Review

The evolution of drought escape and avoidance in natural herbaceous populations

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ABSTRACT

While the functional genetics and physiological mechanisms controlling drought resistance in crop plants have been intensely studied, less research has examined the genetic basis of adaptation to drought stress in natural populations. Drought resistance adaptations in nature reflect natural rather than human-mediated selection and may identify novel mechanisms for stress tolerance. Adaptations conferring drought resistance have historically been divided into alternative strategies including drought escape (rapid development to complete a life cycle before drought) and drought avoidance (reducing water loss to prevent dehydration). Recent studies in genetic model systems such as Arabidopsis, Mimulus, and Panicum have begun to elucidate the genes, expression profiles, and physiological changes responsible for ecologically important variation in drought resistance. Similar to most crop plants, variation in drought escape and avoidance is complex, underlain by many QTL of small effect, and pervasive gene by environment interactions. Recently identified major-effect alleles point to a significant role for genetic constraints in limiting the concurrent evolution of both drought escape and avoidance strategies, although these constraints are not universally found. This progress suggests that understanding the mechanistic basis and fitness consequences of gene by environment interactions will be critical for crop improvement and forecasting population persistence in unpredictable environments.

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1. Introduction

Climate models forecast an increase in both the severity and frequency of drought in the coming 50 years [1]. This change is especially difficult for sessile organisms such as plants, which must be able to respond to wide fluctuations in growing season conditions while still maintaining the ability to correctly time developmental processes in response to environmental cues. At a population level, increasing aridity and drought should lead to strong directional selection for plants with higher fitness under drought conditions (i.e., drought-resistant plants); however, a more nuanced understanding of genes and traits under selection is limited by an incomplete knowledge of the mechanisms that plants use to resist drought stress [2]. Without understanding the innate resistance mechanisms plants possess, it is difficult to accurately assess future population persistence. Determining the prevalence and variation in the mechanisms underlying stress resistance and adaptation is a key goal for plant biologists.

Unlike in natural populations, responses to drought stress have been widely studied in a few major crop plants [3-7]. This literature has resulted in an improved understanding of the physiological pathways involved in drought perception and response as well as identified major-effect genes controlling drought resistance [3]. However, wild populations often harbor large pools of genetic and phenotypic diversity that can provide insights into novel mechanisms of acquiring drought resistance. These insights can range from characteristics new phenotypes to identifying new roles for genes involved in abiotic stress-response pathways. While understanding the diversity and prevalence of mechanisms underlying drought resistance in natural populations clearly benefits evolutionary biologists, these results can also help agronomists more effectively improve or develop crops. Here I synthesize recent progress describing how drought resistance has evolved in natural populations of herbaceous plants. I focus on studies that identify the genetic basis of drought strategies as well as describe the evidence that these strategies are advantageous in natural populations.

Adaptation to soil water availability is common across the ranges of plant species and is associated with the formation of ecotypes [8,9]. Adaptations conferring drought resistance have historically been divided into three alternative strategies: drought escape, drought avoidance, and drought tolerance [10]. Each of these strategies may evolve as a constitutive response that occurs independently of environmental cues such as water deficit, or can evolve as a heritable plastic response that is dependent on one or more environmental cues. Drought escape occurs when plants develop rapidly and reproduce before drought conditions become severe. Cessation of vegetative growth may or may not accompany a drought escape response. In contrast, drought avoidance occurs when plants increase water-use efficiency (WUE) by reducing transpiration, limiting vegetative growth, or increasing root growth, and avoid dehydration during transient periods of drought stress. Drought avoidance has also been referred to as dehydration avoidance in recent literature. Finally, drought-tolerant plants are able to withstand dehydration through osmotic adjustment and production of molecules that stabilize proteins (Fig. 1; [10]). These strategies are coordinated physiological syndromes that involve many physiological and structural traits [11]. For instance, drought avoidance through increased WUE is mediated by lowering stomatal conductance, which in turn can be influenced by a number of different potentially correlated traits such as leaf area, leaf lobing, succulence, or stomatal density. Here, I will focus on recent advances understanding drought escape and avoidance. These advances are largely limited to studies examining flowering time as a measure of drought escape and leaf-level WUE as a measure of drought avoidance as these are the traits that have received the most attention. Mechanisms of drought tolerance have been covered in detail elsewhere [3,12].

