

LETTER

Ecological interactions and the fitness effect of water-use efficiency: Competition and drought alter the impact of natural *MPK12* alleles in *Arabidopsis*

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Abstract

The presence of substantial genetic variation for water-use efficiency (WUE) suggests that natural selection plays a role in maintaining alleles that affect WUE. Soil water deficit can reduce plant survival, and is likely to impose selection to increase WUE, whereas competition for resources may select for decreased WUE to ensure water acquisition. We tested the fitness consequences of natural allelic variation in a single gene (*MPK12*) that influences WUE in *Arabidopsis*, using transgenic lines contrasting in *MPK12* alleles, under four treatments; drought/competition, drought/no competition, well-watered/competition, well-watered/no competition. Results revealed an allele × environment interaction: Low WUE plants performed better in competition, resulting from increased resource consumption. Contrastingly, high WUE individuals performed better in no competition, irrespective of water availability, presumably from enhanced water conservation and nitrogen acquisition. Our findings suggest that selection can influence *MPK12* evolution, and represents the first assessment of plant fitness resulting from natural allelic variation at a single locus affecting WUE.

Keywords

Abiotic-by-biotic interaction, competition, drought stress, fitness, gene-by-environment interaction, *MPK12*, water-use efficiency.

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INTRODUCTION

Water is fundamental to nearly all aspects of plant development, growth, physiology and reproduction (Stebbins 1952), and most terrestrial plants experience soil drying stress to some degree during their lifecycle. This stress, driven by variable precipitation but also by the density of intra- and inter-specific competitors, has therefore likely imposed strong and recurring natural selection on how effectively plants expend their water supply (Bohnert *et al.* 1995). The interaction between the biotic and abiotic environment in determining resource availability is an essential but frequently overlooked determinant of the distribution and abundance of species and, by extension, the composition of communities (Chamberlain *et al.* 2014). Success in a competitive interaction, and thus a species' realised niche, is primarily a function of the basic physiological requirements of each species as they relate to the limited resources available (Chase & Leibold 2003). The extent to which an individual can adjust its physiological strategy as a function of the realised abiotic and biotic environment, termed phenotypic plasticity, is also a key, but underappreciated, determinant of success in ecological settings (Thuiller *et al.* 2013). The interaction of precipitation and competition is expected to result in water availability being highly variable across natural habitats, and hence drive the evolution of a variety of constitutive and plastic water-use strategies within and between species (Schulze 1986; Ludlow 1989). Despite the importance of understanding these varied water-use strategies to both natural and applied situations, and some limited understanding of their genetic basis (Kooyers

2015), we know very little about their ecological and evolutionary dynamics.

Water-use efficiency (WUE) is broadly defined as the ratio of water contributed to metabolic processes (e.g. carbon assimilation) to water lost via transpiration; that is relatively higher WUE plants acquire more biomass per unit of water transpired. As such, WUE describes a fundamental trade-off in land plant evolution: plants can actively transpire and photosynthesise at the biophysical limit, but in doing so they deplete a critical and often limited resource. Plant taxa which exhibit higher WUE typically enjoy greater biomass accumulation and fitness in both natural populations and crop species (e.g. Van Den Boogaard *et al.* 1997; Donovan *et al.* 2007), especially in hot and arid environments (Heschel *et al.* 2002), hence high WUE is often a desirable trait selected for among crop species and cultivars (Boyer 1982; Van Den Boogaard *et al.* 1997). The fitness advantages of high WUE imply that alleles for this trait should be strongly favoured and fixed in nature. However, substantial variation in WUE has persisted within many species (Heschel *et al.* 2002; McKay *et al.* 2003; Donovan *et al.* 2007), and several studies have documented non-random spatial patterns for WUE (e.g. clinal variation; Zhang *et al.* 1993), suggesting that natural selection is at least partially responsible for maintaining both high and low WUE genotypes. The ecological benefits provided by low WUE alleles are not always clear, although several hypotheses have been put forth. For example low WUE individuals may grow more rapidly and attain reproductive maturity prior to the onset of the dry season, as a mechanism for escaping drought (Ludlow 1989; Kooyers 2015). Alternatively,

in environments where water remains unlikely to be limiting, using water liberally may increase productivity by enhancing transpiration and photosynthesis, and/or improving leaf cooling (e.g. Crawford *et al.* 2012). Finally, by rapidly consuming water when it is available, low WUE individuals may gain a competitive advantage by suppressing nearby competitors via a reduction in local water availability (Donovan *et al.* 2007; Gremer *et al.* 2013). Therefore, evaluating the fitness consequences of high or low WUE under different ecological contexts is important for understanding optimal strategies for plant water use.

Differences in WUE can be driven by physiological, phenological, developmental, morphological and molecular trait variation, all likely to be under the genetic control of multiple potentially interacting loci (Chapin *et al.* 1996; McKay *et al.* 2003; Vasseur *et al.* 2014). While a detailed understanding of this complex trait will be extremely valuable for discerning general patterns, most studies that have evaluated the fitness consequences of WUE have done so by contrasting different species with varying levels of WUE or genotypes which carry different allelic combinations for several loci that affect WUE. In this study, we assess the impact of natural allelic variation in a single gene – shown to influence WUE in *Arabidopsis thaliana* (henceforth, *Arabidopsis*) – on fitness under representative ecological conditions. We build on a body of research focused on the genetic architecture of WUE in *Arabidopsis* (McKay *et al.* 2003; Hausmann *et al.* 2005; Juenger *et al.* 2005; Masle *et al.* 2005; Lovell *et al.* 2013; Des Marais *et al.* 2014; Easlon *et al.* 2014; Kenney *et al.* 2014), to explore how natural genetic variation in a single gene affects overall growth and fitness, with the goal of determining the environmental and ecological context under which high and low WUE alleles provides a selective advantage in the presence of realistic abiotic and biotic stressors.

Arabidopsis is an annual distributed across a broad climatic range, with origins throughout Eurasia and Africa, and so different accessions have likely adapted varying tolerance to different climates and levels of water availability (Hancock *et al.* 2011; Lasky *et al.* 2012). Heritable genetic differences in lifetime WUE between *Arabidopsis* accessions were first identified by McKay *et al.* (2003). Juenger *et al.* (2005) studied a relatively high WUE genotype, *Landsberg erecta* (*Ler*) and a relatively low WUE genotype, Cape Verde Island (CVI), and identified several quantitative trait loci (QTL; genomic regions potentially carrying genes responsible for phenotypic variation) that drive genetic variation in WUE among progeny from the *Ler* x CVI recombinant inbred mapping population. Juenger *et al.* (2005) generated a near isogenic line (NIL) capturing the CVI allele for a QTL occurring on chromosome 2 in a *Ler* genetic background, and demonstrated that NILs had reduced WUE, confirming that this QTL underlies WUE variation. Des Marais *et al.* (2014) identified MITOGEN ACTIVATED PROTEIN KINASE 12 (*MPK12*) as a candidate gene underlying the chromosome 2 QTL, and confirmed its functional role in WUE variability by transforming the *Ler-MPK12* alleles back into the NIL, and rescuing the higher WUE phenotype.

Despite the strong molecular and functional evidence for the influence of *MPK12* nucleotide variation on WUE, it remains

unknown whether these allelic variants can have differential effects on performance and lifetime fitness. The genetic lines that were constructed in our previous work (Des Marais *et al.* 2014) offer a unique opportunity to experimentally manipulate a fundamental physiological parameter of a species – water use during photosynthesis – to ask how it balances resource needs during a stressful abiotic and biotic challenge. In the current experiment, we address the following questions: (1) Do the *Ler*- and CVI-*MPK12* alleles lead to constitutive and/or plastic differences in WUE under drought and competitive environments? (2) How do these alleles affect fitness under different water regimes? (3) When competitors are introduced, does this alter the fitness rankings of the focal alleles? And (4) are other traits that contribute to fitness affected by *MPK12* allelic variation? Collectively, we aim to understand how species balance the immediate needs of a resource for survival with the threat of losing this resource to a competitor.

