Competition-by-drought interactions change phenotypic plasticity and the direction of selection on Arabidopsis traits

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Summary

1. Populations often exhibit genetic diversity in traits involved in responses to abiotic stressors, but what maintains this diversity is unclear. *Arabidopsis thaliana* exhibits high within-population variation in drought response. One hypothesis is that competitive context, varying at small scales, promotes diversity in resource use strategies. However, little is known about natural variation in competition effects on Arabidopsis physiology.
2. We imposed drought and competition treatments on diverse genotypes. We measured resource economics traits, physiology, and fitness to characterize plasticity and selection in response to treatments.
3. Plastic responses to competition differed depending on moisture availability. We observed genotype-drought-competition interactions for relative fitness: competitive gradients had little effect on relative fitness under well-watered conditions, while under drought competition caused rank changes in relative fitness. Early flowering was always selected. Higher $\delta^{13}C$ was selected only in the harshest treatment (drought and competition). Competitive context changed the direction of selection on aboveground biomass in both well-watered and drought environments.
4. Our results highlight how local biotic gradients modify abiotic selection, in some cases promoting diversity in abiotic stress response. The ability of populations to adapt to environmental change may thus depend on small-scale biotic heterogeneity.

Keywords: succession, tradeoffs, leaf economics, neighbor interactions, strigolactone

INTRODUCTION

The persistence of genetic variation in phenotypes in spite of the forces eliminating diversity is a central puzzle in biology (Mitchell-Olds *et al.*, 2007). For example, while experiments often show strong selection under putatively harsh abiotic treatments, populations still exhibit diversity in response to such treatments (Hosgood & Parsons, 1968; Sultan & Bazzaz, 1993). This diversity can be surprising given these individuals occupy the same climatic regime. Additionaly, this genetic variation within populations can affect the adaptability of populations to environmental changes, such as directional climate change (Pease *et al.*, 1989; Lynch & Lande, 1993; Jump *et al.*, 2009).

The effects of climate conditions (like precipitation) on organisms occur within the context of fine scale environmental heterogeneity, which can exacerbate or mitigate climatic conditions (Farrior *et al.*, 2013b; Urban *et al.*, 2013; Clark *et al.*, 2014). Organisms within the same population can experience very different environments due to small-scale heterogeneity. This heterogeneity might select for phenotypic plasticity in a single optimal genotype. Alternatively, heterogeneity can promote genetic variation within populations by favoring different genotypes in different environments, *i.e.* a genotype-by-environment (G×E) interaction causing a fitness tradeoff across local environmental gradients (Levene, 1953; Hamrick & Holden, 1979; Schmitt *et al.*, 2003). Substantial theoretical work has addressed how changes in selection within populations may maintain diversity in quantitative traits (Slatkin, 1979; Kondrashov & Yampolsky, 1996; Wittmann *et al.*, 2017). However, there is still little known empirically about the ability of selection to maintain within-population diversity of traits involved in response to abiotic stress.

Limiting resources that are influenced by climate, *e.g.* moisture, may be especially likely to exhibit interactions between climate and competition. In plants for example, different levels of allocation to roots for water acquisition may be favored depending on the presence of neighboring individuals that compete for water (Farrior *et al.*, 2013a). Differing water use efficiencies (WUE) can be favored under moisture limitation depending on whether competitors are present (Campitelli *et al.*, 2016). Specifically, competition can favor more resource acquisitive (as opposed to conservative) strategies when resources are limited (Farrior *et al.*, 2013a). There are many ways biotic and abiotic components of environment may interact to

favor different functional strategies, though these interactions are typically not well studied, especially within species (Blois *et al.*, 2013). Here, we investigate how competitive gradients might promote diversity in traits related to water balance in *Arabidopsis thaliana* (hereafter, Arabidopsis).

Arabidopsis is a useful study system for several reasons. First, it exhibits substantial diversity in drought response within populations. Studies of genetic variation in $\delta^{13}C$, a proxy for water use efficiency (WUE), and stomatal characteristics have found statistically significant but weak relationships with climate of origin (Dittberner *et al.*, 2018) as have studies of survival (Exposito-Alonso *et al.*, 2018). These relatively weak relationships may be surprising given the tendency for local adaptation in Arabidopsis populations (Fournier-Level *et al.*, 2011) and stand in contrast with cold tolerance, which is more closely tied to local winter temperatures (Ågren & Schemske, 2012; Monroe *et al.*, 2016; Gienapp *et al.*, 2017). Second, while Arabidopsis occurs in a wide range of precipitation regimes (Hoffmann, 2002) it also occupies a wide range of microhabitats (Baron *et al.*, 2015), suggesting the opportunity for precipitation-by-microhabitat interactions. Some published data suggest the potential for competition-by-climate interaction effects on performance (Baron *et al.*, 2015; Campitelli *et al.*, 2016) though the physiological basis of these interactions is largely unknown. Third, previous research has yielded insight into genetic and physiological mechanisms of drought adaptation, providing a basis for specific hypotheses about how moisture and competitive gradients might interact to select for different traits (McKay *et al.*, 2003; Juenger *et al.*, 2010; Des Marais *et al.*, 2012; Lovell *et al.*, 2013; Kenney *et al.*, 2014; Lasky *et al.*, 2014, 2018; El-Soda *et al.*, 2015; Bac-Molenaar *et al.*, 2016; Dittberner *et al.*, 2018; Exposito-Alonso *et al.*, 2018, 2019; DeLeo *et al.*, 2019).

Arabidopsis drought responses have been largely characterized into a stereotypical tradeoff between avoidance and escape (McKay *et al.* 2003; Kenney *et al.* 2014). Avoidance involves phenotypes that minimize water loss (*e.g.* stomatal closure) and maximize gain (*e.g.* root growth). Escape involves phenology and rapid development that matches with time periods when moisture is abundant (Ludlow, 1989). Escape may be adaptive when low soil moisture occurs at the end of growing seasons (Kenney *et al.*, 2014) while avoidance may be adaptive when moisture is limited throughout a growing season (Campitelli *et al.*, 2016). Drought escape has been characterized by rapid time to flowering, high specific leaf area (SLA), leaf N, and carbon assimilation rate (*A*), low root:shoot mass ratio and water use efficiency (WUE) (Ludlow,

1989; Des Marais *et al.*, 2012; Kenney *et al.*, 2014; El-Soda *et al.*, 2015) while avoidance is characterized by the opposite values (but see Ferguson *et al.*, 2019). Arabidopsis is a rapidly maturing annual, an early successional strategy, which may be adaptive when neighboring plants are better resource competitors. In disturbed habitats where Arabidopsis frequently occurs, competition likely increases over time as colonists grow in size and number. In this situation, moisture availability may decrease across a season due to greater uptake, favoring Arabidopsis drought escape via faster growth and lower WUE (Cohen, 1970; McLeod *et al.*, 2012; Campitelli *et al.*, 2016). While additional resources vary with competition, *e.g.* light and nutrient availability, we controlled these in our experiments to focus on competition for water.

Despite the importance of Arabidopsis as the model plant, there exists little information on how diverse ecotypes respond physiologically to the presence of neighboring plants. Plant responses to neighbors and potential competitors may use a variety of cues, including nutrient availability (Schenk, 2006), red:far red light ratios (Aphalo *et al.*, 1999), or chemicals (Badri *et al.*, 2012). In our study we prevented nutrient competition and shading, thus we hypothesized responses to neighbors in our experiments could be based on chemical cues. In Arabidopsis, (Campitelli *et al.*, 2016) found large biomass reductions and lower WUE in response to grass neighbors, even under well-watered conditions. These dramatic responses in (Campitelli *et al.*, 2016) could be due to some non-resource cue, though nutrient levels or red:far red light may have changed due to competition with grasses. One additional mechanism for sensing neighbors is perception of root exudate chemicals, which are used by diverse species for belowground sensing of neighboring plants (Badri *et al.*, 2012; Schmid *et al.*, 2013; van Dam & Bouwmeester, 2016). Strigolactones (SLs) are one such type of compound exuded belowground by most plants (Al-Babili & Bouwmeester, 2015) that have been implicated in neighbor avoidance in a moss (Proust *et al.*, 2011). Furthermore, Arabidopsis plants have been shown to suppress inflorescence branching in response to SLs in growth media (Scaffidi *et al.*, 2014) and also to induce stomatal closure (Lv *et al.*, 2018). To the extent that greater apical dominance in the presence of competitors enhances fitness (Baron *et al.*, 2015) it may be adaptive for a plant to suppress branching in response to soil SLs of neighbors.

We studied how competition interacts with moisture inputs to drive trait plasticity and selection on life history and physiology in Arabidopsis. We focused on these specific questions:

1. How does Arabidopsis respond to competitive and moisture gradients via changes in life history and physiology? We hypothesized that drought would induce greater WUE due to stomatal closure, lower performance (biomass and fitness), and greater SLA and leaf N as part of a drought escape strategy (Des Marais *et al.*, 2012). We hypothesized that competition would induce inflorescence elongation (Baron *et al.*, 2015), lower performance (biomass and fitness), and greater investment in resource acquisition (greater SLA, leaf N, lower WUE), possibly due to perception of SLs in soil.
2. Do competitive gradients induce rank-changing G×E in fitness, suggesting that these gradients could promote diversity in response to abiotic stress within populations? We hypothesized that under conditions with ample resources, the presence of neighboring plants would have little influence on the relative fitness of diverse genotypes. By contrast, under moisture limitation we expected that competition would favor genotypes that differ in multiple traits compared to when competitors are absent.
3. What traits explain genetic variation in fitness response to competition (G×E)? We hypothesized that genetic tradeoffs are associated with alternate drought response strategies, such that contrasting treatments change the sign of directional selection coefficients across treatments. Alternatively, consistent genotypes might be favored across competitive environments due to adaptive plasticity.
4. Do any traits exhibit genetic variation in plasticity that is under selection across competitive environments? We hypothesized that there will be some adaptively plastic traits such that trait changes across competitive environments are associated with higher average fitness across competitive environments.

