Species interactions in a changing environment: elevated CO_2 alters the ecological and potential evolutionary consequences of competition

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ABSTRACT

Question: How will global changes impact the ecological and evolutionary outcomes of competition?

Hypothesis: Global changes that alter resource availability, such as rising atmospheric carbon dioxide (CO_2) concentrations, will alter the effects of competition on mean fitness and patterns of natural selection. Because species exhibit different growth responses to elevated CO_2 and because different traits may aid in competition against different taxa, these ecological and evolutionary effects may depend on the identity of the competitor.

Organism: *Arabidopsis thaliana* grown under intraspecific competition or interspecific competition with the C3 grass *Bromus inermis* or the C4 grass *Andropogon gerardii*.

Field site: BioCON (Biodiversity, CO_2, and Nitrogen) experiment at Cedar Creek Ecosystem Science Reserve, Minnesota, USA.

Methods: Manipulate the presence and type of competition experienced by *A. thaliana* populations growing under ambient or elevated CO_2 conditions. Measure the interactive effects of CO_2 and competition on mean fitness and on patterns of natural selection.

Conclusions: Elevated CO_2 reduces the effects of competition on mean fitness, alters the relative fitness effects of different competition treatments, and minimizes the strength of competition as a selective agent.

Keywords: carbon dioxide, competition, effect size, global change, indirect effect, natural selection, resource availability.

INTRODUCTION

Competition is one of the most important biotic interactions affecting plants, and the ecological outcomes of competition have been the subject of considerable empirical and theoretical research (e.g. MacArthur and Levins, 1967; Grime, 1977; Tilman, 1977; Keddy, 1990; Silander and Pacala, 1990; Fargione and Tilman, 2006). This research has led to the development of several ecological

theories that predict competitors' effects on focal plant species. Some of these theories predict that the fitness consequences of competition will be greater when competing taxa are more similar in resource use and, by extension, that differences in resource use are required for species co-existence (e.g. Hardin, 1960; MacArthur and Levins, 1967; Tilman, 1977). Other empirical and theoretical work suggests that the availability of the suite of resources limiting plant growth will determine the competitive rankings of species (e.g. Tilman, 1977; Fynn *et al.*, 2005; Dybzinski and Tilman, 2007). It is plausible that these ecological patterns have evolutionary analogues: that selection is stronger when plants compete with similar competitors and that resource availability affects the intensity of selection on size and other traits that mediate competitive interactions. However, more data are needed to compare the ecological and evolutionary effects of competition.

Understanding how the environment affects both the ecological and evolutionary outcomes of competition is increasingly important given the rapid environmental changes being brought about by global warming, nitrogen deposition, increasing atmospheric carbon dioxide (CO_2) concentrations, and changes in diversity and community composition. In addition, environmental differences may explain some of the apparently inconsistent results observed in studies investigating the evolutionary consequences of competition. While few studies (on either plants or animals) have identified how competitors alter selection on specific traits, those studies that have included rigorous selection analyses reveal few consistent patterns. For example, several studies have detected stronger selection for taller, larger plants in more competitive than less competitive environments (Thomas and Bazzaz, 1993; Miller, 1995; Dudley and Schmitt, 1996; Donohue *et al.*, 2000; Dorn *et al.*, 2000), but some studies investigating selection on other size measures (e.g. leaf size or branch number) or utilizing other fitness components have shown the opposite pattern (Dudley and Schmitt, 1996; Donohue *et al.*, 2000; Dorn *et al.*, 2000). Similarly, studies have detected both stronger and weaker selection for earlier flowering and other phenological traits in more competitive environments (Thomas and Bazzaz, 1993; Dorn *et al.*, 2000; Stanton *et al.*, 2004). In summary, the available data suggest that competition can be a potent modulator of evolution; however, the effects of competition on the evolution of specific plant traits are highly variable, potentially because different taxa of competitors exert different selective pressures or because abiotic environmental conditions influence both the ecological and evolutionary outcomes of competition.

Here, we report on an experiment designed to characterize how biotic and abiotic environmental factors influence the ecological and evolutionary consequences of competition. Specifically, we investigate how competition with different taxa influences plant growth and fitness and the predicted evolution of a synthetic *Arabidopsis thaliana* population under both ambient and elevated atmospheric CO_2 concentrations. In previous studies, we detected little evidence that elevated CO_2 concentrations (eCO_2) directly impact plant evolution in the absence of competition (Lau *et al.*, 2007). Nevertheless, there is ample evidence that changes in CO_2 concentrations impact competitive outcomes (reviewed in Brooker, 2006) and that competition may have a strong influence on evolution (e.g. Miller, 1995). Thus, eCO_2 could indirectly affect plant evolution by altering the outcome of competitive interactions. We grew plants in different competitive environments (no competition, intraspecific competition, or interspecific competition with the C3 grass *Bromus inermis* or the C4 grass *Andropogon gerardii*), under both ambient and elevated CO_2 concentrations to examine the extent to which competitor identity and the abiotic environment affect: (1) mean *A. thaliana* fitness; (2) which accessions are favoured by natural selection and the

expression of phenotypic variation; and (3) patterns of selection on ecologically relevant plant traits. Our main goals were to determine: (1) whether variation in both the ecological and evolutionary consequences of competition can be explained by variation in the abiotic and biotic (e.g. competitor identity) environment, and (2) whether the evolutionary effects of competition parallel ecological effects.

METHODS

Experimental design

We manipulated atmospheric CO_2 concentration (aCO_2) and the competitive environment in a split-plot design. Carbon dioxide treatments were applied to whole plots, and competition treatments were randomly imposed on individual plants within each whole plot. The CO_2 treatments were part of an ongoing FACE (Free Air CO_2 Enrichment) experiment at the Cedar Creek Ecosystem Science Reserve, Minnesota, USA (http://www.lter.umn.edu/biocon/) (Reich *et al.*, 2001). In this experiment, the two CO_2 treatments [aCO_2 ~368 μmol·mol^{-1} or eCO_2 ~560 μmol·mol^{-1}, the predicted concentration of atmospheric CO_2 in 2050 (Houghton *et al.*, 2001)] are applied to six 20-m diameter open-air rings (three rings per treatment). The eCO_2 treatment is maintained by releasing concentrated CO_2 through vertically positioned pipes spaced around the perimeter of the ring (the gas is blown out of the pipes and then moves by diffusion and mass flow across the ring). The control rings (aCO_2) are surrounded by the same pipe structure, but the air blown through these pipes is not enriched in CO_2. The CO_2 treatments were applied during daylight hours over the course of the experiment. FACE systems used to manipulate atmospheric CO_2 concentrations in natural field environments have only minor effects on microclimate or light conditions (Hendrey *et al.*, 1993).

