light. Different qualities of light can therefore play opposing roles in promoting flowering.) FT is a "florigen" produced in leaves and transported to the growing meristem where it stimulates transition from vegetative to floral meristem growth.[49] Thus, the co-occurrence of circadian-determined CO transcript abundance late in the day with the perception of late-day light positively signals for flowering.

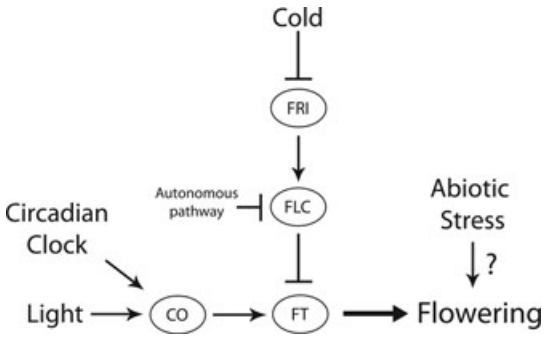

Figure 3. Simplified topology of the *Arabidopsis* flowering time pathway. FRI, Frigida; FLC, Flowering locus C; CO, Constans; FT, Flowering locus T. Arrows indicate positive interaction and T's indicate suppression; these represent flow of information and may not reflect direct interaction.

FT also integrates a second important environmental cue, temperature. In the absence of a cold signal FT transcription, and thus flowering, is inhibited by the Flowering Locus C (FLC) protein.[98] Repression of FLC expression by the autonomous pathway genes leads to early flowering.[99] However, the negative influence of autonomous proteins on FLC expression is overridden by activity of the FRIGIDA (FRI) protein which positively regulates FLC.[38] So the presence of functional FRI and FLC genes inhibits flowering by repressing the florigen FT. Not surprisingly, many accessions that do not require vernalization treatment (e.g., the spring annuals such as Columbia and L*er*) are missing or have inactivated copies of FRI and/or FLC.[38] FRI/FLC-mediated suppression of flowering is broken by several vernalization-pathway genes that accumulate under cold treatment and repress expression of FLC. These vernalization genes, including VIN3, LHP1, and VRN1, modify FLC chromatin and leave a mitotically stabile epigenetic mark on FLC. This modified chromatin suppresses FLC expression, thus freeing activation of FT which signals for the transition to flowering.[100,101]

Flowering time "effector genes"—the downstream targets of FT—are developmental genes involved in floral patterning. After its synthesis in leaves and transport via the vascular system to the shoot apical meristem, FT appears to form a dimer with Flowering Locus D (FD).[102,103] The FT/FD complex then activates floral meristem genes such as *APETALA1* (AP1) and *FRUITFUL* (FUL) which begin the process of forming flowers. It will be interesting to learn where the signal from abiotic stress integrates into this complex pathway.

Cross-talk

Cross-talk refers to the common finding from plant molecular studies that signaling pathways frequently interact with each other. Such interactions

take two general forms. First, seemingly distinct response pathways share common signaling intermediates such as phytohormones and second messengers. Second, signals emerging from distinct environmental perception circuits can be integrated at common network components, such as at the *cis*-regulatory transcriptional apparatus for a key signaling gene.

Cross-talk is apparent in the control of flowering time: the expression of FT, which signals positively for the transition to flowering, is controlled by signals from a light-dependent pathway, a cold-responsive pathway and the internal circadian clock of the plant. Similarly, genes containing DRE/CRT promoter motifs can be activated by an osmotic-responsive ABA-dependent pathway via DREB1D/CBF4, by an ABA-independent osmotic stress responsive pathway via DREB2 or by the DREB1 cold-responsive signaling pathway. Genes which also have an ABRE in their promoter, such as RD29a, add an additional level of signal integration. And perhaps the most striking case of cross-talk comes from one of the best understood plant cell signaling systems: the integration of multiple signals to effect guard cell closure.

A plant stomate is flanked by two guard cells whose plastic size and shape determine whether the stomate is open or closed: turgid cells open the stomate while flaccid cells close the stomate. The turgor of a guard cell is determined by its water status, which in turn is controlled by the concentration of key ions and solutes in the guard cell's cytoplasm. ABA is synthesized primarily in root tissues and then transported in one of several ionic forms to photosynthetically active tissues.[104] We will only outline the critical steps between ABA perception and stomate closure (for a more complete treatment see Refs. 43,44,51,105). ABA stimulates guard cells to generate nitric oxide (NO), inositol-1,4,5 triphosphate (I3P), inositol hexaphosphate (I6P), and reactive oxygen species (ROS) which, in turn, signal via other second messengers for an increase in the intracellular calcium (Ca^{2+}) concentration (Fig. 4). Cytosolic Ca^{2+} is derived from intracellular stores and serves as a major integration point for numerous ABA-dependent and independent signaling cascades. Along with increased cytosolic pH (also apparently stimulated by ABA), the increase in Ca^{2+} concentration depolarizes the cell membrane and activates membrane-bound cation (principally K^+) and anion (principally Cl^-) exporters, causing increased extracellular solute concentration. Water movement out of the cell along this solute concentration gradient then leads to decreased guard cell turgor and stomate closure. ABA also inhibits the reopening of stomates in part by antagonizing the effects of other hormones to increase intracellular solute concentrations. Once the osmotic stress and attendant ABA signal has been relaxed, intracellular accumulation of K^+, Cl^-, malate^{2-}, and sucrose lead to passive movement of water back into the guard cells along a concentration gradient and thus leads to an increase of guard cell size and stomate opening.