METHODS

Plant material

We studied 17 diverse genotypes from across the native range of Arabidopsis and also the reference genotype (Columbia) for a total of 18 genotypes (Table S1). These genotypes mostly represent a panel of diverse parental lines used to create a multi-parent linkage mapping population (Kover *et al.*, 2009). We obtained seeds from the Arabidopsis Biological Resource

Center (ABRC). However, it was later discovered that two ecotypes (Can-0 CS76740, and Wu-0 CS78858) were contaminants from the supplying lab, *i.e.* they were not the original labeled ecotype (Pisupati *et al.*, 2017). Nevertheless, these genotypes represented unique, though unidentified, genotypes (Pisupati *et al.*, 2017) and we included them in the experiments. Seeds received from ABRC were grown for one generation in the lab at 16ºC with a 16-hr photoperiod and the resulting next generation of seed was used in the present experiment. For competitors, we used three genotypes of *Brachypodium distachyon* (Poaceae), a common Mediterranean annual grass. These three genotypes had similar growth form and growth rate and were all simultaneously bulked in a greenhouse, Adi-10, Bd21-3, Bd30-1,

Experimental design and growth conditions

To characterize genetic variation in response to moisture differences and their interactions with competition, we grew these genotypes in a split-plot design with four treatments: (1) well-watered, no competition (*i.e.* a single Arabidopsis plant in a pot), (2) well-watered, with competition, (3) intermittent drought, no competition, and (4) intermittent drought, with competition (Fig. S1). Plants were grown in SC-10 Cone-tainers™ (3.81 cm diameter, 164 ml) in a 98-cell rack (RL98) leaving adjoining cells in the tray empty to prevent shading interaction between neighboring pots (Stuewe and Sons, Tangent, Oregon, USA). Each rack was placed within an individual flow trays. Competition treatments were spaced at every other pot location to prevent among-pot crowding, and each tray was designated a drought or well-watered treatment (Fig. S1). Each Arabidopsis genotype in each treatment had 10 total replicates per treatment, 5 for an initial harvest prior to flowering, and 5 for a post-senescence harvest (total Arabidopsis plants was 720). Genotype pot locations were randomly assigned.

We grew plants in a walk-in Conviron C1210 growth chamber (Conviron Ltd., Winnipeg, Canada). This chamber had constant horizontal airflow to maintain homogenous conditions throughout. We designed a temperature and photoperiod program (Fig. S3, Table S2) to mimic a growing season starting with warm temperatures early in the local growing season, which we reasoned would be a potential site for moisture limitation, and centrally located in the species range. Specifically, we chose conditions for the site of collection of the Lip-0 ecotype near Chrzanów in southern Poland. We used a growing season model (Lasky *et al.*, 2012) with WorldClim data (Hijmans *et al.*, 2005) then extracted a monthly time series of maximum

temperatures and minimum temperatures. We used these temperatures to program the growth chamber, though in the early growing season we were limited to 10ºC nightly temperature because the chamber was unable to generate temperatures <10ºC. Local photoperiod for the growing season was obtained using the 'maptools' package (Bivand & Lewin-Koh, 2019). Weekly conditions were obtained via linear interpolation of the monthly temperature values (Table S2).

Potting composition within each pot consisted of 50% potting mix (Premier pro-mix PGX), 25% medium commercial grade sand, 25% calcined clay (TURFACE® ALL SPORT PRO™, Turface Athletics™, PROFILE Products LLC, Buffalo Grove, IL), with 2.5 cm of the potting mix as a topsoil to assist Arabidopsis plants in germination and initial growth. Glad Press'n Seal Wrap (The Glad Products Company; Oakland, CA) was placed over pots until germination to prevent seed dehydration prior to germination.

Competition and drought treatments

Competition was imposed by sowing six seeds of *Brachypodium distachyon*, along the outside perimeter of each Arabidopsis pot. Germination for *B. distachyon* was near 100%. The three *B. distachyon* genotypes were randomly sorted into competition pots. Preliminary experiments using varying levels of *B. distachyon* in competition with *Arabidopsis* showed that greater than six plants resulted in overcrowding and very poor growth of *B. distachyon,* thus six plants were used as a competition treatment in pots. To focus on potential competition for water with the Arabidopsis individual at the center of the pot, we limited light competition by restraining *B. distachyon* tillers to the outside with a mounted stainless steel ring (Fig. S2).

Drought treatments were imposed by removing bottom water at 16 days after planting (DAP), when all plants had at least the first true leaves fully expanded. Pots in the drought treatment were not watered during 17-19 DAP prior to fertigation (combined watering and fertilizing) treatments to allow for initial drying. To simulate persistent rainfall limitation, starting at 20 DAP, each individual pot in the drought treatment was fertigated from above with 3 ml of full strength Miracle-Gro solution (Miracle-Gro® Water Soluble All Purpose Plant Food, The Scotts Company, LLC., Marysville, OH) every other day. Throughout the entire experiment, all pots in all treatments were fertigated at the same time of day with the same nutrition strength and volume in all treatments to limit nutrient competition and to focus on traits involved in

competition for water. At 29 DAP, fertigation was shifted to 6 ml of ½ strength Miracle Gro solution, and at 50 DAP fertigation was increased to 12 ml of ½ strength Miracle Gro solution. Well-watered pots were kept consistently moist by bottom watering with tap water. We defined the end of the plant life cycle as the time when 50% of siliques were mature (brown and brittle), because at this stage the ecotypes used in this experiment stopped producing new flowers and discontinued development of existing fruits if present. Bottom water in well-watered treatments was removed when at least one plant had 50% mature (brown and brittle) siliques, and the remaining flowering plants were top watered daily with 12 ml of ½ strength Miracle Gro, to maintain the potting mix water and nutrient content of the well-watered plants, until 50% of siliques were mature. Watering in drought treatments was also discontinued for a pot when 50% of siliques were mature. Potting mix volumetric water content for each treatment was monitored throughout the entire experiment with EM-50 dataloggers and EC-5 probes (METER Group Inc., Pullman, WA) within designated non-experimental pots with the reference genotype Col-0 (Fig. S4).

Plant harvest and phenotyping during vegetative stage

To measure phenotypes during vegetative growth that required destructive harvest, we randomly selected five replicates for each genotype and treatment combination for harvest at 55-56 DAP, just prior to the initiation of the first flowering genotypes. In this harvest, *B. distachyon* shoots were cut at soil level, dried at 90 °C, and weighed. Roots of Arabidopsis plants under no competition treatments were washed, dried at 90 °C, and weighed (roots in competition pots were difficult to separate from *B. distachyon* roots and were not weighed). Rosettes were removed from pots 1-2 cm below the soil level and were preserved in 75% ethanol at 4 °C. These rosettes were later measured for total leaf area, dry rosette biomass (excluding root), root diameter at 1 cm below the soil surface, stomatal density using a representative mid-aged leaf, specific leaf area (SLA) using one representative mid-aged leaf, leaf $\delta^{13}C$, leaf $\delta^{15}N$, and total leaf carbon and nitrogen composition. Leaf tissue samples for $\delta^{13}C$, $\delta^{15}N$, total carbon and nitrogen composition were prepared by drying the complete rosettes in coin envelopes for 12-24 hours at 150 °F, then grinding and homogenizing all leaves from the rosette to a fine powder using a mortar and pestle. Based on our estimated nitrogen content, we packaged a 1.5 – 2.0 mg aliquot of finely ground leaf tissue from each rosette into an 8 x 5 mm tin capsule (EA

Consumables, Pennsauken, NJ) and organized packed samples into a flat-bottom 96-well plate. Samples were sent to the UC Davis Stable Isotope Facility (University of California Davis, Department of Plant Science, Davis, CA) for δ13C, δ15N, total carbon and nitrogen analyses using an elemental analyzer interfaced to a continuous flow isotope ratio mass spectrometer (IRMS).

Plant harvest and phenotyping at maturity

Fecundity of those Arabidopsis plants that survived to reproduction was estimated when all siliques reached maturity (dried and brown) and the rosette leaves had senesced. We measured total silique number and silique length of six siliques corresponding to 10, 20, 40, 60, 80, and 90th percentiles of silique positions along the inflorescence in order to represent silique length across the entire inflorescence (*e.g*. the 10th percentile silique was higher up the inflorescence than 10% of all siliques). For each Arabidopsis plant, we also measured inflorescence height and the number of inflorescence branches. After siliques were measured, we dried the mature inflorescence and rosette separately (in addition to rosettes that did not flower) at 90 °C for 24 hours to get aboveground dry biomass.

Response to simulated root exudate strigolactones of neighbors

We used a synthetic strigolactone applied to the soil, $GR24^{rac}$ (ChemPep, Inc., Wellington, FL), to study how SLs from neighboring *B. distachyon,* which exude SLs from their roots (Changenet, 2018), might affect Arabidopsis performance and traits. We asked if genotypes of Arabidopsis most sensitive (in terms of aboveground biomass) to *B. distachyon* neighbors in well-watered conditions were also most sensitive to GR24. Four genotypes from the 18 used in the experiment were selected, two that were highly sensitive to competing grass neighbors (Sf-2 and Ct-1), and two that were less sensitive to neighbors (Wil-2 and Ws-0) under well-watered conditions. Plants were grown alone in pots (no competition). Growing location, temperature, humidity, light level, daylength, and potting mix were as described in the main experiment (see *Experimental design and growth conditions* above). Plants were top watered evenly throughout the top of the potting mix around the rosette using a pipette every day with 3 ml of either 7.8 uM GR24 solution, 0.78 uM GR24 solution, or tap water, with 5 individual replicates per genotype per treatment. All plants received bottom tap water until the first true leaves were expanded, at which point the

bottom water was removed and treatment applications were started. All plants were harvest 52 days after planting, just prior to flowering initiation. Rosette biomass, root biomass, rosette area (Easy Leaf Area) (Easlon & Bloom, 2019), and quantum yield of photosystem II using a MultispeQ V2.0 (Kuhlgert *et al.*, 2016) were measured. Leaf $\delta^{13}C$ and total leaf carbon and nitrogen composition were measured from tissue analyzed at the UC Davis Stable Isotope Facility (University of California Davis, Department of Plant Science, Davis, CA) using sample preparation and analytical methods described in *Plant harvest and phenotyping during vegetative stage*.