METHODS

Plant material

We used seeds from five *Arabidopsis* lines: *Landsberg erecta* (*Ler*-0), Cape Verde Islands (CVI-1), a NIL composed of *Ler* genomic background and an introgression containing CVI-*MPK12*, and two transgenic lines (TG1 and TG3) derived from NIL, but with *Ler-MPK12* re-inserted back into their genome; the production of NIL and TGs are described elsewhere (Juenger *et al.* 2005; Des Marais *et al.* 2014), and Table 1 provides a description. The parental lines, *Ler* and CVI, have climatically divergent origins, and have previously been shown to differ in several important physiological traits related to water-use strategies (e.g. flowering time, stomatal size, gas exchange and WUE: Alonso-Blanco *et al.* 1998; McKay *et al.* 2003; Hausmann *et al.* 2005; Juenger *et al.* 2005; Des Marais *et al.* 2014; Easlon *et al.* 2014; Vasseur *et al.* 2014). Given the physiological differences between *Ler* and CVI, we expected that lines containing either the *Ler*- or CVI-*MPK12* alleles would exhibit high and low WUE respectively (Table 1).

Experimental setup

Germination and establishment

Seeds were cold-stratified in 1.5 mL microcentrifuge tubes at 4°C for 7 days, and subsequently 5–10 seeds were sown using a micropipette into 9 cm² plastic pots (T.O. Plastics, Clearwater, FL, USA) filled with 2 parts Promix Biofungicide (Premier Tech, Rivière-du-Loup, QC, Canada) to 1 part Profile Field and Fairway (Profile, Buffalo Grove, IL, USA). Pots were placed in a growth chamber (22°C days/20°C nights, 14 h light at a photosynthetic photon flux density [PPFD] of 350 μmol m⁻²s⁻¹), then thinned to 1 plant, and transferred into a greenhouse at the University of Texas at Austin on June 21st 2014 when rosettes had achieved 7–8 leaves. Because of the comparatively high light conditions in the greenhouse (PPFD of ca. 1200 μmol m⁻²s⁻¹), we applied shade cloth and mist-watered daily for 5 days to facilitate

Table 1 Summary of Arabidopsis genetic lines used in this experiment

| Genotype* | Name | <i>MPK12</i> alleles | Expected WUE† | Description | Source |
|------------|-------------------------|--|---------------|--|---|
| Ler | Landsberg <i>erecta</i> | <i>Ler</i> only | High | Landsberg, Germany; temperate accession | Stock centre |
| CVI | Cape Verde Islands | CVI only | Low | Cape Verde Islands; sub-tropical accession | Stock centre |
| NIL | Near-isogenic line | CVI only | Low | <i>Ler</i> genetic background with a ~40 kb introgression containing CVI- <i>MPK12</i> alleles | Juenger <i>et al.</i> (2005); Des Marais <i>et al.</i> (2014) |
| TG1 | Transgenic 1 | CVI & <i>Ler</i> ; <i>Ler</i> dominant | High | Same as NIL, inserted <i>Ler</i> - <i>MPK12</i> gene | Des Marais <i>et al.</i> (2014) |
| TG3 | Transgenic 3 | CVI & <i>Ler</i> ; <i>Ler</i> dominant | High | Same as NIL, inserted <i>Ler</i> - <i>MPK12</i> gene | Des Marais <i>et al.</i> (2014) |

*Boldface indicates that these genetic lines were included in the analyses presented in the main text.

†Relative to other lines in the experiment.

acclimation; after which we removed the shade cloth and discontinued mist-watering. We allowed greenhouse conditions to fluctuate with ambient conditions in Austin; daytime temperatures regularly reached ca. 35°C (max 41°C) and nighttime temperatures were typically ca. 21°C (min 19°C), with ca. 14 h days. We began harvesting on day 48 when all individuals were no longer flowering or growing; harvest lasted 3 days, and plants were randomly chosen for harvest on each day.

Experimental treatments

We manipulated both water availability and competition in a fully factored design, giving four treatment combinations; well-watered/no competition (WetNocomp), well-watered/competition (WetComp), water limited/no competition (DryNocomp), water-limited/competition (DryComp). We used 5 genotypes × 4 treatments × 10 biological replicates = 200 plants total. Treatments were initiated upon transfer to the greenhouse. Pots in the competition treatments received 300 mg (ca. 100 seeds per pot) of common Gulf Annual Rye Grass seeds (*Lolium sp.*: Carolina[®], Burlington, VT, USA). Plants in both Wet treatments were bottom-watered every other day, and plants in both Dry treatments were bottom-watered every 6 days. Our goal with this watering regime was to create the following gradient of water availability: WetNocomp, WetComp, DryNocomp and Drycomp. We re-randomised the position of all plants every 6 days to minimise spatial effects within the greenhouse. We measured soil moisture using a time-domain reflectometry (TDR) soil moisture probe (Campbell Scientific Hydrosense, Logan, USA) on day 40 just prior to watering all plants, hence soil moisture differences – and water stress levels – would be at their maximum. We calibrated our probe based on a gravimetric soil moisture experiment (see supplemental material for details). We confirmed that our treatments created the water availability gradient described above (Treat_{water} × Treat_{comp} term in Table 2; Fig. S1).

Traits measured

We collected *Leaf count* as the total number of rosette leaves prior to bolting, as a metric of early vegetative growth. During the experiment we recorded *Bolt day* and *Flowering day* as the number of days to first inflorescence bolt and flower, respectively, and *Soil water content*. At experimental termination, we collected *Height* of the tallest inflorescence branch, *Number of bolts* as the number of inflorescence branches originating at the rosette, *Fruit length* as the mean of three

randomly selected fully developed fruits per plant (taken from separate inflorescences, where possible) and *Fruit number*. We harvested above ground material for both Arabidopsis individuals and the grass competitor, dried them at 55°C for 4 days, and then recorded *Arabidopsis biomass*, *Grass biomass* as a measure of competition, and their sum as a measure of *Community biomass*. We collected 3 mature fruits from 1 plant per genotype per treatment, and counted the number of seeds per fruit to estimate seeds/fruit length; seeds per length of fruit did not differ across treatments ($F_{3,15} = 1.75$, $P = 0.2$), but varied between genotypes ($F_{4,15} = 24.44$, $P < 0.0001$, Fig. S2A). As a result, to estimate seed count as a *Fitness* proxy, we multiplied *Fruits* × *Fruit length* × *Seeds per length* (genotype means) for every plant. We ground whole-dried Arabidopsis, and analysed them for carbon isotope composition (¹³C:¹²C, here termed $\delta^{13}C$) as a proxy for WUE (Farquhar & Richards 1984; Farquhar *et al.* 1989), and nitrogen content, at the University of California at Davis Stable Isotope Facility. We calculated two metrics for nitrogen; the concentration of *N* (mass per tissue weight), and *Total N* of above ground tissue ($N \times Arabidopsis biomass$).