Competition treatments were applied at random to individuals from each of 19 *Arabidopsis thaliana* accessions (ecotypes), under both aCO_2 and eCO_2 conditions. Although local CO_2 concentration is very unlikely to be altered by competitor presence and, therefore, direct competition for this resource is not an issue, CO_2 concentration may indirectly affect competition for other resources such as water, nitrogen, and light as a consequence of effects of eCO_2 on plant growth and water use efficiency (Bazzaz and McConnaughay, 1992; Körner, 2003; Tonsor and Scheiner, 2007; Zak *et al.*, 2007). Four competition treatments were applied: no competition, intraspecific competition, or competition from representative species from two functional groups, C3 and C4 grasses (*Bromus inermis* and *Andropogon gerardii* respectively). Because C3 species typically increase biomass in response to eCO_2 more than C4 species (reviewed in Ackerly and Bazzaz, 1995; Poorter and Navas, 2003), we expected eCO_2 to change competitive rankings. In particular, we hypothesized that: (1) the fitness effects of competition with the C4 species would be reduced under eCO_2 because *A. thaliana* (a C3 species) is likely to proportionally increase biomass more under eCO_2 than the C4 competitor, and (2) competition between *A. thaliana* and the C3 competitors (intraspecific competition and competition with *Bromus*) would be more intense under eCO_2 because both taxa would show strong positive growth responses to eCO_2. While *A. thaliana* is unlikely to compete with *Andropogon* or *Bromus* in natural communities, we use *A. thaliana* as a model for investigating the potential for eCO_2 to indirectly impact the ecology and evolution of plant populations via effects on competitive interactions. *Arabidopsis thaliana* is a useful model for ecological, as well as genetic, studies because of the availability of a wide array of accessions from diverse habitats, previous work on this species demonstrating

physiological, growth, and phenological responses to eCO_2 (e.g. Teng *et al.*, 2006; Tonsor and Scheiner, 2007; Li *et al.*, 2008; Springer *et al.*, 2008), and because we have conducted several other studies investigating direct evolutionary effects of eCO_2 on the predicted evolutionary trajectory of this species (Lau *et al.*, 2007). Our use here of a phenotypically diverse set of accessions increases the statistical power to detect selection.

The factorial design resulted in eight treatment combinations; there were four competitive environments: (1) no competition (control), (2) competition with a second *A. thaliana* individual of the same accession (intraspecific competition), (3) competition with *Bromus*, a C3 grass, and (4) competition with *Andropogon*, a C4 grass, each grown in both aCO_2 and eCO_2. We planted six pots per accession per treatment ($N = 912$ pots in total). Because of low germination of competitors in this experiment, there were more control plants because pots in which competitors did not germinate were treated as no-competition controls, and sample sizes ranged from 11 to 18, 2 to 6, 3 to 6, and 1 to 5 per accession per CO_2 treatment in the no competition (control), intraspecific, *Bromus*, and *Andropogon* competition treatments respectively. *Arabidopsis thaliana* accessions represent populations from both the native European range of *A. thaliana* and the introduced North American range. All accessions were originally obtained from the *Arabidopsis* Biological Resource Center, The Ohio State University, and were provided by J.K. McKay (Colorado State University) and M.T. Brock (University of Wyoming).

For each replicate of each accession, 4–10 seeds were planted into a 164-ml conetainer™ (Ray Leach Conetainers™, Stuewe and Sons, Inc., Corvallis, OR, USA) that had been filled with potting mix (Sunshine Mix #5; Sun Gro Horticulture Canada Ltd., Alberta, Canada) and bottom-watered until saturated. Following planting, conetainers™ were placed in a dark cold-room at 4°C for 2 days to synchronize germination and then moved to a greenhouse where they remained until seeds germinated. In the two interspecific competition treatments, four seeds of the competitor (*Bromus* or *Andropogon*) were planted and later thinned to two competitors per pot. All *A. thaliana* germinants were thinned to one individual per pot, except in the intraspecific competition treatment where germinants were thinned to two *A. thaliana* individuals per pot. It should be noted that the intraspecific and interspecific competition treatments included different plant densities (one competitor in the intraspecific competition treatment, compared with two competitors in the *Bromus* and *Andropogon* treatments). Unequal densities were used in an attempt to equalize competitor biomass across competition treatments, because competitor biomass is often strongly correlated with the magnitude of competitive effect and because total competitor biomass is likely a better indicator of resource use than number of individuals (Goldberg and Werner, 1983). Although final competitor biomasses did differ significantly between competition treatments ($F_{2,465} = 254.13$, $P < 0.0001$), competitor biomasses were roughly equivalent in the intraspecific and *Bromus* treatments (mean ± 1 standard error: 0.25 ± 0.01 g and 0.21 ± 0.01 g respectively). Unfortunately, due to poor growth and establishment, competitor biomass in the *Andropogon* treatments was much lower (0.05 ± 0.01 g).

All plants were moved to the field on 6 June 2006, approximately 5 days after emergence. We watered all plants as needed to prevent drought-induced mortality by placing the potted plants into tubs and bottom-watering (all plants received similar amounts of water). We harvested all *A. thaliana* plants (and the competitors in the same pot) after fruits on the main stem had matured and plants had senesced (between 4 July and 21 July). Although this experiment was conducted in relatively small pots and pot-binding can influence the magnitude of CO_2 responsiveness (e.g. Thomas and Strain, 1991; McConnaughay *et al.*, 1993, 1996), the

plants were also small (mean biomass varied from 0.20 to 0.51 g across treatments) and typically did not become pot-bound over the course of the experiment. Moreover, while the simple system used here limits ecological realism, it does allow for increased control over competitive environments.

