Adaptive Value and Costs of Physiological Plasticity to Soil Moisture Limitation in Recombinant Inbred Lines of *Avena barbata*

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ABSTRACT: Costs are hypothesized to constrain the evolution of adaptive phenotypic plasticity, but they have been difficult to quantify because strong selection should eliminate costly genotypes from natural populations. However, recent studies suggest that crosses between natural populations can recover these genotypes. We determined the adaptive value and costs of, as well as the genetic variation for, physiological and morphological plasticity to soil water limitation in *Avena barbata* recombinant inbred lines (RILs) created by crossing mesic and xeric ecotypes. All traits were plastic, and plasticity in stomatal limitation of photosynthesis and photosynthetic rate before and at reproduction was adaptive. However, we detected a significant cost of plasticity only for stomatal conductance at reproduction, and the mean cost for all traits of *A. barbata* RILs was at least 50% smaller than costs previously estimated using RILs. In addition, heritabilities for plasticity were <0.1 and were significant only for photosynthesis at reproduction and leaf mass per unit area. Our results suggest that costs are less likely to constrain the evolution of adaptive plasticity in *A. barbata* than genetic variation for plasticity.

Keywords: Avena barbata, costs of plasticity, genotype × environment interaction, phenotypic plasticity, photosynthesis, water stress.

Introduction

One constraint on the evolution of adaptive plasticity is the cost of producing a phenotype through plastic rather than fixed development (DeWitt et al. 1998). Plasticity is inferred to be costly when a genotype that produces a phenotype in a given environment through fixed development has higher fitness than a genotype that produces the same phenotype in the same environment through plastic development. Potential costs of plasticity include the energy required to maintain regulatory pathways for producing a phenotype through plastic rather than fixed development, as well as genetic costs caused by epistatic interactions or linkage between plasticity loci and loci that affect fitness (DeWitt et al. 1998). Theory suggests that high costs of plasticity can result in nonplastic specialist genotypes having higher fitness than co-occurring plastic generalist genotypes (van Tienderen 1991; Padilla and Adolph 1996; Sultan and Spencer 2002). However, many empirical tests have failed to detect costs of plasticity (van Kleunen and Fischer 2005; van Buskirk and Steiner 2009), perhaps because genotypes for which plasticity is costly are quickly purged from populations by natural selection (DeWitt et al. 1998).

The magnitude of any costs of plasticity, as well as the statistical power to detect these costs, may be greater in populations of recombinant genotypes for two reasons. First, recombination can recreate genotypes that express genetic costs of plasticity (reviewed in van Kleunen and Fischer 2007). For example, because recombinants contain novel combinations of alleles, they can express costs of plasticity caused by epistatic interactions between loci. If plasticity costs periodically emerge in natural populations after outcrossing and subsequent recombination (Weinig et al. 2006), then experiments using recombinants may provide a more realistic estimate of the magnitude of any costs of plasticity than would experiments using genotypes sampled from natural populations. Second, recombination should recover genotypes that have particularly high or low levels of plasticity relative to genotypes sampled from natural populations (Dechaine et al. 2007; van Kleunen and Fischer 2007). This expansion in the range of phenotypic variation increases the statistical power to detect selection (Schluter 1988) and, thus, plasticity costs.

Because recombination can recover plasticity costs, Callahan et al. (2005) proposed testing for these costs by using populations of recombinant inbred lines (RILs) rather than genotypes sampled from natural populations. To generate

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RILs, genetically distinct parents are crossed to create F_1 heterozygotes. Selfing the F_1 generation results in a population of recombinant F_2 offspring that are propagated by single-seed descent (Lynch and Walsh 1998). Studies that used RILs of *Arabidopsis thaliana* (Callahan et al. 2005; Weinig et al. 2006) and *Brassica rapa* (Dechaine et al. 2007) detected costs of plasticity for more traits, as well as larger costs of plasticity, than did studies that sampled genotypes from natural populations (reviewed in van Kleunen and Fischer 2007). The results of these studies suggest that costs may periodically arise in natural populations and act as a significant constraint on the evolution of plasticity (Weinig et al. 2006).