Although each of these strategies is predicted to evolve in areas of frequent drought stress, they are often viewed as alternative strategies or syndromes that can be optimally employed in specific seasonal contexts for plants with specific life history strategies (Fig. 1; [6,13]). For instance, drought escape may be optimal for annual plants in environments with short growing seasons that are ended by severe terminal drought; whereas drought avoidance may be more optimal if the growing season is punctuated by transient droughts. These strategies are unlikely to evolve together because plants devoting all of their resources to rapid reproduction need to have high rate of carbon fixation and thus also high stomatal conductance. However, plants typically avoid drought by lowering stomatal conductance to conserve water and thus reducing the rate of carbon fixation and growth. The literature has largely supported this view with the most detailed examples pointing toward the independent evolution of drought escape and avoidance strategies [14,15]. There is limited evidence in some systems that suggests that there are not genetic constraints to the concurrent evolution of both strategies within individual populations [16]. The environmental conditions that favor evolution of specific strategies is still an open topic and identifying the genetic constraints and fitness ramifications associated with each strategy is an area of strong interest.

While phenotypes associated with escape or avoidance strategies have often been studied (e.g., [14,17,18]), obtaining a detailed understanding of the genetic and physiological mechanisms that plants use to escape or avoid drought in natural populations has been challenging. Recreating realistic drought conditions in an experimental setting is difficult and may not necessarily reflect field conditions [19]. Drought can combine the effects of water deficit and possible heat stress. Manipulating water availability is complicated in dry-down experiments because water uptake is greater in bigger plants; a problem that can create heterogeneity in the timing of water deficits [20]. An additional challenge is finding species with populations that thrive across a range of aridity conditions and that also possess a genetic toolbox amenable to exploring the genes and pathways responsible for adaptive divergence in morphology and physiology. In model genetic species where the genetic basis of drought escape or avoidance has been characterized, there are often multiple QTL (quantitative trait loci), each of small effect that underlie variation in drought resistance. This makes it difficult to identify the phenotypic effects of a given locus [21,22]. Further, drought escape and avoidance can both be dependent on environmental context where a water deficit or other environmental cue may induce rapid flowering or changes in WUE [23,24]. This inherent plasticity can complicate linkage mapping and make it difficult to predict drought resistance and fitness consequences in a seasonal environment.

Nevertheless, development of new ecological model systems such as Mimulus guttatus, Avena barbata, and Panicum hallii as well as renewed efforts to study Brassica rapa and Arabidopsis sp. in ecological contexts has begun to provide new insights into the genetics underlying adaptive drought escape and avoidance strategies. Specifically, work in these systems has begun to address longstanding questions about ecophysiological traits regarding the prevalence, adaptive value, and genetic architecture underlying variation in these traits in natural populations [6]. Here I review the advances made in the last decade toward identifying the fitness benefits and genetic basis of drought escape and avoidance strategies as well as the constraints that limit concurrent evolution of both strategies. This large body of the literature establishes that variation in drought escape and avoidance traits is prevalent and adaptive, and highlights promising systems where QTLs and genes responsible for this variation are known. In addition, this review
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Fig. 1. Schematic diagram describing the three ways herbaceous plants evolve drought resistance. Each strategy has specific traits and phenotypes that have historically been associated. The phenotypes listed here are examples and do not represent a complete list. Arrows designate the environmental context that would most favor each strategy.

2. The evolution of drought escape in natural populations

2.1. Prevalence

Plants can escape drought by developing rapidly and reproducing before the onset of drought. Thus, flowering time is often the primary trait associated with drought escape in annual populations of herbaceous plants. Earlier flowering during a drought-shortened growing season presumably results in relatively greater fitness through higher seed set or greater seed mass. Extensive variation in flowering time in natural populations has been well documented (e.g., [25]), and several studies have demonstrated that earlier flowering time evolves due to selection caused by drought [26,27]. A central line of evidence for drought escape comes from common garden experiments where plants from xeric areas flower earlier in a common garden than plants from mesic areas. This approach has yielded patterns consistent with drought escape in Arabidopsis lyrata [28], Arabidopsis thaliana [29], Panicum hallii [21], Helianthus anomalous [30], Mimulus guttatus [16], and Boechera holboellii [31]. These results have demonstrated that extensive genetic variation in drought escape exists in natural populations.