Statistical analyses

We employed mixed model ANOVAS using proc mixed (Littell *et al.* 2006) in SAS version 9.4 (SAS Institute, Cary, NC, USA) using the following general model:

$$y = \text{intercept} + \text{Treat}_{\text{water}} | \text{Treat}_{\text{comp}} | \text{Allele} + \text{HarvestDate} + \text{error}$$

where ‘*y*’ is the focal trait, ‘Treat_{water}’ is either Wet or Dry, ‘Treat_{comp}’ is either Comp or Nocomp, ‘Allele’ is for the *MPK12* allele (*Ler* or CVI) and ‘|’ indicates all terms considered both separately and as interaction combinations; we treated genotypes and treatments as fixed effects. To account for the unbalanced design with regard to the number of plants carrying either allele, we implement a Kenward–Roger adjustment (Littell *et al.* 2006). We included the fixed effect ‘HarvestDate’ for all traits that were gathered at harvest to account for multi-day harvesting. We used pairwise contrasts (pdiff statement in SAS) to explore differences between lsmeans of allelic values within each treatment level. All the traits were approximately normally distributed, and transformations did not improve normality, and model residuals were normal; we thus used non-transformed trait values in our analyses.

Table 2 Mixed model ANOVA results for the fixed effects of MPK12 alleles, treatments and their combinations on the fitness components measured here

| Source | Traits (fitness components) | | | | | | | | | | | |
|---|-----------------------------|--------------|---------------|-----------------------|-----------------|----------------|---------------------|----------------|-----------------|-----------------|--------------------|-------------------|
| | Leaf count | Bolt day | Flowering day | $\delta^{13}\text{C}$ | <i>N</i> | Total N | Arabidopsis biomass | Height | Number of bolts | Grass biomass | Soil water content | Community biomass |
| Treat _{water} | NA | NA | NA | 30** | 13.23* | 32.89** | 24.38** | 41.09** | 0.02 | 139.16** | 582.79** | 213.72** |
| Treat _{comp} | NA | NA | NA | 0.53 | 660.87** | 433.9** | 217.04** | 92.81** | 2.28 | NA | 183.18** | NA |
| Allele | 3.37 | 7.41* | 15.1** | 98.2** | 3.89* | 5.78* | 3.83* | 32.84** | 10.13* | 4.63* | 0.37 | 5.24* |
| Treat _{water} × Treat _{comp} | NA | NA | NA | 35.8** | 1.4 | 10.51* | 3.98* | 4.46* | 3.73 | NA | 5.95* | NA |
| Allele × Treat _{water} | NA | NA | NA | 0.67 | 5.82* | 0.7 | 0.03 | 3.86 | 0.04 | 1.96 | 0.01 | 0.37 |
| Allele × Treat _{comp} | NA | NA | NA | 0.02 | 0.02 | 4.23* | 3.42 | 4.14* | 1.16 | NA | 4.45* | NA |
| Allele × Treat _{water} × Treat _{comp} | NA | NA | NA | 2.58 | 3.68 | 0.07 | 1.45 | 0.01 | 4.36* | NA | 3.12 | NA |
| HarvestDate | NA | NA | NA | 4.29* | 40.13** | 4.22* | 0.87 | 1.14 | 0.68 | 31.37** | NA | 32.78** |
| Tray | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | 13.76** | NA |

Boldface alone indicates marginal significance; $0.05 < P < 0.1$.

Asterisk indicates significance; * $P < 0.05$, ** $P < 0.001$.

NA indicates effect not included in model.

Fitness

We were primarily interested in the *allelic* effects of *MPK12* on fitness, and so describe those models here. For results regarding the *genotypic* effects, see the supplementary material. We assessed the fitness effects of *MPK12* alleles by including allele (CVI or *Ler*) as a fixed term, and adding the random term *Genotype* nested in *Allele* to account for inherent differences between lines caused by the introgression in NIL, and positional effects in the transgenics. We elected to remove original CVI individuals from the dataset to ensure that we were directly testing only *MPK12* allelic variation; this provides the purest comparison of *Ler-MPK12* and CVI-*MPK12* alleles because all other genetic variation is removed by using only lines with a *Ler* background. Previous work by Des Marais *et al.* (2014) shows that although transgenic lines contain *Ler*- and CVI-*MPK12* alleles, their phenotype is *Ler*, suggesting that *Ler-MPK12* is dominant.

Fitness components

We explored if *Leaf count*, *Bolt day* and *Flowering day* differed between alleles only; we did not include a treatment term here because all plants had bolted/flowered before treatments began. For the remaining traits, we compared them between treatments and alleles by setting each as the response variable, separately, using the same model structure described above.

We then tested whether *MPK12* alleles significantly altered both competitive ability and water consumption. To test competitive strength and overall effect of WUE on community productivity, we set *Grass biomass* or *Community biomass* as the response variable respectively. Here, we looked for variation between alleles within both competition treatments only; we interpreted a reduction in *Grass biomass* as an increased competitive effect of the focal *MPK12* allele, and increased *Community biomass* as greater productivity. To test water consumption, we set *Soil water content* as the response variable, and interpreted lower *Soil water content* as the focal *Arabidopsis* plant – and its competitors, if present – removing soil

water at a faster rate. In this model we included the fixed effect ‘Tray’ to account for plant position in the greenhouse because we expected greenhouse spatial heterogeneity to impact soil water differently during the 5 days between the previous re-randomisation and the time of measurement.

RESULTS

Fitness

Allelic fitness was strongly impacted by both treatments, especially competition (Fig. 1a and b; Table S1): In DryComp the alleles did not differ for fitness ($t = 1.08$, $P = 0.28$), potentially resulting from the severity of the treatment combination relative to the allelic effect. In DryNocomp, *Ler* alleles performed better, potentially a result of higher WUE ($t = 2.21$, $P = 0.029$). In WetComp, CVI alleles outperformed *Ler* alleles, suggesting a greater competitive ability ($t = 2.05$, $P = 0.042$). Finally, in WetNocomp, *Ler* alleles once again outperformed CVI alleles, suggesting that some factor other than water is now limiting low WUE individuals ($t = 2.26$, $P = 0.026$). There was a strong Allele × Treat_{comp} effect, indicating that *MPK12* alleles significantly change fitness rank depending on the competitive environment; CVI-*MPK12* plants performed better in competition, irrespective of water availability (Fig. 1b; Table S1), suggesting that reduced WUE may be beneficial when facing competition. Although water availability was important for fitness, there was no interaction effect of Allele × Treat_{water} (Fig. 1c; Table S1), indicating that *MPK12* variation may not be important across the degree of soil moisture variation generated by our irrigation timing alone. Given the significant interaction of competition on allelic fitness, we focus many of the following comparisons on this treatment.

$\delta^{13}\text{C}$ and Nitrogen content

Water-use efficiency, measured as $\delta^{13}\text{C}$, was significantly more negative in CVI plants across all environments