Plant measurements

We measured growth, phenological, and fitness traits for the focal *A. thaliana* individual in each pot. On 13 June, we counted the number of leaves and measured rosette diameter to the nearest 1 mm. On 22 June, when most plants were just beginning to flower, we measured rosette diameter. Flowering began on 13 June; we checked plants for flowering every other day for the remainder of the season. Both *A. thaliana* and competitors were harvested as *A. thaliana* vegetative material began to senesce and fruits on the main stem began to dehisce. After harvest, we recorded plant height, number of flowering stems, and silique (fruit) number. The dry weights of the total above-ground portion of each *A. thaliana* plant and all competitors were obtained after drying tissue at 60°C. Seed counts from three representative fruits per plant revealed that fruit number was highly correlated with estimated seed production (fruit number × mean seed number per sampled fruit) ($r = 0.87$, $P < 0.0001$, $N = 158$, based on individuals in the *Bromus* and no competition treatments). Strong correlations also have been reported by Mauricio and Rausher (1997) and Westerman and Lawrence (1970), supporting the use of fruit number as a basis of inferences about fecundity in this species. Fecundity is only one aspect of fitness, however, and using fruit or seed number as an estimate of lifetime fitness does not account for potential trade-offs between seed or fruit number and seed quality.

Statistical analyses

Phenotypic effects and genotype × environment interaction

To evaluate effects of competition treatment, CO_2 environment, variation among accessions, and variation in accession response to competition and CO_2 treatments, we performed mixed-model nested analysis of variance (ANOVA) on each trait [PROC MIXED (SAS Institute, Inc., 2001)]. In these analyses, competition, CO_2, accession, and all interactions were included as fixed factors, and each plant trait was the response variable. Ring nested within the CO_2 treatment was included as a random factor, and initial plant diameter (measured before moving to the field) was included as a covariate. Analyses of effects on fitness measures (fruit number and biomass) were conducted on both raw and natural log-transformed data because analyses on log-transformed data test whether the proportional reductions in fitness due to competition are equivalent across CO_2 environments (multiplicative model), whereas analyses of untransformed data show whether the absolute reduction in fitness due to competition is equivalent across CO_2 treatments (additive model). Although residuals from analyses of both natural log-transformed and non-transformed data of fruit number and biomass differed significantly from normal distributions, Kruskal-Wallis statistics were high in all cases (0.87 and 0.89 for log-transformed biomass and log-transformed fruit number respectively, and 0.98 for both non-transformed biomass and fruit number), and residual plots did not show egregious deviations from normality. The distribution of residual values from the analyses on

log-transformed data, however, approximated less closely a normal distribution, and these residual values were also more leptokurtic than residuals from analyses on non-transformed values.

Significant $CO_2 \times$ competition interactions affecting fitness indicate that the resource environment influences competitive outcomes. Significant competition $\times$ accession interactions indicate that competitive environments affect natural selection among accessions or the opportunity for selection (i.e. variation in fitness), and significant competition $\times$ accession $\times CO_2$ interactions provide evidence that the CO_2 environment alters the magnitude of these evolutionary effects. When a significant effect of competition was detected, we performed pairwise contrasts between all competition treatment combinations to identify which competition treatments were responsible for the effect. To further investigate how competitive effects vary across resource environments, we performed an analysis of covariance (ANCOVA) where we replaced the competition treatment term with competitor biomass (using only plants in the *Bromus* and *Andropogon* competition treatments). Plants in the intraspecific competition treatment were excluded from this analysis because each accession was competed against a second plant of the same accession and, therefore, the competitor's accession varied in a non-random manner, making it impossible to differentiate between competitor biomass and accession effects. For both types of analyses, significance of random factors was tested with ln-likelihood ratio tests.

As mentioned above, significant competition $\times$ accession interactions could result from heterogeneous variances across treatments. Therefore, to identify patterns in the expression of variation across treatments, we calculated coefficients of variation (CV) and broad-sense heritabilities of fitness and growth traits in each environment. Coefficients of variation were calculated from accession means and are relevant to both ecological and evolutionary outcomes because increased coefficients of variation are indicative of both increased asymmetric competition and increased opportunity for selection. Broad-sense heritabilities were calculated in each treatment as the proportion of variation in each trait explained by accession.

Patterns of selection

We conducted phenotypic selection analyses (Lande and Arnold, 1983) to estimate selection on all traits measured on our population. Phenotypic selection analysis is a regression approach used to estimate both selection differentials and selection gradients. Selection differentials, which we estimated by performing separate univariate regression analyses to evaluate the relationship between fitness and each trait (Price, 1970), provide estimates of net selection acting on a trait – that is, selection acting directly on that trait plus selection acting on correlated traits. Selection gradients, which provide estimates of the strength of selection acting directly on each trait by accounting for selection on correlated traits included in the analysis (i.e. indirect selection) (Lande and Arnold, 1983), were estimated using multiple regression. Five traits were included in the multiple regression (selection gradient) analyses: biomass, rosette size, height, stem number, and flowering date. These growth and phenological traits were chosen for the selection analyses because both competition and eCO_2 might be expected to exert selection on growth traits (e.g. taller plants may be favoured when light competition is intense) and because competition often influences selection on phenological traits such as flowering time (Thomas and Bazzaz, 1993; Dorn *et al.*, 2000; Stanton *et al.*, 2004). In addition, eCO_2 and competition might be predicted to alter selection on flowering time because both factors are expected to alter soil moisture availability, and early flowering is a common

drought avoidance strategy in many species, including *A. thaliana* (McKay *et al.*, 2003). Quadratic and interaction gradients were not included because of the large sample sizes required, and only linear selection gradients are presented. In both the simple and multiple regressions, individual relative fitness was the response variable, and the morphological and phenological traits were predictor variables. Relative fitness was calculated as fruit number divided by mean fruit number in that competition $\times$ CO_2 treatment. All predictor variables were standardized by their within-treatment standard deviation (Lande and Arnold, 1983; Arnold and Wade, 1984). We tested for between-treatment differences in selection by including the measured traits, CO_2 and competition treatments, and interactions between traits and the CO_2 and competition treatments in an ANCOVA (Price, 1970; Lande and Arnold, 1983). Ring nested within CO_2 treatment was included as a random factor. In addition to the ANCOVA, we also bootstrapped the regression coefficients (1000 re-samples) to obtain 95% confidence intervals for each selection differential and gradient [using the %BootCI macro (SAS Institute, Inc., 2001)].

Phenotypic selection analyses can be biased by environmentally induced covariances between fitness and measured traits (Rausher, 1992; Scheiner *et al.*, 2002; Winn 2004; Stinchecombe *et al.*, 2002). To determine whether such biases influenced our results, we performed a genotypic selection analysis (*sensu* Rausher, 1992) on accession mean trait and fitness values. Estimates of selection differentials and gradients obtained from the genotypic selection analyses were qualitatively similar to those obtained from the phenotypic selection analyses, and similar results were observed in the ANCOVA testing for effects of competition and CO_2 treatments on patterns of selection (results not shown). Here, we present results from the phenotypic selection analyses alone because only 19 accessions were included in this study, resulting in low power for the genotypic selection analyses.