Evolution of drought escape occurs not only through a constitutive difference in developmental timing, but also through changes in the mechanisms by which populations respond to environmental cues, i.e., a plastic drought escape response. Plant time the transition to flowering through temperature, vernalization, photoperiod, and water availability cues [32–34]. Adaptive drought escape responses occur through temperature and photoperiod cues [35,36]. The role of water availability as an environmental cue for the transition to flowering is typically considered secondary to photoperiod and vernalization cues; but may be the most important for breeding a drought escape response because it provides the most accurate environmental assessment of drought. Evidence for an adaptive drought escape response to water deficit in natural populations is limited. For example, the degree of plasticity in flowering time between wet and dry experimental environments was constant for several Arabidopsis lyrata populations that spanned a latitudinal gradient indicating that enhanced drought escape response has not evolved within any of these populations [28].

2.2. Fitness consequences

Evidence for drought escape as an adaptive strategy comes from temporally replicated series of observations examining changes in populations following drought events (longitudinal studies; e.g., [26]), phenotypic selection analyses under water-limiting conditions (e.g., [18]), and reciprocal transplant experiments (e.g., [37]). Adaptive drought responses have been well-characterized in multiple species. For instance, there was strong selection for earlier flowering in two Brassica rapa populations following drought that resulted in flowering an average of 1.9 days and 8.6 days earlier in each population [26]. However, the general importance of a plastic drought escape response in natural populations is debatable given the limited number of species with a documented drought escape response. The drought escape response in the same Brassica rapa populations described above was also examined, and there was no evidence for increased plasticity for flowering time after the aforementioned drought event [38]. While these experiments do not identify plastic drought escape responses as adaptive, few experiments have explicitly tested the adaptive value of plastic responses to drought. Further empirical and theoretical studies are necessary to identify conditions when such acclimation may be advantageous. For instance, evolving a highly plastic drought escape response may be more likely where severe droughts have occurred infrequently over the evolutionary history of a lineage, whereas evolving a constitutive drought escape response may occur if droughts have occurred frequently over the same timespan.

2.3. Genetic basis

The genetic architecture underlying variation in drought escape has been elucidated in a number of species, although the specific genes and their physiological effects remain relatively uncharacterized. One promising system is Panicum hallii, a switchgrass relative found along an aridity gradient in the U.S. desert southwest. Both a xeric ecotype (P. hallii var. hallii) and a mesic ecotype (P. hallii var. filipes) exist. There is extreme divergence in flowering time between these ecotypes where var. hallii flowers in approximately half the time (~44 days) it takes var. filipes (~85 days) in a common mesic environment. QTL mapping experiments have identified two regions that account for small but significant portions of this difference [21]. This extensive variation in flowering time between geographically close populations provides a promising system to study the ecological genetics of drought resistance.
Studies in more established systems have progressed to identify the molecular genetic basis and physiological mechanisms underlying drought escape. For instance, *Mimulus guttatus* is quickly becoming a model plant for studying drought escape and avoidance in natural populations [39]. The growing season of annual ecotypes of *M. guttatus* is ended by terminal drought; however, there is extensive variation in the timing and duration of the growing season. A survey of flowering time in a common environment that utilized 51 populations collected along 10 separate altitudinal transects demonstrated that populations with shorter growing seasons flower earlier under inductive conditions [16]. Reciprocal transplant experiments between annual and perennial *M. guttatus* ecotypes reveal a clear connection between earlier flowering and fitness in a population with a short growing season typically ended by drought [40]. QTL mapping following up on these reciprocal transplant experiments identified several regions contributing to fitness through earlier flowering and higher survival, including the candidate gene *DIV2* [41]. Subsequent work demonstrated that a single inversion that includes *DIV2* is responsible for a large portion of divergence in flowering time and fitness between ecotypes and that this inversion is widespread throughout the range of *M. guttatus* [42]. Interestingly, this QTL represented only 1 of 6 QTL found for flowering time, indicating that many additional loci of smaller effect also contribute to drought escape in this system [41]. Collectively, this work demonstrates a clear fitness advantage for rapidly developing plants in populations with drought-shortened growing seasons, as well as identifies an inversion as the molecular genetic basis for variation in drought escape. QTL studies such as the ones described above provide information about which genes or locations in the flowering regulatory network can be successfully tweaked to induce earlier flowering without producing negative tradeoffs in growth or reproduction.

