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FAMILY- AND POPULATION-LEVEL RESPONSES TO ATMOSPHERIC CO_2 CONCENTRATION: GAS EXCHANGE AND THE ALLOCATION OF C, N, AND BIOMASS IN *PLANTAGO LANCEOLATA* (PLANTAGINACEAE)[1]

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To ascertain the inheritance of responses to changing atmospheric CO_2 content, we partitioned response to elevated CO_2 in *Plantago lanceolata* between families and populations in 18 families in two populations. Plants were grown in 35 Pa and 71 Pa partial pressure of CO_2 (pCO_2) in open-top chambers. We measured above- and belowground mass, carbon (C), nitrogen (N), hexose sugar, and gas exchange properties in both CO_2 treatments. Families within populations differed in mass, mass allocation, root : shoot ratios, aboveground percentage N, C : N ratio, and gas exchange properties. The CO_2 × family interaction is the main indicator of potential evolutionary responses to changing CO_2. Significant CO_2 × family interactions were observed for N content, C : N ratio, and photosynthetic rate (*A*: instantaneous light-saturated carbon assimilation capacity), intercellular CO_2 concentration, transpiration rate (*E*), and water use efficiency (WUE = *A*/*E*), but not for stomatal conductance. Families differed significantly in acclimation across time. The ratio of *A* in elevated vs. ambient growth CO_2, when measured at a common internal CO_2 partial pressure was 0.79, indicating downregulation of *A* under CO_2 enrichment. Mass, C : N ratio, percentage, C (%C), and soluble sugar all increased significantly but overall %N did not change. Increases in %C and sugar were significant and were coincident with redistribution of N aboveground. The observed variation among populations and families in response to CO_2 is evidence of genetic variation in response and therefore of the potential for novel evolutionary trajectories with rising atmospheric CO_2.

Key words: biomass allocation; elevated CO_2; gas exchange; genetic variation; nitrogen assimilation; photosynthesis; *Plantago lanceolata*; Plantaginaceae.

Many studies have shown that whole-plant mass in C_3 species typically increases in response to elevated CO_2 (reviewed by Poorter, 1993; Curtis and Wang, 1998). However, as the variety of species studied and the length of experiments have increased, considerable interspecific variation in the magnitude and duration of this ''typical'' response to elevated atmospheric CO_2 has been documented (Bazzaz, Coleman, and Morse, 1990; Garbutt, Williams, and Bazzaz, 1990; Hunt et al., 1995). Substantial interspecific variation has also been observed in the physiological responses to elevated CO_2, in changes in nitrogen acquisition, and in the partitioning of overall mass, carbon, and nitrogen among plant parts and functions (Sage, 1994; Curtis and Wang, 1998).

While interspecific differences in responses to elevated CO_2 can have important implications for ecological interactions, they also indicate that genetic variation in responses exists, at least at a macroevolutionary level. Intraspecific differences arise and are translated into interspecific variation through the actions of drift and divergent selection. The extent of intraspecific variation indicates, at least in a qualitative way, the potential for rapid adaptation to elevated CO_2. Thus while extant interspecific differences in response to elevated CO_2 demonstrate that evolution in CO_2 responses can occur on a geologic time scale, the extent of intraspecific variation indicates the likelihood of near-term genetic adaptation to shifting atmospheric CO_2 composition on something closer to ecological time scales. Yet the presence and nature of that intraspecific variation is largely unexplored.

To understand the potential evolutionary consequences of increasing atmospheric CO_2, we need to examine the extent of genetic variation in relevant traits both at the level of populations and at that of families. In some species, genetic variation is distributed mainly among rather than within populations, largely as a function of dispersal and mating system effects (Loveless and Hamrick, 1984). When genetic variation is distributed mainly among populations, and the within-population heritabilities are low, the movement of genes between populations can be as important a source of genetic variance as mutation and recombination, especially in small populations (Weber, 1990, 1992). Variation at the family level within populations in contrast is the classic quantitative genetic measure of genetic variation, among-sibling variance being the basis of commonly used heritability estimates (Hallauer and Miranda, 1981). It is therefore important to assess genetic variation both within and among populations.

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In the small number of studies that have examined intraspecific variation in the context of elevated CO_2 atmospheres, both growth (Wulff and Alexander, 1985; Poorter, Pot, and Lambers, 1988; Curtis et al., 1996) and reproductive output (Cure and Acock, 1986; Curtis, Snow, and Miller, 1994; Curtis et al., 1994) exhibit genetic variation. For example, Curtis, Snow, and Miller (1994) showed genotype-specific effects of elevated CO_2 on fecundity, and Curtis et al. (1996) demonstrated that both *Raphanus raphanistrum* and *Plantago lanceolata* varied physiologically among sibships in 71 Pa CO_2. However, none of these studies determined if families or genotypes *respond differently* when grown in a high CO_2 environment than they do when grown in a conventional CO_2 environment. This is important for understanding the evolutionary implications of the observed variation. If families or genotypes respond similarly, i.e., maintain the same relative ranks across environments, then directional selection will favor the same genotypes in both environments. Thus any resultant evolution is not CO_2-environment specific, but is a general evolutionary response to characteristics that are common to both CO_2 environments.