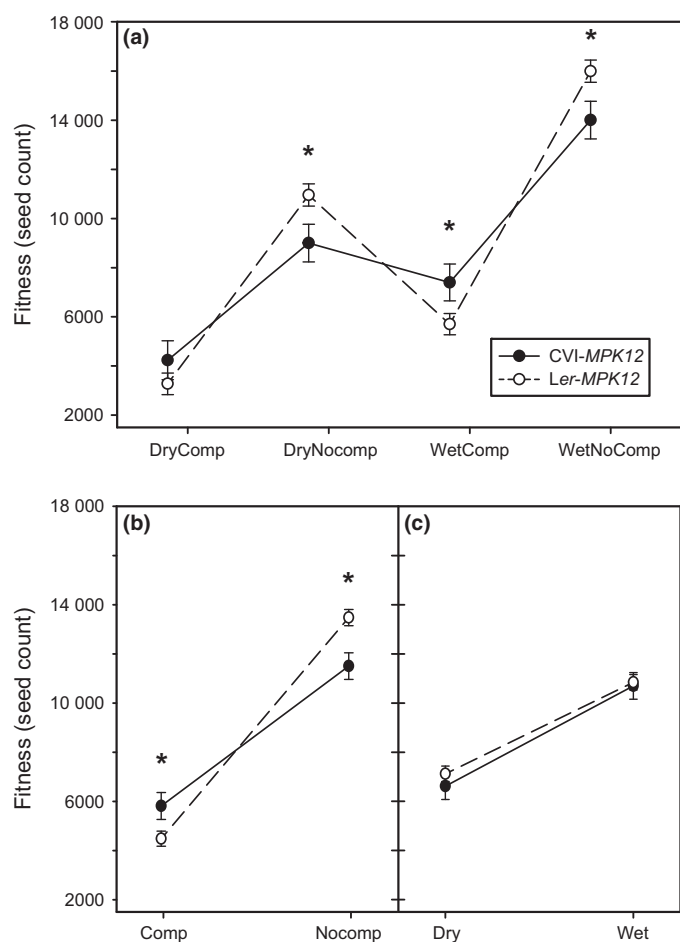


Figure 1 (a) Fitness, estimated as a seed count (lmeans \pm 1 SE), in the four different experimental treatments grouped by *MPK12* allelic class. Estimated fitness for *MPK12* allelic classes when considering (b) competition treatments and (c) water availability treatments, separately. Asterisks represent significant pairwise comparisons at the $P = 0.05$ level. Only *Arabidopsis* lines with *Ler* genetic backgrounds are presented.

(Fig. 2a, Table 2), by a magnitude consistent with previous findings (Juenger *et al.* 2005; Des Marais *et al.* 2014), confirming that CVI-*MPK12* alleles exhibit reduced lifetime integrated WUE. In the presence of competitors, water availability strongly influenced $\delta^{13}\text{C}$ across both allelic classes, with both *Ler* and CVI being most conservative in dry conditions and least conservative in wet competition (indicated by a significant $\text{Treat}_{\text{comp}} \times \text{Treat}_{\text{water}}$ term; Table 2). This pattern suggests that the combination of infrequent watering and competition generates dry enough soils to induce stomatal closure and reduce water loss. Interestingly, we found that the ranking of WUE across treatments did not mirror the pattern expected from the ranking of soil moisture content across treatments. In particular, we found that the lowest WUE occurred in well-watered pots with competition rather than in the absence of competition, suggesting a key role of competition in the regulation of *Arabidopsis* physiology.

N and $\text{Total } N$ were strongly affected by both treatments, especially competition (Fig. 2b; Table 2). In the DryNocomp

treatment, *Ler-MPK12* plants had more nitrogen, and after accounting for treatment, *Ler-MPK12* had overall greater N (Allele term; Table 2), suggesting that the *Ler* allele enhances nitrogen uptake. The presence of competition affected nitrogen uptake more severely than water deficit, likely resulting from the competitors acquiring much of the available nitrogen. On a per tissue basis, there was a significant interaction of allele and water availability (Allele \times $\text{Treat}_{\text{water}}$; Table 2) with *Ler* alleles having greater N in dry soils, but no difference in wet soils. On a whole plant level, there was a significant allele by competition interaction (Allele \times $\text{Treat}_{\text{comp}}$; Table 2) with *Ler* alleles having greater $\text{Total } N$ when free of competition, but no difference when in competition.

Fitness components

Both early vegetative growth rate and the onset of reproduction were affected by allelic variation; CVI-*MPK12* plants produced slightly more rosette leaves and exhibited an accelerated reproductive phenology (Fig. 3a and b; Table 2), suggesting that differences in growth and phenology can be driven by *MPK12* allelic variation. The two alleles differentially affected *Arabidopsis* biomass in that *Ler-MPK12* plants had significantly greater biomass (Allele term in Table 2). While both treatments influenced biomass, the presence or absence of competition marginally interacted with allelic variation for biomass, with *Ler* having significantly greater biomass in the absence of competitors, and no difference between alleles in the presence of competition (Fig. 3c; Table 2). CVI-*MPK12* individuals produced more primary bolts when in competition, whereas *Ler-MPK12* individuals were overall taller (Fig. 3b and c; Table 2).

Competitive strength and soil moisture

CVI-*MPK12* individuals were stronger competitors, especially in a wet environment, indicated by the reduced biomass of the grass competitors (Fig. 4a; Allele term in Table 2). Interestingly, although CVI plants exhibit relatively lower WUE in all treatments (Fig. 2), when facing competition CVI plants in combination with their competitors did not dry their pots faster than *Ler* individuals (competition treatment; Fig. 4b). This finding is likely the result of a reduced total community biomass produced by CVI and their competitors compared to *Ler*, especially in wet conditions (Fig. 4c, Allele term in Table 2). When competitors were absent, *Ler* individuals consumed less soil water, supporting greater WUE than CVI, and leading to a significant Allele \times $\text{Treat}_{\text{comp}}$ interaction for soil water content (Fig. 4b; Table 2).

DISCUSSION

Habitat variation in the degree of both water availability and competition has likely imposed natural selection on different water-use strategies. For example high WUE may be favoured in water limited conditions as a method for maintaining water status, and low WUE genotypes may be more resistant to competition by effectively stealing water resources

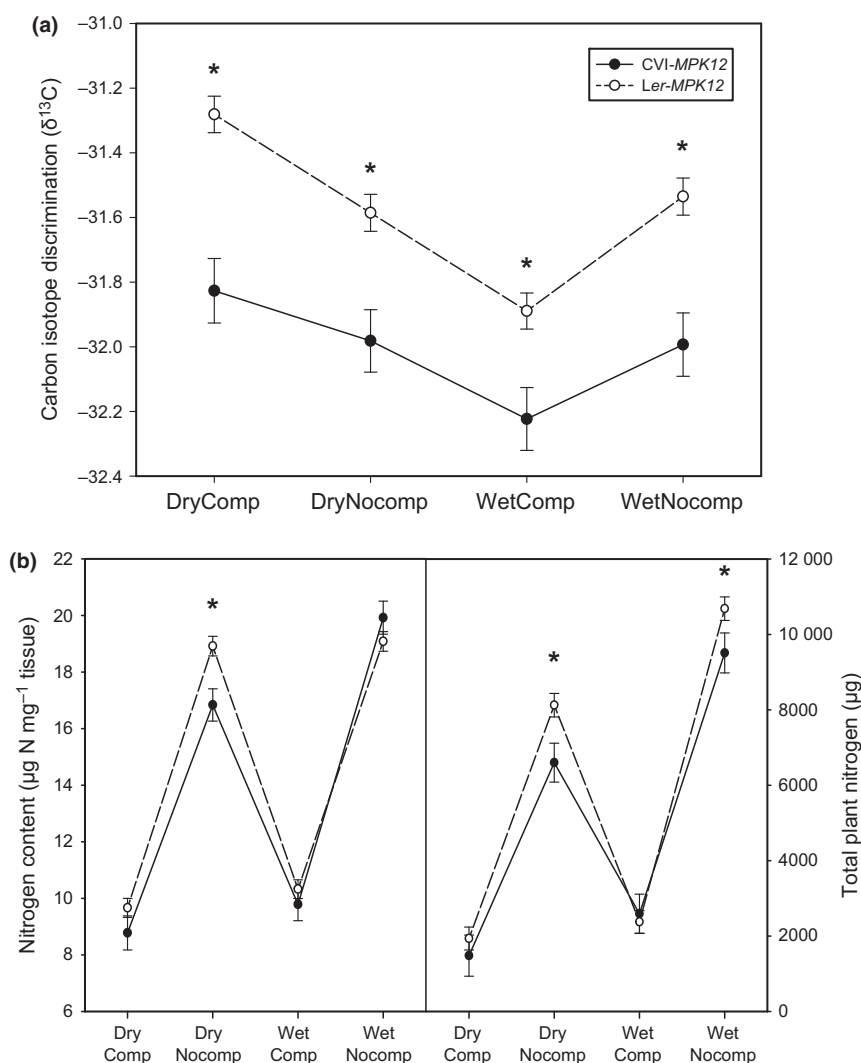


Figure 2 (a) Carbon isotope discrimination of alternative *MPK12* alleles for above ground biomass only. More negative carbon discrimination values indicate lower integrated lifetime water-use efficiency. (b) Nitrogen content per plant tissue weight and whole plant nitrogen content of above ground biomass only. Points represent lsmeans \pm 1 SE, and asterisks represent significant pairwise comparisons at the $P = 0.05$ level. Only Arabidopsis lines with *Ler* genetic backgrounds are presented.