The above examples target the genes involved in constitutive differences in drought escape, but less is known about drought escape as a plastic response to water deficit. Two experiments utilizing genetic knockouts in *Arabidopsis thaliana* have connected water availability cues to abscisic acid (ABA) signaling as well as to several genes in the flowering gene regulatory network [33,43]. Water deficit is largely perceived by the roots and promotes an increase in ABA levels throughout the plant. ABA promotes transcriptional upregulation of *FT*, *TSF*, and *SOC1* leading to flowering only under long days [33]. The expression of *GIGANTEA* (*Gl*), a member of the photoperiod pathway, is key in ensuring that flowering escape response only occurs under long day conditions. Under long day conditions, *Gl* is expressed and interacts independently of other photoperiod pathway members such as *FKP1*, *ZTL*, or *CO* to enable ABA-mediated expression of *FT* and *SOC1*. It is hypothesized that *Gl* regulates chromatin accessibility and blocks repressors of *FT* and *SOC1* (such as *SVP* and *FLC*) to allow drought escape responses to occur [33]. However, *Gl* does have other roles in starch metabolism and carbon signaling in *Arabidopsis* as well as interactions with the gibberellic acid signaling pathway that could promote expression of *FT* and lead to a drought escape response [44,45]. These links between the ABA signaling network, the flowering gene regulatory network, and water availability signals have not been fully synthesized into a coherent framework. Development of this connection is a major priority for understanding how drought escape responses are elicited and able to vary in natural populations.

While the role for heritable genetic variation in the evolution of constitutive and plastic drought escape is well established, the role of epigenetic variation is more controversial. There are numerous examples detailing how DNA methylation, histone modification, and chromatin remodeling regulate gene expression constitutively and in response to environmental cues such as drought [46]. However, researchers have yet to identify an epigenetic variant that underlies phenotypic differences in phenology and fitness differences within natural populations. In a set of *Arabidopsis* epirILs (lines with nearly identical genomes but contrasting DNA methylation profiles), variation in DNA methylation has been associated with heritable variation in flowering time as well as several other important ecological traits [47,48]. This experiment demonstrates the potential of epigenetic variation as an underlying molecular basis for adaptive evolution. Additionally, drought may act to environmentally induce epigenetic changes and lead to a drought escape response. Evidence that epigenetic variants create adaptive variation in drought escape in natural populations is currently nonexistent, but this does not mean it does not exist. Identifying the role of this variation is an open and important question in the drought literature given the significant role that epigenetic modification plays in eliciting stress responses [46].

### 3. The evolution of drought avoidance in natural populations

#### 3.1. Prevalence

Drought avoidance adaptations can occur through a myriad of morphological and physiological traits. Accessions adapted to xeric conditions may have lower specific leaf area [49], greater succulence [50], increased leaf reflectance [51], accentuated xeric lobing [52], and altered stomatal size and density [53], although these traits do not necessarily coordinate evolve in a drought avoidance syndrome. Many of these traits are expected to contribute to variation in transpiration efficiency, with drought-avoiding plants from xeric populations having greater WUE through higher photosynthetic capacity and lower transpiration. Transpiration efficiency is a measure of performance, and is approximated at leaf-level through both instantaneous WUE and an integrated measure of life-long transpirational efficiency (613C; [54] for review). In addition to reducing water loss, plants may slow or cease growth in response to incipient drought as a method of avoiding dehydration [55]. Adaptations conferring drought avoidance may also involve higher ratios of root to shoot growth prior to drought conditions [31]; this adaptation may be more advantageous for longer-lived perennial plants as a larger root system may provide a cost-effective advantage in multiple growing seasons [56,57]. Unfortunately, root to shoot ratio has proven difficult to measure in natural populations and better methodology is a key need for deciphering the importance of this phenotype. Here I focus on a drought avoidance strategy achieved by regulating WUE as this phenotype has constituted the majority of studies.

Similar to drought escape strategies, drought avoidance can evolve as a constitutive change in WUE or as a plastic response to water deficit or other environmental cues. Despite strong evidence that traits conferring drought avoidance are prevalent in populations located in xeric areas, there are few cases where xeric-adapted ecotypes have consistently higher WUE than mesic-adapted ecotypes across several environments [17,31,37,39]. Instantaneous WUE appears to be a more plastic phenotype than flowering time with a generally low broad sense heritability (h<0.1) [6]. Some plants with high instantaneous WUE in adequately watered conditions have low instantaneous WUE in water-limited conditions [23,58]. Indeed, accessions from xeric areas are frequently reported to have higher WUE than accessions from mesic areas only under drought conditions indicating that improved WUE is the product of a gene by environment interaction contingent on the presence of a water deficit [17,37,59]. Thus, although many studies have measured water-use efficiencies in lines from natural populations, few conclusions can be drawn about the prevalence of drought avoidance adaptations in nature. Future experiments need to examine the degree of plasticity in natural populations to draw more
nuanced conclusions about the frequency of drought avoidance adaptations.