To demonstrate inherited differences *in response to elevated CO_2,* one must show a change in the rank order among genotypes across CO_2 environments. When the phenotypic ranks of a set of genotypes (or families, or populations) change across environments, then selection for high phenotypic value will select on different genotypes in the two environments. Thus any response to selection will be through increase in the frequencies of different genotypes in the two environments.

In this study we tested for genetic variation in the response to elevated CO_2 in the short-lived perennial herb *Plantago lanceolata,* using maternal families from two natural populations. *Plantago lanceolata* has been used extensively in physiological, ecological, and genetic studies (Teramura and Strain, 1979; Tonsor, 1985, 1989; Kuiper and Bos, 1992; Tonsor and Goodnight, 1997). Populations of *P. lanceolata* grow in a variety of habitats and have been found to exhibit both local adaptation (Teramura and Strain, 1979; Teramura, 1983; Tonsor, 1985, 1990) and phenotypic plasticity (Teramura and Strain, 1979; Antonovics and Primack, 1982; van Tienderen, 1990). The species is known to be genetically variable for a broad variety of physiological, morphological, and life history traits (Teramura and Strain, 1979; Teramura, 1983; Wolff and Van Delden, 1987; Tonsor and Goodnight, 1997) and is capable of undergoing rapid evolutionary change (Wu and Antonovics, 1976; Wolff and Van Delden, 1989). Studies of *P. lanceolata* grown in elevated CO_2 have revealed genetic variation in allelochemical content (Fajer, Bowers, and Bazzaz, 1992) and early growth parameters (Wulff and Alexander, 1985). However, these studies did not address genetic differences in genotype or family ranks between CO_2 environments.

The goal of the study was to determine the extent to which there is a change in rank using the genotype × CO_2 interaction in mass gain and in a number of morphological and physiological traits that are known to change in mean value in response to elevated CO_2. We measured a hierarchy of traits including total plant mass, mass allocation, tissue sugar, carbon (C) and nitrogen (N) content. Changes in mass allocation patterns provide a measure of the extent to which plants alter their investment in roots or shoots under CO_2 enrichment. Changes in sugar content can indicate the extent to which a plant is able to down-regulate its carbon assimilation machinery sufficiently to keep N and C in balance in the plant tissue (Sage, Sharkey, and Seemann, 1988). Since N is often the most limiting resource in terrestrial ecosystems, shifts in tissue-specific nitrogen content can be a critical factor in optimizing N economy and fitness in an elevated CO_2 environment.

This study measured the extent to which variation in responses to an elevated CO_2 environment can be partitioned among families and populations. Inherited variation is the basis for adaptive evolutionary responses to selection, and this study establishes whether inherited variation exists in traits known to exhibit plastic responses atmospheric carbon content. However, predicting the extent of a future adaptive responses also requires careful field estimation of selection on these traits, which was beyond the scope of this study.

MATERIALS AND METHODS

We studied two populations of *P. lanceolata.* The Ely Lake (EL) population (Allegan County, Michigan, USA) grows on exposed sandy soil on a sunny lakeshore, experiencing high irradiance at midday (and midday water stress), but partial shade from the surrounding oak–pine forest early and late in the day. The Kellogg Field (KF) population (Kalamazoo County, Michigan, USA) grows in a sandy loam in the partial shade, intermixed in a canopy of tall herbaceous vegetation, on the edge of a mown field. Seeds from 25–30 maternal plants (families) in each population were collected in the fall of 1991. Ten families from KF and eight families from EL were selected randomly for use in the experiment.

In early June 1992 all of the seeds from each of the eighteen families were planted in flats and placed in an open-top chamber at the CO_2 level in which they were to be grown (either ambient or twice-ambient CO_2). After germination, seedlings were transplanted into 10 cm diameter × 30 cm high pots with mesh screen bottoms, one seedling per pot. Pots were filled with 2.45 L of 50 : 50 mixture of native field soil (Kalamazoo loam) and sand. Low seed numbers for some families resulted in an average within-family sample size of 8.5 seedlings. All seedlings were assigned randomly to one of eight 0.5-m^3 outdoor open-top chambers (Curtis and Teeri, 1992). The chambers were placed adjacent to the Terrestrial Field Laboratory at the Kellogg Biological Station (Kalamazoo County, Michigan, USA). The plants were watered as needed, usually twice daily. There was no fertilizer supplementation.

Pure CO_2 was dispensed via manual flow meters into input blowers and then into the four elevated CO_2 chambers to increase CO_2 concentration. The four ambient chambers received no additional CO_2. Chamber CO_2 levels were monitored continuously with an infra-red gas analyzer (LI-6251, LI-COR, Lincoln, Nebraska, USA) (see Curtis and Teeri, 1992). Quantum sensors and thermocouples within each chamber were used to record irradiance levels and temperature. During daytime (0700–1900) CO_2 was 72.1 ± 5.8 Pa (mean ± 1 SD) inside the elevated chambers, and 36.4 ± 2.6 Pa inside the ambient chambers. Elevated chamber CO_2 was allowed to follow diurnal fluctuations, so that both nightime and daytime CO_2 concentrations were appropriately elevated. Daytime temperatures were 1.70 ± 0.6°C higher inside the chambers than outside, with no significant difference in temperature between ambient and elevated chambers. After 3 wk of growth, plants exhibited symptoms of light stress (prostrate growth and red pigmentation of the leaf bases) and all chambers were covered with shade cloth. The shade cloth reduced incident light 68%, and plants recovered their normal phenotype. Since both populations experienced partial shade in their native habitats (although with different variances), this seemed an appropriate amendment to the treatment.