RESULTS

Ecological effects

As expected, plants grown in eCO_2 were larger than plants grown in aCO_2, and plants grown in the absence of competitors were larger and produced more fruits than plants grown with competitors (Tables 1, 2). Individuals of the C4 grass *Andropogon* were much smaller than either *Bromus* or *A. thaliana* competitors, however, and their competitive effects were correspondingly less pronounced and not statistically significant (averaged across CO_2 treatments, *Andropogon* reduced *A. thaliana* biomass by 2% and fruit production by 6%).

Competitive effects on *A. thaliana* fruit number depended on the CO_2 environment, as indicated by the significant $CO_2 \times$ competition interaction on fruit number ($F_{3,799} = 3.75$, $P < 0.011$; on ln-transformed fruit number, $F_{3,799} = 5.55$, $P < 0.001$). Both the absolute and proportional reductions in fitness caused by interspecific competition tended to be greater in aCO_2 than eCO_2 environments (Tables 1, 2). Competition with *Bromus* reduced *A. thaliana* fruit production by 35% (31 fruits) in aCO_2 and by 28% (30 fruits) in eCO_2. Competition with *Andropogon* reduced *A. thaliana* fruit production by 11% (9 fruits) in aCO_2 and increased fruit production by 2% (3 fruits) in eCO_2, although the effects of competition with *Andropogon* were not statistically significant in either CO_2 environment ($P > 0.16$). Similar results were observed when competitor biomass, rather than competitor presence, was included in the analyses. We observed that the negative correlations between *A. thaliana* fruit production and *Bromus* or *Andropogon* biomass were less in eCO_2 than
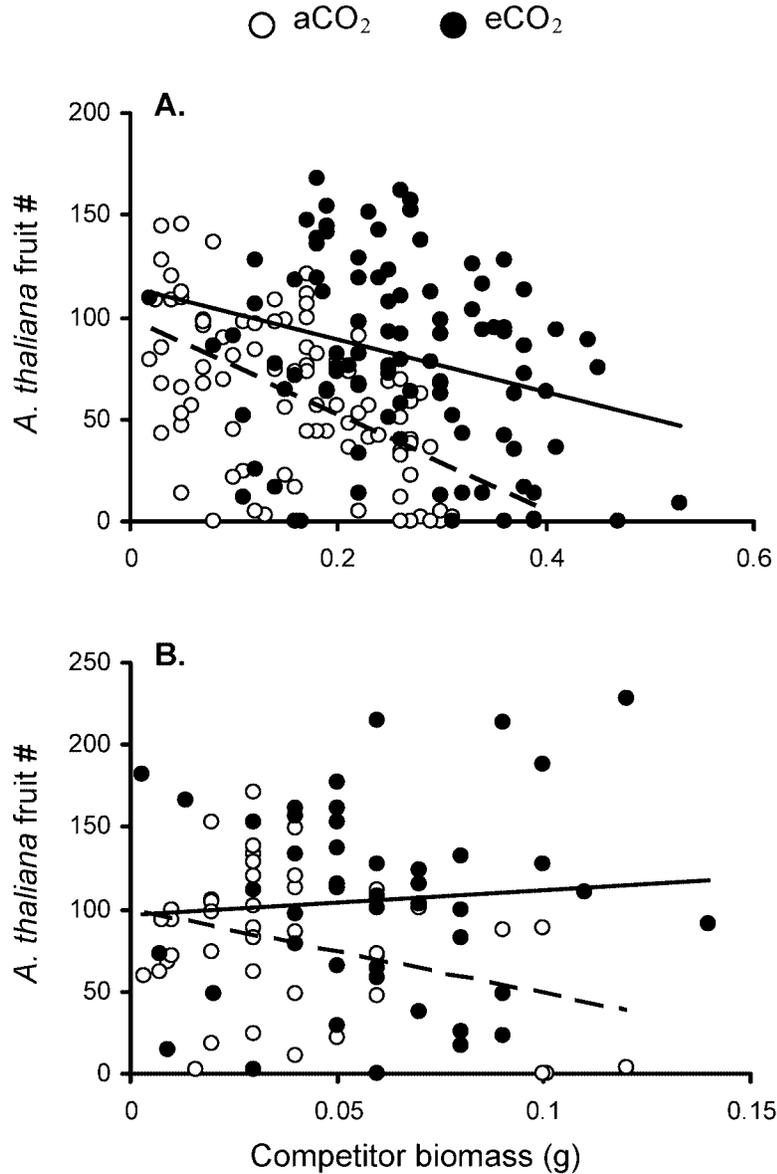
Fig. 1. Relationship between *A. thaliana* fruit production and *Bromus* (A) or *Andropogon* (B) biomass under aCO_2 (open symbols, dashed line) and eCO_2 (filled symbols, solid line).

aCO_2 (significant interactions between competitor biomass and CO_2 on *A. thaliana* fruit production: $F_{1,66} = 7.15$, $P = 0.009$; Figure 1). Thus, eCO_2 increased *A. thaliana* fruit production by reducing competitive effects (per unit biomass), despite increasing the biomass of the competitor. Elevated CO_2 had the opposite effect on intraspecific competition: intraspecific competition reduced fruit production more in eCO_2 (48 fruits or 38%) than in aCO_2 (28 fruits or 32%) environments. Thus, CO_2 concentration altered the
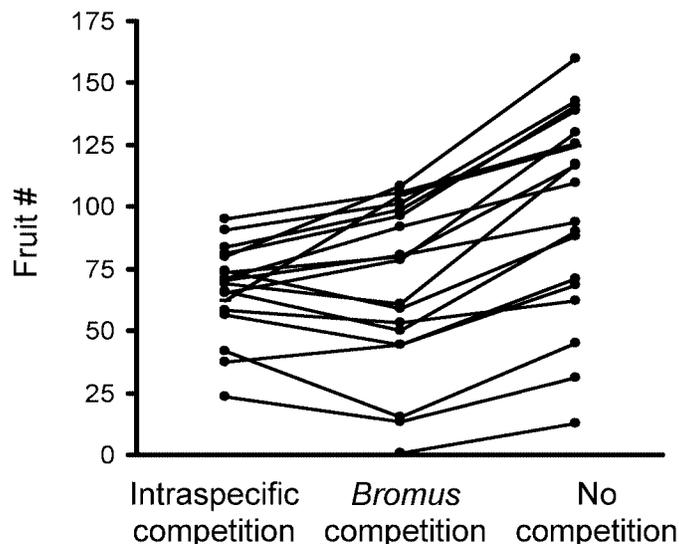
Fig. 2. Reaction norms showing the mean fruit production of each accession in three competition treatments (intraspecific competition, competition with *Bromus*, no competition).

