

Predicting adaptive evolution under elevated atmospheric CO₂ in the perennial grass *Bromus erectus*

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Abstract

Increasing concentrations of CO₂ in the atmosphere are likely to affect the ecological dynamics of plant populations and communities worldwide, yet little is known about potential evolutionary consequences of high CO₂. We employed a quantitative genetic framework to examine how the expression of genetic variation and covariation in fitness-related traits, and thus, the evolutionary potential of a species, is influenced by CO₂. In two field experiments, genotypes of the dominant grassland perennial *Bromus erectus* were grown for several years in plots maintained at present-day or at elevated CO₂ levels. Under noncompetitive conditions (experiment 1), elevated CO₂ had little impact on plant survival, growth, and reproduction. Under competitive conditions in plots with diverse plant communities (experiment 2), performance of *B. erectus* was reduced by elevated CO₂. This suggests that the effect of CO₂ was largely indirect, intensifying competitive interactions. Elevated CO₂ had significant effects on the expression of genetic variation in both the competitive and noncompetitive environment, but the effects were in opposite direction. Heritability of plant size was generally higher at elevated than at ambient CO₂ in the noncompetitive environment, but lower in the competitive environment. Selection analysis revealed a positive genotypic selection differential for plant size at ambient CO₂, indicating selection favoring genotypes with high growth rate. At elevated CO₂, the corresponding selection differential was nonsignificant and slightly negative. This suggests that elevated CO₂ is unlikely to stimulate the evolution of high biomass productivity in this species.

Keywords: adaptation, competition, elevated CO₂, global change, grassland, heritability, quantitative genetics, selection

Introduction

Human activities continue to alter terrestrial and aquatic ecosystems worldwide at an unprecedented rate. Of the many components of global climate change the rise in atmospheric CO₂ is probably the most predictable one. Current projections forecast a doubling or tripling of preindustrial CO₂ concentrations by the end of the 21st century (IPCC, 2001). In the last two decades, an

enormous body of research has demonstrated that elevated CO₂, the primary substrate for photosynthesis, can have profound effects on plant growth and reproduction (Jablonski *et al.*, 2002; Poorter & Navas, 2003), species interactions and community dynamics (Niklaus *et al.*, 2001; Marissink & Hansson, 2002; Polley *et al.*, 2003; Poorter & Navas, 2003), and ecosystem processes (Körner, 2000; Niklaus & Körner, 2004). Surprisingly little work has been done, however, on the potential consequences of rising CO₂ for plant evolution (see e.g. Schmid *et al.*, 1996; Steinger *et al.*, 1997; Roumet *et al.*, 2002; Ward & Kelly, 2004; Wieneke *et al.*, 2004).

Evolutionary processes are fundamental in plant adaptation to changing environments and can be of critical importance for the persistence of species in the face of global change (Geber & Dawson, 1993). Furthermore, evolutionary processes may influence how the

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functioning of ecosystems is affected by environmental change, especially when dominant or keystone species are involved. For example, it was suggested that CO₂-forced selection for increased biomass accumulation in dominant plant species may considerably alter our current predictions of primary productivity in a future CO₂ atmosphere, which only consider short-term plastic responses to CO₂, but neglect potential evolutionary changes occurring in the longer term (Bazzaz *et al.*, 1995; Ward & Kelly, 2004).

How can we study the consequences of rising CO₂ for plant evolution? One approach is to perform a selection experiment and grow plant lines for a number of generations under different scenarios of climate change (Potvin & Tousignant, 1996; Ward *et al.*, 2000; Collins & Bell, 2004). This approach is usually only feasible with species that have a short generation time. Such short-lived species, however, are rarely dominant components of natural plant communities and, thus, may have relatively low impacts on community and ecosystem processes. A second approach, also applicable to perennial plants, is to predict phenotypic evolution based on well-developed theory of quantitative genetics (Falconer & Mackay, 1996). This theory has proven successful in predicting the evolutionary trajectories of phenotypes over a few generations and is used in many plant and animal breeding programs. At the heart of the theory is the so-called breeder's equation: $R = Sh^2$, which states that the evolutionary rate of change of a phenotypic trait (R) is directly proportional to the strength of selection (S) on that trait and its heritability (h^2). Heritability is the proportion of total phenotypic variance that is genetically additive (V_A/V_P). Heritability estimates are specific for given environments because the expression of additive genetic variance (V_A), as well as other components of nonadditive genetic (dominance and epistasis) and environmental variance are all sensitive to environmental conditions (Falconer & Mackay, 1996; Hoffmann & Merilä, 1999). Note that V_P is the sum of all these variance components, including the additive genetic one.

To apply the quantitative genetic framework to an understanding of phenotypic evolution under global climate change, experimental data are needed on how components of global change affect the expression of heritable variation and thus the rate with which natural populations will adapt to changing environments. Whereas a number of studies have examined intraspecific differences between populations or genotypes in their mean phenotypic response to CO₂ (genotype \times environment interactions; Curtis *et al.*, 1994; Norton *et al.*, 1995; Schmid *et al.*, 1996; Steinger *et al.*, 1997; Ward & Strain, 1997; Andalo *et al.*, 2001; Roumet *et al.*, 2002; Mohan *et al.*, 2004), there is so far little

information on how elevated CO₂ might influence the expression of genetic variation and covariation in ecologically relevant traits within species (but see Bazzaz *et al.*, 1995; Thomas & Jasienski, 1996). Notice that the presence of significant genotype \times environment interactions can, but need not imply a change in genetic variation or heritability between environments; it is possible that only the ranking of genotypes changes but not the spread of mean values represented by them.

Current theory is insufficient to make predictions about if and in what direction elevated CO₂ might affect the expression of genetic variation and covariation in fitness-related traits. Recent reviews summarizing empirical studies about environmental effects on heritability classified environments in terms of whether they are favorable or unfavorable, and equate unfavorable environments with rare or novel environments (Jenkins *et al.*, 1997; Hoffmann & Merilä, 1999). Although changes in genetic variance across environmental conditions were found to be common in these studies, the direction of these changes was highly variable (see Paschke *et al.*, 2003). In agricultural studies with crop plants, heritability for yield tends to be lower under unfavorable than under favorable conditions, presumably due to higher environmental variance (V_E) under unfavorable conditions (Blum, 1988, but see Ceccarelli, 1994). The proposition that unfavorable environments equate evolutionarily novel environments (Lynch & Lande, 1993) may not be applicable to elevated CO₂. Double than present-day CO₂ levels typically favor plant growth (Poorter & Navas, 2003), but these concentrations represent an evolutionary novel environment, to which plants have not been exposed for the last 20 million years (Thomas & Jasienski, 1996, see also Pearson & Palmer, 2000).