Instantaneous carbon assimilation capacity (A), transpiration (E), stomatal conductance (g_s), and leaf internal CO_2 concentration (C_i) measurements were made twice on all plants, from 9 to 13 August and from 4 to 10 September 1992. Each plant was measured at both CO_2 growth treatment concentrations at saturating light levels (1300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetically active radiation [PAR]). Measurements were made on the youngest fully expanded leaf. We used two infra-red gas analyzers (Model LCA3, Analytical Development Corp., Hoddeson, UK) for these measurements. The CO_2 source (35 Pa or 71

Pa CO_2) was rotated to a different instrument each day to allow us to estimate instrument effects.

Plants were harvested after 127 d growth, above- and belowground parts separated, dried at 60°C, and weighed. No plants flowered during this experiment. To determine the extent to which tissue quality was affected by the CO_2 treatments, three families were arbitrarily selected for biochemical analysis. The available funds for CHN analysis limited the total sample size for this analysis to $N = 36$: (6 plants per family) × (3 families) × (2 CO_2 treatments). Shoot and root tissue of each plant was analyzed separately for percentage C (%C) and percentage N (%N) with a CHN analyzer (Carlo Erba, Paramus, New Jersey, USA). Soluble carbohydrate concentration was determined enzymatically (see Hurry et al. 1995). Tissue was ground and the supernatent was centrifuged prior to being divided into two samples. In the first sample, hexokinase was added to convert glucose to glucose-6-phosphate (G-6-P). Phosphoglucose isomerase was then used to drive the conversion of fructose-6-phosphate to G-6-P. Glucose-6-Phosphate was oxidized to 6-phosphogluconate using glucose-6-phosphate dehydrogenase, and the concentration of the NADPH formed in the process was measured spectrophotometrically at A_{340}.

Statistical analysis—In evolutionary genetic studies, changes in genotypic rank across environments are typically assessed through tests for heterogeneity of slopes in a regression of genotypic responses across environments or by a test for an interaction between genotype and environment in an analysis of variance (ANOVA) or multivariate analysis of variance (MANOVA). We used ANOVAs and MANOVAs, because our environmental treatments consisted of two discrete CO_2 concentrations.

Physiological variables—The effects of block, instrument, and date of measurement were removed from the four gas exchange variables by subtracting their respective deviations from the grand mean summed over block, instrument, and measurement dates (Tonsor and Goodnight, 1997). Because the four gas exchange variables were expected to be correlated with each other (Tonsor and Goodnight, 1997), they were initially analyzed using a multivariate analysis of variance (MANOVA), to determine overall, main, and interaction effects. The MANOVA was followed by univariate mixed model ANOVAs for the four primary physiological variables plus water use efficiency (WUE), defined as A/E. The overall mixed models for the analysis of the physiological variables included four fixed effects: the CO_2 treatment at which plants were grown (growth CO_2), month of measurement, the CO_2 concentration at which gas exchange was measured (measurement CO_2), and population of origin. The models also included one random effect (family nested within population) and two-way interactions for all main effects. Interaction terms containing the random effect were also treated as random effects.

Significance levels in the multivariate analysis were interpreted using Roy's Greatest Root because of its statistical power and suitability for post hoc statistical comparisons (Scheiner, 1993). Sample size for each family in each CO_2 treatment ranged from 3 to 6 individuals. All variables in the analysis were normally distributed except E, which was $\log_{10}$ transformed, and g_s, which was square-root transformed.

Since each plant was measured at two CO_2 levels in each of 2 mo, we performed two separate repeated-measures analyses: one to test the response of the physiological variables to measurement CO_2 and another to test how these responses differed between August and September. The physiological variables were initially tested together in multivariate repeated measures ANOVAs and then were considered separately for their contributions to the multivariate results. The model was identical to the one used in the MANOVA described above.

Mass and tissue biochemistry—Mass data from the main experiment and tissue biochemistry data from the subset of three families were statistically analyzed using ANOVA. The model for the analysis of the main experiment was: $y_{ijkl} = y...\alpha_i + \beta_j + \delta_k + \gamma_{l(k)} + \beta\delta_{jk} + \beta\gamma_{jk} + \epsilon_{ijkl}$, where y_{ijkl} is them measurement for the $ijkl$th individual, $y...$ = grand mean, α_i = mean effect of block i, β_j = mean effect of CO_2 level j, δ_k = the mean effect of population k, γ_l = mean effect of the lth family within population k, and the paired, doubly subscripted terms are the two-way interactions. The three-way interactions and the block interactions were never significant, and so they were omitted from the final analyses. The SAS MODEL statement read: *variable* = BLOCK CO_2 POPULATION FAMILY(POP) (CO_2 × POPULATION) (CO_2 × FAMILY) (SAS, 1989). Target sample size for each family in each CO_2 environment was $n = 6$, although this target was not always met, due to a paucity of seeds. Of the 24 families used in the main experiment, six families were excluded from analysis because two or fewer individuals were present in one or both CO_2 growth environments.