Photosynthesis

We measured whole-plant gas exchange on a separate set of plants in an experiment that replicated the temperature, soil, and photoperiod conditions in the main experiment. However, we did not include *B. distachyon* competitors and we covered topsoil in clay. We did not use competition treatments due to the challenges of measuring whole plant gas exchange on the focal Arabidopsis plant when surrounded by *B. distachyon* competitors. We used the clay cap as suggested by the gas exchange analyzer manufacturer (LI-COR Inc., Lincoln, NE, USA) to limit soil respiration from interfering with photosynthetic gas exchange measurements. Prior to planting, the top soil was covered with a 0.5 cm layer of potting clay (Msanne *et al.*, 2011; Souza *et al.*, 2015) with a 0.5 cm hole in the center for plant growth. Even with the clay and initial soil sterilization, we observed low, non-zero soil respiration from well-watered pots, and as a result below we do not focus analysis on changes in photosynthesis between treatments, but instead use photosynthesis measurements in selection analyses within moisture treatments.

Five replicates per genotype per water treatment were planted in a randomized block design. Photosynthesis (*A*) was measured between 8:00 AM and 12:30 PM using a LiCor 6400XT with a LI6400-17 Whole Arabidopsis Chamber (LI-COR Inc., Lincoln, NE, USA) modified to fit SC-10 Ray Leach Cone-tainers™ (Stuewe and Sons Inc., Tangent, OR) by widening the opening that holds the Cone-tainer to fit a size -223 O-ring (McMaster Carr part number 1170N95, Elmhurst, IL). This modification created an air-tight seal around the circumference of the Cone-tainer approximately 4 mm from the top of the Cone-tainer, to prevent gas leaks from the surrounding environment and to allow for continuous air flow and even concentrations of gasses throughout the chamber during measurements.

Data analysis

Broad-sense heritability within treatments was calculated as the proportion of variation in phenotypes explained by genotype identity within each treatment, using a general linear model. We also implemented linear mixed models, where drought and competition treatments were fixed effects and genotype effects, genotype-by-treatment interactions, and trays were random effects. Using these mixed models, we tested the hypothesis that fixed effects were zero and also calculated variance components for genotype random effects, genotype × moisture random effects, genotype × competition random effects, and genotype × moisture × competition random effects, using the R package 'VCA' (Schuetzenmeister & Dufey, 2018). We calculated 95% confidence intervals on variance components (constrained to be non-negative - negative components are set to zero) and report as significant components where the interval excludes zero. We calculated genotypes' breeding values for each trait in each treatment using similarly constructed linear mixed models, using the R package 'lme4' (Bates *et al.*, 2013). To assess whether there were genetic correlations in traits often thought to represent coordinated strategies, we calculated Spearman's rank correlations between breeding values for flowering time, SLA, leaf C:N, $\delta^{13}C$, and *A* (assimilation rate).

We tested for evidence of selection within each treatment by fitting linear regression models of relative fitness (absolute fitness / mean fitness among genotypes within a treatment) as a function of breeding values for individual phenotypes. Flowering time was a trait under strong selection in all treatments and thus we also tested trait correlations with fitness in multiple regression while also including a flowering time covariate, which allowed us to get better estimates of direct selection acting on a trait while accounting for flowering time selection (Lande & Arnold, 1983).

We tested for changing selection across treatments, using an approach to deal with variance inhomogeneity in fitness among treatments. We used permutations to generate a null model as in (Kooyers *et al.*, 2017). Specifically, we permuted trait values among genotypes within treatments 1,000 times to generate a null distribution of selection coefficients to then compare observed coefficients between different competition treatments.

We also tested for selection on plasticity in response to competition. We linear models with breeding values tested how plasticity (trait change) in response to competition within a

given moisture treatment was associated with absolute fitness in each individual competition treatment, in a multiple regression that also included trait means averaged across competition treatments (Stinchcombe *et al.*, 2004). This allowed us to test for selection on plasticity across competitive contexts while accounting for differences among genotypes in their average traits.

RESULTS

Treatment effects on demography

Survival to reproduction was generally high across treatments, with 95% of plants surviving until flowering (only one plant survived but did not flower by the end of the experiment). There was no pre-flowering mortality in either no-competition treatment (100% survival). By contrast, with competition, survival was 98% under well-watered conditions and 84% under drought, indicating that competition combined with drought was the harshest treatment. As expected, mean genotype absolute fitness (mean estimated total mature fruit length per initial seedling following thinning) was highest under well-watered conditions without crowding (1900 mm, standard deviation among all individuals, SD = 599), followed by drought without crowding (656 mm, SD = 264), well-watered and crowding (387 mm, SD = 181), and drought and crowding combined (131 mm, SD = 95). We did not measure fecundity on *B. distachyon*, though its total aboveground biomass was greater in well-watered treatments compared to drought treatments at the mid-experiment harvest (mean pot total biomass, well-watered = 0.36 g, SD = 0.05; drought = 0.22 g, SD = 0.04).

Genetic variation in phenotypes and plastic response to drought and competition

Heritability

Broad-sense heritability within treatments differed widely among traits, with flowering time having the highest heritability in every treatment (ranging from 0.88 in drought and competition to 0.96 to well-watered and competition, Table S3). Heritability averaged among traits was highest in the well-watered no competition treatment, followed by well-watered competition, drought no competition, and drought competition, respectively. Heritability was higher for leaf

C:N (averaged across four treatments $h^2 = 0.41$) compared to leaf proportion N (averaged across four treatments $h^2 = 0.36$) in most treatments and thus we below focus results on C:N.

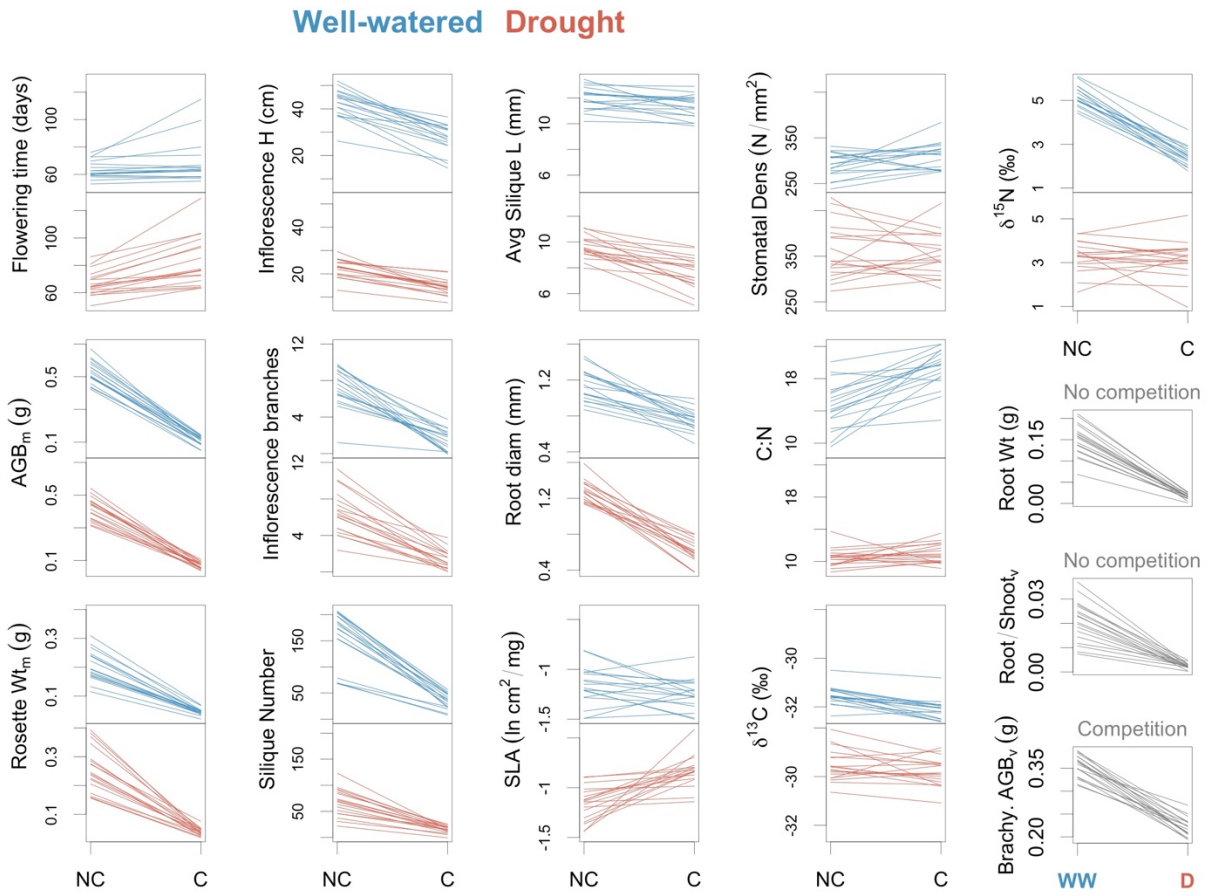
Trait plasticity

Drought and competition treatments generally had consistent effects on performance measures: under drought or competition plants had significantly smaller rosettes at mid-experiment harvest (rosette $\text{weight}_{\text{vegetative}}$ and total leaf $\text{area}_{\text{vegetative}}$), lower inflorescence weight, shorter inflorescences, fewer total mature fruits (siliques), shorter fruits, and less aboveground biomass ($\text{AGB}_{\text{mature}}$ *i.e.* end of experiment harvest; linear mixed-effects models LMMs, all $p<0.05$, Table S4, Figure 1). A notable exception was that plants had larger rosette $\text{weight}_{\text{mature}}$ (at final harvest) under drought treatments compared to well-watered (LMM, drought fixed effect estimate = 55.3 mg, $p < 10^{-6}$). There was a significant interaction between competition and drought for $\text{AGB}_{\text{mature}}$, inflorescence weight, inflorescence height, fruit number, average fruit length, such that under the combination of drought and competition these traits were greater than expected from the additive contribution of the two treatments (LMM fixed effects all $p<0.05$, Table S4). However, rosette $\text{weight}_{\text{mature}}$ and average fruit length were lower than expected under combined drought and competition (Figure 1), while rosette size at mid-experiment harvest (rosette $\text{weight}_{\text{vegetative}}$ and total leaf $\text{area}_{\text{vegetative}}$) showed no significant treatment interaction (Table S4). The number of inflorescence branches was strongly reduced under competition (LMM fixed effect, $p < 10^{-8}$), without any significant drought or interaction effects.