3.2. Fitness consequences

The innate plasticity in WUE has made it difficult to characterize relationships between WUE and fitness in natural populations. Results from those that have measured fitness are mixed. Boechera holboellii from xeric environments had higher instantaneous WUE through lower stomatal conductance as well as increased investment in root biomass and lower leaf mass per unit area [31]. Plants from the xeric area were more fit in their native environment in a reciprocal transplant experiment suggesting that these phenotypic differences led to higher fitness in xeric conditions. Phenotypic selection analyses also suggest that plants with lower stomatal conductance are more fit in xeric environments [17]. However, in the natural populations where these experiments were conducted, plants from the xeric environment do not necessarily have higher WUE or lower stomatal conductance than plants from the mesic environments. This suggests that while higher WUE may be selected in one particular year, directional selection is not consistently strong enough to cause adaptive differentiation between populations from xeric and mesic areas. The results from other systems are more nuanced and context dependent. There is evidence for selection on high WUE varieties of Impatiens capensis during a normal relatively dry growing season [60], but low WUE plants that flower earlier were selected during an early season drought in a subsequent experiment [61]. This suggests that the type of drought strategy under selection depends on when drought stress occurs in development and the intensity of the drought. The fitness consequences of having higher WUE are often unintentional in annual plants. A series of papers contrasting xeric- and mesic-adapted ecotypes of Avena barbata find that plants that are able to acquire resources, develop quickly, and flower early are favored in both environments [62–64]. Notably, plants with lower stomatal conductance, such as that found in the xeric ecotype, had no advantage in any environment, but those with elevated rates of carbon fixation acquire resources and flower more quickly in both environments. Perhaps the xeric ecotype would be favored during years with more extreme drought conditions, and reciprocal transplant experiments failed to catch this variation. Collectively, these experiments suggest that WUE is not a simple performance phenotype that can be used easily for breeding.

3.3. Genetic basis

Given the phenotypic complexity of WUE it is not surprising that the physiological mechanisms connecting genetic variation with phenotypic variation are just beginning to be elucidated [53]. A limited number of genes underlying variation in WUE in natural populations have been identified. Two notable examples of genes underlying variation in WUE have recently been identified in divergent Arabidopsis thaliana accessions, Tus-1 and Kas-1. These accessions have distinctly different physiological strategies where Tus-1 plants flower earlier and have lower WUE than Kas-1 plants. This physiological difference is caused by loss of function allele at FRI (FRIDIGA) in Tus-1 plants conferring an enhanced drought escape strategy. The loss of a function FRI allele also causes decreased WUE through increased stomatal conductance [15,65,66].

A separate cross between divergent Arabidopsis lines Landsberg erecta (Ler) and Cape Verde Islands (CVI) have uncovered several small-effect QTL that underlie variation in stomatal conductance [22]. One of these QTL has a single non-synonymous substitution at a highly conserved glycine residue in the gene MPK12, and is responsible for a sizable portion of variance in stomatal conductance [59]. MPK12 encodes a protein that regulates stomatal guard cell physiology. Plants with this substitution have larger guard cells and stomatal openings. Interestingly, this substitution also has environment-dependent effects; when subjected to ABA treatment (as occurs following perception of water deficit), plants with this substitution are not able to retain stomatal closure as long as plants without this substitution. This suggests a partially impaired ability to respond to a critical environmental cue. All of the work conducted on drought avoidance has demonstrated the need for a greater understanding of gene by environment interactions [24]. Future progress in identifying the adaptive significance and genetic basis of drought avoidance will rely on developing a better understanding about how water deficit is perceived, as well as the pathways involved in signal transduction and developmental responses. A solid understanding of both the stomatal developmental pathway [67] and the ABA response pathway [68] already exists and connections are rapidly being synthesized. This framework is necessary for candidate gene approaches, such as those utilized in the above Arabidopsis research.