TABLE 1. Multivariate analysis of variance for the main effects of growth CO_2 concentration (GCO_2), population (POP), measurement CO_2 concentration (MCO_2), month of measurement (MONTH), and family nested within population (FAM), and their two-day interactions. The three-way interaction was not significant.

Source	Roy's greatest df	Roy's greatest root	F	$P > F$
GCO_2	4,618	0.15	23.6	0.0001
POP	4,618	0.02	3.4	0.009
MCO_2	4,618	47.52	7342.8	0.0001
MONTH	4,618	0.09	14.0	0.0001
FAM	16,621	0.12	4.7	0.0001
GCO_2 × POP	4,618	0.02	2.4	0.048
GCO_2 × CO_2	4,618	0.09	13.2	0.0001
GCO_2 × MONTH	4,618	0.03	5.0	0.0006
GCO_2 × FAM	16,621	0.08	3.1	0.0001
POP × MCO_2	4,618	0.002	0.2	0.9
POP × MONTH	4,618	0.003	0.4	0.8
MCO_2 × MONTH	4,618	0.01	2.2	0.07
MCO_2 × FAM	16,621	0.02	0.9	0.5
MONTH × FAM	16,621	0.06	2.3	0.002

The model for the analysis of tissue content in the three families was: $y_{ij} = y...\alpha_i + \beta_j + \alpha\beta_{ij} + \epsilon_{ij}$, where y_{ij} is them measurement for the ijth individual, $y..$ = grand mean, α_i = mean effect of CO_2 treatment i, β_j = mean effect of family j, and $\alpha\beta_{ij}$ = the CO_2 × family interactions. In SAS language (SAS, 1989), the model statement was: *variable* = CO_2 FAMILY (CO_2 × FAMILY). Since only one family from the EL population was included, a population effect could not be included. All variables were normally distributed except root : shoot ratio. A log transformation of root : shoot ratio to achieve normality did not change significance levels and the untransformed data were used in the overall ANOVA.

Since a comparison of family means was intended initially, all main effects and interactions in the model were considered to be fixed (Gill, 1978). A statistically significant POPULATION or FAMILY mean square demonstrates that these populations or families differ genetically from each other. A statistically significant interaction term ((POPULATION × CO_2) or (FAMILY × CO_2)) indicates that the populations or families respond differentially to the CO_2 growth environment.

RESULTS

Physiology—*Population-level effects*—The MANOVA showed that populations were significantly different overall (Table 1), but in the ANOVAs, no single physiological variable showed significant variation between the two populations (Table 2). There was also a significant overall population by growth CO_2 interaction detected in the MANOVA, although none of the individual physiological factors had significant population by growth CO_2 interactions in the ANOVAs.

Family-level effects—Families showed significant overall physiological variation (Table 1); however, no single physiological trait showed significant family-level variation (Table 2). The family × growth CO_2 interaction was significant in the MANOVA, and for A, E, C_i and WUE but not for g_s in

TABLE 2. Summary of probabilities from univariate mixed-model ANOVAs, with growth CO_2 concentration (GCO_2), population (POP), measurement CO_2 concentration (MCO_2), month of measurement (MONTH), and family nested within population (FAM), and their two-way interactions. The three-way interaction was not significant.

Source	df	*A*	g_s	*E*	WUE	C_i
GCO_2	1	0.002	0.23	0.11	0.0001	0.0001
POP	1	0.17	0.54	0.64	0.09	0.17
MCO_2	1	0.0001	0.0001	0.007	0.0001	0.0001
MONTH	1	0.006	0.13	0.74	0.0001	0.0001
FAM	16	0.38	0.45	0.27	0.28	0.13
GCO_2 × POP	1	0.51	0.65	0.31	0.104	0.42
GCO_2 × MCO_2	1	0.003	0.01	0.09	0.0001	0.0001
GCO_2 × MONTH	1	0.003	0.03	0.88	0.0001	0.15
GCO_2 × FAM	16	0.005	0.44	0.04	0.02	0.05
POP × MCO_2	1	0.90	0.66	0.18	0.58	0.45
POP × MONTH	1	0.76	0.87	0.81	0.87	0.44
MCO_2 × MONTH	1	0.01	0.96	0.45	0.03	0.97
MCO_2 × FAM	16	0.92	0.99	0.99	0.42	0.80
MONTH × FAM	16	0.03	0.02	0.003	0.44	0.33

the ANOVAs. These interactions were not due simply to increased spread among families in elevated CO_2, but result from changes in rank. As an example, this family × growth CO_2 interaction is illustrated for *A* in the style typical in reports of genotype × environment interactions (Fig. 1). The other significant interactions are similar in the extent to which the norms of reaction for families or populations cross each other between environments. Families did not respond differently to measurement CO_2. There was a significant MANOVA family × month interaction, and this was reflected in significant ANOVA family × month interactions for *A*, *E*, and g_s Leaf internal CO_2 concentration and WUE did not show significant ANOVA family level interactions.

