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.

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 interspecific 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 genetic 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 provide 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 contribute 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 is 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, Riviere-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
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 (WetComp), water limited/no competition (DryNocomp), well-watered/competition (WetNocomp), water limited/competition (DryNocomp). 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 (Treatwater×Treatcomp 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 ($F_{4,15} = 24.4, P < 0.0001$, Fig. S2A). As a result, to estimate seed count as a **Fitness proxy**, we multiplied **Fruits x Fruit length x Seeds per length** (genotype means) for every plant. We ground whole-dried Arabidopsis, and analysed them for carbon isotope composition ($^{13}$C:$^{12}$C, 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 x 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:

$$y = \text{intercept} + \text{Treatwater} | \text{Treatcomp} | \text{Allele} + \text{HarvestDate} + \text{error}$$

where ‘$y$’ is the focal trait, ‘Treatwater’ is either Wet or Dry, ‘Treatcomp’ 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

<table>
<thead>
<tr>
<th>Source</th>
<th>Leaf day</th>
<th>Bolt day</th>
<th>Flowering day</th>
<th>δ¹³C</th>
<th>N</th>
<th>Total N</th>
<th>Arabidopsis biomass</th>
<th>Height</th>
<th>Number of bolts</th>
<th>Grass biomass</th>
<th>Soil water content</th>
<th>Community biomass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatwater</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>30**</td>
<td>13.23*</td>
<td>32.89**</td>
<td>24.38**</td>
<td>41.09**</td>
<td>0.02</td>
<td>139.16**</td>
<td>582.79**</td>
<td>213.72**</td>
</tr>
<tr>
<td>Treatcomp</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>0.53</td>
<td>660.87**</td>
<td>433.9**</td>
<td>217.04**</td>
<td>92.81**</td>
<td>2.28</td>
<td>NA</td>
<td>183.18**</td>
<td>NA</td>
</tr>
<tr>
<td>Allele</td>
<td>3.37</td>
<td>7.41*</td>
<td>15.1**</td>
<td>98.2**</td>
<td>3.89*</td>
<td>5.78*</td>
<td>3.83*</td>
<td>32.84**</td>
<td>10.13*</td>
<td>4.63*</td>
<td>0.37</td>
<td>5.24*</td>
</tr>
<tr>
<td>Treatwater ×</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>35.8**</td>
<td>1.4</td>
<td>10.51*</td>
<td>3.98*</td>
<td>4.46*</td>
<td>3.73</td>
<td>NA</td>
<td>5.95*</td>
<td>NA</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Allele × Treatwater</td>
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<td>NA</td>
<td>NA</td>
<td>0.67</td>
<td>5.82*</td>
<td>0.7</td>
<td>0.03</td>
<td>3.86</td>
<td>0.04</td>
<td>1.96</td>
<td>0.01</td>
<td>0.37</td>
</tr>
<tr>
<td>Allele × Treatcomp</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>0.02</td>
<td>0.02</td>
<td>4.23*</td>
<td>3.42</td>
<td>4.14*</td>
<td>1.16</td>
<td>NA</td>
<td>4.45*</td>
<td>NA</td>
</tr>
<tr>
<td>Allele</td>
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<td></td>
<td></td>
<td>2.58</td>
<td>3.68</td>
<td>0.07</td>
<td>1.45</td>
<td>0.01</td>
<td>4.36*</td>
<td>NA</td>
<td>3.12</td>
<td>NA</td>
</tr>
<tr>
<td>Treatwater ×</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>4.29*</td>
<td>40.13**</td>
<td>4.22*</td>
<td>0.87</td>
<td>1.14</td>
<td>0.68</td>
<td>31.37**</td>
<td>NA</td>
<td>32.78**</td>
</tr>
<tr>
<td>Treatcomp</td>
<td></td>
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</tr>
<tr>
<td>HarvestDate</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>4.29*</td>
<td>40.13**</td>
<td>4.22*</td>
<td>0.87</td>
<td>1.14</td>
<td>0.68</td>
<td>31.37**</td>
<td>13.76**</td>
<td>NA</td>
</tr>
<tr>
<td>Tray</td>
<td></td>
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<td></td>
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</tbody>
</table>

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 transgensics. 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 weather 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×Treatcomp 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×Treatwater (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.

δ¹³C and Nitrogen content

Water-use efficiency, measured as δ¹³C, was significantly more negative in CVI plants across all environments...
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×Treatwater; 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×Treatcomp; Table 2) with Ler alleles having greater 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×Treatcomp 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

(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 δ13C 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 Treatcomp×Treatwater 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 Total N were strongly affected by both treatments, especially competition (Fig. 2b; Table 2). In the DryNocomp
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 x 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.
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 & Riggins 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
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 $\delta^{13}$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

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**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 lsmeans ± 1 SE, and asterisks indicate significant pairwise contrasts at the $P = 0.05$ level. Only Arabidopsis lines with Ler genetic backgrounds are presented.

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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 × 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 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.

ACKNOWLEDGEMENTS

The authors are grateful to Jesse Lasky (Pennsylvania State University), Brian Enquist (University of Arizona) and three anonymous reviewers for comments that greatly improved an earlier version of this manuscript. We thank Shane Merrell (University of Texas) for greenhouse support. Funding was provided through a National Science Foundation Plant Genome Research Program award (IOS-0922457).

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.

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Editor, Brian Enquist
Manuscript received 12 October 2015
First decision made 17 November 2015
Manuscript accepted 5 January 2016

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