3.4. Resource reserves as drought avoidance adaptations

Much of the drought avoidance research has focused on transpiration efficiency despite the existence of other drought avoidance mechanisms. The production of compounds that act as stress protectants or stabilize critical proteins during water deficits are normally associated with the evolution of a drought tolerance strategy. However, plants may rely on either developing large stores of nutrients in prime conditions and/or diverting resources maintaining homeostasis during initial water deficits as a drought avoidance strategy. For example, Lupinus alba diverts organic nitrogen and carbohydrate resources from stems to seed pods at the first sign of water deficit. This reallocation results in similar seed set as plants that have not been subjected to drought stress [69]. Enhanced carbohydrate storage may be beneficial to all plants that frequently undergo drought stress as closing stomata to reduce water loss also reduces CO2 uptake and growth. Whether these short-term reserves are used to maintain homeostasis or support a reduced level of metabolism over longer stretches may depend on the history of drought intensity and frequency within a given population. Populations adapted to frequent short-term droughts may be able to maintain homeostasis, while those accustomed to longer droughts may reduce growth rates and adjust metabolism accordingly.

During the beginning stages of drought, nitrogen also becomes limited due to diminished nitrogen reductase activity. Thus, developing basic reserves of usable plant nitrogen would be useful during short-term droughts. Cyanogenic glucosides may play this nitrogen reserve role in white clover (Trifolium repens). The presence of cyanogenic glucosides is polymorphic in white clover, and the presence of cyanogenic glucosides has long been thought to act as an herbivore deterrent. New evidence suggests that the ability to produce cyanogenic glucosides also confers a fitness advantage under drought conditions [70]. Cyanogenic glucosides have been identified as a nitrogen transport or storage molecules in several other species [71], and the regulatory network detailing the breakdown of cyanogenic glucosides in useable plant nitrogen has been characterized in sorghum [72]. Further work must demonstrate that concentrations of cyanogenic glucosides decrease during drought, and levels of NO3− are maintained throughout short-term droughts. Other common molecules may serve a similar reservoir or buffering role. For instance, trehalose, a previously identified osmoprotectant, may serve as a reserve carbohydrate [73]. Identification of other such short-term reserves may serve as useful untapped variation for introducing into crop-breeding programs.
3.5. Genetic tradeoffs in the evolution of drought resistance

While drought escape and drought avoidance are frequently viewed as alternative strategies for adapting to drought stress, phenotypic selection analyses in the field suggest that directional selection acts to both increase WUE and reduce time to flowering if this variation is present [74]. Empirical studies have often provided evidence of negative genetic correlations between drought escape and avoidance traits, where plants that have evolved rapid development and earlier flowering often have reduced WUE and capacity for drought avoidance [14,15,17,22,66]. Physiologically, this negative correlation makes sense: if a plant closes its stomata to reduce stomatal conductance, there should be a reduction in photosynthetic rate due to limited CO₂ uptake that slows growth and development. Empirically, a negative genetic correlation between flowering time and WUE has been shown in Arabidopsis thaliana. This correlation is attributable to antagonistic pleiotropy at the FRI locus where a loss of function allele is associated with earlier flowering and lower WUE [15,65,66]. This result suggests that the evolution of drought resistance is subject to a genetic constraint that may prevent breeding programs from developing lines that have high WUE as well as rapid generation time.

However, several counterexamples to this trend have reported associations between flowering time and either δ¹³C or instantaneous WUE where earlier flowering plants also have higher WUE [16,29,37,75]. For instance, in a study examining Arabidopsis thaliana from populations along an aridity gradient in Northeastern Spain, populations that bolt earlier also have lower transpiration rates and higher WUE [29]. Notably, this result was opposite of the well-documented negative genetic correlation in A. thaliana described above (i.e. [15]). This discrepancy could be due to timing of when gas exchange rates were measured, the environmental context that the measurements were taken under or, in this specific example, a lack of the FRI allele from all populations sampled. A similar negative correlation between flowering time and WUE (δ¹³C) was found for Mimulus guttatus populations distributed across a series of aridity gradients (Fig. 2; [16]). This study also examined several traits (suculence, stomatal density, trichome number, and specific leaf area) often associated with WUE, and found little evidence for genetic correlations between traits associated with drought escape and avoidance within the most arid populations surveyed. A caveat to this study is that the common garden was conducted under well-watered conditions and not under water-limiting conditions or in a reciprocal transplant design. Other studies in the M. guttatus conducted under both wet and dry conditions demonstrate that the correlation between WUE and flowering time switches direction depending on environment [58]. An additional explanation for these results is that another unmeasured tradeoff axis may exist. For instance, plants that are able to escape or avoid drought may invest very little into vegetative growth or drought tolerance.