Guard cell behavior is influenced by other external cues, such as ambient CO_2 levels[106] and light,[107] as well as a complex internal environment including intercellular calcium concentrations[108] and multiple phytohormones.[44] In *Arabidopsis*, jasmonic acid, brassinosteroids, and salicylic acid generally act synergistically with ABA in promoting stomate closure. Interestingly, the role of salicylic acid in stomate closure may represent its broader function in pathogen response signaling: closure of stomates restricts bacterial access to the interior spaces of leaves. This cross-talk between abiotic and biotic stress response pathways is an active and exciting area of research.[109] By contrast, auxins and cytokinins generally inhibit stomate closure and/or signal for reopening following relaxation of osmotic stress. The role of ethylene in osmotic stress response is harder to define, and seems to vary by species.[44] Besides playing small roles in guard cell behavior, each of these phytohormones has essential roles in other aspects of plant function, from seedling germination throughout development, during tropic and pathogen responses and on to organ senescence. For a plant to accurately control stomatal aperture, all of these signals must be interpreted, integrated, and acted on by guard cells.

Our understanding of *Arabidopsis* biology is not yet to the point where we have identified complex protein interaction networks which might allow us to quantify levels and kinds of signal cross-talk. A major feature of yeast and other microbial protein networks is the uneven distribution of interaction partners: a small number of proteins interact with many other proteins whereas most proteins have a small number of interaction partners (e.g., Ref. 110). In *Arabidopsis* abiotic stress signaling, we can begin

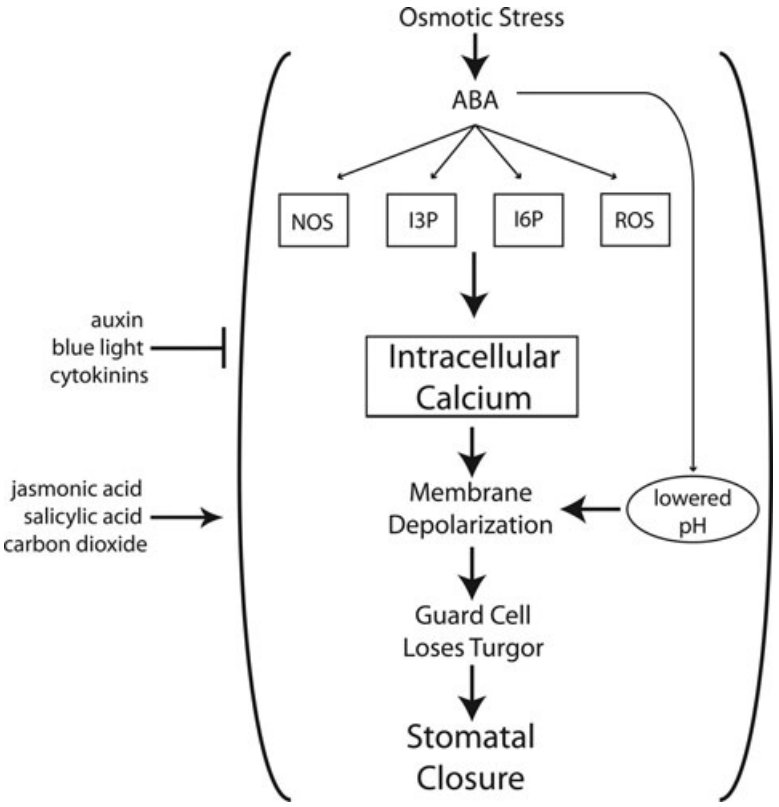

Figure 4. **Simplified topology of signaling steps involved in stomate closure and some of the internal and external cues that also control stomate behavior. The precise mechanisms of action of the stimuli shown outside the brackets are unknown.**

to see a pattern similar to this, particularly with respect to transcriptional regulation. As already discussed, diverse environmental signals are perceived by FT, MPK6, a particular DREB/CBF family member or the proteins that regulate ABA biosynthesis. These signalers, in turn, interact with many downstream partners. Such convergent regulatory mechanisms may allow for highly specific control of important fitness components under strong selection, such as flowering time, or they may economize genome and proteome space by using a single effector protein to ameliorate a cellular stress common to multiple environmental perturbations. We will discuss possible evolutionary explanations for the abundance of cross-talk in the next section.