In contrast to whole plant performance measures, traits associated with life history or water balance showed more diverse responses to water and competition (Figure 1). For example, mean flowering time showed a significant competition by drought interaction, such that flowering was significantly delayed only under combined competition and drought (LMM, competition×drought fixed effect $p=0.0007$, Table S4). Competition resulted in significantly decreased root diameter (LMM, fixed effect $p<10^{-8}$) and reduced $\delta^{13}C$ (a proxy for water use efficiency, $p<10^{-5}$) across moisture treatments (Table S4). Under drought, competition resulted in even greater reductions in root diameter (LMM, competition×drought fixed effect $p<10^{-6}$) and greater SLA ($p = 0.0002$), while SLA was not affected by competition in well-watered conditions. Under well-watered conditions, competition resulted in greater leaf C:N (LMM,

competition×drought fixed effect $p<10^{-6}$) and reduced $\delta^{15}N$ ($p<10^{-8}$), while these were not affected by competition under drought (Table S4, Figure 1).

Figure 1. Reaction norms for traits across 18 Arabidopsis genotypes (each line represents one genotype). Lines show breeding values estimated from linear mixed models. 'NC' = no competition, 'C' = competition, 'WW' = well-watered, 'D' = drought. At right in gray are three traits that were only measured in a single competition/no-competition treatment.



Drought treatments (apart from competition effects) resulted in reduced root weight, root:shoot ratio, greater stomatal density, lower leaf C:N, greater $\delta^{13}C$, and lower δ15N (all traits from mid-experiment vegetative harvest, LMM, all drought fixed effects $p < 0.004$, Table S4, Figure 1).

The four ecotypes we tested for response to a strigolactone (SL, $GR24^{rac}$) under well-watered conditions showed saturating reductions in aboveground vegetative biomass with increased SL levels (Figure S5, ANOVA on log proportional biomass with ranked GR24 treatment covariate, $p < 10^{-15}$, genotype-GR24 interaction $p = 0.7398$). Similarly, log rosette canopy area was significantly reduced by GR24 (ANOVA with ranked GR24 treatment

covariate, $p < 10^{-12}$, genotype-GR24 interaction p = 0.6306). Genetic variation in vegetative rosette weight response to *B. distachyon* was weakly correlated to their response to SL treatment, the latter estimated from a rank correlation between log rosette biomass and the range of SL concentrations tested (Spearman's rho = 0.4). The genotype least sensitive to *B. distachyon* neighbors was also the least sensitive to SL treatment.

The SL treatment affected other traits. GR24 caused increased $\delta^{13}C$ (ANOVA $\delta^{13}C$ response to ranked GR24 treatment covariate, p = 0.0004, genotype-GR24 interaction p = 0.1588) and decreased leaf C:N (ANOVA, $p < 10^{-6}$, genotype-GR24 interaction p = 0.2508, Figure S5). GR24 had genotype-specific effects on the quantum yield of photosystem II, with two genotypes (Ct-1 and Sf-2) having greater PSII yield with increasing GR24 and Wil-2 having decreased PSII yield with increasing GR24 (ANOVA quantum yield of PSII response to ranked GR24 treatment covariate, p = 0.4047, genotype-GR24 interaction p = 0.0088). Reductions in biomass in response to GR24 were genetically correlated with positive responses in PSII yield to SL treatment (Spearman's rho = -0.80, Pearson's r = -0.99). There were no clear effects of the SL treatment on leaf thickness (ANOVA with ranked GR24 treatment covariate, p = 0.7546, genotype-GR24 interaction p = 0.5629).

Genetic correlation in traits

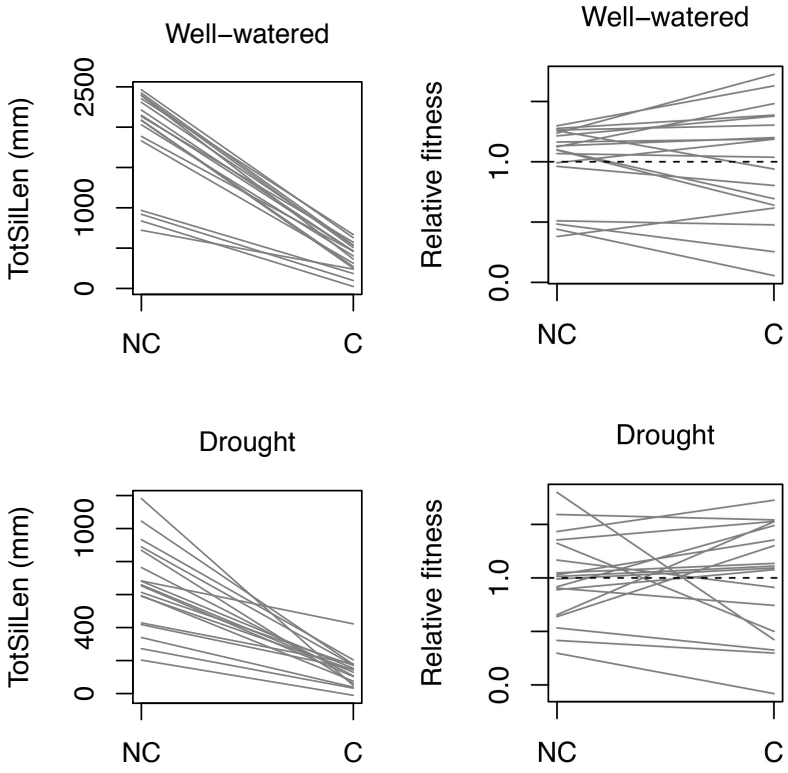
We generally found that flowering time and $\delta^{13}C$ were negatively correlated: later flowering genotypes had lower $\delta^{13}C$ (well-watered, competition Spearman's rho = -0.66; drought, no competition rho = -0.45, drought, competition rho = -0.47) with the exception well-watered, no competition treatments where the two were unrelated (rho= -0.08). As expected, SLA and leaf C:N were always negatively correlated (Figure S6). These traits were not closely related to *A* (assimilation rate) in well-watered conditions but under drought and no competition we observed that genotypes with greater *A* had greater SLA (rho = 0.38), lower C:N (rho = -0.38) and lower $\delta^{13}C$ (rho = -0.42).

Genetic and genotype-environment interaction effects on traits

Total fitness (Figure 2) and total silique number showed significant genotype-by-competition-by-water treatment interactions but not significant genotype interactions with single treatment axes (95% CIs of variance components excluded zero, Table S5), suggesting the potential for genetic

tradeoffs in performance due to drought-competition interactions. Flowering time exhibited a strong genetic variance component and also a significant genotype-by-competition-by-treatment interaction but no genotype interaction with single treatment axes. Vegetative stage (mid-experiment harvest) and mature (end of experiment harvest) rosette weights both showed significant genotype-by-competition interactions but other traits did not. Root:shoot, root weight, and $\delta^{15}N$ (vegetative harvest) showed significant genotype variance components but no significant genotype-by-treatment interactions. Inflorescence height, inflorescence branch number, silique length, root diameter, SLA, stomatal density, C:N, d13C, total rosette leaf area (vegetative harvest) did not show significant genotype-by-treatment variance components or significant genotype variance components (Table S5).

Figure 2. Reaction norms of absolute fitness (*i.e.* total silique length, left columns) and relative fitness (standardized by dividing by mean fitness within each treatment, right columns) across competition treatments for the 18 studied genotypes (each line represents a genotype). Lines show breeding values estimated from linear mixed models. The well-watered (top row) and drought (bottom row) treatments are shown (note their changing scales).



To assess the existence of tradeoffs across environments, we calculated the correlation in relative fitness between competitive treatments. Under well-watered conditions, competition did

not have a strong effect on the relative fitness of genotypes (absolute fitness correlation across environments, $r = 0.82$, $p < 10^{-5}$, Figure 2). However, under drought, competition had a strong impact on relative fitness, such that fitness was weakly related across competitive contexts ($r = 0.38$, $p = 0.1170$, Figure 2). Comparing moisture treatments under no competition, relative fitness was weakly correlated across environments, suggesting possible tradeoffs ($r = 0.33$, $p = 0.1813$). Under competition, fitness was more correlated across moisture treatments ($r = 0.52$, $p = 0.0263$).