The combined results of these studies suggest that the adaptation to drought is not ubiquitously constrained to alternative strategies, and suggest that we have a limited understanding of the physiological constraints involved in evolving drought resistance. Notably, few studies examine genetic correlations between strategies within individual populations, where these constraints may actually impact the evolutionary trajectory of populations under drought stress. The role of genetic constraints in adaptation to drought stress require further study, particularly in identifying how often QTL underlying variation for flowering time and WUE colocalize, how much of this variation occurs within populations, and whether these correlations result in constraint to evolutionary responses. Future work should ideally utilize populations that both undergo frequent drought stress and where substantial variation in flowering time and WUE exist. It is also clear that future studies must test for genetic correlations in multiple environments as WUE is subject to gene by environment interactions.

4. A path toward understanding drought resistance adaptations in natural populations

Our understanding of the genetic mechanisms and the fitness tradeoffs associated with drought resistance adaptation has made impressive strides during the last decade. Notably, QTLs that identify the genetic basis of variation in drought escape and avoidance strategies have been found in many species (e.g. [21,22,41,37]) and have provided insight into the significant roles that genetic constraint and gene by environment interactions play in evolving drought resistance. Further, greenhouse and field studies have begun to elucidate the fitness ramifications of variation in drought escape and avoidance mechanisms. These discoveries have been made primarily utilizing natural populations, and suggest that wild populations offer a diverse resource for identifying novel mechanisms of drought resistance for both ecologists and agronomists. These studies have also highlighted areas where further research is needed for both evolutionary biologists and agronomists. It is clear that enhanced drought escape and avoidance is triggered via environmental cues. However, we currently lack a detailed understanding of how cues are integrated into physiological responses, and we have not identified genes or epigenetic variation responsible for variation in drought escape or avoidance responses. While these are concerns that plague genetic engineers, breeders should also consider the ramifications of environment-dependent effects. An enhanced drought escape or avoidance response may come at an unknown fitness or yield cost in other environments. While increased drought resistance could increase yield in during a drought year, it could decrease yield in a more typical year. Although this concern is not novel in traditional, trait-based, or molecular-assisted breeding programs,
explicitly assessing these costs across a range of environments is clearly necessary given the gene by environment interactions associated with these traits.

Future progress is also needed in determining how seasonal context and intensity of drought influences evolution of drought strategies and fitness of plants in natural populations. While Fig. 1 provides ecologically predictions describing the drought resistance strategy that should evolve, these predictions are largely untested and need to be addressed through experimental manipulation in field conditions. Much of this review has focused on terminal droughts that end the growing season as these droughts are often associated with strong selection for drought escape or avoidance. Less research has been devoted to transient or intermittent drought events, and understanding how plants respond and adapt in response would provide an interesting contrast. Given that intermittent drought events take place within a single generation, plastic responses limiting plant growth and increasing WUE should be highly beneficial. As greater fluctuations in precipitation become normal, understanding which drought resistance strategies are most efficient will obtain greater importance. This includes relatively understudied drought avoidance phenotypes such as enhanced root:shoot ratios and buffering molecules. If these phenotypes are responsible for large increases in fitness during droughts in natural populations, perhaps these hard to measure phenotypes would be worth pursuing for breeders and engineers.

A continuing priority is determining the importance of adaptive constraints in the evolution of drought resistance. Natural populations represent excellent systems to study whether genetic constraints between drought resistance syndromes exist and when they are adaptive. In natural populations different levels of genetic correlation exist in different populations, and nature provides an arena to study when these combinations are adaptive. Discovery of these constraints will provide insights into the indirect costs of selecting for certain traits such as flowering or heading earliness, and could identify new directions for breeding drought-resistant crops that also have high yield. It is naive to suggest that an ideal crop plant should employ both drought escape and avoidance strategies. An ideal variety allocates the maximum amount of resources to produce the highest yield in optimal conditions, and is capable of switching to a drought strategy during incipient drought conditions. The optimal drought strategy depends on the specific drought conditions of a region. Thus, focus should be directed to synthesizing or finding varieties that are able to respond to incipient drought conditions without sacrificing yield during optimal growing conditions. These conclusions indicate that understanding the benefits, costs, and basis of gene by environment interactions is critical for developing the next generation of crops and determining how populations will respond to unpredictable environments.

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