Evolutionary processes driving abiotic stress response

As just discussed, a key perspective emerging from molecular work is that abiotic stress pathways may be functionally (pleiotropically) linked through sensing, signaling, and adaptive responses across a host of stresses. At face value, the extreme degree of cross-talk and functional linkage may seem an insurmountable constraint on future evolution. How can populations evolve fine-tuned or specialized adaptive responses to drought if these evolutionary responses will have indirect effects on cold or salt tolerance? How promising are breeding or genetically modified organism (GMO) strategies for plant improvement if all manipulations result in trade-offs or costs associated with other abiotic or biotic stress pathways? These questions are fundamentally issues about the evolvability of stress tolerance traits. In addition, many of the molecular responses identified can be considered plastic responses to the environment. For example, both the induction of a transcription factor and the increase of an osmoticum in response to a stress are "plastic" responses of the transcriptome and metabolome, respectively. As such, two obvious links between the fields of molecular biology and evolutionary

genetics lie in the notions of evolvability and of adaptive plastic responses.

Genetic correlations and evolvability

Quantitative genetics focuses on understanding the factors determining phenotypic variation in continuously distributed traits. In general, these types of traits are controlled by both environmental variation and the segregation of many genes, often of small effect. Traditional quantitative genetic approaches rely on resemblance among relatives (usually families generated by breeding) to partition the total phenotypic variation for a trait into environmental and genetic components of variance[111] and result in estimates of parameters like the additive genetic variance (V_a) and narrow-sense heritability (h^2). This approach can be easily extended to explore genetic associations among traits with the calculation of the additive genetic covariance (Cov_a) between traits. Genetic covariances can be caused by pleiotropic gene action, genetic linkage, or genetic linkage disequilibrium between alleles at genes affecting multiple traits. Overall, genetic variability in a set of traits can be characterized by the so-called **G**-matrix depicting the additive genetic variances and covariances for a suite of traits (see Ref. 112 for an excellent review of basic multivariate quantitative genetics).

Evolutionary biologists and plant breeders have long been interested in estimating quantitative genetic parameters as a way to "predict" short-term evolution in response to artificial or natural selection. In the simple univariate case, the breeder's equation ($R = h^2 s$) can be used to predict the evolutionary change in a trait mean (R) in a focal population as the product of the narrow-sense heritability for that trait (h^2) and the relationship between the trait and reproductive fitness (s, the selection differential). Here, heritability can be thought of as a weighting factor rendering the degree of selection response experienced by a population. Using matrix notation, the breeder's equation can be extended to model the evolution of multiple traits by

$$\Delta \mathbf{Z} = \mathbf{G}\beta,$$

where $\Delta \mathbf{Z}$ is a vector of the change in the mean values of the traits after one generation of selection, **G** is the genetic variance/covariance matrix, and β is the multivariate selection gradient.[113] In this case, the vector response can be influenced not only by the degree of genetic variability for each trait but also the genetic correlations among traits as embodied in the **G**-matrix. Depending on the sign of the additive genetic covariance, selection can result in both direct and indirect responses to selection. As such, the **G**-matrix can be thought of as a central determinant of the evolutionary potential of a trait.

Genetic correlations can slow the rate and alter the pattern of adaptation in natural populations depending on the magnitude and sign of the genetic correlations and direction of selection.[113,114] Over short time scales genetic correlations can lead to the evolution of maladapted phenotypes. Under the extreme case of perfect genetic correlations ($r_G = 1$ or -1) populations may be constrained from reaching a global adaptive peak. The range of conditions leading to strong constraints may be considerably relaxed when multivariate phenotypes are considered.[115,116] For example, Dickerson[117] showed that a set of individual traits could all display genetic variance and yet a response to selection for increasing values may not be possible as a result of the genetic correlation among traits. In this case, the pattern of genetic correlation results in axes in multivariate space that lack genetic variation despite the fact that each univariate trait had significant heritable variance. As such, genetic correlations caused by pleiotropic architecture can clearly interfere with adaptive evolution of complex systems.

One clear example of genetic constraints in Arabidopsis ecological genetics is the pleiotropic link between flowering time and whole-plant physiology. Strong positive genetic correlations have been observed between date of first flowering and measures of water-use efficiency among natural accessions of *Arabidopsis*: early flowering accessions exhibit low WUE and late flowering accessions exhibit high WUE[30,32,34] (Fig. 1). In this case, there is much reduced genetic variation in the bivariate space for early flowering/high WUE and late flowering/low WUE trait combinations. As such, selection toward these trait combinations would show little evolutionary response. Selection on early flowering time may also be constrained by pleiotropic costs of underlying alleles directly on fitness. Kover *et al.*[118] selected for early flowering time in spring and winter-simulated environments and successfully recovered lines that flowered earlier under each environment. However, the early-flowering spring lines had lower fruit set than control plants, suggesting

that early flowering does not necessarily increase fitness. One possible mechanism for this trade-off is the apparent negative effect of early-flowering FRI alleles on the number of branches and nodes on the plant.[119]