outcomes of inter- versus intraspecific competition. In aCO_2, *A. thaliana* grown with *Bromus* tended to be smaller and produce fewer fruits than plants grown in competition with a second *A. thaliana* plant. In contrast, in eCO_2 environments, intraspecific competition tended to decrease *A. thaliana* growth more than interspecific competition from *Bromus* (Table 1). The effects of CO_2 on competitive rankings were not entirely driven by differences in biomass responses of the competitor to eCO_2. In fact, eCO_2 caused a greater increase in *Bromus* than in *A. thaliana* biomass (*Bromus* plants were 69% heavier in eCO_2 than aCO_2, whereas *A. thaliana* individuals were only 41% heavier in eCO_2) – the opposite of what would be expected if the change in relative competitive effects resulted entirely from differences in CO_2 growth responsiveness.

Genotype × environment interactions

We detected differences between accessions for all measured traits (significant accession effects, Table 2). We also found evidence that competition altered the relative fitness of the different accessions (accession × competition interaction on fruit number, $P < 0.0001$; Table 2, Figure 2); however, these effects were likely due to differences in variance between treatments rather than crossing reaction norms, since genetic correlations in fruit production between competitive environments were significant and high (pairwise Pearson correlations ranged from $r_g = 0.95$ to 0.99). Therefore, the accessions favoured in one competitive environment are generally favoured in all competitive environments, but the opportunity for selection (measured as the coefficient of variation in fruit production among accession means) may depend on the competitive environment. Intraspecific competition typically decreased the opportunity for selection, especially in eCO_2 conditions, while competition with *Bromus* increased the opportunity for selection, especially in aCO_2 environments (*Bromus*: CV = 0.58 in aCO_2, 0.46 in eCO_2; *Andropogon*: CV = 0.54 in aCO_2,

Table 1. Least-square means (1 standard error) for each trait in each competition treatment in each CO_2 environment

Trait	aCO$_2$				eCO$_2$			
	No competition	A. thaliana	Bromus	Andropogon	No competition	A. thaliana	Bromus	Andropogon
Leaf number	6.49^a (0.22)	6.42^a (0.24)	6.44^a (0.23)	6.42^a (0.26)	6.53^a (0.22)	6.37^a (0.24)	6.69^a (0.23)	6.61^a (0.25)
Diameter 13 June (mm)	16.50^a (0.29)	16.02^a (0.39)	16.43^a (0.36)	16.38^a (0.48)	16.98^{ab} (0.29)	16.15^a (0.40)	17.46^b (0.36)	16.96^{ab} (0.45)
Diameter 22 June (mm)	20.03^a (0.33)	19.34^{ab} (0.48)	17.73^b (0.45)	19.89^a (0.62)	22.46^a (0.34)	21.6^{ab} (0.50)	20.90^b (0.45)	22.54^{ab} (0.58)
Flowering date (days since germination)	25.13^a (0.30)	24.35^a (0.37)	25.07^a (0.35)	25.54^a (0.42)	25.57^a (0.31)	24.96^a (0.36)	25.56^a (0.35)	25.24^a (0.40)
Plant height (cm)	20.84^a (0.40)	18.47^b (0.49)	16.70^c (0.47)	20.75^a (0.57)	24.02^a (0.41)	21.54^b (0.49)	20.2^c (0.46)	24.18^a (0.55)
Stem number	5.49^a (0.13)	3.88^b (0.22)	3.78^b (0.20)	4.96^a (0.28)	6.08^a (0.13)	4.11^b (0.21)	4.52^b (0.19)	5.98^a (0.27)
Biomass (g)	0.36^a (0.01)	0.23^b (0.02)	0.20^b (0.02)	0.35^a (0.02)	0.51^a (0.01)	0.29^b (0.02)	0.30^b (0.02)	0.51^a (0.02)
Fruit number	87.23^a (4.05)	59.34^b (5.08)	56.61^b (4.83)	77.91^a (6.08)	108.73^a (4.07)	67.21^b (5.07)	78.78^b (4.76)	111.44^a (5.84)

Note: Superscript letters indicate treatment means that differ significantly within each CO_2 environment ($P < 0.05$), following a Tukey correction for multiple comparisons within each test.

Table 2. *F*-values and statistical significance of the effects of CO_2 and competition treatments, accession, and all possible interactions, and χ^2 values for random effects as estimated with ln-likelihood ratio tests

Source	Leaf number	Diameter 13 June	Diameter 22 June	Flowering date	Height	Stem number	Biomass	Fruit number
CO_2	0.15	**33.71****	1.97	0.02	**35.31****	10.02*	**42.47****	13.81*
Accession (Acc)	**6.61*****	**3.13*****	**2.90*****	**314.58*****	**89.15*****	**8.66*****	**8.85*****	**40.55*****
Competition (Comp)	0.56	**13.61*****	2.83*	**4.46****	**113.81*****	**63.27*****	**295.55*****	**75.56*****
$CO_2 \times$ Acc	0.94	1.82*	0.69	**2.30****	1.70*	0.79	2.01**	1.70*
$CO_2 \times$ Comp	0.85	0.73	0.88	0.79	0.55	0.74	**13.44*****	3.75*
Comp $\times$ Acc	1.06	0.86	0.82	**1.87****	**1.85****	1.47*	1.21	**2.00*****
$CO_2 \times$ Comp $\times$ Acc	0.71	0.96	0.80	0.58	1.13	0.94	0.96	0.78
Size covariate	**95.98*****	**99.68*****	**279.85*****	**38.25*****	6.06*	0.00	**32.90*****	**37.20*****
Random effects								
Ring (CO_2)	**66.0*****	4.3*	**10.0****	**22.9*****	**23.4*****	1.0	**21.4*****	**16.3*****

Note: The Satterthwaite approximation was used to estimate degrees of freedom to assess the significance of *F*-values. Values shown in **bold** are statistically significant ($P < 0.05$) after accounting for multiple comparisons with a Bonferroni correction. Asterisks indicate statistical significance (prior to Bonferroni correction) as follows: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$.