In the present study, we examined the quantitative genetic consequences of elevated CO₂ in a long-term field experiment to predict the potential for adaptive evolution in a CO₂-rich atmosphere. To our knowledge, this is the first study examining CO₂ effects on quantitative genetic variation and covariation under realistic field conditions and over several years. Our study species is the perennial grass *Bromus erectus*, the dominant plant species in the semi-dry calcareous grassland community of our research site. Significant CO₂ responses in this species would very likely have pertinent effects on community and ecosystem processes. We conducted two parallel experiments in the same set of field plots. In the *tube experiment*, replicated genotypes of *B. erectus* were grown for several growing seasons under low competition in plastic tubes buried in the soil. In the *community experiment*, a second set of genotypes was grown under competitive conditions in experimental plant communities assembled from the local

species pool. We asked the following specific questions: (1) what is the plastic response of *B. erectus* to elevated CO₂ when grown under low and high interspecific competition? (2) How does elevated CO₂ affect heritable variation in fitness-related traits? (3) Does elevated CO₂ alter selection on plant vegetative growth?

Materials and methods

Study site and species description

The study site was located in the Jura mountains, 20 km southwest of Basel, Switzerland. The site is situated on a southwest exposed, moderately steep slope (inclination 20°) at an elevation of 520 m altitude. The vegetation at the site is typical for nutrient-poor, semidry soils overlaying calcareous bedrock and is composed of over 120 perennial plant species (Joshi *et al.*, 2006). In this habitat, *B. erectus* forms extensive swards in which other plant species are embedded.

B. erectus Huds. is a perennial, polycarpic grass with an obligate outcrossing breeding system. The plant grows as a tussock, a dense clump of tillers connected by short rhizomes. It is the matrix-forming species in semidry and dry grasslands on calcareous soils throughout Europe. At the study site, *B. erectus* contributed ca. 50% to total aboveground community biomass. Although *B. erectus* has a rather slow innate

growth rate and is a weak competitor against more productive grasses, it can attain local dominance due to its capacity to tolerate drought, which occurs regularly in these grasslands.

Experimental design

The experiment comprised a total of 24 field plots arranged in four blocks along the slope of the study site (Fig. 1). The 1.27 m² plots were planted with experimental plant communities assembled from the native species pool and had three levels of diversity: 5, 12, and 31 plant species (see Niklaus *et al.*, 2001 for details). Plots with different species diversities were exposed to either ambient CO₂ concentrations (ca. 360 ppm) or elevated CO₂ concentrations (ca. 600 ppm) in a factorial design. Stable CO₂ concentrations were maintained using open-top, open-bottom Screen-Aided CO₂ Control units (SACC; Leadley *et al.*, 1997).

Community experiment. In May 1993, tussocks of *B. erectus* were haphazardly collected from the study site at a minimum distance of 10 m, hence, every tussock most likely represents a different genotype. Tussocks were split into single tillers and vegetatively propagated in the greenhouse. In September 1993, a total of 31 genotypes were transplanted into the experimental communities at the study site. Fifteen

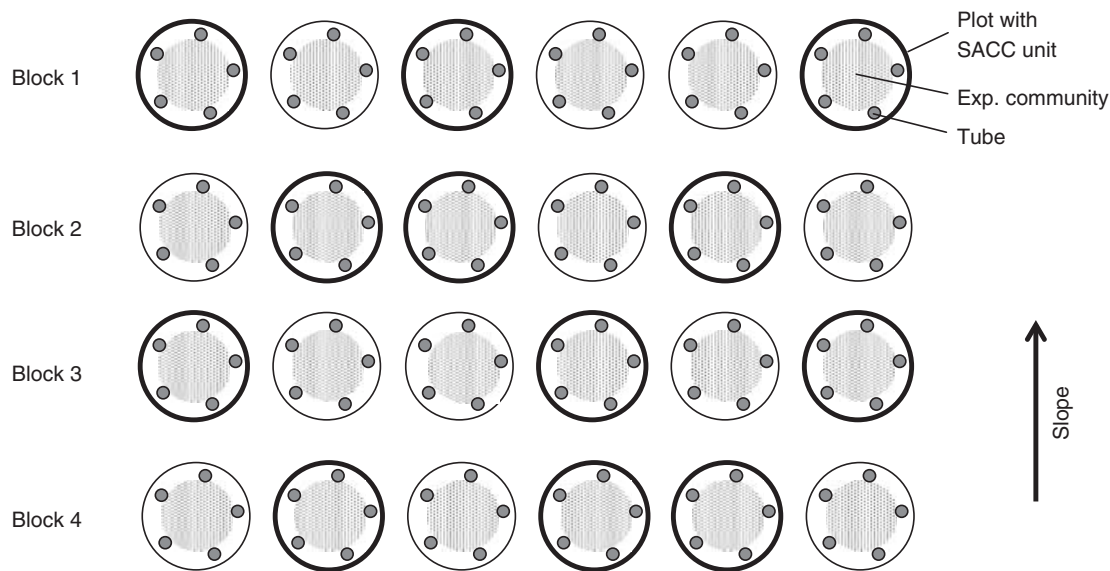


Fig. 1 Schematic representation of the experimental design of the community and the tube experiment. Twenty-four plots (1.27 m²) equipped with screen-aided CO₂ control (SACC) units were arranged in four blocks along the slope of the field site in a calcareous grassland. Plots were exposed to either ambient (thin lines) or elevated CO₂ concentrations (thick lines). In the *community experiment*, 31 vegetatively propagated genotypes of *Bromus erectus* were grown in experimental plant communities (hatched area); 15 genotypes were grown in block 1+2, 16 genotypes in block 3+4 (one replicate genotype in each plot). In the *tube experiment*, a second set of 30 genotypes was grown in special tubes (gray circles) aimed to reduce plant competition; 15 genotypes were grown in block 1+2, 15 genotypes in block 3+4 (one replicate genotype in each of two blocks).

randomly selected genotypes were grown in blocks 1 and 2, and the remaining 16 genotypes in blocks 3 and 4. Each genotype had initially one randomly positioned plant individual (replicate) in each of 12 plots (i.e. there were normally six replicates per genotype in each CO₂ treatment). As the positions of the other plant species within each plot were also randomized, the experimental *B. erectus* plants experienced a variable neighborhood in terms of species identity, mimicking the natural situation in the field. CO₂ exposure started with the beginning of the growing season in early April 1994 (i.e. 6 months after transplantation of the genotypes).