It has long been known that highly pleiotropic systems with contrasting selective pressures could reduce the evolvability of a system over the short-term.[113,120,121] More recently, theoreticians have considered the links between genetic architecture and evolvability by asking what patterns of genetic variances and covariance facilitate or constrain long-term evolutionary responses. In particular, a key conceptual advance has come by considering how the **G**-matrix itself can evolve as a result of mutation, drift, and response to selection.[122–124] One important determinant of the G-matrix evolution is the nature of pleiotropic effects themselves. For example, complementary pleiotropy occurs when alleles have effects in a common direction on two traits ($++$ or $--$) while antagonistic pleiotropy occurs when alleles have opposing effects on two traits ($+-$ or $-+$). There has been a growing realization of the potential impact and importance of "hidden pleiotropy," where the pleiotropic effects of loci cancel out and on average result in no observable genetic correlation between traits.[125]

Several studies have suggested that the highest evolvability or adaptive potential over long-term evolutionary timescales may exist in systems with intermediate levels of pleiotropy and nonadditive genetic variance.[125–129] This hypothesis is based on several arguments. First, a hierarchical structure of restricted pleiotropy that results in relatively independent "modules" may allow selection to act independently at the module level.[128–132] The idea is that over evolutionary time traits sets that function together may evolve to be bundled into pleiotropic gene networks, and those with no functional overlap will evolve independent genetic control. For instance, Berg[133] hypothesized that natural selection to maintain proper fit between flowers and their pollinators would lead to increased correlations between floral organs and decreased correlations between flowers and the rest of the plant. Here, genetic modularity would allow flowers and leaves to follow independent adaptive paths over evolutionary time. Indeed, a number of quantitative genetic studies have found evidence for large positive genetic correlations among sets of either flower or leaf traits, but low and generally nonsignificant genetic correlations between flower and leaf traits.[32,134–136]

As described above, many abiotic stresses signal for activation of genes loosely organized into modules. DRE/CRT-containing genes can be activated either by ABA-dependent (via DREB1d/CBF4) or independent (CBF1,2,3/DREB1a,b,c) pathways (see Fig. 2) and there is some evidence that the combination of different transcription factors in various configurations leads to activation of subsets of the DRE/CRT-containing target genes. RD29a and its paralog RD29b are apparently under this sort of differential control, as RD29a has both DRE/CRT and ABRE elements, whereas RD29b lacks DRE/CRT elements.[59] This modularity may increase the degrees of freedom available for the evolution of novel or unique stress response.

Several theoretical studies have considered the role of effects sizes for pleiotropic loci on evolvability. Griswold[126] found an enhanced evolvability with increasing pleiotropy under models with a mixture of directional and stabilizing selection on traits. Here, beneficial mutations, on average, have larger selective effects when the number of characters pleiotropically affected by selection increases. In the case of stress tolerance, pleiotropic modules associated with several functionally related stresses (cold, water deficit, salt) may allow and facilitate the adaptive evolution of specialized responses or increase adaptability by increasing the selective effects of stress genes. The *Arabidopsis* MAP Kinases and SNF1-Related Protein Kinases may be interesting candidates in this context.

Hansen[127,137] has argued that intermediate levels of pleiotropy can increase evolvability because evolvability depends not only on the occurrence of pleiotropy, but also on patterns of variability in pleiotropic effects. For example, if we imagine that drought and cold tolerance are both affected by a large set of common genes, but the pleiotropic effects between drought and cold include both positive and negative patterns, the two tolerance strategies may be largely autonomous despite high levels of pleiotropy. Hansen[127] has shown with conditional evolvability models that such traits exhibit higher evolvabilities because both trait sets have larger mutational target sizes. This type of outcome may be particularly likely in systems

exhibiting many gene interactions, especially differential epistasis that modifies pleiotropic relationships among traits.[130,138,139]

Evolution of adaptive plasticity

Many of the abiotic stress responses studied by molecular biologists can be considered phenotypically plastic responses. One simple definition of plasticity is any organismal change that is induced by the environment, though such a change may not necessarily represent an adaptive response in either the physiological or evolutionary sense of "adaptation." Evolutionary ecologists define phenotypic plasticity as the ability of a single genotype to produce multiple phenotypes in response to environmental variation.[140] Plants are famous for the degree of phenotypic plasticity that they exhibit. One important notion is that plants, especially as sessile organisms, have evolved plastic responses that modulate their phenotypes to the challenges imposed by the specific environment they experience. This is a particularly attractive hypothesis, in part due to the notion of cost savings: plants only produce expensive phenotypes when needed. This notion has played an important role in applied plant biology too, with genetic engineering strategies aimed at employing inducible stress tolerance systems.[141] In this case, the hope has been to improve stress tolerant crops with little or no yield drag in good agronomic environments.