0.50 in eCO_2; intraspecific competition: CV = 0.41 in aCO_2, 0.30 in eCO_2; no competition: CV = 0.44 in aCO_2, 0.41 in eCO_2). In contrast, the proportion of phenotypic variation in fruit number explained by accession (an estimate of broad-sense heritability in highly selfing species such as *A. thaliana*) was reduced in *both* intraspecific and *Bromus*-competition treatments (*Bromus:* $H^2 = 0.60$ in aCO_2, 0.55 in eCO_2; *Andropogon:* $H^2 = 0.72$ in aCO_2, 0.58 in eCO_2; intraspecific competition: $H^2 = 0.49$ in aCO_2, 0.26 in eCO_2; no competition: $H^2 = 0.68$ in aCO_2, 0.54 in eCO_2). As with the coefficient of variation results, these changes in heritability were greatest in the eCO_2 treatment for intraspecific competition and were greater in the aCO_2 treatment for competition with *Bromus*.

We also detected significant accession $\times CO_2$ effects on fruit number (Table 2); however, cross CO_2 environment genetic correlations were once again very high ($r_g > 0.98$), suggesting that the same accessions will be favoured by natural selection in both current and future CO_2 environments. In addition, CO_2 did not detectably influence how accessions differed in their response to the competition treatments (no significant $CO_2 \times$ competition $\times$ accession interaction; Table 2).

Patterns of natural selection

Selection differentials

Univariate analyses revealed evidence for selection favouring plants that grew taller, had greater above-ground biomass, and flowered earlier in all CO_2 and competitive environments (Table 3). Selection also favoured plants with more stems in most environments. While the direction of selection was consistent across environments, competition treatments influenced the magnitude of selection (significant competition $\times$ trait interactions; Table 4). Interspecific competition increased selection on biomass more than intraspecific competition (compared with the no competition treatment), but intraspecific competition reduced selection on flowering date, while interspecific competition had no effect on selection on this trait (Table 3). These competition effects were much greater, and typically only statistically significant, in aCO_2 (Table 3). Compared with the effects of competition, evolutionary effects of CO_2 were relatively weak. Carbon dioxide only influenced the magnitude of selection on stem number, and this effect depended on the competitive environment (significant $CO_2 \times$ competition $\times$ trait interaction; Table 4). Elevated CO_2 tended to increase selection for more stems when competitors were absent, but tended to reduce selection on stem number when plants experienced competition (Table 3).

Selection gradients

We used multiple regression analyses to estimate selection gradients, which account for correlations between the traits included in the model and, therefore, estimate the direct selection acting on a trait. The selection gradient analysis also indicated that earlier flowering individuals with larger biomass were favoured in most environments (Table 3). Results from the ANCOVA suggested that competition impacts the magnitude of direct selection on flowering date and biomass (significant competition $\times$ trait interactions: flowering date, $F_{3,870} = 3.91$, $P < 0.01$; biomass, $F_{3,872} = 3.60$, $P < 0.05$; see Appendix 1). Pairwise comparisons show that the magnitude of the competition effect depended on competitor identity; intraspecific competition reduced selection for earlier flowering relative

Table 3. Phenotypic selection differentials and gradients in each competition $\times$ CO_2 environment

	aCO$_2$				eCO$_2$			
Trait	No competition	*A. thaliana*	*Bromus*	*Andropogon*	No competition	*A. thaliana*	*Bromus*	*Andropogon*
Selection differentials								
Biomass	**0.17**[a]	**0.24**[a]	**0.37**[b]	**0.30**[ab]	**0.21**[a]	**0.18**[a]	**0.27**[a]	**0.27**[a]
Flowering date	**−0.36**[a]	**−0.25**[b]	**−0.35**[a]	**−0.39**[a]	**−0.30**[ab]	**−0.21**[a]	**−0.32**[ab]	**−0.39**[b]
Rosette diameter	0.06[a]	0.04[a]	−0.03[a]	−0.04[a]	0.06[a]	0.03[a]	0.05[a]	0.12[b]
Stem number	0.02[a]	**0.24**[b]	**0.29**[b]	0.17[ab]	**0.13**[ab]	**0.22**[a]	0.19[ab]	0.02[b]
Height	**0.25**[a]	**0.28**[a]	**0.43**[b]	**0.36**[ab]	**0.29**[a]	**0.18**[b]	**0.33**[a]	**0.34**[b]
Selection gradients								
Biomass	0.04[a]	**0.16**[ab]	**0.24**[b]	0.10[ab]	**0.11**[a]	**0.17**[a]	**0.19**[a]	**0.15**[a]
Flowering date	**−0.39**[a]	**−0.21**[b]	**−0.29**[ab]	**−0.44**[a]	**−0.29**[a]	**−0.20**[a]	**−0.31**[a]	**−0.34**[b]
Rosette diameter	0.03[a]	−0.00[a]	−0.05[a]	−0.03[a]	−0.00[a]	0.01[a]	**0.10**[a]	0.06[a]
Stem number	**0.06**[a]	0.06[a]	0.01[a]	0.11[a]	**0.07**[a]	0.07[a]	0.03[a]	−0.03[a]
Height	−0.06[a]	0.06[a]	0.09[a]	−0.14[a]	−0.01[a]	0.05[a]	−0.01[a]	0.02[a]

Note: Selection differentials and gradients whose 95% confidence intervals do not overlap 0 are indicated in **bold**. Letters indicate significant differences between competition treatments (no competition, intraspecific competition, *Bromus* competition, or *Andropogon* competition) within each CO_2 environment, as indicated by pairwise comparisons in the ANCOVA.

Table 4. *F*-values and chi-square statistics (for random factors) from univariate ANCOVAs where each trait, CO_2, competition, and all interactions were included as predictor variables, and relative fruit number was the response variable

Source	Rosette diameter	Flowering date	Height	Stem number	Biomass
Trait	2.06	**468.09******	**323.62******	**66.13******	**181.38******
$CO_2 \times$ Trait	1.23	0.98	1.70	1.34	0.93
Competition $\times$ Trait	0.41	**4.23****	**4.86****	**7.43******	**4.30****
$CO_2 \times$ Competition $\times$ Trait	0.68	0.13	1.79	3.02*	0.90
Random effects					
Ring(CO_2)	$\chi^2 = \mathbf{6.6}$*	$\chi^2 = \mathbf{18.3}$****	$\chi^2 = 4.7$*	$\chi^2 = \mathbf{9.1}$**	$\chi^2 = 5.5$*

Note: CO_2, competition, and the $CO_2 \times$ competition interactions are not presented here because fitness was relativized by treatment (as a result, these main effects were always non-significant). Significant interactions between the traits and CO_2 or competition treatments indicate that the CO_2 or competition treatments alter selection differentials. Significance is indicated as follows: $^{\top}P < 0.1$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$ before Bonferroni correction. Values in **bold** remained significant after the Bonferroni correction was applied.

to the no competition controls, and competition with *Bromus* increased selection for increased biomass relative to the no competition control. As with the selection differentials, the effects of competition on selection gradients were greater and only statistically significant in aCO_2 environments (Table 3). These results should be interpreted with caution, however – the bootstrapped 95% confidence intervals for the selection gradients in each competition treatment overlapped.