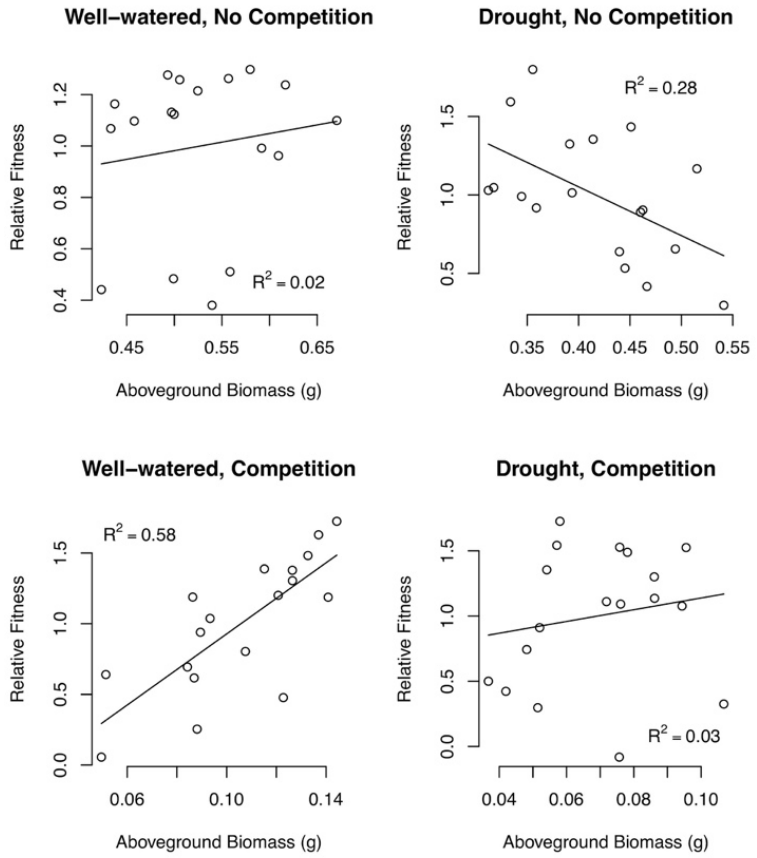
Selection on phenotypes within and across environments

To identify traits affecting fitness variation, we tested for univariate and multivariate selection on traits within each environment. Earlier flowering time (FT) was favored in all four environments (linear models, well-watered, no competition $p <10^{-6}$, well-watered, competition $p=0.0020$, drought, no competition $p = 0.0162$, drought, competition $p = 0.0231$, Table S6). Greater inflorescence height (linear model $p = 0.0001$) and inflorescence branch number ($p = 0.0337$) was linked to higher fitness in well-watered competition treatments but not in other treatments (all $p > 0.05$). Inflorescence height in well-watered competition treatments was selected even after conducting multiple regression also including a flowering time effect (inflorescence height $p = 0.0214$, flowering time $p = 0.0469$). Smaller rosettes (mature harvest) were selected under no competition treatments of both moisture regimes (well-watered, no competition $p = 0.0095$, drought, no competition $p = 0.0030$), though these patterns were not statistically significant after including flowering time in multiple regression (Table S6). AGB_{mature} was positively selected under well-watered competition treatments ($p = 0.0002$), negatively selected under drought no competition ($p = 0.0223$), but unrelated with fitness in well-watered no competition treatments ($p = 0.5625$) and drought competition ($p = 0.4926$). Even after including flowering time in multiple regression, AGB_{mature} was positively selected in well-watered treatments with competition (AGB_{mature} $p = 0.0020$, FT $p = 0.0159$) but not so in drought treatments without competition (AGB_{mature} $p = 0.4383$, FT $p = 0.2785$). In the harshest environment, drought and competition, we found that $\delta^{13}C$ was positively selected ($p = 0.0291$) though this was not significant after including FT ($\delta^{13}C$ $p = 0.1830$, FT $p = 0.1433$). Stomatal density, SLA, root:shoot, root biomass, photosynthetic rate, C:N, $\delta^{15}N$, and root diameter did not show significant selection in any environment (Table S6).

To determine the traits responsible for genotype-level tradeoffs across treatments, we tested for changes in the coefficients of selection across competition treatments within a given moisture environment. We found significant changes in selection depending on the competitive environment for inflorescence height and AGB_{mature} under well-watered conditions, with plants with taller inflorescences (two-tailed permutation test $p = 0.024$) and AGB_{mature} ($p = 0.022$) being more fit under competitive environments but with no detectable effect on fitness under no competition (Table S7, Figure 3). These changes in selection on these traits contrast with the strong correlation (*i.e.* weak tradeoffs) between relative fitness of genotypes in well-watered conditions when comparing plants growing alone or with competitors (Figure 2). By contrast, we had observed changing ranks of relative fitness across competition treatments under drought (Figure 2). We did not find significant changes in selection on any phenotypes under drought across competition treatments, though AGB_{mature} showed the most substantial change (permutation test $p = 0.0540$, Figure 3). To further investigate the fitness tradeoffs across competition treatments under drought, we tested change in relative fitness between treatments and found it to be associated with AGB_{mature} under drought and competition (relative fitness of drought under no competition - relative fitness under competition vs. AGB_{mature} under drought and competition, linear model, $p = 0.0118$) while AGB_{mature} under drought no competition did not explain the fitness changes ($p = 0.8333$).

When testing for evidence of selection on traits averaged across competitive environments and independently on plasticity, we found significant selection under well-watered conditions for a plastic increase in inflorescence height under competition compared to no competition ($p = 0.0034$) while average inflorescence height across treatments was not under significant selection. For other traits where the average value was under selection across competitive environments, we found no independent selection on plasticity (Table S8).

Figure 3. Changing selection on aboveground biomass of mature (AGB_{mature}) plants among treatments. In well-watered environments, Arabidopsis with greater AGB_{mature} are selected under competitive environments, but weakly when competitors are absent. Under drought environments, *lower* AGB_{mature} is selected when competitors are absent, but not when competitors are present.



DISCUSSION

Organismal responses to abiotic conditions can be influenced by biotic interactions, such as interactions that affect general organismal health (*e.g*. parasitism, Price, 1991), change the balance of optimal allocation to abiotic stress tolerance (*e.g*. facilitation in arid systems Bertness & Callaway, 1994), or directly change the availability of abiotic resources (*e.g*. competition for water Cohen, 1970). Given rapidly global climate change we are particularly interested in limiting resources that are influenced by climate, *e.g*. moisture. Such resources may be especially likely to exhibit interactions between climate and competition in their effects on organisms. As environments change, understanding the diversity of organismal responses may be important for forecasting ecological and evolutionary trajectories (Bay *et al.*, 2017). Although abiotic stress

and biotic interactions are a burgeoning topic in community ecology (Clark *et al.*, 2014; Alexander *et al.*, 2015), less is known about intraspecific genetic variation in phenotypes that influence responses to combined abiotic-biotic conditions (Baron *et al.*, 2015; Campitelli *et al.*, 2016). Here, we studied the model plant Arabidopsis to identify traits involved in plastic responses to the combination of drought and competition as well as to determine how competition can change selection for traits related to water balance. We found strong plastic responses to neighboring plants that differed between drought and well-watered conditions. Furthermore, we found that under drought there are major rank changes in relative fitness depending on the presence of competitors and that competition led to changing phenotypic selection.

Plastic responses to competitive and moisture gradients

We found plastic changes in most phenotypes studied across the different treatments imposed, with strong interactions between competition and moisture (Campitelli *et al.*, 2016). Competition and drought generally resulted in reduced measures of performance across the board in both well-watered and drought treatments, although plants in drought and competition had higher performance (biomass and total silique length) than expected from the additive contribution of each stressor. This may indicate multiplicative growth responses to increased resources available in the absence of competition or with sufficient water. Additionally, plants under drought without competition generally had larger rosettes at maturity than in other conditions, perhaps signifying an adaptive conservative response that reduces reliance on inflorescence (cauline) leaves for photosynthesis (Figure 1).

By contrast, life history and water balance phenotypes showed a variety of plastic responses to these environmental gradients. Generally, plastic responses to drought showed a strategy partly indicative of adaptive escape, *i.e.*a resource acquisitive strategy: lower leaf C:N, and greater stomatal density, perhaps allowing plants to rapidly increase photosynthesis and take advantage of pulses of water. At the same time, higher $\delta^{13}C$ under drought indicated higher water use efficiency and stomatal closure as conditions dried. (Des Marais *et al.*, 2012) found that Arabidopsis genotypes tend to plastically increase SLA and decrease C:N under drought, which the authors also interpreted as a drought escape response. However, several non-vernalization requiring genotypes in (Des Marais *et al.*, 2012) showed the opposite or mixed patterns: greater

C:N and lower SLA under drought. Our study genotypes were non-vernalization requiring, and we found that drought led to increased SLA only when Arabidopsis were in competition; there was no drought effect when plants were alone. These drought-by-competition interactions suggest the drought escape strategy might be specific to certain life history strategies or competitive contexts.

Plastic competitive responses differed between drought and well-watered conditions, demonstrating how responses to abiotic and biotic conditions are interdependent. Responses under well-watered conditions with competition were most strongly characterized by higher leaf C:N, suggesting a decrease in a photosynthetic rate per leaf mass, although this was coupled with a consistent but slight decrease in $\delta^{13}C$, the latter of which suggests an acquisitive growth strategy via greater stomatal or mesophyll conductance to escape competition and is consistent with findings by (Campitelli *et al.*, 2016). By contrast, competition under drought resulted in substantially greater SLA, with some genotypes also reducing $\delta^{13}C$, perhaps indicating an acquisitive escape strategy (allowing greater whole-plant carbon assimilation) (Des Marais *et al.*, 2012). Indeed, we found that under drought genetic variation in SLA was positively associated with whole plant carbon assimilation (Figure S6).

$\delta^{15}N$ was only elevated in well-watered no-competition treatments, which we interpreted as a shift in Arabidopsis use of N pools among treatments. Synthetic fertilizers (*e.g.* Miracle Grow used here) cause lower leaf $\delta^{15}N$ relative to organic sources like peat humus (used in our growth media), as fertilizer has more ammonium, which requires a single assimilation event (in roots) versus multiple assimilation events (in roots and shoots) for nitrate sources (Evans *et al.*, 1996; Bateman *et al.*, 2005; Trandel *et al.*, 2018). We interpret our results as showing that well-watered Arabidopsis plants without competition used more N from the peat humus versus fertilizer. Under well-watered conditions with competitors, the fertilizer N source may have been favored by the Arabidopsis plants due to smaller root systems for exploitation of soil N, causing low leaf $\delta^{15}N$. Under drought, N uptake may have been restricted to brief periods following fertigation, favoring the fertilizer source and causing low leaf $\delta^{15}N$.