The effects of CO_2—The overall treatment effects for plants grown and measured in both ambient and elevated CO_2 at two dates in the growing season are presented in Table 3. The MANOVA of physiological variables (Table 1) detected a highly significant effect of growth CO_2 on the combined set of physiological variables. The univariate ANOVAs (Table 2) showed significant overall growth CO_2 effects on *A*, WUE, and C_i but not on g_s or *E* (Table 1). The CO_2 concentration of the growth environment (growth CO_2) affected only *E* and WUE in August. However, by September substantial differences between plants grown at the two atmospheres had developed and were manifested as between growth-environment differences in *A* and WUE manifested in both measurement atmospheres. September differences in g_s due to the growth CO_2 environment were only observed in the 71 Pa measure-

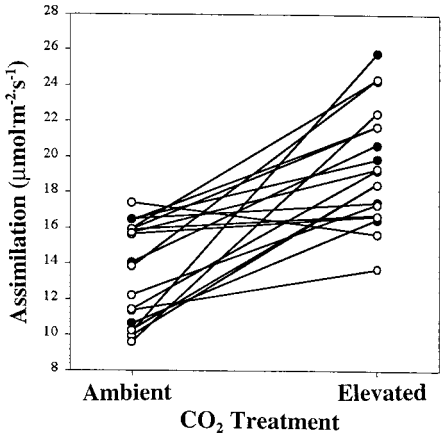

Fig. 1. Norms of reaction for carbon assimilation rates across 18 families measured in September. Open symbols are KF population; closed symbols are EL population.

TABLE 3. Effects of CO_2 treatments on net CO_2 assimilation (*A*) stomatal conductance (g_s), transpiration rate (*E*), water use efficiency (WUE), and internal CO_2 concentration (C_i) in *Plantago lanceolata* in August and September, mean (SE), $N = 89$–99 for each variable. CO_2 treatment means were compared using univariate one-way ANÖVÄs. Different superscript letters indicate significant differences between means across treatments. Lowercase a's and b's indicate differences between growth CO_2 means, both measured at 35 Pa CO_2. Uppercase A's and B's indicate differences between growth CO_2 means, both growth treatments measured at 71 Pa CO_2. Uppercase X's and Y's indicate differences between growth environment means when measured in their respective growth CO_2 environments (e.g., 35 Pa-grown measured at 35 Pa, vs. 71 Pa-grown, 71 Pa measured). Differences were considered to be significant at the $P < 0.025$ level (Bonferroni correction for multiple comparisons of means).

Variable	Measurement CO_2 (Pa)	Growth CO_2 (Pa) 35	Growth CO_2 (Pa) 71
AUGUST			
A (μmol·m^{-2}·s^{-1})	35	12.86 (0.37)^{a,X}	13.27 (0.33)^{a}
	71	26.17 (0.52)^{A}	24.48 (0.58)^{A,Y}
g_s (mmol·m^{-2}·s^{-1})	35	1.23 (0.07)^{a}	1.09 (0.06)^{a}
	71	0.87 (0.06)^{A}	1.05 (0.05)^{A}
E (mmol·m^{-2}·s^{-1})	35	7.37 (0.17)^{a}	7.56 (0.20)^{a}
	71	6.94 (0.15)^{A}	7.31 (0.17)^{A}
WUE	35	1.76 (0.04)^{a,X}	1.84 (0.04)^{a}
	71	3.81 (0.06)^{A}	3.33 (0.07) ^{B,Y}
C_i	35	251.16 (1.74)^{a,X}	247.01 (1.85)^{a}
	71	523.10 (4.38)^{A}	552.82 (4.09)^{B,Y}
SEPTEMBER			
A (μmol·m^{-2}·s^{-1})	35	13.00 (0.50)^{a,X}	11.39 (0.47)^{b}
	71	24.8 (0.71)^{A}	19.59 (0.80)^{B,Y}
g_s (mmol·m^{-2}·s^{-1})	35	1.14 (0.07)^{a,X}	1.02 (0.07)^{a}
	71	0.91 (0.06)^{A}	0.85 (0.05)^{B,Y}
E (mmol·m^{-2}·s^{-1})	35	7.29 (0.22)^{a}	7.28 (0.24)^{a}
	71	6.84 (0.20)^{A}	7.16 (0.27)^{A}
WUE	35	1.78 (0.05)^{a,X}	1.51 (0.04)^{b}
	71	3.68 (0.07)^{A}	2.76 (0.10)^{B,Y}
C_i	35	259.65 (2.41)^{a,X}	264.92 (2.35)^{a}
	71	529.35 (4.39)^{B}	568.51 (8.42)^{A,Y}

TABLE 4. Analysis of variance for components of mass. The components are: aboveground mass (M_A), belowground mass (M_B), total mass (M_T), and the ratio of root mass to shoot mass (R : S). For each mass variable, the mean square (MS), F ratio (F), and probability of a larger F (P) from the ANOVAs are displayed. Significance levels: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

		M_A		M_B		M_T		R : S	
Source	df	MS	F	MS	F	MS	F	MS	F
Growth CO_2 concentration	1	62.6	2.5	86.6	15.0***	296.5	6.1*	0.3	13.0***
Population	1	333.6	13.4***	68.3	11.8***	703.8	14.5***	0.0005	0.03
Block	3	64.5	2.6	1.9	0.3	61.7	1.3	0.1	5.3**
Family (Population)	16	56.6	2.3*	15.2	2.6*	119.9	2.5**	0.05	2.4**
CO_2 × Population	1	6.3	0.3	2.6	0.5	17.1	0.4	0.0006	0.03
CO_2 × Family (Population)	16	19.9	0.8	4.7	0.8	39.1	0.8	0.01	0.7
Error	152	24.8		5.8		48.6		0.02	

ment atmosphere, while September differences in E C_i were only observed at 35 Pa (Table 3).