Most studies of costs of plasticity in plants have focused on morphological traits (reviewed in van Kleunen and Fischer 2005), but plasticity in physiological traits also contributes to fitness (Ackerly et al. 2000). Physiological traits directly control resource acquisition and can therefore be responsible for morphological responses to environmental variation (Sultan 1995; Ackerly et al. 2000; Pigliucci 2001). For example, increased photosynthesis is necessary to provide carbon for adaptive plastic increases in morphological traits such as internode length (e.g., Donohue et al. 2000) and leaf size (e.g., Dorn et al. 2000). Recent reviews find that natural selection is rarely estimated on physiological traits of either plants or animals (Kingsolver et al. 2001; Geber and Griffen 2003), despite the importance of these traits for growth and reproduction.

Relative to plasticity in morphological traits, the evolution of plasticity in physiological traits may be more likely to be constrained by a lack of genetic variation. Heritabilities for photosynthetic traits of plants are ~50% lower than heritabilities for morphological traits (reviewed in Geber and Griffen 2003). Given that there tends to be less genetic variation for trait plasticity than for trait means within environments (reviewed in Scheiner 1993), heritabilities for plasticity in physiological traits may be particularly low. However, most studies of plasticity in plants have focused on morphological traits (van Kleunen and Fischer 2005), and there are relatively few estimates of genetic variation in plasticity of physiological traits (Sultan and Bazzaz 1993; Heschel et al. 2004*a*, 2004*b*).

We examined the adaptive value and costs of, as well as genetic variation for, physiological and morphological plasticity in response to soil moisture limitation in a population of *Avena barbata* Pott. ex. Link RILs. These RILs were created by crossing mesic and xeric ecotypes from California, which differ in morphological and physiological traits associated with adaptation to contrasting rainfall environments (Hamrick and Allard 1975; Latta et al. 2004; Sherrard and Maherali 2006). Individuals from dry sites (xeric ecotype) are fixed for one set of alleles at each of five allozyme loci, whereas individuals from moist sites (mesic ecotype) are homozygous for the alternate set of alleles (Hamrick and Allard 1972). Although *A. barbata* primarily self-fertilizes, there is infrequent outcrossing between ecotypes in regions where they overlap (Latta et al. 2007). Consequently, mesic × xeric RILs can provide a realistic estimate of the magnitude of costs of plasticity in natural *A. barbata* populations.

Physiological plasticity is often assumed to be adaptive, but tests of this assumption are rare (Caruso et al. 2006). In Mediterranean annuals, there is strong selection for drought escape through earlier flowering over dehydration avoidance through water conservation (Stanton et al. 2000; Volis et al. 2002, 2004; Sherrard and Maherali 2006). Earlier flowering is facilitated by rapid growth, which in turn may be caused by high rates of photosynthesis and transpiration (Bazzaz 1979; Geber and Dawson 1997; McKay et al. 2003). For example, comparative studies indicate that genotypes and species with short life spans often have higher photosynthetic capacity than those with long life spans (Mooney et al. 1976; Geber and Dawson 1997; Reich et al. 1999; McKay et al. 2003). If plasticity in *A. barbata* facilitates drought escape, then genotypes should flower earlier and have higher photosynthetic capacity when growing in a dry versus a wet soil environment. If this plasticity is adaptive, then genotypes that respond to water availability in the predicted direction should have higher fitness than less plastic genotypes. We tested for these predicted plastic responses, assessed whether they were adaptive and/or costly, and estimated genetic variation for plasticity by growing a population of *A. barbata* RILs in contrasting watering treatments.

Methods

Study Species

Avena barbata is a highly selfing (>95%) European winter annual grass that has invaded the Mediterranean region of the southwestern United States since its introduction over 200 years ago (Garcia et al. 1989). Both neutral genetic markers and measurements of quantitative traits indicate that the parental mesic and xeric ecotypes are genetically homogeneous monomorphic lineages (Gardner and Latta 2008). The initial cross between a single mesic individual and a single xeric individual, followed by one generation of selfing, produced 188 F_2 individuals. The F_2 individuals were selfed for four generations through single-seed descent, resulting in F_6 individuals that were 96.75% homozygous (Gardner and Latta 2008). This cross mimics a population of progeny from a hybridization event between the mesic and xeric ecotypes in regions where they overlap (Gardner and Latta 2006). Because 90% of *A. barbata* growing in California are of either the mesic or the xeric

ecotype (Garcia et al. 1989), this cross captures a substantial portion of the standing genetic variation in California populations of this species. The RILs express a broader range of variation in morphological and physiological traits than does either parental ecotype (Gardner and Latta 2008; Maherali et al. 2008).