In well-watered conditions, Arabidopsis responded to competition with greatly reduced performance, including reduced inflorescence branching, despite being well-watered, fertilized, and a lack of shading. Such a strong response suggests Arabidopsis plants were using a chemical cue to sense the neighboring Brachypodium (Badri *et al.*, 2012; Schmid *et al.*, 2013). Studies in a

variety of systems suggest that plant responses to neighboring plant root exudates could be responsible for such signaling (Pierik *et al.*, 2013). Previous studies have shown that SLs in growth media can suppress inflorescence branching by Arabidopsis (Scaffidi *et al.*, 2014) and cause stomatal closure (Lv *et al.*, 2018). Our treatments with a synthetic strigolactone resulted in a strong reduction in vegetative rosette biomass, suggesting SL signaling could help explain Arabidopsis response to neighbors. Genetic variation in response to *B. distachyon* in well-watered conditions was modestly related to genetic variation in response to SL treatment, indicating variation in SL signaling might also play a role in genetic variation in response to neighbors. The yield of PSII was greater for two genotypes under SL treatment, consistent with findings in tomato that GR24 led to greater expression of light-harvesting genes (Mayzlish-Gati *et al.*, 2010). However, two other Arabidopsis genotypes showed *lower* PSII yield in response to SL, and these genotypes had lower reduction in biomass in response to the SL, for reasons that are unclear to us. We also found that experimental SL addition caused an increase in $\delta^{13}C$ (likely signaling stomatal closure, Lv *et al.*, 2018) and a decrease in C:N, patterns that were opposite the Arabidopsis response to their grass neighbors under our well-watered treatment. This indicates that other non-SL cues are required to explain the responses to grass neighbors that we observed under putative non-resource limited conditions (our well-watered treatment).

Genetic variation and genotype-by-environment interactions

Our findings indicate that water limitation selects for different Arabidopsis genotypes depending on whether competitors are present or absent. We observed substantial genetic variation in most traits in each environment (Table S3) and we observed significant genotype-by-competition-by-moisture treatment interactions for performance related traits (fitness components and aboveground biomass) and flowering time. The genotype-by-competition-by-moisture effects on fitness indicate that the effects of competition on relative fitness are conditional on moisture environment. This competition-by-moisture effect on selection could be seen in the substantial changes in relative fitness depending on competition under drought, but the fairly consistent relative fitness despite the presence of neighbors in well-watered conditions (Figure 2). Rank changes in relative fitness across competitive gradients were observed among natural genotypes at a moist site (Baron *et al.*, 2015) and among genotypes segregating for a single water-use-efficiency locus under drought (Campitelli *et al.*, 2016). Here we show selection among natural

genotypes under drought. This suggests that in nature, heterogeneity of competitive context may select for alternate Arabidopsis traits in water limited environments, suggesting a potential mechanism promoting the high genetic diversity in drought responses in natural populations of Arabidopsis (Dittberner *et al.*, 2018; Exposito-Alonso *et al.*, 2018).

We found that genetic variation in flowering time was consistently negatively associated with leaf $\delta^{13}C$, suggesting higher intrinsic leaf-level WUE due to lower stomatal conductance or higher assimilation rate *A* at a given stomatal conductance for early flowering plants (Ferguson *et al.*, 2019). The positive genetic correlation we found between flowering time and $\delta^{13}C$ might be a result of maladapted late-flowering genotypes having lower *A* for a given stomatal conductance. Our findings contrast with some previous studies that found the early flowering plants had low leaf $\delta^{13}C$ (McKay *et al.*, 2003; Lovell *et al.*, 2013; Kenney *et al.*, 2014). Our panel of natural genotypes was lacking genotypes that flower late without vernalization, thus these genotypes might partly be responsible for the discrepancies with these publications. Additionally, (Ferguson *et al.*, 2019) did not find a flowering time relationship with leaf $\delta^{13}C$ in comparisons of NILs at major flowering time QTL.

Selection under competition and changes in selection across competitive gradients

We identified several traits associated with fitness in specific competitive contexts. Inflorescence height and AGB_{mature} showed the strongest evidence for changing selection depending on competitive context, both in well-watered conditions. Under well-watered competitive conditions, plants with greater AGB_{mature} and taller inflorescences had greater fitness, but not in the absence of competition. Selection for AGB_{mature} may indicate that the investment in leaves for resource capture (comprising a large portion of non-reproductive of AGB, and including both rosette and cauline leaves) is adaptive when surrounded by competitors, while allocation to reproductive parts may be more favored when competitors are absent. The lack of selection for greater AGB_{mature} and taller inflorescences in drought may indicate that aboveground vegetative investments are less favored when competitors are smaller aboveground. We also found positive selection for the plastic increase in inflorescence height under well-watered competition treatments. We interpret this as evidence that inflorescence height is only a good measure of total fecundity under the treatment with the most competitor biomass (well-watered), where

competitors lead to suppressed inflorescence branching (Schmitt *et al.*, 2003; von Wettberg *et al.*, 2008).

Under drought and competition, plants with greater $\delta^{13}C$ had higher fitness, indicating greater leaf-level water use efficiency was adaptive, but not in other treatments. We expected lower WUE would be favored under drought with competitors to rapidly complete development before competitors depleted resources too low (Campitelli *et al.*, 2016). Instead, higher WUE may have been favored because conditions were too dry to permit success of a rapidly developing drought escape strategy, thus higher WUE may be necessary in these environments. Alternatively, despite the dramatically lower performance of plants in response to competition under drought, the intermittent watering may have been enough to favor conservative drought avoidance strategies (high $\delta^{13}C$). Our findings contrast with those of (Kenney *et al.*, 2014) who found terminal drought and well-watered treatments both selected for lower $\delta^{13}C$ and plants with greater AGB_{mature}, suggesting acquisitive escape strategies were favored. Unsurprisingly, early flowering was favored in competitive treatments as it was in all treatments, similar to the consistently observed selection for earlier flowering in many experiments (Austen *et al.*, 2017) including those on Arabidopsis under terminal drought (Kenney *et al.*, 2014).

Conclusions

The effects of abiotic stress on phenotypic plasticity and natural selection depend on biotic context, as we demonstrated in this study of Arabidopsis responses to drought and competition. Arabidopsis showed strong plastic responses to competition and changes in phenotypic selection that were contingent on moisture environment. Our results suggest Arabidopsis exhibits characteristics of both drought and competitor escape, but also traits and selection indicative of adaptive drought avoidance. Arabidopsis represents an important model system for plants with growing interest in its mechanisms of adaptation to local environments. Our results indicate that natural variation and its geographic distribution in Arabidopsis should be interpreted in light of biotic interactions.

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Author contributions

CML carried out experiments, analyzed data, and wrote the manuscript. JRL planned the experiments, analyzed data, and wrote the manuscript.

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SUPPLEMENTAL FIGURES AND TABLES

Figure S1. Experimental treatments, including (A) drought treatment under competition and no competition, and (B) well-watered treatment under competition and no competition. Photographs were taken 53 days after planting, prior to the first harvest.

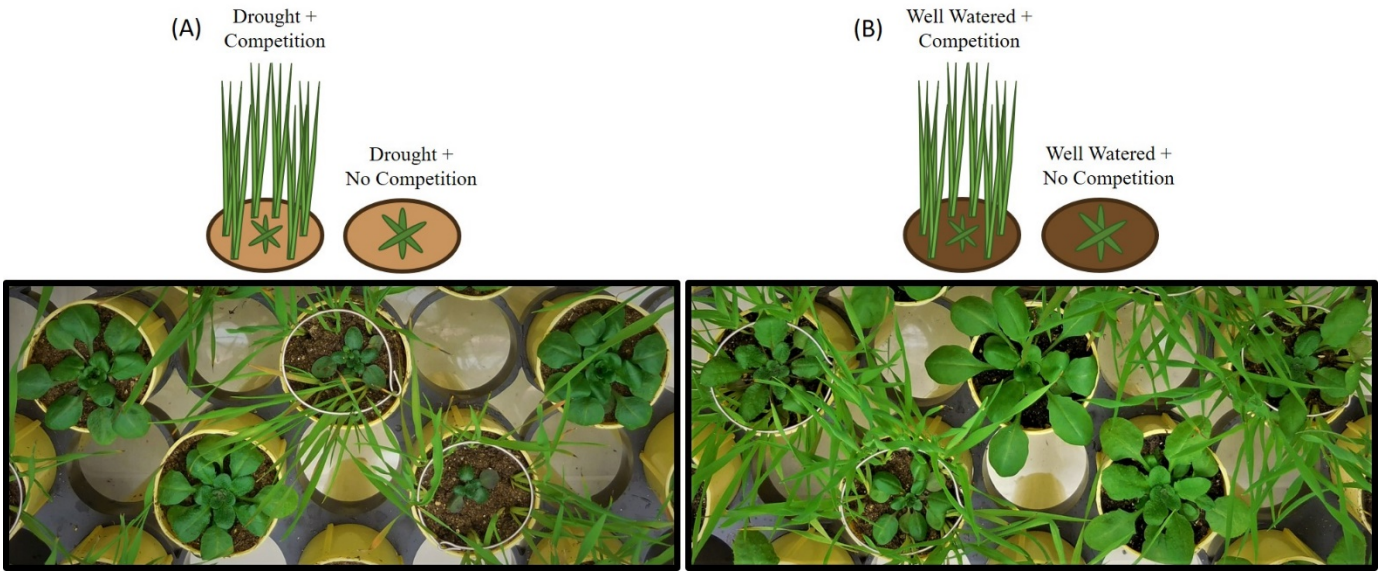

Figure S2. Experimental plant with stainless steel wire installed to prevent *B. distachyon* shading.

Figure S3. Time series of temperature (°C) conditions using a HOBO data logger (HOBO®, Onset Computer Corporation). Temporary spikes in temperature were due to brief power outages, and did not result in any clear detrimental effects on experimental plants.