Measurement CO_2 concentration (MCO_2) had a highly significant effect overall (Table 1), and all physiological variables showed significant responses to measurement CO_2 (Table 2). Water use efficiency increased when measured at 71 Pa CO_2. Stomatal conductance declined at the higher measurement CO_2, indicating that reduced stomatal aperture is the cause for the decline in E and increase in WUE. Leaf internal CO_2 concentration approximately doubled from ~25 Pa at 71 Pa CO_2 to ~54 Pa at 35 Pa CO_2, with smaller effects of growth CO_2 or month of measurement.

Mass allocation—*Population- and family-level effects*—There were significant differences between populations for all mass variables except root : shoot ratio (Table 4). Population KF always had significantly larger mass measures than population EL, and there were no significant growth CO_2 × population interaction terms (Table 4). There were significant effects of family in all four mass variables (Table 4), but no growth CO_2 × family interactions were detected for the mass variables.

Growth-CO_2 effects—There were significant CO_2 effects on belowground mass, total mass, and root : shoot ratio (Table 4), with elevated-grown means significantly greater than ambient-grown means (Table 5). Total mass increased in elevated-grown plants by 15% and root : shoot ratio by 19%. Although early CO_2 effects on aboveground size (leaf number) were detected, (data not shown) these differences disappeared by the end of the experiment. Final aboveground mass did not show a significant growth-CO_2 response.

Tissue biochemistry—*Family-level effects*—The three families varied significantly for four of the eight tissue content traits; aboveground %N, aboveground and belowground C : N, and belowground soluble sugar. There were significant growth CO_2 × family interactions detected for two tissue content traits: aboveground C : N and aboveground %N (Table 6).

The effects of growth CO_2—There were significant differences overall between CO_2 treatments for all but belowground %C and soluble sugar (Table 6). Whole-plant %C was slightly higher in the elevated than in the ambient CO_2 treatment (Table 7). Whole-plant soluble sugar was 15% higher in elevated compared to ambient-CO_2 grown plants (Table 7).

Whole plant %N did not respond significantly to growth CO_2 (Table 7), but N distribution shifted to aboveground tissues and away from belowground tissues. Aboveground C : N is lower in the elevated CO_2 environment than in the ambient (Table 7), while belowground the C : N is higher in the elevated CO_2 environment than the ambient CO_2 environment (Table 7). Since whole plant %C content differs only slightly between the two CO_2 environments (data not shown), the shift in C : N ratios must result from the shifts in N allocation between CO_2 growth environments (Table 7).

TABLE 5. Mass means by treatment and population, with standard errors in parentheses. Asterisks indicate significance levels in tests for differences in means between growth CO_2 environments, within populations. Significance levels: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Population	Growth CO_2 (Pa)	Aboveground mass (g)	Belowground mass (g)	Root : shoot ratio
EL	35	10.8 (0.7)	5.5 (0.2)	0.51 (0.02)
	71	11.5 (0.7)	6.6 (0.3)*	0.59 (0.03)**
KF	35	13.2 (0.8)	6.3 (0.4)	0.49 (0.03)
	71	14.7 (0.9)	7.9 (0.4)***	0.59 (0.02)***
Overall	35	12.1 (0.6)	5.9 (0.2)	0.49 (0.02)
	71	13.1 (0.6)	7.4 (0.3)***	0.59 (0.02)***

DISCUSSION

The organization of inherited variation is a critical factor in determining the tempo and mode of evolution. The partitioning of variation among families and populations plays a critical role in determining the magnitudes of responses to selection. The environmental dependency of genetic effects on the phenotype is also critical, although much less well understood. To a large extent, genotype–environment interactions have been treated by quantitative geneticists as noise that obscures the predictive ability of standard quantitative genetic approaches to predicting responses to selection (Roff, 1997). In the context of changing environmental conditions, understanding genotype–environment interactions is critical to understanding the probable evolutionary responses to the new environmental regime. When genotypes produce qualitatively different phenotypes in new environments (e.g., shifted genotypic ranks), simply measuring variation in the conventional environment may not adequately describe the nature of the inherited variation which natural selection will ''see'' in the new environment. In this study, we were therefore interested in both the structuring of variation at the population and family levels and in the way in which that variation changed in its expression across CO_2 environments. In keeping with the conventional biometric approaches to the partioning of phenotypic variation across levels of population structure and environments, we first discuss

TABLE 6. ANOVA for tissue composition components for three families (EL 15, KF 32, KF 35), analyzed separately for aboveground and belowground tissues. Probabilities < 0.05 are shown in bold type.

Source	df	Soluble sugar		Percentage carbon		C : N ratio		Percentage nitrogen	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Aboveground									
CO_2	1	7.0	**0.0135**	9.8	**0.0039**	6.5	**0.0160**	4.4	**0.0438**
Family	2	1.5	0.25	1.7	0.20	12.5	**0.0001**	11.1	**0.0003**
CO_2 × Family	2	1.9	0.17	0.7	0.52	6.0	**0.0065**	4.8	**0.0156**
Error	30								
Belowground									
CO_2	1	3.3	**0.0792**	0.3	0.59	6.0	**0.0203**	11.7	**0.0019**
Family	2	5.7	**0.0078**	1.8	0.18	2.1	0.14	5.1	**0.0122**
CO_2 × Family	2	0.4	0.70	0.1	0.88	0.0	0.98	0.4	0.70
Error	30								

the main effects of family- and population-level variation, then consider the population × CO_2 and family × CO_2 interactions, the true focus of this study.