The effects of competition on both selection gradients and differentials were similar, although the selection gradients for height, stem number, and in some treatments biomass typically were much smaller than the differentials, suggesting that a portion of the selection acting on these traits is indirect, resulting from selection acting on correlated traits. We acknowledge that phenotypic analyses can be biased by environmental variation that affects both fitness and phenotype (Mitchell-Olds and Shaw, 1987; Rausher, 1992; Stinchcombe *et al.*, 2002; Winn, 2004). However, the results obtained here are relatively consistent with both genotypic selection analyses performed on the same data set (results not shown) and also with genetic selection differentials and gradients estimated in a separate study that included 60 distinct *A. thaliana* genotypes grown in similar no competition and *Bromus* competition treatments (J.A. Lau *et al.*, unpublished manuscript).

DISCUSSION

The effects of competition on plant populations and community structure are well known, and many studies have shown that the ecological effects of competition are influenced by both competitor identity and the abiotic environment (reviewed in Goldberg, 1996). How environmental variation influences the evolutionary consequences of plant competition is less well understood. Consistent with previous work, we found that both competitor identity and the abiotic environment influenced the magnitude of the effects of competition on the mean fitness of an experimental *Arabidopsis thaliana* population. Elevated atmospheric CO_2 concentrations tended to reduce the fitness consequences of interspecific

competition, but increased the fitness effects of intraspecific competition. Competition also affected predicted evolutionary responses, and these effects depended on both competitor identity and the abiotic environment. Elevated CO_2 generally weakened the selective effects of competitors: competition altered the intensity of natural selection on flowering date, height, stem number, and biomass but the differences between competition treatments were typically greater in aCO_2 than eCO_2 environments. Thus, the role of competition as an agent of selection might be expected to be reduced under predicted future eCO_2 conditions, although this may not apply similarly in conditions where soil resources are more strongly limited than was likely in this study. Just as competitors differed in their effects on mean fitness, the competitor taxa also differed in their effects on predicted evolutionary responses. Competition with *Bromus* increased selection for larger biomass, but intraspecific competition had greater impacts on selection on flowering date. This result implies that the traits contributing to high fitness depend on the identity of the competitor, and that mean plant phenotypes might be expected to change in response to changes in community composition.

Ecological effects of competition and CO_2

Taxa with more similar resource requirements are expected to compete more intensely (MacArthur and Levins, 1967; Stubbs and Wilson, 2004). As a result, the fitness effects of intraspecific competition are expected to exceed those of interspecific competition, although differences in plant size also may strongly influence fitness outcomes as evidenced by the weak competitive effects of *Andropogon* compared with *Bromus* in this study. Our results were consistent with this prediction in the eCO_2 environment but not in the aCO_2 environment. In the eCO_2 environment, intraspecific competition caused greater reductions in *A. thaliana* growth and fitness than competition with *Bromus*, even though competitor densities were higher in the interspecific competition treatments (two *Bromus* competitors were planted into each pot while only a single *A. thaliana* competitor was added to each pot). In contrast, in aCO_2 conditions *Bromus* had a greater competitive effect on *A. thaliana* than did intraspecific competition. While our experimental design precludes us from determining exact competitive rankings (because number of competitors was not held constant across competitor treatments), our results do suggest that eCO_2 alters the relative fitness effects of different competition treatments. In both environments, competition with the C4 grass *Andropogon* had little effect on growth and fitness, likely because of the slow inherent growth of this competitor and its small size throughout this experiment, rather than its ecophysiological characteristics of particular relevance to eCO_2. The reversal in the magnitude of intra- versus interspecific competitive effects across CO_2 treatments is not due to differences in biomass responsiveness to eCO_2; the effects of intraspecific competition were greater in eCO_2 than aCO_2 even though eCO_2 caused greater increases in the biomass of *Bromus* than *A. thaliana*. Rather, the effect of CO_2 on competitive rankings may be due to: (1) changes in which resource most limits *A. thaliana* growth in the different CO_2 environments [e.g. water and/or carbon may be limiting in aCO_2 but increased water use efficiency and carbon availability in the eCO_2 environment may have resulted in nitrogen- (e.g. Reich *et al.*, 2006) or light-limiting growth in eCO_2], or (2) changes in the strength of competition for a limiting resource. Interestingly, these results are also consistent with recent work showing that communities consisting of taxa with more similar phenotypic traits are less able to use limiting resources or respond to increases in limiting resources, including CO_2 (Reich *et al.*, 2004).

Effects of competition and eCO$_2$ on patterns of natural selection

Given the strong ecological effects of competition, it might be expected that competition also has a strong influence on evolutionary change. Documenting that competition is the selective agent responsible for adaptive differences among populations can be difficult. Studies that combine selection analyses with experimental manipulations of competition, however, can be useful for identifying how the competitive environment alters predicted evolutionary responses, and studies investigating the potential evolutionary consequences of competition in different abiotic environments can shed light on how anthropogenic environmental changes might alter the evolutionary (in addition to the ecological) consequences of competitive interactions. Surprisingly few studies have investigated how plant–plant competition alters predicted evolutionary trajectories, and even fewer have attempted to do so under multiple environmental conditions (but see Stanton *et al.*, 2004). Here we have demonstrated that the effects of competition on predicted evolutionary trajectories are likely to be reduced in the elevated CO$_2$ environments predicted in the future.