Experimental Design and Data Collection

As part of a larger study of the evolution of physiological traits in *A. barbata* (Sherrard and Maherali 2006; Maherali et al. 2008, 2009; Sherrard et al. 2009), we grew 26 RILs and the two parental ecotypes in a greenhouse environment. The RILs were selected to match the range and frequency distribution of fitness for all 188 lines when grown under well-watered greenhouse conditions (Gardner and Latta 2006). The mean fitness of our sample of lines was not significantly different from the mean fitness of all 188 lines, and our sample included RILs in both the fifth and ninety-fifth percentiles of the fitness distribution. To allow enough time for physiological measurements and to ensure that all traits were measured on plants at the same life stage, we used a randomized complete block design, with four temporal blocks of 56 plants (eight plants in each of 26 RILs and the two parental ecotypes; $N = 224$). To create the temporal blocks, four groups of seeds were germinated 12 days apart in February–March 2004 by removing the palea and lemma and placing them on moist filter paper for 96 h at 4°C. Seeds were then returned to room temperature and placed in the dark for 24 h. Each seedling was planted in a 4.1-L pot filled with Pro-Mix BX (Premier Tech, Rivière-du-Loup, Quebec, Canada) and placed on a greenhouse bench.

After 21 days, when the seedlings had their first true leaves, half of the plants from each parental ecotype and RIL were randomly assigned to the dry treatment, and half were assigned to the wet treatment. Volumetric water content (VWC) of the soil was monitored using a moisture probe (Hydrosense CD620, Campbell Scientific, Edmonton, Alberta, Canada). Plants in the wet treatment were watered daily to saturation (mean VWC $\pm$ 1 SD = 31.1% $\pm$ 9.9%). Plants in the dry treatment were provided with 50 mL of water every 2 days, which maintained the VWC below 5% and is the equivalent of 132 mm of precipitation for the duration of the treatment (5.5 mm week^{-1}). This was comparable to the total rainfall during the driest growing season at the xeric site since 1963 (165 mm; October 1976–March 1977) and is 69 mm less than that of any growing season at the mesic site since 1953 (Sherrard and Maherali 2006). Because the amount of watering was held constant, the severity of water stress experienced by plants in the dry treatment increased as the plants grew. In contrast, plants in the wet treatment experienced a consistently moist soil environment.

Every 2 weeks, plants were fertilized with 100 mL of 20%-20%-20% NPK fertilizer (Plant Products, Brampton, Ontario, Canada) at a concentration of 2.5 g L^{-1}. The fertilizer also contained the micronutrients B (0.02%), Cu (0.05%), Fe (0.1%), Mn (0.05%), Mo (0.0005%), and Zn (0.05%). Plants were provided with supplemental light from high-intensity discharge lamps to maintain a photoperiod of 16 h of light per day. On regularly scheduled watering days, plants were watered after leaf gas-exchange data were collected, rather than before. This ensured that gas exchange reflected the long-term differences in water availability between treatments, rather than a short-term water pulse (Schwinning and Sala 2004).

Trait and Fitness Measurements

To determine how photosynthesis and water use of *A. barbata* responded to drought, we measured leaf gas exchange and estimated the carboxylation activity of ribulose 1,5-bisphosphate carboxylase-oxygenase (Rubisco) and stomatal limitation of photosynthesis from these measurements. Photosynthesis and transpiration were measured on the youngest fully expanded leaf for all 224 plants in the experiment using an open gas-exchange system (LI-6400, Li-Cor, Lincoln, NE). Preliminary data indicated that *A. barbata* maintained relatively constant gas exchange during the morning (M. E. Sherrard and H. Maherali, unpublished data). As a result, steady-state leaf gas exchange was measured between 0830 and 1230 hours EST. Plants were measured in a random order each day. Because gas exchange is sensitive to variation in light, temperature, and atmospheric humidity, we held these environmental variables constant throughout the measurements. We used red-blue light-emitting diodes to provide a saturating incident irradiance (1,500 μmol m^{-2} s^{-1}) comparable to what *A. barbata* experiences in the field (Jackson et al. 1995). Leaf temperature was held at ~26°C with a Peltier cooling module and cuvette, and leaf-to-air vapor pressure deficit (D) was maintained at 1.9–2.0 kPa to reflect prevailing ambient conditions. Stomatal conductance (g_s) was calculated from transpiration using a boundary layer conductance of 3.54–4.82 mol m^{-2} s^{-1}, which was determined on the basis of fan speed and leaf area using the energy balance algorithms of the LI-6400. Leaf area was calculated from leaf dimensions.