Tube experiment. An additional set of 30 genotypes, haphazardly collected from the same site in summer 1994, was grown in specially designed polyethylene tubes. The tubes were buried in the soil in a circle around the experimental plant communities described above (see Fig. 1). The tubes (19 cm high, 9 cm diameter) had large holes drilled into the wall and contained a nylon mesh bag filled with sieved topsoil from the study site. These tubes were designed to allow the plants to grow under reduced competition pressure, while still assuring a good contact between the plants and the surrounding abiotic and biotic environment. The nylon mesh (pore size 60 µm) prevented roots from growing into or out of the tubes. However, at the beginning of the second growing season, two holes were punched into the bottom of the mesh bags to allow the roots of the experimental plants to grow deeper into the soil. This was done to avoid a potential limitation of the CO₂ response due to limited root space as the plants grew bigger. In April 1995, four replicates of each genotype (two for each CO₂ treatment) were transplanted randomly into the tubes. Fifteen randomly selected genotypes were transplanted into blocks 1 and 2, the remaining 15 genotypes were planted into blocks 3 and 4.

Measurements

To assess fitness within each CO₂ environment, we recorded survival, number of tillers (a proxy for vegetative plant size) and number of flowering culms (a proxy for reproductive output) of each individual during several censuses over the course of the 5-year (community experiment) or 3-year (tube experiment) observation period. We estimated overall plant fitness as the cumulative number of flowering culms produced by an individual over the observation period. Number of flowering culms was positively related with seed number ($R^2 = 0.46$, $P < 0.0001$, $n = 186$, data from the 1996 census of the tube experiment). Only very few

individuals failed to reproduce and fitness of these individuals was recorded as zero. Plants were cut each year in summer after seed set and in autumn at 5 cm aboveground. This cutting regime was intended to simulate cattle grazing, a management practice that was carried out at this site during more than a century.

Data analysis

In both the tube and the community experiment, we pooled data from the three diversity treatments to maximize the statistical power to detect CO₂ effects on trait means and variances. Pooling was justified, because analyses with diversity included as a factor in the model revealed no significant effects (tube experiment) or only main effects of diversity (community experiment). Interactions between diversity and CO₂ or between diversity and genotype were not significant. For the community experiment, diversity was retained in the model as an additional blocking factor, but results are not reported here. We also tested a more complicated model, which accounts for the fact that genotypes were split into two block groups (block 1/2 vs. block 3/4; see above). This model yielded very similar results, so the simpler model was used. We used the procedure MIXED of the statistical software package SAS V9 (SAS Institute, Cary, NC, USA) for all analyses.

Plant performance. To analyze the effects of elevated CO₂ on trait means we used mixed-effects models of the form:

$$\begin{aligned} \text{variable} = & \text{grand mean} + \text{block} + \text{diversity} + \text{CO}_2 \\ & + \text{plot}(\text{block}, \text{CO}_2, \text{diversity}) + \text{genotype} \\ & + \text{genotype} \times \text{CO}_2 + \text{error} \end{aligned}$$

CO₂ and diversity were treated as fixed factors, and block, plot (nested within CO₂, diversity, and block), genotype, and genotype \times CO₂ were treated as random factors. Repeated-measures analysis [REPEATED statement in PROC MIXED with TYPE = SP(POW) option] was used to model the temporal dynamics of tiller numbers per plant. Akaike's information criterion was used to choose among several covariance structures (spatial power law, spherical, Gaussian) suitable for unequally spaced data. Tiller number was square-root transformed to better satisfy the assumptions of normality and homoscedasticity. The effect of CO₂ on plant survival was analyzed using cumulative mortality at the end of the experiment as the response variable. As this is a binary variable, we used a generalized linear mixed model (GLIMMIX procedure in SAS) with a binomial error distribution and a logit link function. It was not possible to conduct a survival time analysis, because standard statistical

packages do not incorporate random effects in these models.

Opportunity for selection. Novel environmental conditions such as elevated CO₂ can influence the potential of evolution in many ways. One component of this potential is the opportunity for selection, which is defined as variation in relative fitness (Arnold & Wade, 1984). The opportunity for selection sets an upper bound on the strength of selection. To determine the effect of elevated CO₂ on opportunity for selection, we calculated the variance in relative fitness (total fitness of an individual divided by mean fitness) in each CO₂ environment.

Genetic variation and covariation. The use of clonally propagated genotypes in quantitative genetic experiments only allows the estimation of genetic parameters in the broad sense. In addition to additive genetic components of variance and covariance, broad-sense estimates may also contain variation that is due to dominance, epistasis, and common parental or juvenile environment, and can therefore be upwardly biased (Lynch & Walsh, 1998). We made several attempts to minimize the influence of common environment: (1) genotypes were precultivated during several months under uniform growing conditions in the greenhouse, (2) during precultivation, newly emerging tillers were repeatedly split and planted back into growing trays at random positions, and (3) replicates were trimmed back to a single tiller before transplantation into the field plots in order to standardize plant size (community experiment only).

We estimated components of genetic variance and covariance with REML as implemented in the MIXED procedure in SAS (Littell *et al.*, 1996). For univariate analyses within CO₂ treatment levels, models consisted only of the random effects genotype, block, and plot. The broad-sense heritabilities (H^2) were then estimated from genetic variance components using the formula $H^2 = V_G / (V_G + V_R)$, where V_G represents the variance among genotypes and V_R represents the residual variance (Falconer & Mackay, 1996). The residual variance is likely to reflect mainly small-scale environmental variance in the field and was therefore used to calculate the coefficient of environmental variation $CV_E = \sqrt{(V_R)/x}$, where x represents the trait mean. Similarly, the coefficient of genetic variation was calculated as $CV_G = \sqrt{(V_G)/x}$ (Houle, 1992). SE of heritabilities were estimated by the delta method (Lynch & Walsh, 1998). Likelihood-ratio tests were used to determine whether V_G differed from zero. Twice the difference in log-likelihoods between a full model including genotype effects and a reduced model,

in which genotype effects were constrained to zero, was used as a test statistic and compared with a χ^2 distribution with one degree of freedom (Littell *et al.*, 1996). As in this case variance components were estimated on the boundary of the parameter space, the resulting error probability was halved (Littell *et al.*, 1996, p. 44). A similar procedure was used to test if V_G differed between CO₂ treatments: we ran a model in which V_G was allowed to vary between CO₂ treatments (TYPE = UN in RANDOM statement of PROC MIXED in SAS) and a restricted model, in which V_G was constrained to be equal (TYPE = TOEP). Again, twice the difference in log-likelihoods between the two models was used for significance testing. We used the same procedure to check if the two sets of genotypes (block 1/2 vs. block 3/4, see above) differed in V_G within CO₂ environments, but we found no significant difference.