from competitors. As a result, divergent natural selection can potentially act on genetic variation for WUE in nature and maintain locally adapted genotypes due to spatially or temporally varied selection pressures. Here, we tested the ecological importance of natural allelic variation at a single gene shown to affect WUE in Arabidopsis, under two interacting ecologically relevant conditions: competition and drought. Our experiment revealed three major findings: (1) The CVI-*MPK12* allele constitutively drives a reduction in WUE under all conditions; (2) Low WUE is beneficial when plants were faced with competition, whereas the opposite pattern was observed in the absence of competition, irrespective of our irrigation treatment; (3) This fitness interaction appears to be driven by the enhanced ability of CVI allele to suppress its neighbouring competitors, whereas the *Ler* allele increases biomass and nutrient accumulation in the absence of competitors. We elaborate on our findings in the following sections.

Low WUE leads to greater competitive performance and drought escape

The enhanced competitive ability of low WUE plants is perhaps not surprising given the common association between liberal water use and high growth rates (Angert *et al.* 2007; Huxman *et al.* 2008; Kimball *et al.* 2013, 2014), which typically confers stronger competitive ability, as has been found in competition experiments that utilise the CVI \times *Ler* cross (Fakheran *et al.* 2010). However, patterns can vary under different water regimes. For example in their work on desert annual species, Gremer *et al.* (2013) demonstrated that a relatively lower WUE species exerted the strongest competitive effect on its higher WUE neighbours in well-watered conditions, but was most strongly affected by – and had a much weaker competitive effect on – its neighbours in dry soils. Similarly, we found that in competition the low WUE allele (CVI) conferred higher fitness as compared to the *Ler* allele

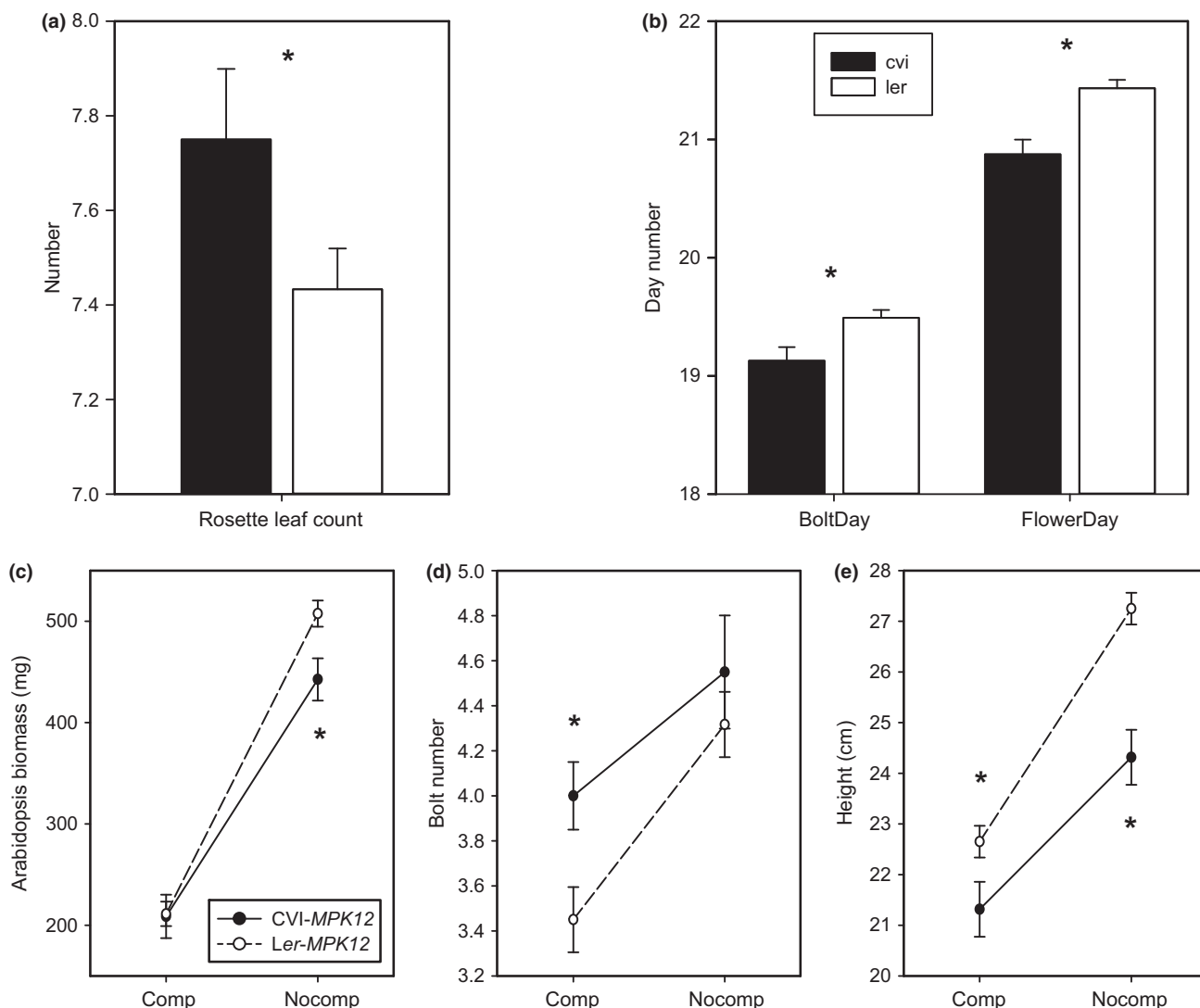


Figure 3 The effect of *MPK12* alleles on (a) early vegetative growth, and (b) reproductive phenology. (c–e) Comparison of the interaction between alternative *MPK12* alleles and competition on Arabidopsis above ground biomass, the number of primary bolts produced and height of the tallest bolt. All bars and points are ± 1 SE, and asterisks indicate a significant pairwise comparison at the $P = 0.05$ level. Only Arabidopsis lines with *Ler* genetic backgrounds are presented.

(Fig. 1b), and had a greater competitive effect (defined by Goldberg 1996) on their neighbouring grass (Fig. 4a and c), particularly in wet conditions. Furthermore, although CVI had greater fitness overall in competition, we found that the competitive benefit of *CVI-MPK12* was greatly diminished under dry conditions, as illustrated by a non-significant fitness difference in the dry-competition treatment. Our results further suggest that low WUE enabled CVI plants to consume more water, grow rapidly, flower early and increase branchiness, potentially giving them an advantage in competition (Fig. 3). We did not expect to find phenological differences between *Ler* and CVI, given that *MPK12* does not overlap with known flowering time QTL (Juenger *et al.* 2005; Vasseur *et al.* 2014). However, Guilbaud *et al.* (2015) present a model that proposes flowering time cues may also be triggered by growth rate changes, which our results support. In addition, our findings indicate that *CVI-MPK12* may also contribute to

a drought escape strategy (rapid growth and earlier reproduction: Ludlow 1989; Kooyers 2015), reflecting patterns observed across Arabidopsis accessions (e.g. McKay *et al.* 2003; Juenger *et al.* 2005; Lovell *et al.* 2013; Kenney *et al.* 2014) and genotypes that experience early-season drought in other taxa (e.g. Dudley 1996; Geber & Dawson 1997; Franks *et al.* 2007).