The first set of gas-exchange measurements was made 70 days after germination and 49 days after the treatments were initiated, just before flowering. We made a second set of instantaneous gas-exchange measurements under ambient CO_2 concentration 110 days after germination on

leaves attached to flowering stalks. We recorded instantaneous light-saturated photosynthetic rate (A) and stomatal conductance to water vapor (g_s) at ambient CO_2 concentration (400 μL L^{-1}). The leaf segment used for the second set of gas-exchange measurements was harvested, dried at 70°C for 48 h to constant mass, and weighed. We calculated leaf mass per unit area (LMA), which is correlated with gas exchange (Reich et al. 1999), as dry leaf mass (g) divided by fresh leaf area (cm^2). Plants with higher LMA values have thicker leaves.

We determined the degree to which water availability influenced the biochemical regulation of photosynthesis by measuring the response of photosynthesis to variation in intercellular CO_2 concentration (C_i). Photosynthesis is primarily limited by ribulose 1,5-bisphosphate (RuBP) regeneration when C_i is high and by Rubisco activity when C_i is low (Sharkey 1985; Geber and Dawson 1997). To determine the maximum rate of carboxylation (V_{cmax}), which represents Rubisco activity, we measured the response of light-saturated photosynthesis (A) to the manipulation of intercellular CO_2 concentration (A/C_i curve). An A/C_i curve was constructed for each individual in the experiment within 6 days of the first set of gas-exchange measurements, before flowering. The A/C_i curves were constructed by varying the concentration of CO_2 in the cuvette chamber from 50 to 1,800 μL L^{-1} at 100–200 μL L^{-1} intervals in a specific sequence (e.g., Long and Bernacchi 2003). We assumed that A was limited solely by Rubisco at intercellular $[CO_2] < 250$ μL L^{-1} (e.g., Wullschleger 1993; Geber and Dawson 1997). Therefore, V_{cmax} was calculated as the regression slope of this portion of the A/C_i data fitted to a linearized version (Long and Bernacchi 2003) of a mechanistic biochemical model of photosynthesis (Farquhar et al. 1980; Sharkey 1985).

In addition to biochemical limitations, photosynthesis can be limited by the diffusion of CO_2 into the leaf through stomata. Stomatal closure reduces water loss, but it also restricts CO_2 diffusion into the leaf (Cowan 1977). Relative stomatal limitation of photosynthesis (l_g) is an index of how much potential carbon gain is lost because of a stomatal restriction of CO_2 supply (Farquhar and Sharkey 1982; Jones 1985). To determine how photosynthesis can be influenced by the interplay between the biochemical demand for CO_2 by Rubisco relative to the supply of CO_2 through stomata, we estimated l_g in each moisture treatment. To do this, the A/C_i curve data were fitted to a nonlinear model:

$$A = a(1 - \exp(-bC_i)) + c, \quad (1)$$

where c is the Y-intercept, $1/b$ is the rate constant, and a is the slope (Jacob et al. 1995; Reid and Fiscus 1998). We calculated l_g, using the differential method of Jones (1985):

$$l_g = \frac{r_g}{r_g + r^*}, \quad (2)$$

where r_g is the gas-phase resistance to CO_2 uptake (the supply function) and r^* is the slope of the A/C_i curve (demand function). We calculated r^* as the first derivative of equation (1) at the operating C_i and r_g as $(C_a - C_i)/A$ at the operating C_i (Jones 1985). The value of l_g varies between 0 and 1, with high values indicating that photosynthesis is more limited by gas-phase CO_2 diffusion through stomata (Jones 1985).