Estimates of V_G and heritabilities were calculated using untransformed data, and likelihood-ratio tests were done after square-root transformation for the variables tiller number and number of flowering culms. For mortality data, heritability on the underlying scale was calculated following the formula given in Lynch & Walsh (1998, p. 735). We used a permutation procedure, in which individual plants were randomly assigned to genotypes, to test for significant genetic variation in mortality within each CO₂ environment (Mitchell-Olds, 1986).

Genotypic selection differentials for plant growth were estimated as the genetic covariance between tiller number and relative fitness within each CO₂ environment (Rausher, 1992). Selection differentials were standardized by dividing the estimate by the SD of the trait. Tests whether selection differentials within CO₂ environments differed from zero were done by comparing likelihood ratios of an unconstrained model with a model where the genetic covariance was constrained to zero (TYPE = UN(1) in RANDOM statement). Selection differentials were estimated from untransformed data.

Results

Community experiment

CO₂ effects on plant performance. Approximately one third of the 354 plants surveyed at the start of the experiment in spring 1994 died before the end of the experiment in summer 1998 (Table 1). Mortality rate was rather uniform over the 5-year period in both CO₂ treatments (data not shown). Generalized linear mixed model analysis revealed no significant CO₂ effect on cumulative mortality at the end of the experiment ($F_{1,19.4} = 0.6$, $P > 0.4$).

Table 1 Mean values (± 1 SE) and estimates of quantitative genetic parameters (± 1 SE) for plant mortality at the end of the experiment and fitness (total number of flowering culms produced during the experiment) of *Bromus erectus* grown at ambient and elevated CO₂

Traits	CO ₂ treatment	Mean	Heritability	Genetic variance	CV _g	Environmental variance	CV _e
<i>Community experiment</i>							
Mortality	Ambient	29%	0.16 \pm 0.11	0.018	–	0.178	–
	Elevated	35%	0.06 \pm 0.10	0.008	–	0.206	–
Fitness	Ambient	3.39 \pm 0.30	0.20 \pm 0.077	1.55 \pm 0.70	0.37	6.23	0.74
	Elevated	2.77 \pm 0.30	0.08 \pm 0.068	0.42 \pm 0.36	0.23	4.69	0.78
<i>Tube experiment</i>							
Mortality	Ambient	18%	0	0	–	0.145	–
	Elevated	18%	0	0	–	0.152	–
Fitness	Ambient	5.50 \pm 0.54	0.54 \pm 0.166	4.88 \pm 2.22	0.40	4.12	0.37
	Elevated	4.45 \pm 0.54	0.42 \pm 0.143	2.56 \pm 1.45	0.36	3.48	0.42

For mortality data, heritability was estimated on the underlying continuous scale. CV_g and CV_e refer to the coefficient of genetic and environmental variation, respectively.

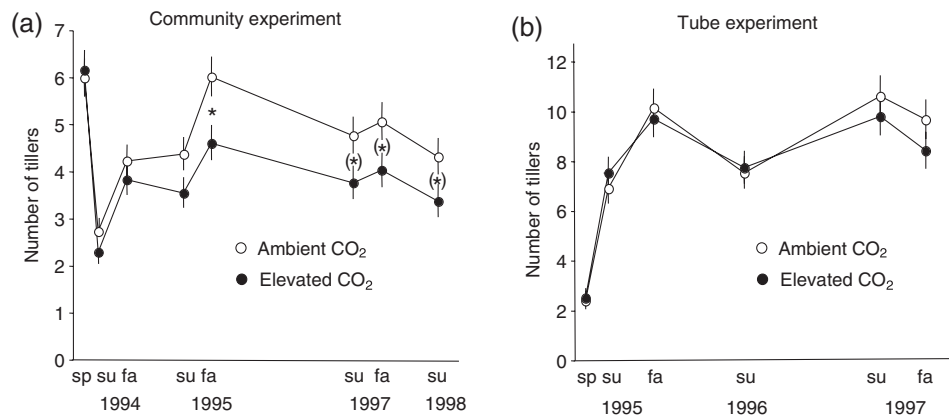


Fig. 2 Temporal dynamics of tillering of *Bromus erectus* grown at ambient or elevated CO₂ in the field. In the community experiment (a) plants were grown under competitive conditions in experimentally established mixed plant communities. In the tube experiment (b) plants were grown in tubes designed to reduce competition. Circles represent least-square means (± 1 SE) from a repeated-measures ANOVA with the terms CO₂, plot and time in the model. sp, spring (April); su, summer (June); fa, fall (October). *Significant differences between CO₂ environments (* $P < 0.05$; *) $P < 0.1$).

The development of tiller populations of individual plants, a measure of plant size and thus performance, was characterized by a marked decrease in tiller number from spring to summer of the first year, and a recovery thereafter (Fig. 2a). The initial decline in the first growing season was probably due to increasing aboveground competition in the experimental communities as they established. Soil nutrient levels were probably high in the first year, as a consequence of soil disturbance when field plots were prepared. Indeed, many plant species grew very vigorously in the first season (Niklaus *et al.*, 2001) leading to the suppression of the competitively inferior *B. erectus* grass. Repeated-measures analysis revealed a significant negative effect of elevated CO₂ on tiller

number ($F_{1,17.5} = 4.87$, $P < 0.05$), and no significant CO₂ \times time interaction ($F_{7,207} = 0.99$, $P > 0.4$; Fig. 2a).

We used the cumulative number of flowering culms produced by a plant during the experimental period as a measure of reproductive performance. This measure integrates plant survival, size, and reproductive allocation and can, thus, be considered as a good indicator of plant fitness. We found that elevated CO₂ had no significant effect on fitness ($F_{1,17.3} = 2.03$, $P > 0.17$; Table 1).

CO₂ effects on variance in fitness. Opportunity for selection (phenotypic variance in relative fitness) was very similar across CO₂ environments (0.66 and 0.67 at ambient and elevated CO₂, respectively). Thus, there is

no indication that the upper bound of selection strength is influenced by CO₂.