High WUE is favourable in non-competitive environments

Under a non-competitive environment, higher WUE was favoured, irrespective of soil water availability (Fig. 1). This pattern is commonly observed in environments that experience water deficit (Dudley 1996; Heschel *et al.* 2002; Heschel & Riginos 2005; Donovan *et al.* 2007; McKay *et al.* 2008); if an individual has exclusive access to the available soil water, conserving water enables high WUE plants to maintain functional

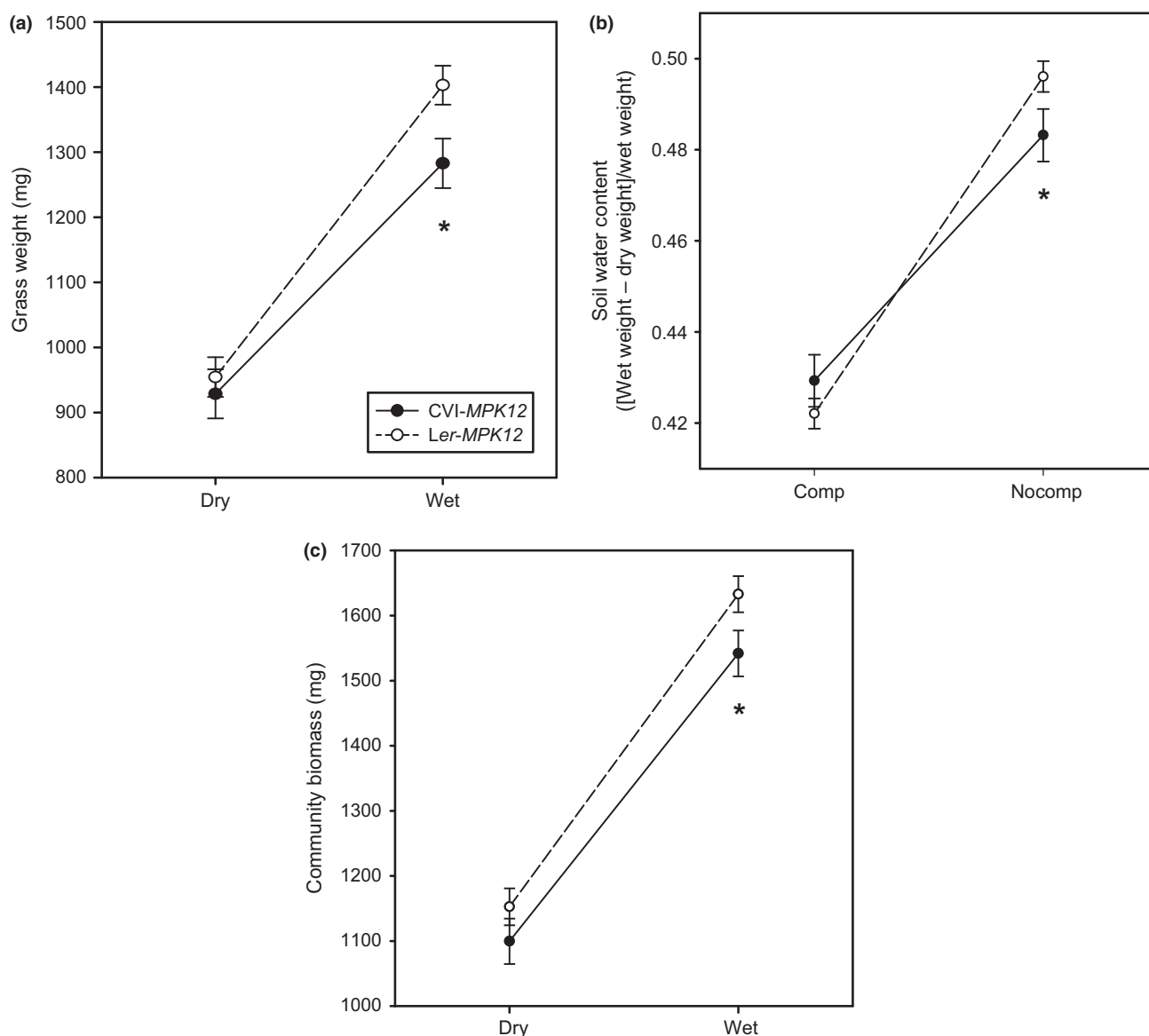


Figure 4 (a) Competitive strength of *Arabidopsis MPK12* alleles, as estimated by the biomass of grass competitors; a reduction in grass weight indicates stronger competitive ability. (b) Comparison of soil moisture between *MPK12* alleles in competition or non-competitive environments. (c) The effect of water availability on total community biomass (*Arabidopsis* biomass + Grass biomass) within the competition treatment only. All points represent 1smeans \pm 1 SE, and asterisks indicate significant pairwise contrasts at the $P = 0.05$ level. Only *Arabidopsis* lines with *Ler* genetic backgrounds are presented.

water status and avoid dehydration until the next bout of precipitation. To our surprise, however, high WUE plants also performed better in WetNocomp conditions. Contrary to our finding, we expected low WUE plants to exhibit enhanced transpiration and nutrient uptake in WetNocomp, which typically leads to a greater photosynthetic capacity (Field *et al.* 1983; Donovan *et al.* 2011; Brouillette *et al.* 2014). While we did find evidence for greater transpiration in low WUE lines (lower $\delta^{13}\text{C}$ and soil moisture; Figs. 2a and 4b), we did not observe greater nutrient uptake. Instead, the high WUE allele enabled individuals to obtain more nitrogen in both non-competition treatments (Fig. 2b). This result suggests that *Ler-MPK12* individuals are more efficient at acquiring nutrients from the soil when there are no competitors, potentially

improving their photosynthetic performance, overall biomass and reproductive output.

It is currently unclear how *Ler-MPK12* increases nutrient uptake. *MPK12* is expressed in roots and has been shown to play a role in root development (Lee *et al.* 2009). Hence, one possibility is that *Ler-MPK12* increases initial root investment, and hence greater overall access to soil N. *CVI-MPK12* may allocate more early growth to above ground tissue and flowering, and hence fails to effectively explore the soil for N. Alternatively, *CVI-MPK12* plants may deplete their soil moisture rapidly or often enough that it hinders their ability to acquire soil N, either by reduced mobilisation of N, or because N becomes difficult to extract from dry soils due to physical property changes of the soil (e.g. Clarke & Barley

1968; Hu *et al.* 2009). Future work exploring the N-use strategies of alternative *MPK12* alleles will help disentangle these hypotheses. To our knowledge, no study has documented a functional role for *MPK12* in biomass accumulation, growth architecture or nitrogen uptake/utilisation. As such, our study extends the pleiotropic role of natural variation in *MPK12* in Arabidopsis growth and development.