For phenotypic plasticity to evolve there must be genetic variation in phenotypic responses to the environment. Genetic variation in phenotypic plasticity is also known as genotype-environment interaction. Genotype by environment interactions (GEIs) are often studied through common garden experiments replicating germplasm (e.g., varieties, clones, genotypes, transgenic lines) under different environmental conditions (see Ref. 7 for a general review of GEI in Arabidopsis). These data are analyzed in a factorial ANOVA framework testing for constitutive differences among replicated genotypes (genetic variation), environmental effects (plasticity), and their interaction (variability in the response of genotypes to the same environmental effects). Importantly, GEIs can be driven by several different genetic architectures (see Fig. 5). For example, a common interpretation of rank changing (crossover) GEI is the occurrence of genetic tradeoffs through antagonistic pleiotropy. In this case, a genotype may have high performance in one environment and low performance in another owing to a sign change in the phenotypic effects of a gene for a key trait. This is an important genetic architecture as it can be a strong limit to responses to artificial or natural selection. In contrast, differential sensitivity occurs when the magnitude of phenotypic effect of a gene depends on the environment. Conditional neutrality occurs when a gene has a phenotypic effect in one environment, but no effect in another. Finally, environment-specific epistasis, perhaps related to sensory perception and signal transduction, can lead to a diversity of GEI patterns at the phenotypic level.[32]

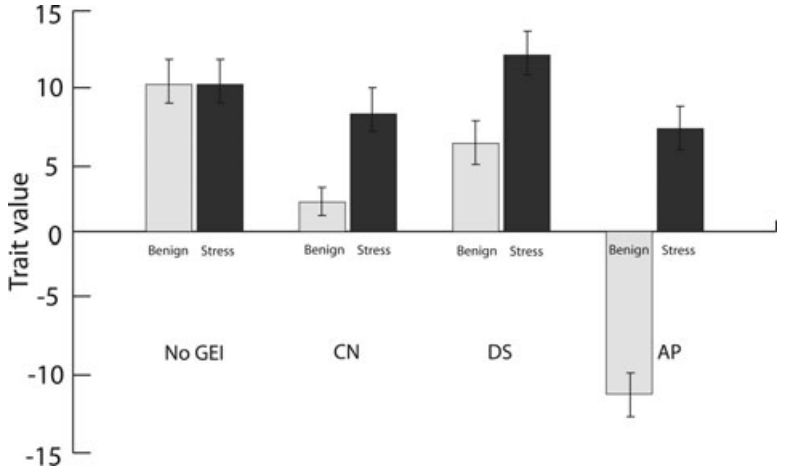

Figure 5. **The genetic architecture of genotype-by-environment interaction, including depictions of no GEI, conditional neutrality (CN), differential sensitivity (DS), and antagonistic pleiotropy (AP) at the gene level between benign and stressful abiotic conditions. Trait value could indicate fitness, response level in response to stress, or any other phenotypic metric.**

Several different lines of evidence point toward the existence of GEI in abiotic stress response. Juenger *et al.*[57] found significant treatment by genotype interaction in the expression of hundreds of genes in an experiment that imposed mild water stress on *Arabidopsis* Tsushima and Kashmir accessions. Genetic variation in transcriptional response to salt stress[142] and cold stress[143] has also been documented. GEIs in morphological and life-history responses to abiotic stress have also been demonstrated. Hausmann *et al.* reported strong GEI for water stress on fruit production, shoot biomass, leaf nitrogen content, number of branches and fitness.[35] GEI has also been observed for flowering time under multiple temperature or day-length treatments (e.g., Ref. 118) and root morphology in relation to water stress.[144] Recombinant inbred line populations derived from several of these accessions are available, lending to the prospect of dissecting the genetic basis of GEI for many abiotic stress response traits in Arabidopsis.

At a mechanistic level, GEIs can be driven by a host of phenomena. For example, genetic variation in environment-sensing receptors could be expected to result in differential phenotype responses across environmental gradients. However, a growing number of studies suggest that many other mechanisms can underlie GEI,[145] including environmental regulation of gene expression,[146,147] environment-specific epigenetics (e.g., vernalization induced methylation and flowering time[148–150]), and even environment-specific alternative splicing, miRNA production, or posttranslational modifications.[151–154]

A number of possible mechanisms of GEI have been implicated in studies of abiotic stress tolerance. Perhaps the most intriguing among these are the growing number of documented cases of environmental sensors which, if variable in the natural environment, could be a source of variability in phenotypic plasticity in abiotic stress response. Several potential osmotic sensors have been identified, including AtHK1/AHK1, which acts as a positive regulator of osmotic stress signaling in *Arabidopsis*[155] and can serve as an osmotic sensor in yeast.[156] The *Nicotiana* NtC7 protein is upregulated in response to osmotic stress and when overexpressed it provides plants with enhanced water stress tolerance, but not salt stress.[157] Kumar and Wigge[94] recently reported a possible histone-based mechanism through which a special type of histone become destabilized by increasing temperature and thereby makes possible the expression of nearby genes. Variation in any of these proteins, their expression, modification, or degradation, represents exciting candidates for GEI interactors.