Quantitative genetic variation at ambient and elevated CO₂. Mortality significantly differed among genotypes at ambient ($P < 0.05$, one-tailed permutation test), but not at elevated CO₂ ($P > 0.3$), indicating heritable variation in mortality only under present-day CO₂ conditions (Table 1). In a direct comparison of genetic variance components, however, the difference between CO₂ environments was not significant ($P > 0.14$, two-tailed permutation test). We also detected significant genetic variation for tiller number at most census dates and in both CO₂ environments. During the first two growing seasons, heritability estimates for tiller number were always lower at elevated than at ambient CO₂ (Fig. 3a). Genetic variance significantly differed ($P < 0.01$) between CO₂ treatments at the three census dates between fall 1994 and fall 1995. As the CO₂ treatment also had a negative impact on the mean values of tiller number, the mean-standardized coefficient of genetic variation may be more appropriate than heritability for comparing the amount of genetic variation expressed in each CO₂ environment (Houle, 1992). The difference between ambient and elevated CO₂ remained positive and significant (analysis not shown). It seems, therefore, that the lower heritability at elevated CO₂ was primarily due to a reduced expression of genetic variance and to a lesser extent due to increased environmental variance.

Genetic variance of the cumulative number of flowering culms per individual produced during the experiment, used as a fitness measure, was significantly larger than zero both in ambient ($\chi^2 = 13.28$; $P < 0.001$)

and elevated CO₂ ($\chi^2 = 7.21$; $P < 0.01$; Table 1). The magnitude of genetic variance in this trait was not significantly different between the two CO₂ treatments ($\chi^2 = 0.69$; $P > 0.4$).

Genotypic selection on plant growth at ambient and elevated CO₂. To examine if CO₂ influenced selection on plant vegetative growth, we estimated genotypic selection differentials for plant size (tiller number) in each CO₂ environment. We used only first-year growth data to protect against increasing bias as plants started to die over subsequent years (notice that for these individuals the number of flowering culms was counted as zero). To obtain a single measure of early growth from data of tiller number in spring, summer, and fall, we conducted a principal component analysis and extracted the first principal component (PC1). PC1 scores were then used for estimation of selection differentials (PC1 explained 78% and 72% of variation in tiller number at ambient and elevated CO₂, respectively). At ambient CO₂, we found a significant positive selection differential for growth of 0.19 ($\chi^2 = 4.8$; $P = 0.028$). At elevated CO₂, the selection differential was -0.06 and nonsignificant ($\chi^2 = 0.3$; $P > 0.5$). Similar results were obtained when selection differentials were calculated separately for each census date.

Tube experiment

CO₂ effects on plant performance. Until fall 1997, three growing seasons after transplantation to the field, 18% of the plants had died in both ambient and elevated CO₂ plots. Plants in the tubes attained much higher tiller numbers compared with plants grown in the

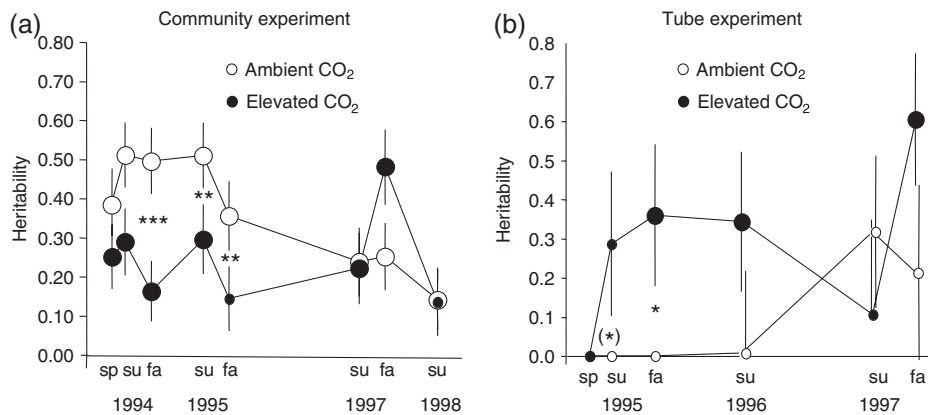


Fig. 3 Heritability (± 1 SE) for number of tillers of *Bromus erectus* exposed to ambient (open circles) or elevated CO₂ (closed circles). In the community experiment (a) plants were grown under competitive conditions in mixed plant communities, while in the tube experiment (b) plants were grown in tubes designed to reduce competition. Large circles indicate that genetic variance was significantly ($P < 0.05$) larger than zero (log-likelihood test). *Genetic variance significantly differed between CO₂ environments (log-likelihood test; $***P < 0.001$, $**P < 0.01$, $*P < 0.05$, $(*) P < 0.1$). sp, spring (April); su, summer (June); fa = fall (October).

mixed communities, suggesting that the tubes indeed reduced competition from neighboring plants. The temporal dynamics of tillering is shown in Fig. 2b. Repeated-measures ANOVA detected no significant CO₂ main effect ($P > 0.7$) or CO₂ × time interaction ($P > 0.3$) for the number of tillers. Plants in the tubes did not set seeds in the first year, so fitness was estimated as the cumulative number of flowering culms produced in the second and third year. Fitness did not significantly differ between the CO₂ environments ($F_{1,14.4} = 2.99$, $P > 0.1$; Table 1).

CO₂ effects on variance in fitness. Overall, opportunity for selection was considerably smaller in the tube experiment (0.28 and 0.33 at ambient and elevated CO₂, respectively) than in the community experiment. The difference between CO₂ treatments was not significant ($\chi^2 = 0.3$; $P > 0.6$).

Quantitative genetic variation at ambient and elevated CO₂. We found no evidence that genotypes differed in mortality at either ambient or elevated CO₂. Genetic variability for tiller number was zero at the start of the experiment in both CO₂ environments, but sharply increased to statistically significant or marginally significant ($P < 0.1$) levels at high CO₂ from the second census onwards (except summer 1997) (Fig. 3b). In contrast, heritability of tiller number remained low at ambient CO₂ in the first 2 years and increased to a marginally significant level ($P < 0.1$) in the third year. The difference between CO₂ environments was marginally significant in fall of the first year ($\chi^2 = 3.16$; $P = 0.08$; Fig. 3b). Fitness exhibited significant genetic variation at both ambient ($\chi^2 = 3.36$; $P < 0.05$) and elevated CO₂ ($\chi^2 = 3.25$; $P < 0.05$; Table 1). The difference between CO₂ environments was not significant ($\chi^2 = 0.01$; $P > 0.9$).

Genotypic selection on plant growth at ambient and elevated CO₂. As mortality in the tube experiment was lower than in the communities, we estimated genotypic selection differentials for growth data (tiller number) from the first and second year (censuses in summer 1995, fall 1995, and summer 1996). In a principal components analysis, PC1 accounted for 64% and 73% of variation in tiller number at ambient and elevated CO₂, respectively. The genotypic selection differential for growth could not be estimated for the ambient-CO₂ treatment, because no genetic variation was expressed in this environment (Fig. 3b). At elevated CO₂, the selection differential was -0.06 , which was not significantly different from zero ($\chi^2 = 0.18$; $P > 0.6$).