Leaf gas exchange is influenced by the size and density of stomata on the leaf surface (Nobel 1991), traits that can respond plastically to soil moisture (Xu and Zhou 2008). To measure stomatal length and density, we made a mold with polyvinylsiloxane dental impression material (Extrude Medium, Kerr Manufacturing, Orange, CA) of the adaxial and the abaxial surfaces of one fully expanded leaf per individual, and used the hardened mold as a cast for clear nail polish. Molds were made after measurements of A/C_i curves were completed, before flowering. We measured stomatal length on the nail polish impression as the average distance in micrometers between the junctions of the guard cells (Malone et al. 1993; Maherali et al. 2002) for eight randomly selected stomates per leaf side. We measured stomatal density as the average number of stomates in two randomly selected 1-mm^2 viewing areas per leaf side. The measurements and counts were made using a light microscope interfaced with a Nikon Coolpix 4500 digital camera and ImageJ software (Abramoff et al. 2004; U.S. National Institutes of Health; http://rsb.info.nih.gov/ij/). We report stomatal length and density as averages of the adaxial and abaxial surfaces.

We recorded the day when spikelets first appeared as the date of first flower. We ended the experiment 165 days after germination to simulate a growing season in the field. Each *A. barbata* spikelet produces two single-seeded florets. We estimated the proportion of seeds aborted as the number of empty florets in a random selection of 100 florets per individual. Fitness was expressed as total seed number, which was calculated as total spikelet number × 2 × proportion of unaborted seeds.

Statistical Analysis

We used a two-way ANOVA to determine whether fitness and 10 other traits (table 1) responded plastically to soil water availability in our 26 *A. barbata* RILs. Treatment (wet vs. dry) and RIL were included as fixed factors. However, the results of the ANOVA were qualitatively similar

Table 1: Effects of soil water availability and recombinant inbred line on 10 phenotypic traits and fitness of *Avena barbata*

	Term			
Trait	*T*	*L*	*T* × *L*	*B*
Prereproductive *A* (μmol CO_2 m^{-2} s^{-1}):				
MS	336.256	17.291	6.668	65.466
F	31.292	1.609	.621	6.092
P	<.001[a]	.043	.919	.001[a]
Prereproductive g_s (mol m^{-2} s^{-1}):				
MS	1.904	.006	.005	.009
F	275.526	.854	.684	1.287
P	<.001[a]	.667	.867	.281
Reproductive *A* (μmol CO_2 m^{-2} s^{-1}):				
MS	26.887	18.022	20.3908	51.798
F	2.257	1.513	1.705	4.348
P	.135	.068	.027	.006[a]
Reproductive g_s (mol m^{-2} s^{-1}):				
MS	3.133	.016	.013	.080
F	254.412	1.291	1.058	6.520
P	<.001[a]	.176	.399	.001[a]
V_{cmax} (μmol m^{-2} s^{-1}):				
MS	2.767×10^5	2.441×10^3	1.312×10^3	9.873×10^3
F	210.352	1.855	.998	7.505
P	<.001[a]	.012	.474	<.001[a]
l_g:				
MS	8.481	.008	.011	.044
F	900.856	.838	1.157	4.719
P	<.001[a]	.689	.288	.004[a]
LMA (g cm^{-2}):				
MS	8.468×10^{-6}	1.848×10^{-6}	9.141×10^{-7}	3.339×10^{-6}
F	16.211	3.538	1.750	6.393
P	<.001[a]	<.001[a]	.022	<.001[a]
Stomatal density (no. per mm^2):				
MS	2,029.688	259.171	86.048	157.211
F	24.382	3.113	1.034	1.889
P	<.001[a]	<.001[a]	.428	.134
Stomatal length (μm):				
MS	212.443	22.802	3.715	14.894
F	33.917	3.640	.593	2.378
P	<.001[a]	<.001[a]	.937	.072
Date of first flower:				
MS	3.272×10^3	1.077×10^3	129.966	721.825
F	38.103	12.550	1.513	8.405
P	<.001[a]	<.001[a]	.068	<.001[a]
Seed production:				
MS	4.152×10^6	3.987×10^5	1.557×10^5	3.548×10^6
F	24.051	2.310	.902	20.551
P	<.001[a]	.001[a]	.602	<.001[a]