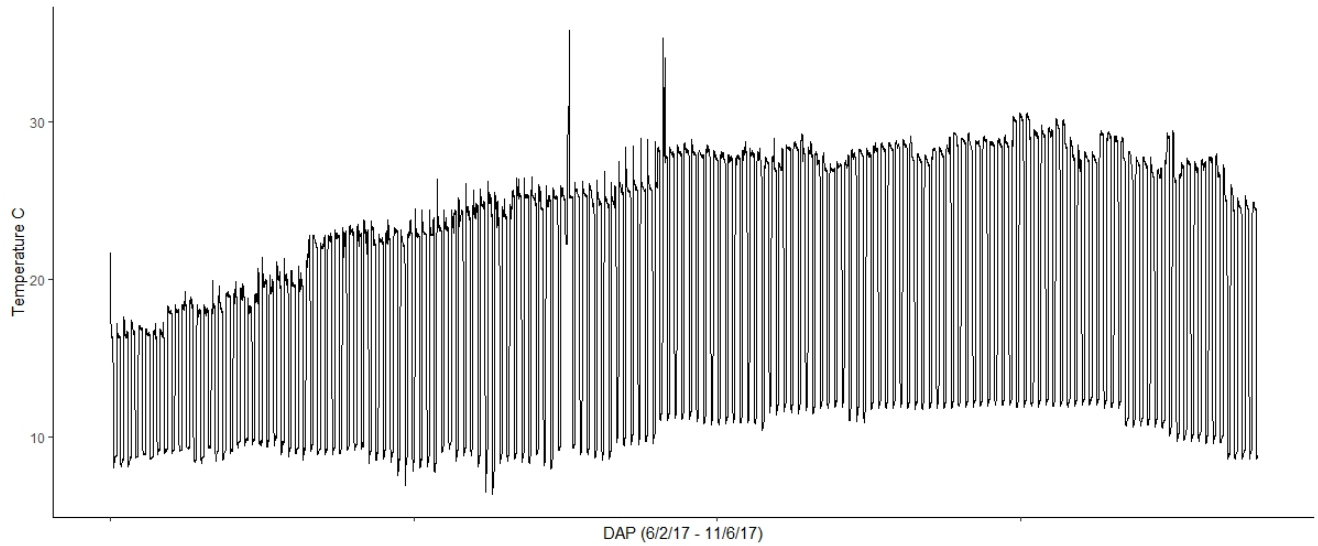

Figure S4. Percent volumetric water content (%VWC) of potting mix within pots for each treatment was monitored using EM-50 dataloggers and EC-5 probes (METER Group Inc., Pullman, WA).

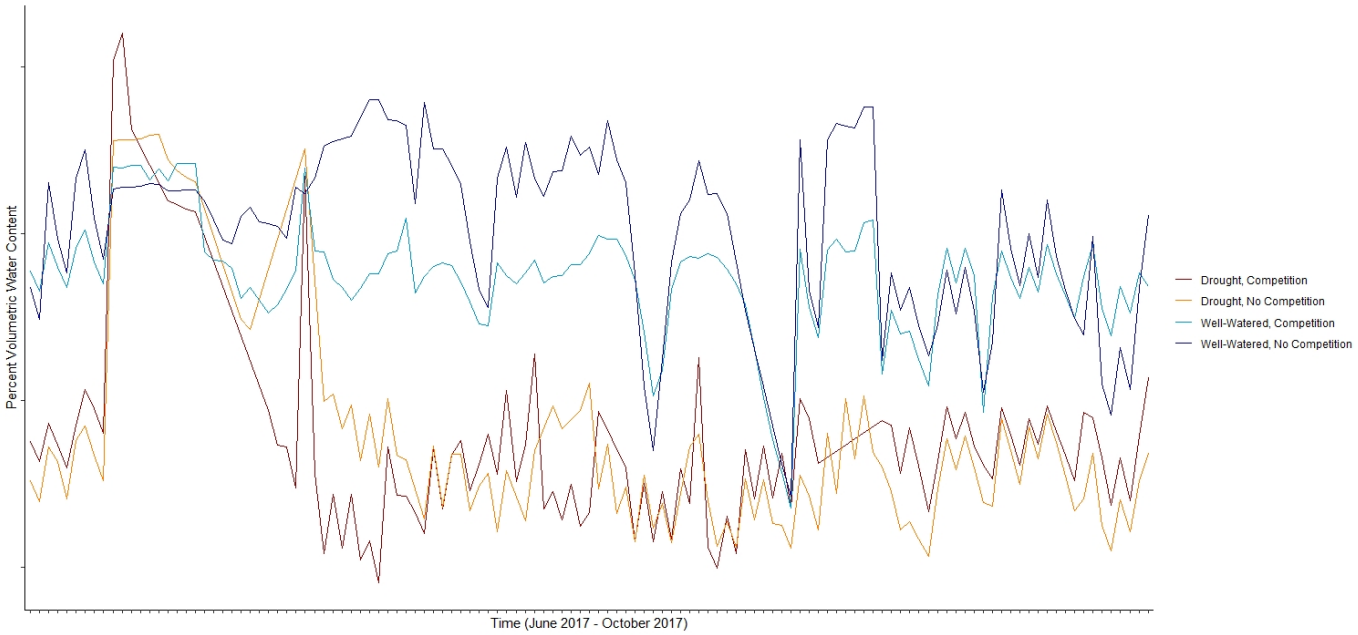

Figure S5. Response of four genotypes to increasing concentrations of GR24 applied to soil.

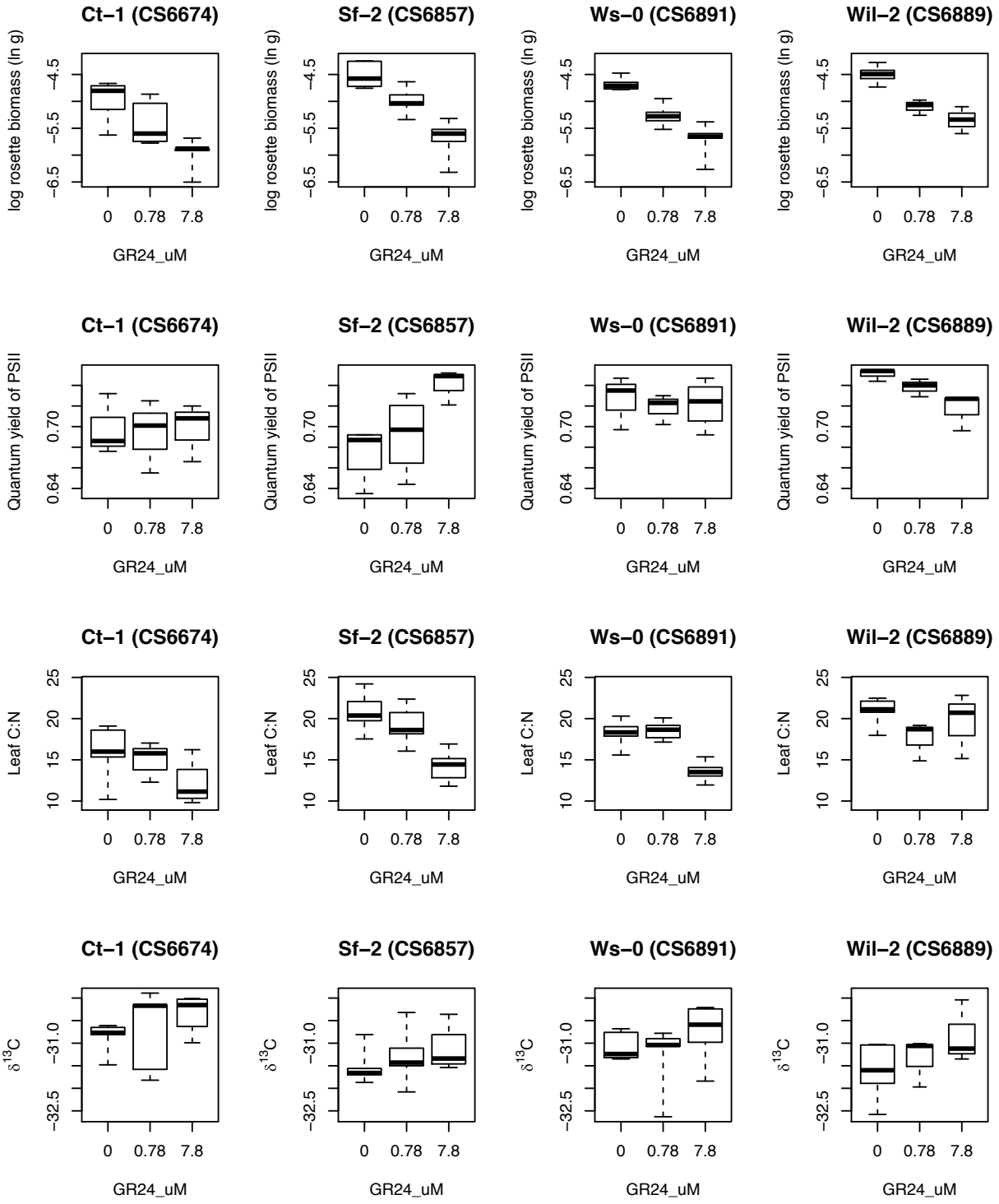

Figure S6. Bivariate relationships between traits often thought to be exhibit coordinated variation. Note that gas exchange (used to caculate *A*) was only measured in the absence of competitors, these estimates are used in the comparisons with traits under competition.