Discussion

Plants may respond to global climate change by adjusting their phenotype to the prevailing environmental conditions (phenotypic plasticity) or by evolving adaptations in response to altered selection by the new environmental conditions (Geber & Dawson, 1993; Schmid *et al.*, 1996). While literally hundreds of studies have addressed the question of how elevated CO₂ alters plant growth and development within a single generation (Körner, 2000), very little is known whether CO₂ can act as a selective agent and influence the evolution of plant phenotypes (Kohut, 2003; Ward *et al.*, 2000). Most CO₂ studies interested in evolutionary questions have adopted a norm of reaction approach characterizing how the average phenotype of a genotype changes as a function of the atmospheric CO₂ concentration, and examining whether there is genetic variation in reaction norms (genotype × environment interactions) within or among natural populations (reviewed in Ward & Kelly, 2004). This approach has been widely used in evolutionary ecology, mainly to examine trade-offs in the performance of genotypes occurring in spatially heterogeneous environments (Via *et al.*, 1995). In contrast, the concentration of CO₂ is spatially highly uniform but continuously increasing over time at the global scale. An interesting question with regard to plant evolution is then whether the predicted increase in CO₂ concentration, to which all genotypes and populations will be exposed in a similar way, will influence the expression of genetic variation in fitness-related phenotypic traits and, thus, the pace of evolutionary change. In the following, we will first discuss the effect of an experimental doubling of the CO₂ concentration on the mean performance *B. erectus*, and then discuss the observed changes in the expression of genetic variation and covariation in performance traits.

Plastic response in performance to elevated CO₂

A surprising result of this long-term field study was that *B. erectus* did not respond to high CO₂ by increasing growth (tiller number) or other performance traits (survival, reproduction) both under competitive (community experiment) and noncompetitive conditions (tube experiment). We also found no evidence for an increase in individual tiller size in high CO₂ (data not shown). Literature data compiled by Poorter & Navas (2003) indicate that probably less than 20% of all noncultivated herbaceous species do not respond positively to elevated CO₂ when grown individually. The observed lack of a positive response in *B. erectus* is surprising because it cannot be explained by a physiological incapacity of the species to respond to elevated CO₂. Stocker *et al.*

(1997) observed a sustained increase in photosynthetic rate of 42% at less than doubled CO₂ concentrations in *B. erectus* measured at the same field site as the present study. Moreover, in a greenhouse experiment we found large CO₂-induced stimulation of vegetative growth (>80%, unpublished data; see also Steinger *et al.*, 2000). Thus, either the CO₂-induced growth stimulation may be downregulated in the longer term (Körner, 2000) or counteracted by increased dissimilation processes (e.g. respiration).

As *B. erectus* was nonresponsive to elevated CO₂ when grown individually, we might expect CO₂-forced competitive suppression of the grass when grown in mixed communities, assuming other species to respond positively to high CO₂. Indeed, we found a significant decrease in tiller number at elevated CO₂ in the community experiment, although total reproduction and survival were not significantly reduced. Species exhibiting large and sustained positive CO₂ responses in the experimental communities included *Carex flacca*, *Hieracium pilosella*, and *Sanguisorba minor* (Niklaus *et al.*, 2001). However, these species were of low abundance in the communities and, consequently, total community aboveground biomass was only slightly increased by elevated CO₂. Nevertheless, we can predict from our data that the lack of a positive plastic CO₂ response under field conditions may lead to future declines in abundance of *B. erectus*. This grass is already under present conditions a relatively weak competitor in the more fertile and humid habitats of its geographic range and can only reach dominance at unfertile sites prone to drought (Ellenberg, 1986). In a future CO₂-rich atmosphere, it could be outcompeted at these sites by more positively responding species, unless rapid evolutionary responses would mitigate competitive suppression.

With regard to the following discussion of the micro-evolutionary consequences of elevated CO₂, we conclude that elevated CO₂ may represent an indirectly stressful environment for *B. erectus*, resulting from intensified competitive interactions.

Genetic variation in performance traits at ambient and elevated CO₂

Although CO₂ effects on mean values of fitness-related traits were absent or small, there were surprisingly large effects on the amount of genetic variation expressed in these traits. For example, in the low-competition environment of the tube experiment, significant genetic variance (heritability) in plant size (tiller number) was expressed in the first 2 years at elevated but not at ambient CO₂, but CO₂ had virtually no effect on the mean value of this trait. CO₂ also affected the expression of genetic variance in the community experi-

ment, but the effect was opposite to that observed in the tube experiment: under competitive conditions genetic variance of plant size was often higher at ambient than at elevated CO₂, particularly in the first 2 years of the experiment.

The best measure of fitness in our study is the total number of flowering culms produced until the end of the two experiments, because it integrates survival, plant size and reproduction over the entire experimental period. Traits that are closely related to fitness are expected to exhibit low heritabilities, because strong selection might have depleted genetic variance (Fisher, 1930). However, in the tube experiment we found a rather large heritability for number of flowering culms both at ambient ($H^2 = 0.54$) and elevated CO₂ ($H^2 = 0.42$). In the community experiment significant heritability for this fitness measure was only detected at ambient ($H^2 = 0.20$) but not at elevated CO₂ ($H^2 = 0.08$). We can multiply the heritability with the phenotypic variance in relative fitness (opportunity for selection I) to obtain an estimate of the response of fitness to selection ($R = H^2 I$). For the community experiment, this yields an estimate of 0.13 and 0.05 (in units of SD) at ambient and elevated CO₂, respectively. This would therefore suggest that elevated CO₂ might slightly decrease the rate of evolutionary change. For the tube experiment, predicted rates of evolutionary change were more similar across CO₂ environments (0.15 at ambient CO₂, 0.14 at elevated CO₂), and of similar magnitude as the ones of the community experiment, despite the markedly lower opportunity for selection in the tubes.

We know of only one study that examined CO₂ effects on the expression of genetic variation in a quantitative genetic framework (Bazzaz *et al.*, 1995), and ours is the first to pursue such an approach in the field. A number of quantitative genetic studies adopting the norms of reaction approach reported significant genetic variation in the responsiveness to CO₂ (genotype \times environment interactions, $G \times E$) within or among populations. The occurrence of $G \times E$ interactions, however, does not provide a direct test of whether genetic variances change across CO₂ environments. In addition, the analysis of $G \times E$ by ANOVA may be inappropriate when among-genotype variance strongly differs between environments violating the homoscedasticity assumption of the analysis. Using heterogeneous variance models, we found significant changes in genetic variances even though $G \times E$ interactions in ANOVA were not significant (analysis not shown). In the greenhouse study of Bazzaz *et al.* (1995) elevated CO₂ increased the predicted evolutionary response to selection (R) on seed number. This effect was significant for individually grown plants and was due to a larger heritability at elevated CO₂, which

more than compensated the lower opportunity for selection (I) at elevated CO_2 .