Selection on genetic polymorphisms

We have presented a novel demonstration of environmentally variable selection acting on a natural genetic polymorphism, leading to an allele \times environment interaction. This finding suggests an ecological mechanism for the maintenance of variation in WUE. The evolutionary history of *MPK12* natural variation is, however, not well understood, and further research exploring *MPK12* population genetics, especially in Cape Verde Islands (where the rare CVI allele is found), will be necessary for confirming our hypotheses. Given that Cape Verde Islands are sub-tropical, and the CVI accession originates from a habitat that receives relatively high precipitation, we hypothesise that there is relaxed selection for high WUE, and increased selection for more competitive low WUE alleles. However, it is presently unknown if CVI-*MPK12* has fixed across its natural Cape Verde Islands range, and if this resulted from selection or genetic drift, or if this mutation is rare even within CVI populations. Our previous work suggested that this mutation is unique to CVI (Des Marais *et al.* 2014), and a survey of additional Arabidopsis accessions recently sequenced for the 1001 genomes project (<http://signal.salk.edu/atg1001/index.php>) currently confirms this. However, the CVI-*MPK12* allele is not the only natural variant in *MPK12*. Indeed, we observe evidence of spatially structured genetic variation in *MPK12* – measured as F_{ST} – in a single nucleotide polymorphism (SNP) in the intergenic sequence immediately upstream of *MPK12* (Horton *et al.* 2012). This SNP is also correlated with minimum precipitation during the driest month in a climate genome wide association study (Hancock *et al.* 2011). (Note: this SNP is not necessarily a functional variant, and is most likely genetically linked to one or more variants actually driving phenotypic differences among accessions.) Collectively, these results suggest that *MPK12* may be a recurring target of selection for local adaptation to climate.

Competition for water, WUE variation and community productivity

An interesting outcome of our experiment was that low WUE individuals were very effective at suppressing their competing neighbours through individual competition for water, so much so that both community biomass and overall water consumption by the community was low relative to their high WUE counterparts (see Fig. S3). This finding could have important implications when considering total productivity and/or biodiversity in natural systems under water limitation and in terms of the overall density of competitors. For example dense communities consisting of low WUE plants are likely to experience elevated and intense competition, potentially causing the overall suppression productivity and less total water consump-

tion, whereas high WUE genotypes may increase community productivity but consume more total water. Our results highlight the importance of considering how changes in the abiotic environment may affect biotic interactions, and vice versa, supporting the necessity of incorporating both abiotic and biotic components into ecosystem biodiversity models (Clark *et al.* 2014a,b). However, our experiment compares the influence of low and high WUE alleles on competitive ability, and fitness only indirectly, as we draw our conclusions based on competition assays involving a non-related grass species. Our future work in this system will compete the Arabidopsis lines outlined here directly against one another, with the goal of assessing how WUE variation impacts fitness under conspecific competition.

The recent ecological literature has seen considerable interest in understanding how species interactions can change under varying abiotic conditions (Chamberlain *et al.* 2014), and how the biotic and abiotic environments may interact to generate new conditions that affect these interactions, and in turn influence community composition (Clark *et al.* 2014a,b). The research we have presented here examined the consequences of competition, water deficit and their combination on plant fitness, and how genetic variation for WUE can interact with these environmental conditions and impact the magnitude of their consequences. Our work revealed that variation in even a single gene can have a major effect on fitness, mediated through its interaction with the biotic and abiotic environments, representing a novel contribution in support of the notion outlined above. We believe further research in this area will provide valuable insight into understanding the processes that structure communities, and how they may respond to future predicted environments.

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AUTHORSHIP

BEC, DLD and TEJ conceived the idea. BEC carried out the experiment. BEC and TEJ analysed the data. BEC wrote the first draft, and all authors contributed substantially to revisions.

REFERENCES

- Alonso-Blanco, C., El-Assal, S.E., Coupland, G. & Koornneef, M. (1998). Analysis of natural allelic variation at flowering time loci in the Landsberg *erecta* and Cape Verde Islands ecotypes of *Arabidopsis thaliana*. *Genetics*, 149, 749–764.
- Angert, A.L., Huxman, T.E., Barron-Gafford, G.A., Gerst, K.L. & Venable, D.L. (2007). Linking growth strategies to long-term population dynamics in a guild of desert annuals. *J. Ecol.*, 95, 321–331.