The pattern of genetic variation across environments can be investigated by estimating either the GEI effect on one trait or the genetic correlation (r_G) among character states of the same trait expressed in different environments.[158] The genetic correlation approach is very useful in evolutionary studies because—just as genetic correlations among traits in a single environment can alter the direction and magnitude of evolutionary responses to selection[114]—genetic correlations across environments can influence the evolution of plasticity. For example, the magnitude and sign of r_G across environments may strongly influence whether evolution in a heterogeneous environment will produce ecologically general traits or traits adapted to specific niches.[159–162] If character states across environments have perfect correlations ($+1$, or -1) evolution in each environment is fundamentally coupled because of complete genetic dependence (influenced by the same genes and alleles). In this case, r_G often favors the short-term evolution of the generalist strategy. A genetic correlation of zero across environments means that character states are genetically distinct (are influenced either by alleles at different loci or by the same alleles through environmentally dependent pleiotropy) and can evolve independently, thus allowing at least some potential for the evolution of specialization.[161] Finally, negative genetic correlations across environments measures the extent of trade-offs or constraints between traits expressed in alternative environments. Negative genetic correlations are particularly useful in evaluating the evolutionary costs of specialization or generalization in a variable environment. More generally, we can envision genetic constraints imposed by genetic correlations as singularities, or zero determinants, of the additive genetic variance/covariance matrix for a set of evolving traits or character states of traits in multiple environments[115,116,163] (but see Ref. 164 for caveats on interpreting genetic correlations).

Several quantitative models have been developed for exploring the evolution of phenotypic plasticity, including extensions of response to selection

equation models framed upon discrete environmental patches.[158,160,161] For example, the Via/Lande model focuses on the evolution of "character states" of a trait expressed across environmental heterogeneity and considers both the direct evolutionary response to selection within an environment, along with the indirect (correlated) response due to selection in an alternative environments.

These models have been a powerful heuristic framework for considering the relative importance of the frequency of environments, strength and direction of selection, patterns of genetic variability, and dispersal on the evolution of adaptive plasticity. For example, Via[163] explored the dynamic evolution of plasticity in response to selection on character states by investigating a variety of environmental and genetic scenarios and the degree to which these factors shape the relative importance of direct and indirect responses. For instance, models considering a common and a rare environment show that adaptive evolution occurs more rapidly in the common environment than in the rare environment, all else being equal. Similar models can be use to explore the impacts of novel environments (by changing the strength of selection on traits in each environment), complex habitats (by increasing the number of environments), or by altering the patterns of trait trade-off (by changing the across environment genetic correlation), or connection between alternative habitat types (by changing the magnitude or direction of gene flow). In general, these exercises demonstrate that any factor that increases the magnitude of the correlated response to selection that is opposite in sign to the direct response will constrain the rate of the evolution of adaptive plasticity. In the case of abiotic stress tolerance, one could use this framework for considering the evolution of candidate tolerance traits in natural populations or crop species in response to natural or artificial selection.

One characteristic prevalent from the molecular literature is the occurrence of negative pleiotropic fitness effects of abiotic stress genes in the benign environment (e.g., yield drag). WUE is tightly linked with biomass and seed production. Selection for low water use efficiency in wheat leads to greater biomass and grain yields under drought stress. But the yield advantage of these low-WUE as compared to normal-WUE lines decreases as water becomes less limiting such that under well-watered conditions the low-WUE lines are relatively poorer-performing.[165] Yield drag may be partially ameliorated through transgenic mechanisms to mimic inducibility. Such a strategy was employed by Kasuga *et al.*[166] who reasoned that abiotic stress tolerance could be enhanced by overexpressing the DREB1a transcription factor in *Arabidopsis*, which is osmotic-stress responsive. By placing DREB1a under the strong constitutive CaMV 35S promoter the authors recovered plants showing significantly enhanced survival under the predicted stressful environments. However, under benign conditions most lines were considerably smaller than wild-type plants and several of the overexpressing lines produced fewer than 1% of the number of seeds produced by wild type. The authors then reasoned that by placing DREB1a under a stress-inducible promoter, in this case the native promoter of the RD29a gene, they could ameliorate some of the negative effects associated with a constitutive stress phenotype. The RD29a::DREB1a transgenic plants did, in fact, show enhanced survival under stressful conditions with smaller fitness effects in the benign environment.

Results such as these lead us to predict a prevalence of negative across environment correlations when considering the adaptation of populations across benign and stressful conditions. As such, we might expect to see natural populations exhibiting compromised or maladapted phenotypes rather than finding populations at adaptive peaks.[137,167] This might be indicated by strong directional selection for physiological traits that relate to performance under stressful environmental conditions, as populations may be far from adaptive peaks.