Increased heritability at elevated CO_2 , as was also found for plant size in the tube experiment, is consistent with the hypothesis that more genetic variation should be expressed in evolutionary novel environments (Hoffmann & Merilä, 1999). Several explanations have been proposed to account for this pattern. For example, strong selection in the native environment might have eliminated mutations with slightly deleterious effects (Service & Rose, 1985; Holloway *et al.*, 1990; Kawecki *et al.*, 1997). Alternatively, stabilizing selection in the native environment might have favored mechanisms that reduce phenotypic differences among genotypes (canalization) (Pál, 1998).

The result that elevated CO_2 affected heritability of plant size in the community experiment in the opposite direction (decrease) to that observed in the tube experiment (increase) is puzzling. We do not believe that this is due to the use of two different sets of genotypes, because both sets were haphazardly sampled from the same site and precultivated in the greenhouse under the same conditions. One possible explanation for the decreased heritability in the community experiment might be that intensified competitive interactions at elevated CO_2 operated to amplify small-scale environmental variation, thereby inflating the denominator of the heritability equation (Thomas & Bazzaz, 1993; Thomas & Jasienski, 1996). However, although environmental variance was indeed slightly higher at elevated CO_2 , decreased heritability was mainly due to a lower expression of genetic variance. A second possibility would be that intensified competition at elevated CO_2 might have reduced heritability by increasing plant stress (Jenkins *et al.*, 1997).

Natural selection under elevated CO_2

An important, yet unresolved question with regard to the global carbon cycle is whether plants will adapt to elevated CO_2 by evolving higher rates of carbon assimilation and biomass accumulation (Bazzaz *et al.*, 1995; Ward & Kelly, 2004). Our findings of the selection analysis of the community experiment predict that such adaptive responses are unlikely to occur in *B. erectus*. While selection at ambient CO_2 favored genotypes of large early size, selection at elevated CO_2 did not target early plant size. Plant size (in terms of tiller number) is probably a good proxy for vegetative biomass accumulation, because dry mass per tiller was unaffected by CO_2 (data not shown). To the extent that the estimated selection differentials for the first year extrapolate to the plants' entire life time, we expect no evolutionary shift

toward increased biomass productivity with increasing CO_2 levels.

It is remarkable to note that the few studies addressing the question of genetic shifts towards increased biomass production at high CO_2 drew very similar conclusions, even though they studied plants with different life histories and employed different experimental approaches. In a selection experiment with the annual plant *Arabidopsis thaliana*, Ward & co-workers (2000) reported that, after five generations of selection for high seed number, *Arabidopsis* plants selected at elevated CO_2 exhibited either similar or lower biomass production relative to plants selected at ambient CO_2 , which was attributed to a reduction in the length of the life cycle. Similarly, selection lines of the unicellular green alga *Chlamydomonas* failed to evolve higher growth rates at elevated CO_2 even after 1000 generations of selection (Collins & Bell, 2004). Based on results of a greenhouse experiment with the annual plant *Abutilon theophrasti* and using a quantitative genetic approach similar to the one of our study, Bazzaz & co-workers (1995) predicted very low evolutionary increases in biomass productivity with rising CO_2 levels. In sum, the limited evidence so far suggests that selection in a future CO_2 -rich atmosphere will not operate to increase plant growth above the level observed in a single generation.

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References

- Andalo C, Goldringer I, Godelle B (2001) Inter- and intragenotypic competition under elevated carbon dioxide in *Arabidopsis thaliana*. *Ecology*, **8**, 157–164.
- Arnold SJ, Wade MJ (1984) On the measurement of natural and sexual selection – theory. *Evolution*, **38**, 709–719.
- Bazzaz FA, Jasienski M, Thomas SC, Wayne P (1995) Microevolutionary responses in experimental populations of plants to CO_2 -enriched environments – parallel results from two model systems. *Proceedings of the National Academy of Sciences of the United States of America*, **92**, 8161–8165.
- Blum A (1988) *Plant Breeding for Stress Environments*. CRC Press, Boca Raton.
- Ceccarelli S (1994) Specific adaptation and breeding for marginal conditions. *Euphytica*, **77**, 205–219.