- Bohnert, H.J., Nelson, D.E. & Jensen, R.G. (1995). Adaptations to Environmental Stresses. *Plant Cell*, 7, 1099–1111.
- Boyer, J.S. (1982). Plant productivity and environment. *Science*, 218, 443–448.
- Brouillette, L.C., Mason, C.M., Shirk, R.Y. & Donovan, L.A. (2014). Adaptive differentiation of traits related to resource use in a desert annual along a resource gradient. *New Phytol.*, 201, 1316–1327.
- Chamberlain, S.A., Bronstein, J.L. & Rudgers, J.A. (2014). How context dependent are species interactions? *Ecol. Lett.*, 17, 881–890.
- Chapin, F.S. III, Autumn, K. & Pugnaire, F. (1996). Evolution of suites of traits in response to environmental stress. *Am. Nat.*, 142, S78–S92.
- Chase, J.M. & Leibold, M.A. (2003). *Ecological Niches: Linking Classic and Contemporary Approaches*. University of Chicago Press, Chicago.
- Clark, J.S., Bell, D.M., Kwit, M.C. & Zhu, K. (2014a). Competition-interaction landscapes for the joint response of forests to climate change. *Glob. Chang. Biol.*, 20, 1979–1991.
- Clark, J.S., Gelfand, A.E., Woodall, C.W. & Zhu, K. (2014b). More than the sum of the parts: forest climate response from joint species distribution models. *Ecol. Appl.*, 24, 990–999.
- Clarke, A.L. & Barley, K.P. (1968). The uptake of nitrogen from soils in relation to solute diffusion. *Soil Res.*, 6, 75–92.
- Crawford, A.J., McLachlan, D.H., Hetherington, A.M. & Franklin, K.A. (2012). High temperature exposure increases plant cooling capacity. *Curr. Biol.*, 22, R396–R397.
- Des Marais, D.L., Auchincloss, L.C., Sukamtoh, E., McKay, J.K., Logan, T., Richards, J.H. et al. (2014). Variation in MPK12 affects water use efficiency in *Arabidopsis* and reveals a pleiotropic link between guard cell size and ABA response. *Proc. Natl Acad. Sci. USA*, 111, 2836–2841.
- Donovan, L.A., Dudley, S.A., Rosenthal, D.M. & Ludwig, F. (2007). Phenotypic selection on leaf water use efficiency and related ecophysiological traits for natural populations of desert sunflowers. *Oecologia*, 152, 13–25.
- Donovan, L.A., Maherali, H., Caruso, C.M., Huber, H. & de Kroon, H. (2011). The evolution of the worldwide leaf economics spectrum. *Trends Ecol. Evol.*, 26, 88–95.
- Dudley, S.A. (1996). Differing selection on plant physiological traits in response to environmental water availability: a test of adaptive hypotheses. *Evolution*, 50, 92–102.
- Easlon, H.M., Nemali, K.S., Richards, J.H., Hanson, D.T., Juenger, T.E. & McKay, J.K. (2014). The physiological basis for genetic variation in water use efficiency and carbon isotope composition in *Arabidopsis thaliana*. *Photosynth. Res.*, 119, 119–129.
- Fakheran, S., Paul-Victor, C., Heichinger, C., Schmid, B., Grossniklaus, U. & Turnbull, L.A. (2010). Adaptation and extinction in experimentally fragmented landscapes. *Proc. Natl Acad. Sci. USA*, 107, 19120–19125.
- Farquhar, G.D. & Richards, A. (1984). Isotopic Composition of Plant Carbon Correlates with Water-use Efficiency of Wheat Genotypes. *Australian Journal of Plant Physiology*, 11, 539–552.
- Farquhar, G.D., Ehleringer, J. & Hubick, K. (1989). Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology*, 40, 503–537.
- Field, C.B., Merino, J. & Mooney, H.A. (1983). Compromises between water-use efficiency and nitrogen-use efficiency in five species of California evergreens. *Oecologia*, 60, 384–389.
- Franks, S.J., Sim, S. & Weis, A.E. (2007). Rapid evolution of flowering time by an annual plant in response to a climate fluctuation. *Proc. Natl Acad. Sci. USA*, 104, 1278–1282.
- Geber, M.A. & Dawson, T.E. (1997). Genetic variation in stomatal and biochemical limitations to photosynthesis in the annual plant, *Polygonum arenastrum*. *Oecologia*, 109, 535–546.
- Goldberg, D.E. (1996). Competitive ability: definitions, contingency and correlated traits. *Philos. Trans. R. Soc. B*, 351, 1377–1385.
- Gremer, J.R., Kimball, S., Keck, K.R., Huxman, T.E., Angert, A.L. & Venable, D.L. (2013). Water-use efficiency and relative growth rate mediate competitive interactions in Sonoran Desert winter annual plants. *Am. J. Bot.*, 100, 2009–2015.
- Guilbaud, C.S.E., Dalchau, N., Purves, D.W. & Turnbull, L.A. (2015). Is “peak N” key to understanding the timing of flowering in annual plants? *New Phytol.*, 205, 918–927.
- Hancock, A.M., Brachi, B., Faure, N., Horton, M.W., Jarymowycz, L.B., Sperone, F.G. et al. (2011). Adaptation to climate across the *Arabidopsis thaliana* Genome. *Science*, 334, 83–86.
- Hausmann, N.J., Juenger, T.E., Sen, S., Stowe, K.A., Dawson, T.E. & Simms, E.L. (2005). Quantitative trait loci affecting $\delta^{13}\text{C}$ and response to differential water availability in *Arabidopsis thaliana*. *Evolution*, 59, 81–96.
- Heschel, M.S. & Riginos, C. (2005). Mechanisms of selection for drought stress tolerance and avoidance in *Impatiens capensis* (Balsaminaceae). *Am. J. Bot.*, 92, 37–44.
- Heschel, M.S., Donohue, K., Hausmann, N. & Schmitt, J. (2002). Population differentiation and natural selection for water-use efficiency in *Impatiens capensis* (Balsaminaceae). *Int. J. Plant Sci.*, 163, 907–912.
- Horton, M.W., Hancock, A.M., Huang, Y.S., Toomajian, C., Atwell, S., Auton, A. et al. (2012). Genome-wide patterns of genetic variation in worldwide *Arabidopsis thaliana* accessions from the RegMap panel. *Nat. Genet.*, 44, 212–216.
- Hu, T., Kang, S., Li, F. & Zhang, J. (2009). Effects of partial root-zone irrigation on the nitrogen absorption and utilization of maize. *Agric. Water Manag.*, 96, 208–214.
- Huxman, T.E., Barron-Gafford, G., Gerst, K.L., Angert, A.L., Tyler, A.P. & Venable, D.L. (2008). Photosynthetic resource-use efficiency and demographic variability in desert winter annual plants. *Ecology*, 89, 1554–1563.
- Juenger, T.E., McKay, J.K., Hausmann, N.J., Keurentjes, J.J.B., Sen, S., Stowe, K.A. et al. (2005). Identification and characterization of QTL underlying whole-plant physiology in *Arabidopsis thaliana*: $\delta^{13}\text{C}$, stomatal conductance and transpiration efficiency. *Plant, Cell Environ.*, 28, 697–708.
- Kenney, A.M., McKay, J.K., Richards, J.H. & Juenger, T.E. (2014). Direct and indirect selection on flowering time, water-use efficiency (WUE, $\delta^{13}\text{C}$), and WUE plasticity to drought in *Arabidopsis thaliana*. *Ecol. Evol.*, 4, 4505–4521.
- Kimball, S., Gremer, J.R., Huxman, T.E., Lawrence Venable, D. & Angert, A.L. (2013). Phenotypic selection favors missing trait combinations in coexisting annual plants. *Am. Nat.*, 182, 191–207.
- Kimball, S., Gremer, J.R., Barron-gafford, G.A., Angert, A.L., Huxman, T.E. & Venable, D.L. (2014). High water-use efficiency and growth contribute to success of non-native *Erodium cicutarium* in a Sonoran Desert winter annual community. *Conserv. Physiol.*, 2, 1–13.
- Kooyers, N.J. (2015). The evolution of drought escape and avoidance in natural herbaceous populations. *Plant Sci.*, 234, 155–162.
- Lasky, J.R., Des Marais, D.L., McKay, J.K., Richards, J.H., Juenger, T.E. & Keitt, T.H. (2012). Characterizing genomic variation of *Arabidopsis thaliana*: the roles of geography and climate. *Mol. Ecol.*, 21, 5512–5529.
- Lee, J.S., Wang, S., Sritubtim, S., Chen, J.G. & Ellis, B.E. (2009). *Arabidopsis* mitogen-activated protein kinase MPK12 interacts with the MAPK phosphatase IBR5 and regulates auxin signaling. *Plant J.*, 57, 975–985.
- Littell, R., Milliken, G., Stroup, W., Wolfinger, R. & Schabenberger, O. (2006). *SAS for Mixed Models*. J. Chem. Inf. Model. Second. SAS Institute Inc., Cary, NC, USA.
- Lovell, J.T., Juenger, T.E., Michaels, S.D., Lasky, J.R., Platt, A., James, H. et al. (2013). Pleiotropy of FRIGIDA enhances the potential for multivariate adaptation Pleiotropy of FRIGIDA enhances the potential for multivariate adaptation. *Proc. R. Soc. B Biol. Sci.*, 280, 20131043.
- Ludlow, M.M. (1989). Strategies of response to water stress. In: *Structural and Functional Responses to Environmental Stresses* (eds Kreeb, K.H., Richter, H. & Hinckley, T.M.). SPB Academic Publishing, The Hague, The Netherlands, p. 269.
- Masle, J., Gilmore, S.R. & Farquhar, G.D. (2005). The ERECTA gene regulates plant transpiration efficiency in *Arabidopsis*. *Nature*, 436, 866–870.

- McKay, J.K., Richards, J.H. & Mitchell-Olds, T. (2003). Genetics of drought adaptation in *Arabidopsis thaliana*: I. Pleiotropy contributes to genetic correlations among ecological traits. *Mol. Ecol.*, 12, 1137–1151.
- McKay, J.K., Richards, J.H., Nemali, K.S., Sen, S., Mitchell-Olds, T., Boles, S. *et al.* (2008). Genetics of drought adaptation in *Arabidopsis thaliana* II. QTL analysis of a new mapping population, KAS-1 x TSU-1. *Evolution*, 62, 3014–3026.
- Schulze, E.D. (1986). Carbon dioxide and water vapor exchange in response to drought in the atmosphere and in the soil. *Annu. Rev. Plant Physiol.*, 37, 247–274.
- Stebbins, G.L. (1952). Aridity as a stimulus to plant evolution. *Am. Nat.*, 86, 33–44.
- Thuiller, W., Münkemüller, T., Lavergne, S., Mouillot, D., Mouquet, N., Schifffers, K. *et al.* (2013). A road map for integrating eco-evolutionary processes into biodiversity models. *Ecol. Lett.*, 16, 94–105.
- Van Den Boogaard, R., Alewijnse, D., Veneklaas, E.J. & Lambers, H. (1997). Growth and water-use efficiency of 10 *Triticum aestivum* cultivars at different water availability in relation to allocation of biomass. *Plant, Cell Environ.*, 20, 200–210.
- Vasseur, F., Bontpart, T., Dauzat, M., Granier, C. & Vile, D. (2014). Multivariate genetic analysis of plant responses to water deficit and high temperature revealed contrasting adaptive strategies. *J. Exp. Bot.*, 65, 6457–6469.
- Zhang, J., Marshall, J. & Jaquish, B. (1993). Genetic differentiation in carbon isotope discrimination and gas exchange in *Pseudotsuga menziesii*. *Oecologia*, 93, 80–87.

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