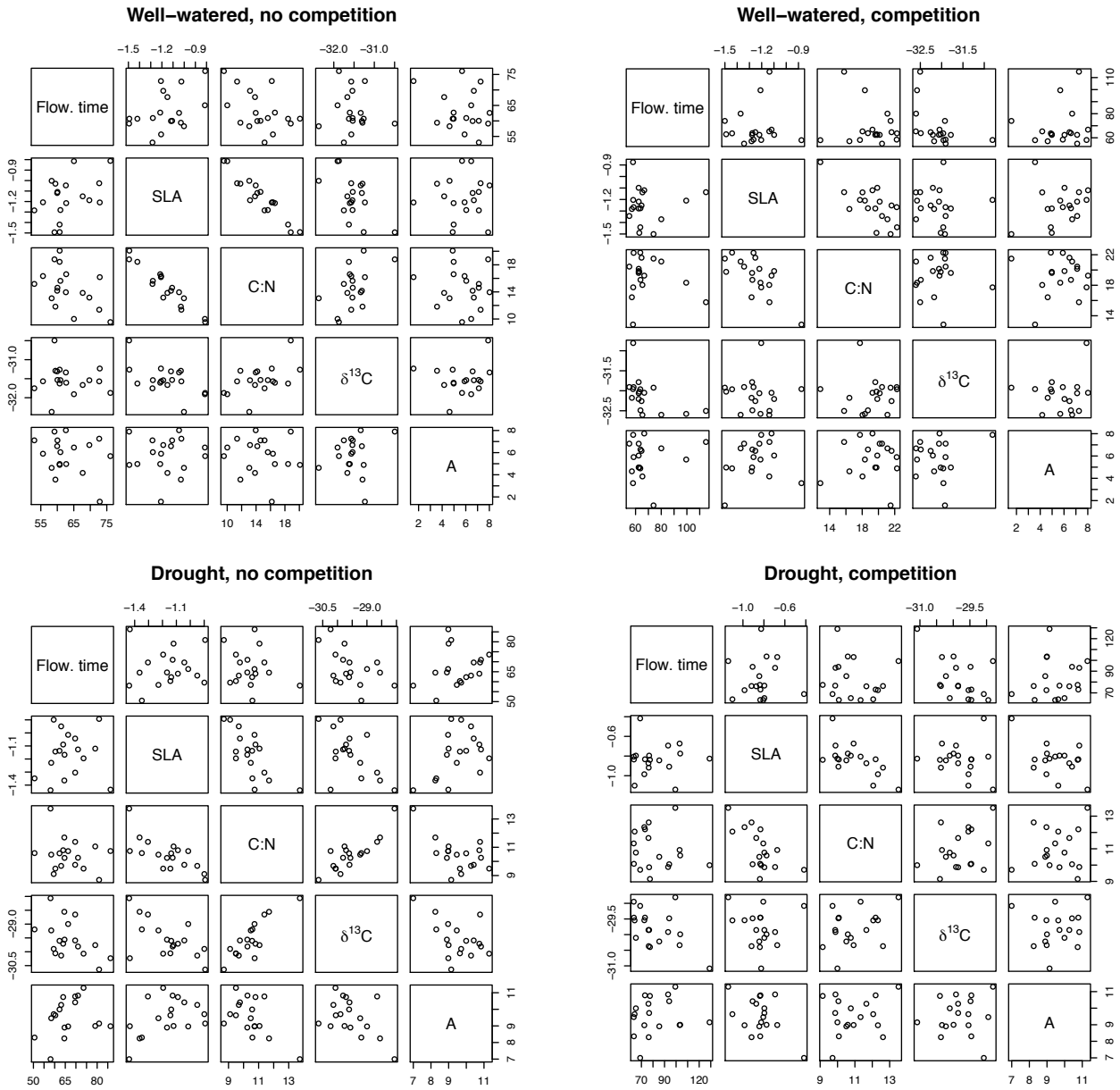

Table S1. The 18 parent genotypes from the Multiparent Advanced Generation Inter-Cross (MAGIC) used in this study.

ABRC Stock #	Accession
76734	Bur-0
76740	Can-0*
76778	Col-0
76786	Ct-1
76831	Edi-0
76969	Kn-0
77020	Ler-0
1380	Mt-0
77128	No-0
77156	Oy-0
6839	Po-0
77222	Rsch-4
77247	Sf-2
77389	Tsu-0
78856	Wil-2
78857	Ws-0
78858	Wu-0*
78880	Zu-0

* Stock was identified as incorrect by ABRC after seed was obtained (Pisupati *et al.*, 2017).

Table S2. Temperature and photoperiod program for Conviron growth chamber, to simulate early spring germination for a spring annual.

Week	Night °C	Day °C	Photoperiod (Hours:Minutes)
1	10	15	13:04
2	10	15	13:30
3	10	15	13:54
4	10	16	14:20
5	10	17	14:42
6	10	18	15:06
7	10	19	15:26
8	10	20	15:44
9	10	21	16:00
10	10	21	16:12
11	11	22	16:18
12	12	23	16:22
13	12	23	16:20
14	13	24	16:16
15	13	24	16:06
16	13	24	15:52
17	13	24	15:34
18	13	24	15:16
19	13	24	14:54
20	13	24	14:32
21	12	23	14:08
22	11	22	13:42
23	10	21	13:18
24	10	20	12:52

Table S3. Broad sense heritability of traits within each treatment. 'm' suffixes indicate mature end stage harvest, while 'v' indicates the pre-flowering vegetative stage harvest.

	WW NC	WW C	D NC	D C
Absolute fitness	0.7880	0.7337	0.5693	0.2797
AGB_m	0.6026	0.5489	0.6739	0.4255
Rosette wt_m	0.8592	0.5178	0.7970	0.4030
Inflorescence wt	0.6081	0.6419	0.3673	0.4387
Flowering time	0.8932	0.9568	0.8873	0.8798
Inflorescence height	0.8378	0.7176	0.4076	0.3531
Inflorescence branches	0.6920	0.4795	0.5927	0.3241
Silique number	0.7972	0.6835	0.5705	0.3024
Average silique length	0.5640	0.6601	0.4642	0.2772
Root wt_v	0.3012		0.1627	
Root diam_v	0.2952	0.2444	0.1254	0.4691
Root:shoot	0.3087		0.2019	
Total leaf area_v	0.3101	0.3695	0.2286	0.3768
Stomatal density	0.2026	0.2080	0.3614	0.3554
Rosette wt_v	0.6003	0.4206	0.2611	0.3754
log(SLA)	0.3924	0.2513	0.3884	0.2936
Leaf prop N	0.5285	0.3096	0.2566	0.3425
Leaf prop C	0.2960	0.2139	0.2566	0.2951
Leaf C:N	0.5785	0.4204	0.3269	0.3335
$\delta^{13}C$	0.3825	0.4691	0.3838	0.2766
$\delta^{15}N$	0.2820	0.2496	0.3640	0.3437
A (whole plant assimilation)	0.1325		0.1633	
Brachypodium AGB		0.2780		0.3606

Table S4. Results of treatment fixed effects on phenotypes from a linear mixed-effects model where genetic and tray effects were modeled as random effects.

See attached files.

Table S5. Variance components estimates for linear mixed-effects model where genotype, genotype-treatment interactions, and tray effects were modeled as random effects and treatments were fixed effects. 'est' refers to the variance component estimate and 'LCL' and 'UCL' refer to the lower and upper 95% confidence limits for the estimate. Variance components estimated and fixed at zero report 'NA' for the 95% CI.

See attached files.

Table S6. Selection on traits in different treatments

See attached files.

Table S7. P-values for two-sided tests of changing selection on traits across competition treatments within a given moisture treatment. Null models based on permutations of trait values among genotypes within each treatment.

Trait	Well-watered	Drought
AGB_m	0.0234	0.0540
Rosette wt_m	0.5724	0.1924
Inflorescence wt	0.1128	0.7846
Flowering time	0.7300	0.7428
Inflorescence height	0.0120	0.9152
Inflorescence branches	0.0510	0.2610
Silique number	0.2676	0.6670
Average silique length	0.1474	0.2240
Root diam_v	0.3632	0.4636
Stomatal density	0.8092	0.0912
Rosette wt_v	0.5802	0.2506
log(SLA)	0.5902	0.4428
A (whole plant assimilation)	0.7372	0.7238
Leaf C:N	0.9894	0.6186
$\delta^{13}C$	0.3030	0.1606
$\delta^{15}N$	0.7570	0.6918

Table S8. Selection on mean and plasticity of trait values across competition treatments. Plasticity was calculated as no competition – competition treatments. For example, selection for an increase in inflorescence height in well-watered competition treatments is indicated by a negative coefficient.

	Well-watered				**Drought**			
Trait	*Trait avg. coeff.*	*Trait avg. p*	*Trait plast. coeff.*	*Trait plast. p*	*Trait avg. coeff.*	*Trait avg. p*	*Trait plast. coeff.*	*Trait plast. p*
AGB_m	0.2650	0.0308	-0.2166	0.0705	-0.3588	0.0209	0.1413	0.3254
Rosette wt_m	-0.1773	0.4933	-0.0116	0.9638	-0.6082	0.0057	0.3485	0.0848
Inflorescence wt	0.3517	0.0019	-0.1323	0.1775	-0.0133	0.9222	0.2081	0.1414
Flowering time	-0.3877	0.0004	-0.1145	0.1950	-0.4004	0.0105	-0.2510	0.0870
Inflorescence height	0.1203	0.0602	-0.2056	0.0034	-0.0746	0.4506	0.0756	0.4450
Inflorescence branches	0.0818	0.4116	-0.1058	0.2915	-0.0956	0.4823	-0.0801	0.5552
Silique number	0.3852	0.0000	-0.0667	0.1681	0.3362	0.0002	0.0323	0.6357
Average silique length	0.1113	0.1363	-0.1480	0.0537	0.2355	0.0135	-0.0003	0.9970
Root diam_v	0.0491	0.5826	-0.1139	0.2125	-0.0649	0.5133	0.0386	0.6958
Stomatal density	-0.1108	0.1869	-0.0565	0.4912	-0.0602	0.5424	0.1225	0.2241
Rosette wt_v	0.1423	0.5122	-0.0109	0.9597	0.0963	0.6021	-0.1698	0.3622
log(SLA)	-0.0364	0.6589	-0.1200	0.1582	0.0799	0.4150	0.0448	0.6451
Leaf C:N	0.0995	0.2216	0.0905	0.2638	-0.0327	0.7434	0.0112	0.9108
$\delta^{13}C$	0.0964	0.2224	-0.1133	0.1553	0.1617	0.0817	-0.1151	0.2039
$\delta^{15}N$	0.0346	0.6815	0.0762	0.3714	0.0688	0.4971	0.0356	0.7241