- Collins S, Bell G (2004) Phenotypic consequences of 1000 generations of selection at elevated CO₂ in a green alga. *Nature*, **431**, 566–569.
- Curtis PS, Snow AA, Miller AS (1994) Genotype-specific effects of elevated CO₂ on fecundity in wild radish *Raphanus raphanistrum*. *Oecologia*, **97**, 100–105.
- Ellenberg H (1986) *Vegetation Ecology of Central Europe*, 4th edn. Cambridge University Press, Cambridge.
- Falconer DS, Mackay TFC (1996) *Introduction to Quantitative Genetics*, 4th edn. Addison Wesley Longman, Harlow, Essex, UK.
- Fisher RA (1930) *The Genetical Theory of Natural Selection*. Oxford University Press, Oxford, UK.
- Geber MA, Dawson TE (1993) Evolutionary responses of plants to global change. In: *Biotic Interactions and Global Change* (eds Kareiva PM, Kingsolver JG, Huey RB), pp. 179–197. Sinauer Associates, Sunderland, MA.
- Hoffmann AA, Merilä J (1999) Heritable variation and evolution under favourable and unfavourable conditions. *Trends in Ecology and Evolution*, **14**, 96–101.
- Holloway GJ, Povey SR, Sibly RM (1990) The effect of new environment on adapted genetic architecture. *Heredity*, **64**, 323–330.
- Houle D (1992) Comparing evolvability and variability of quantitative traits. *Genetics*, **130**, 195–204.
- IPCC *Climate Change 2001: The Scientific Basis*. Cambridge University Press, Cambridge, UK.
- Jablonski LM, Wang XZ, Curtis PS (2002) Plant reproduction under elevated CO₂ conditions: a meta-analysis of reports on 79 crop and wild species. *New Phytologist*, **156**, 9–26.
- Jenkins NL, Sgro CM, Hoffmann AA (1997) Environmental stress and the expression of genetic variation. In: *Environmental Stress, Adaptation and Evolution* (eds Bijlsma R, Loeschcke V), pp. 79–96. Experientia Supplementum, Basel.
- Joshi J, Stoll P, Rusterholz H, Schmid B, Dolt C, Baur B (2006) Small-scale experimental habitat fragmentation reduces colonization rates in species-rich grasslands. *Oecologia*, **148**, 144–152.
- Kawecki TJ, Barton NB, Fry JD (1997) Mutational collapse of fitness in marginal habitats and the evolution of ecological niches. *Evolutionary Ecology*, **9**, 38–44.
- Kohut R (2003) The long-term effects of carbon dioxide on natural systems: issues and research needs. *Environment International*, **29**, 171–180.
- Körner C (2000) Biosphere responses to CO₂ enrichment. *Ecological Applications*, **10**, 1590–1619.
- Leadley PW, Niklaus PA, Stocker R, Körner C (1997) Screen-aided CO₂ control (SACC): a middle ground between FACE and open-top chambers. *Acta Oecologica*, **18**, 207–219.
- Littell RC, Milliken GA, Stroup WW, Wolfinger RD (1996) *SAS System for Mixed Models*. SAS Institute Inc, Cary, NC.
- Lynch M, Lande R (1993) Evolution and extinction in response to environmental change. In: *Biotic Interactions and Global Change* (eds Kareiva PM, Kingsolver JG, Huey RB), pp. 234–250. Sinauer Associates, Sunderland, MA.
- Lynch M, Walsh JB (1998) *Genetics and Analysis of Quantitative Traits*. Sinauer Associates, Sunderland, MA.
- Marissink M, Hansson M (2002) Floristic composition of a Swedish semi-natural grassland during six years of elevated atmospheric CO₂. *Journal of Vegetation Science*, **13**, 733–742.
- Mitchell-Olds T (1986) Quantitative genetics of survival and growth in *Impatiens capensis*. *Evolution*, **40**, 107–116.
- Mohan JE, Clark JS, Schlesinger WH (2004) Genetic variation in germination, growth, and survivorship of red maple in response to subambient through elevated atmospheric CO₂. *Global Change Biology*, **10**, 233–247.
- Niklaus PA, Körner C (2004) Synthesis of a six-year study of calcareous grassland responses to *in situ* CO₂ enrichment. *Ecological Monographs*, **74**, 491–511.
- Niklaus PA, Leadley PW, Schmid B, Körner C (2001) A long-term field study on biodiversity × elevated CO₂ interactions in grassland. *Ecological Monographs*, **71**, 341–356.
- Norton LR, Firbank LG, Watkinson AR (1995) Ecotypic differentiation of response to enhanced CO₂ and temperature levels in *Arabidopsis thaliana*. *Oecologia*, **104**, 394–396.
- Pál C (1998) Plasticity, memory and the adaptive landscape of the genotype. *Proceedings of the Royal Society of London Series B*, **265**, 1319–1323.
- Paschke M, Bernasconi G, Schmid B (2003) Population size and identity influence the reaction norm of the rare, endemic plant *Cochlearia bavarica* across a gradient of environmental stress. *Evolution*, **57**, 496–508.
- Pearson PN, Palmer MR (2000) Atmospheric carbon dioxide concentrations over the past 60 million years. *Nature*, **406**, 695–699.
- Polley HW, Johnson HB, Derner JD (2003) Increasing CO₂ from subambient to superambient concentrations alters species composition and increases above-ground biomass in a C-3/C-4 grassland. *New Phytologist*, **160**, 319–327.
- Poorter H, Navas ML (2003) Plant growth and competition at elevated CO₂: on winners, losers and functional groups. *New Phytologist*, **157**, 175–198.
- Potvin C, Tousignant D (1996) Evolutionary consequences of simulated global change: genetic adaptation or adaptive phenotypic plasticity. *Oecologia*, **108**, 683–693.
- Rausher MD (1992) The measurement of selection on quantitative traits – biases due to environmental covariances between traits and fitness. *Evolution*, **46**, 616–626.
- Roumet C, Laurent G, Canivenc G, Roy J (2002) Genotypic variation in the response of two perennial grass species to elevated carbon dioxide. *Oecologia*, **133**, 342–348.
- Schmid B, Birrer A, Lavigne C (1996) Genetic variation in the response of plant populations to elevated CO₂ in a nutrient-poor, calcareous grassland. In: *Carbon Dioxide, Populations, and Communities* (eds Körner C, Bazzaz FA), pp. 31–49. Academic Press, San Diego, USA.
- Service PM, Rose MR (1985) Genetic covariation among life-history components – the effect of novel environments. *Evolution*, **39**, 943–945.
- Steinger T, Gall R, Schmid B (2000) Maternal and direct effects of elevated CO₂ on seed provisioning, germination and seedling growth in *Bromus erectus*. *Oecologia*, **123**, 475–480.
- Steinger T, Lavigne C, Birrer A, Groppe K, Schmid B (1997) Genetic variation in response to elevated CO₂ in three grassland perennials – a field experiment with two competition regimes. *Acta Oecologica*, **18**, 263–268.

- Stocker R, Leadley PW, Körner C (1997) Carbon and water fluxes in a calcareous grassland under elevated CO₂. *Functional Ecology*, **11**, 222–230.
- Thomas SC, Bazzaz FA (1993) The genetic component in plant size hierarchies – norms of reaction to density in a *Polygonum* species. *Ecological Monographs*, **63**, 231–249.
- Thomas SC, Jasienski M (1996) Genetic variability and the nature of microevolutionary responses to elevated CO₂. In: *Carbon Dioxide, Populations, and Communities* (eds Körner C, Bazzaz FA), pp. 51–81. Academic Press, San Diego, USA.
- Via S, Gomulkiewicz R, De Jong G, Scheiner SM, Schlichting CD, Van Tienderen P (1995) Adaptive phenotypic plasticity: consensus and controversy. *Trends in Ecology and Evolution*, **10**, 212–217.
- Ward JK, Antonovics J, Thomas RB, Strain BR (2000) Is atmospheric CO₂ a selective agent on model C-3 annuals? *Oecologia*, **123**, 330–341.
- Ward JK, Kelly JK (2004) Scaling up evolutionary responses to elevated CO₂: lessons from *Arabidopsis*. *Ecology Letters*, **7**, 427–440.
- Ward JK, Strain BR (1997) Effects of low and elevated CO₂ partial pressure on growth and reproduction of *Arabidopsis thaliana* from different elevations. *Plant, Cell and Environment*, **20**, 254–260.
- Wieneke S, Prati D, Brandl R, Stocklin J, Auge H (2004) Genetic variation in *Sanguisorba minor* after 6 years *in situ* selection under elevated CO₂. *Global Change Biology*, **10**, 1389–1401.