Plantago lanceolata has been shown to exhibit variation among populations or among families or genotypes in a wide variety of traits. For example, Wu and Antonovics (1976) and Tonsor (1990) documented the existence of variation in lead tolerance along roadsides that received input from car exhaust when leaded gasoline was used for fuel. Wolff and Van Delden (1987, 1989) found significant broadsense heritabilities for 18 morphological and developmental traits (significant in some populations and not in others). Wulff and Alexander (1985) observed genetic variation in early life history stages in *P. lanceolata.* Tonsor and Goodnight (1997) detected narrow-sense heritable variation in *A*, *E*, and a set of morphological traits.

Similarly, at either the population or the family level or both, this study detected variation in many of the traits measured (4 out of 10 at the population level, 9 of 18 at the family level). In some traits, particularly those associated with mass, the same population, and the same families within populations, had highest trait values regardless of the CO_2 concentration in the atmosphere of growth. Thus, population KF had greater aboveground mass, belowground mass, and total mass than population EL, and both populations responded to CO_2 enrichment by increasing mass. While population-level variation was not examined for tissue biochemistry, four of the eight biochemical traits evidenced significant family-level variation that was maintained across CO_2 environments. For physiological variables, the MANOVA indicated both population- and family-level variation, although no single physiological trait had significant variation at either the population or family level when examined in separate ANOVAs. It is not uncommon for a MANOVA to indicate overall significance for a suite of attributes with partially shared causes, such as the physiologically integrated traits measured here, without corresponding significance for the individual traits in univariate ANOVAs (Manly, 1986).

Thus, in about half of the traits examined, families within populations differ significantly in their average trait values, regardless of the CO_2 environment in which they are examined. There would therefore appear to be the potential for evolutionary change in the average values of these traits. This inference is further supported by the discovery of significant differences between populations in four of the ten traits for which population-level variation was examined; these two populations have evolved different mean trait values for mass and its partitioning as well as in their overall physiological properties as measured in the MANOVA.

The family ranks in mass and its partitioning did not change across CO_2 environments. The lack of family × CO_2 interaction provides no variation on which evolutionary responses to high-CO_2 environments could be based. Root : shoot ratio responses are highly dependent on pot size in elevated CO_2 (Sage, 1994) and should therefore be interpreted with caution.

However, this study found significant family × CO_2 interactions in belowground C : N ratio and belowground soluble sugar. Thus, there is potential for a within-population, between-family evolutionary response to CO_2 through selection on family variation in responses of %N and aboveground C : N ratio.

This study did not incorporate measures of fitness, which would have required a second season of growth. However, %N and C : N ratio are closely related to N-use efficiency. When

TABLE 7. Mean (SE) from the analysis of tissue biochemistry. Soluble sugar = soluble sugar concentration. CO_2 = growth, CO_2 partial pressure. Significance levels for means compared across growth CO_2 environments: † $P < 0.10$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Family	CO_2 (Pa)	Aboveground				Belowground			
		Soluble sugar (mg/g)	N (%)	C (%)	C : N	Soluble sugar (mg/g)	N (%)	C (%)	C : N
EL15	35.5	44.9 (5.8)	2.02 (0.10)	38.9 (1.2)	19.5 (1.6)	47.4 (3.0)	1.30 (0.05)	34.8 (1.4)	26.6 (1.0)
	71	62.7 (5.3)†	1.83 (0.09)	40.2 (0.8)	22.4 (1.4)	50.0 (3.0)	1.10 (0.04)	35.4 (1.0)	30.7 (1.9)†
KF 32	35.5	60.4 (5.8)	1.21 (0.10)	40.1 (0.3)	38.0 (4.7)	54.4 (2.6)	1.30 (0.04)	36.0 (1.0)	30.6 (1.4)
	71	60.4 (5.4)	1.72 (0.11)**	42.0 (0.7)*	25.9 (1.1)**	62.2 (4.8)	1.20 (0.10)	37.1 (0.8)	35.4 (1.4)*
KF 35	35.5	47.1 (0.9)	1.62 (0.10)	38.9 (0.7)	24.6 (2.1)	56.1 (4.3)	1.20 (0.10)	37.2 (1.5)	29.3 (1.6)
	71	60.4 (3.2)**	2.03 (0.10)†	41.5 (0.7)*	21.1 (1.0)	61.7 (3.1)	1.10 (0.05)	37.4 (0.5)	32.0 (1.1)
Overall	35.5	51.1 (4.1)	1.61 (0.10)	39.0 (0.5)	27.1 (2.8)	53.0 (1.8)	1.30 (0.10)	36.3 (0.7)	28.9 (0.7)
	71	61.3 (3.3)**	1.82 (0.10)*	41.1 (0.5)*	23.0 (0.7)*	57.8 (2.6)†	1.20 (0.05)*	36.7 (0.5)	32.8 (0.9)**

C limitations are removed by increased atmospheric carbon content, it is quite likely that N will be the limiting factor in many ecosystems. In addition, N uptake and N-use efficiency may drive competitive interactions and therefore structure plant communities in many N-limited ecosystems (Tilman, 1993). Therefore, differences in N-use efficiency are likely to have fitness consequences, although this remains to be demonstrated in the context of elevated CO_2 in *P. lanceolata.*

Both population $\times$ CO_2 and family $\times$ CO_2 variation was detected in the MANOVA. In the univariate ANOVAs, only WUE showed a near-significant population $\times$ CO_2 interaction. At the family level, all traits except g_s showed significant CO_2 interactions. It is clear therefore that the necessary inherited variation exists for within-population, between-families evolutionary response to elevated CO_2.