In a seminal review, Dewitt *et al.*[3] raised the important issue of costs and limits of plasticity and their role in adaptive evolution. A cost of plasticity is the reduction in fitness or yield of a genotype as a consequence of expressing a certain phenotype through a plastic rather than a fixed physiology or development. From a conceptual perspective, costs might be associated with maintaining receptors necessary for sensing the environment, the cost of the regulatory mechanisms allowing plastic responses, or though the specific production cost of making a phenotype through plastic rather fixed strategies. Dewitt *et al.*[3] also noted that there are several potential limits to the utility of plastic

responses in contrasts to fixed strategies. For example, plasticity relies on informational cues from the environment and it is possible that these cues are unreliable, can be "misunderstood" or interpreted in the wrong context, or lag with respect to the stressful conditions. An inappropriate response to cues, poor cues, or long lag times may lead to the expression of maladaptive phenotypes.

Several of these hypotheses regarding costs associated with maintaining plastic-response machinery are difficult to test in *Arabidopsis* because we have little information about naturally occurring variation in plasticity for abiotic stress response traits. We can, however, make several inferences about costs associated with misperceiving environmental cues by considering the effects of several known hypersensitive or insensitive mutants. As described earlier, overexpressing positive regulators of abiotic stress signaling generally leads to plants with increased survival under stressful conditions, but at significantly reduced growth rates. The smaller stature of these plants may reflect adaptive inhibition of growth, or side effects of investing considerable energy into stress-response machinery.

Reconciling the molecular and evolutionary paradigms

The main goal of our article has been to review the molecular biology advances in the field of abiotic stress tolerance and, where possible, to draw links from these studies to the field of ecological genetics. We hope that the perspectives gained from considering the evolution of plant stress tolerance can help interpret and guide both future molecular and ecological genetic research. We also hope that our review helps generate hypotheses concerning the evolution of abiotic stress tolerance and a framework for testing alternative predictions.

One of the key observations from the molecular work is that there are many genes impacting plant stress tolerance. On the face of it, these results suggest that the mutational target for stress tolerance may be high and therefore substantial genetic variation in physiology and abiotic stress responses may be found in natural populations. Unfortunately, we do not know if results from mutagenesis, knockout or overexpression studies are good indicators of levels of standing variation.[168] In fact, it is possible that these studies are misleading in that they tend to identify genes that have large effects, many pleiotropic effects, and are "hubs" in gene networks. The phenotypes of laboratory-induced mutations in the ABI1 protein are indicative of such strongly pleiotropic effects. ABI1 is a negative regulator of ABA signaling and plays an important role in guard cell signaling. The widely studied dominant negative mutant abi1–1 shows ABA insensitivity and high stomatal conductance leading to a "wilty" phenotype. This mutant also has reduced sensitivity to ABA during seedling germination that can lead to seed germination while still in the silique.[169] Presumably, any individuals harboring mutations of this type would suffer considerable fitness deficits. The many pleiotropic effects of MPK6 knockouts, described above, may also overstate the effects of naturally segregating mutations in abiotic stress genes.[88–90] It is possible that these and other such genes are under strong purifying selection, are not polymorphic in natural populations, and as such are not involved in adaptation to local abiotic stresses. Or, alternatively, such genes may harbor adaptive variation related to expression polymorphisms that lead to more restricted pleiotropic effects. In the case of *Arabidopsis*, efforts to resequence the entire genome of a large number of accessions from a diversity of habitats and climatic regions may provide one avenue for exploring standing genetic variation in stress related genes.[170]

A number of studies have identified natural variation in candidate traits or operationally defined abiotic stress tolerance, especially in crops and model species.[8,141] However, in few cases do we know the actual loci controlling this quantitative genetic variation. Furthermore, few studies have jointly explored sets of abiotic stresses and as such we do not have a good sense for the pattern of genetic correlation (the abiotic environment **G**-matrix) across drought, cold, salt, or other common stresses. This is in contrast to many biotic systems, where genetic correlations across resistance to insect herbivores (reviewed in Ref. 171) and bacterial/fungal pathogens have been explored.[172] These data are sorely needed to evaluate the importance of pleiotropy in constraining or facilitating the evolution of stress tolerances.

A predominant viewpoint in the molecular literature is that seemingly diverse abiotic stresses are actually similar from a mechanistic perspective at the cellular level. This interpretation leads to the viewpoint that cross-talk or pleiotropic links may

be "adaptive," reinforcing, and lead to plants with general vigor. However, it is also possible that existing protocols and methods for imposing stress (e.g., PEG generated water-deficit) are poor representations of naturally occurring abiotic stress. For example, many drought studies focus on unrealistic levels of water-deficit imposed over time courses that allow little opportunity for plant acclimation. Similarly, many salt assays impose ecologically unrealistic treatment levels to plants that have no history of evolving on salt-contaminated soils. As such, it is possible that much of the molecular work has more correctly identified genetic mechanisms related to "shock-responses" rather than to ecologically relevant stress. Similarly, several recent studies have noted remarkable differences in plant responses to "joint stress treatments" when compared to single stress treatments, for example the imposition of simultaneous drought and heat stress.[173,174] We desperately need additional research using molecular tools but with experiments incorporating more realistic stress treatments and a better appreciation for the frequency and co-occurrences of stress in the field.