In addition to examining how the role of competition as a selective agent might change under future elevated CO$_2$ conditions, we also wished to determine whether the evolutionary effects of competition follow the same general rules as the ecological effects of competition. In particular, we tested whether intraspecific competition was a greater selective agent than interspecific competition and whether competition was a stronger agent of selection in the resource environment where competition had the greatest effects on mean fitness. Although our observed effects of competition on mean fitness were generally consistent with predictions based on ecological rules, different evolutionary effects were observed for different traits. For example, as predicted, intraspecific competition had greater effects on selection for earlier flowering than interspecific competition, but in contrast to expectations, interspecific competition significantly influenced selection on biomass and other growth traits, whereas intraspecific competition did not. These differential effects of intra- versus interspecific competition on different traits may result from many possible mechanisms, one of which would posit that the most limiting resource for intraspecific competitors is not the same as the resource most limiting under interspecific competition and traits likely differ in their contribution to acquisition of different resources (Huston and Smith, 1987; Tilman, 1988, 1990; Goldberg, 1996; Fynn *et al.*, 2005; but see Grime, 1977).

The effects of both intra- and interspecific competition on patterns of selection were typically reduced under eCO$_2$. As a result, the effects of resource availability on both the ecological and evolutionary effects of interspecific competition were similar (reduced effects of competition both on mean fitness and the magnitude of selection differentials and gradients in eCO$_2$). However, the ecological and evolutionary effects of intraspecific competition were not in accord, since intraspecific competition had stronger ecological effects (reductions in mean fitness) under eCO$_2$, but stronger evolutionary effects (selection on flowering time) in aCO$_2$. This result highlights that factors causing large changes in mean fitness do not necessarily produce large changes in the relative fitness rankings of different accessions or the associations between traits and fitness (see also Strauss *et al.*, 2008). It is also important to note that both the ecological and evolutionary effects of competition (and CO$_2$) might be, and perhaps are likely to be, different in a more realistic mixed community setting with greater total biomass and greater resource competition. Moreover, while selection on different traits depended on competitor identity, genetic correlations

for fruit production in the different competitive environments were close to one, suggesting that the same accessions were favoured when competing with both conspecifics and heterospecifics.

Effects of competition and eCO$_2$ on opportunity for selection and competitive asymmetries

In addition to changing the relationship between traits and fitness, environmental conditions can change the amount of variation in fitness and growth traits. Variation in size traits has implications for competition (whether competitive interactions are symmetric or asymmetric with respect to size) and stand structure, and variation in fitness traits implies natural selection. Opportunity for selection, as measured by the coefficient of variation in fitness (fruit number), may differ among environments, resulting in differences in evolutionary change even if the fitness rankings of accessions are consistent. In our study, variation in fruit number [and biomass, height, and branch number (results not shown)] was typically greater under interspecific competition than in the absence of competition or under intraspecific competition. Other studies also have found that more intense competition (e.g. increased plant density) increases phenotypic variation (e.g. Boyden et al., 2009), in part because asymmetric competition amplifies variation in early size (e.g. Thomas and Bazzaz, 1993). When such environmental conditions cause increased phenotypic variation, rather than enhanced expression of genetic variation, little impact on the rate of evolutionary change is expected, despite dramatic changes in stand structure. This appears to be the case in our experiment. The proportion of variation in fruit number due to variation among accessions (i.e. broad-sense heritability) was lower in the presence of competitors. Because the rate of adaptation depends on both the severity of selection and heritability, decreases in heritability will further reduce evolutionary responses in flowering time in competitive environments (intraspecific competition reduced selection for earlier flowering) and will limit the evolutionary response of biomass (interspecific competition increased selection for biomass).

We also found that eCO$_2$ reduced the coefficient of variation in fitness (and other growth traits) in most competition treatments and also reduced the proportion of variation explained by accession, especially when competitors were present. This implies reduced opportunity for selection and lower heritabilities for fitness under elevated CO$_2$, which will reduce rates of evolutionary change and also will result in greater uniformity of stand structure. Other studies have found that eCO$_2$ increases phenotypic variation in some environments (e.g. Bazzaz and McConnaughay, 1992) and decreases variation in other environments (e.g. Wayne and Bazzaz, 1997). Interestingly, the effects of CO$_2$ on phenotypic (and potentially, genotypic) variation may depend on the limiting resource and CO$_2$-induced changes in plant phenotypes. For example, Wayne and Bazzaz (1997) suggest that decreased coefficients of variation in eCO$_2$ environments results from reduced seedling size inequalities because eCO$_2$: (1) reduced size-asymmetric competition for light because of differences in CO$_2$-use efficiency of taller versus shorter competitors, and (2) reduced leaf area, allowing more light to penetrate to shorter competitors. Similar processes in our study could explain why eCO$_2$ reduced coefficients of variation under most competition treatments, although we suspect that competition for water may be more important than for light in *A. thaliana*, given its rosette-forming architecture.

CONCLUSIONS

Our results indicate that two factors that influence the ecological effects of competition (competitor identity and resource availability) also influence the evolutionary effects of competition. Moreover, eCO_2 altered the relative fitness effects of different competitors and reduced the effects of competition on predicted evolutionary trajectories. Thus, our results also illustrate how changing environmental conditions, such as rising atmospheric CO_2 concentrations, can influence both ecological and evolutionary processes by altering fundamental interactions between species, such as competition. The effects of competition on evolutionary processes were trait specific, however, and the patterns did not suggest general evolutionary principles of competition.

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Appendix 1. *F*-values and chi-square statistics (for random factors) from ANCOVAs on relative fruit number: CO_2, competition, and the $CO_2 \times$ competition interaction were also included in the model, but are not presented here because fitness was relativized by treatment (as a result, these main effects were always non-significant). Significant interactions between the traits and CO_2 or competition treatments indicate that the CO_2 or competition treatments alter selection gradients. Significance is indicated as follows: $^\top P < 0.1$, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$.

Source	d.f.	*F*-value
Biomass	1,872	**42.35******
Flowering date	1,872	**269.25******
Rosette diameter	1,872	0.23
Stem number	1,871	**6.96****
Height	1,871	0.03
Biomass $\times CO_2$	1,872	0.22
Flowering date $\times CO_2$	1,872	1.22
Diameter $\times CO_2$	1,872	1.96
Stem $\times CO_2$	1,871	0.76
Height $\times CO_2$	1,871	0.11
Biomass $\times$ Competition	3,872	**3.60***
Flowering date $\times$ Competition	3,870	**3.91****
Diameter $\times$ Competition	3,870	0.07
Stem $\times$ Competition	3,870	0.47
Height $\times$ Competition	3,871	1.31
Biomass $\times CO_2 \times$ Competition	3,872	0.32
Flowering date $\times CO_2 \times$ Competition	3,870	1.05
Diameter $\times CO_2 \times$ Competition	3,870	1.94
Stem $\times CO_2 \times$ Competition	3,870	0.94
Height $\times CO_2 \times$ Competition	3,871	0.99