Although this study made no attempt to assess the fitness functions associated with the measured traits, Tonsor and Goodnight (unpublished data) found that there was a strong positive effect of *A* on fitness in *P. lanceolata* (1 SD increase in *A* predicted 0.18 SD increase in reproductive dry mass), while *E* had a negative effect on fitness (1 SD increase in *E* predicted 0.11 SD decrease in reproductive dry mass), when measured in present-day atmospheres. Since plants were only grown for one season in the present study, they did not reproduce. However, if there are equally strong fitness effects in elevated CO_2 as in ambient CO_2 environments, we might expect the family $\times$ CO_2 interactions to lead to selection favoring differing genotypes in an elevated CO_2 world. This type of response has been found in other species. In *Arabidopsis thaliana,* Tonsor and Vandermeulen (1998) found that $G \times E$s similar to those found in the present study do not affect vegetative mass, but have significant effects on reproductive mass. For this reason, although we have first-season mass data, we are hesitant to use mass as a surrogate for fitness.

The growth responses to elevated CO_2 we found are in accord with the general pattern of response often reported in other studies (e.g., Bazzaz, 1990; Stitt, 1991). Despite a near-doubling of *A* for much of the growing season, mass only increased by ~15% overall. Despite the observed down-regulation of *A,* tissue sugar content remained 15% higher in 71-Pa-grown plants compared to those grown at 35 Pa. This is consistent with the idea that down-regulation is not always sufficient to maintain homeostasis in nonstructural carbohydrates (Sage, Sharkey, and Seemann, 1988; Sage, 1994; Cheng, Moore, and Seemann, 1998).

Although leaf %N often declines in elevated CO_2 growth environments (Bassirirad, 1997; Chu, Coleman, and Mooney, 1992), our plants maintained approximately constant whole-plant %N, and leaf %N content increased at 71 Pa. Therefore, C sink capacity was the likely cause of photosynthetic down-regulation. High hexose sugar content is known to be directly involved in down-regulation in some plants (Jang and Sheen, 1994; Jang et al., 1997).

Fajer, Bowers, and Bazzaz (1989), and Fajer (1989; see also Fajer, Bowers, and Bazzaz, 1989, 1992) observed decreases in leaf %N in *Plantago lanceolata* grown at 70 Pa CO_2, in contrast to our observed increase at 71 Pa CO_2. Overall, the mass accumulation in Fajer et al. (1992; mass not presented in the 1989 papers) was remarkably similar to our results (e.g., mean mass in the elevated treatment 20.9 and 19.6, in Fajer and this study, respectively), eliminating the possibility of size-dependent differences (see Coleman, McConnaughay, and Bazzaz, 1993). Neither light regime nor fertilizer treatment appears to explain the difference, and a definitive cause for the differences in N response cannot be ascertained. The differences suggest that the relationship between growth CO_2 and N allocation may be very sensitive to growth conditions. The genotypes used in the two studies were of course different as well, and this may have also contributed to the observed differences in N uptake and partitioning.

Finally, the interaction term in an ANOVA or MANOVA may not adequately determine the presence of genetic variation that can lead to an adaptive response to elevated CO_2, although it remains the best assessment method available. Analysis of responses to elevated CO_2 family by family indicated that a few families showed responses significantly differing from the ''typical'' or average family responses (Klus, 1995). The families exhibiting extreme responses (either very large or in a direction opposite of expectation) were more prevalent in the elevated CO_2 environment. These rare families may provide more information about the possible evolutionary consequences of elevated atmospheric CO_2 than families that exhibit more ''typical'' responses. When exposed to a novel environment, the favored genotypes may be in low frequency initially. This can result in an insignificant $G \times E$ interaction in an analysis of variance, despite the fact that these rare genotypes can contribute to a response to selection when the population is placed in the new environment. This is illustrated by the rarity of metal-tolerant genotypes in normal pasture populations, yet rapid adaptation when metal is introduced to the environment (McNeilly, 1968; Tonsor, 1990). In the present study, it is possible that adaptation to elevated CO_2 could proceed even in those traits in which no family or population $\times$ CO_2 interaction was observed. Evolution would initially proceed very slowly in such traits, increasing in rate as the genes responsible for the rare interaction types increased in frequency in response to selection. This is because the favored genes contribute little to additive genetic variance when present in low frequency and increase in their contribution to the additive genetic variance and heritability as their frequency increases (see Falconer, 1981).

The extent to which ecological vs. evolutionary processes will determine the fate of individual species as atmospheric CO_2 rises remains unclear. This study demonstrates that significant genetic variation exists in traits that may have causal links to the ecological responses of the plant to changing carbon availability and suggests that a better understanding of within-species genetic responses is necessary before a complete picture of community and ecosystem responses can be obtained.

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