Quantitative genetic models of phenotypic plasticity provide a useful heuristic tool for considering how spatially varying abiotic stresses can result in the evolution of tolerance. They highlight the ways that selection, genetic variation, and the frequency of the environment can drive the evolution of generalist or specialist plants. However, these models make very simple assumptions about the genetic basis of plasticity and generally do not explicitly incorporate possible costs or limits to plasticity (but see Ref. 159). In contrast, molecular studies are teasing apart a host of possible mechanisms underlying the genetics of plasticity, including proteins involved in environmental sensing and those at key signal integration or "decision-making" steps regarding which of several response modules to activate. Here is an ideal opportunity for theoretical work to benefit from a better molecular understanding of gene-by-environment interaction. To what extent do standard quantitative genetic models based on additive genetic effects represent genetic variation in plasticity? How important are epistatic networks in generating plasticity, how might these networks vary in natural populations, and how can they be incorporated in new modeling efforts? What characteristics lead to "costly" plastic responses? Undoubtedly both fields will benefit from new empirical data and more realistic modeling efforts.

From an applied perspective, plant biologists hope to engineer plants with higher intrinsic yield or plants that exhibit better yield responses under a variety of abiotic stresses (e.g., Refs. 175,176). A key hurdle is yield drag or the occurrence of antagonistic pleiotropy for tolerance alleles when expressed in the absence of stress. As such, much of the molecular work has focused on transcriptional regulators that would be cued to the occurrence of stress and thus sidestep pleiotropic drag. There are potentially important limitations to this approach, including the adequacy of the plant to sense stress in an agronomic setting, the timescales of plant response relative to agronomic practices (e.g., periods of drought or the timing of harvest), or high intrinsic costs associated with overexpression of natural inducible systems (e.g., through the toxic buildup of an osmoticum). There are, of course, other strategies that could be employed. For example, conditionally neutral alleles that increase fitness under stress but have no apparent cost in the absence of stress would be ideal loci for crop improvement. Interestingly, several recent studies of local adaptation have identified largely conditionally neutral alleles increasing fitness in one habitat with no measurable cost in others.[177–180] As we have argued above, the genes identified through genetic screens likely represent large-effect genes that are under strong purifying selection in nature. By studying naturally occurring functional variants in key abiotic stress pathways, molecular biologists may be able to identify candidates that will enhance crop productivity without compromising other components of plant fitness under variable environments. These allelic variant swaps—similar to a genetic common garden experiment—would also be an interesting approach for studying epistatic interactions and the effects of small perturbations on network function, because the extent of pleiotropy of key signaling proteins are likely dependent on their genetic background.

A final exciting and important point of conceptual overlap between molecular and ecological genetic perspectives deals with epistatic relations among genes and gene products. Evolutionary and quantitative geneticists have largely thought of epistasis as nonadditive effects that complicate a direct mapping of genotype to phenotype.[181] By contrast, biochemists and molecular geneticists use

"epistasis" in a more functional sense, denoting interaction of gene products. These two usages might be more accurately denoted as genetic epistasis and functional epistasis, respectively. While genetic epistasis is frequently treated as "extra" or even unidentifiable genetic variation, there exists a biological connection between genetic and functional epistasis. An important first step in understanding the role of functional epistasis in the evolution of abiotic stress traits will be identifying natural variation in functional epistasis. Fine-mapping approaches combined with detailed knowledge of gene location and annotation gained from genome sequencing now allow evolutionary biologists to identify epistatic interactors, and the effects of these interactors can be quantified by introgression into different genetic backgrounds using either transgenic or introgression lines. QTL mapping of expression traits (eQTL) is another avenue that may identify such variation.

Concluding remarks

A meaningful synthesis of molecular biology with evolutionary and quantitative genetics is fast becoming a reality, with exciting opportunities for reciprocal enlightenment between the two traditions of inquiry. As we move forward, it will be essential that molecular biologists appreciate that the genes and networks which are often studied in isolation have complex evolutionary histories and exist in a many-dimensional genetic and environmental context. Students of evolutionary and quantitative genetics have often assumed—as a simplifying assumption in models—that the molecular mechanisms underlying quantitative traits under selection are unknowable or irrelevant. We hope that our attempt at a synthetic treatment of these two fields has made clear that these two sides have much to learn from each other, and that the equations of quantitative genetics and "alphabet soup" of molecular genetics are not so scary after all.

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Conflicts of interest

The authors declare no conflict of interest.

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