Note: Traits were analyzed using a two-way ANOVA with treatment (*T*; wet vs. dry) and recombinant inbred line (*L*) as main effects. A term (*B*) was also included to control for variation among temporal blocks. For treatment, df = 1, 150–153; for line and treatment × line, df = 25, 150–153; for block, df = 3, 150–153. *A* = photosynthetic rate; g_s = stomatal conductance; V_{cmax} = maximum velocity of carboxylation of ribulose 1,5-bisphosphate carboxylase-oxygenase (Rubisco); l_g = relative stomatal limitation of photosynthesis; LMA = leaf mass per unit area; MS = mean squares.

[a] Remained significant after sequential Bonferroni correction by the Dunn-Sidak method.

if RIL was included as a random factor (analyses not shown). Block was included as a fixed factor to control for variation among the four temporal cohorts. When there was a significant treatment × line interaction, we estimated the Spearman rank correlation (r_s) between mean trait values of the same lines growing in the wet and dry treatments. If r_s was not significant ($P > .05$), then we inferred that the interaction was caused by a change in the rank order of lines between treatments. Because analyses of absolute and relative fitness can lead to different conclusions about genotype × environment interactions (Stanton and Thiede 2005), we analyzed both absolute and relative seeds per plant. These two analyses were qualitatively similar (data not shown), and so we present only the ANOVA for absolute fitness. To determine whether gas exchange varied within each 4-h measurement period, we included time of measurement as a covariate in the ANOVAs for A, g_s, l_g, and V_{cmax}. Because this covariate did not explain significant variation in any gas-exchange trait (analyses not shown), we present the ANOVA without time of day included in the model.

The assumption of normality of residual variance was tested using Lilliefor's test (Wilkinson 1997). We tested the assumption of homogeneity of variances by examining residuals for each treatment group. If these assumptions were violated, we log-transformed the data. Even after transformation, g_s, l_g, and date of first flower were heteroscedastic. However, these traits differed between treatments in both the ANOVA (table 1) and the nonparametric tests (analyses not shown). Consequently, we present the results of the ANOVA for these traits.

To determine whether and how parental mesic and xeric ecotypes responded plastically to soil water availability, we compared 10 traits (table 1) between wet and dry treatments, using paired, two-tailed *t*-tests. A separate *t*-test was performed for each combination of ecotype and trait. Plants were paired within each of the four temporal blocks, resulting in *t*-tests with 3 degrees of freedom.

To compare heritabilities for plasticity in *A. barbata* RILs with estimates for other species, we calculated the intraclass correlation $\tau = \sigma_g^2/(\sigma_g^2 + \sigma_e^2)$, where σ_g^2 is the between-line variance in plasticity and σ_e^2 is the within-line environmental variance (Scheiner and Lyman 1989). Because *A. barbata* RILs are 96.75% homozygous (Latta et al. 2007), variation within each line is caused almost entirely by random environmental effects. Therefore, τ is equivalent to the broad-sense heritability (H^2; Falconer and McKay 1996). The broad-sense heritability estimates all genetic contributions to phenotypic variation, including additive genetic, dominance, epistatic, and maternal effects (Lynch and Walsh 1998). However, dominance effects can be discounted because RILs are homozygous. In addition, RILs were grown in a common environment in the generation before our experiment, which should minimize maternal effects. Terms for τ were calculated using the mean squares from the same ANOVAs that we used to determine whether traits responded plastically to soil water availability (table 1). The mean squares error (MSe) term is equivalent to the within-line environmental variance (σ_e^2). The mean squares for treatment × line (MSg) is equivalent to $\sigma_e^2 + n\sigma_g^2$, where n is the number of individuals per genotype (Lynch and Walsh 1998). Thus, to calculate H^2, $\sigma_e^2 = \text{MSe}$ and $\sigma_g^2 = (\text{MSg} - \text{MSe})/n$. We used the P value for the RIL term in the ANOVA to determine whether H^2 for plasticity in a trait was significant. If σ_g^2 was < 0, then we set H^2 to 0.

We used two approaches to determine whether plasticity was adaptive. First, we regressed each line's mean fitness across treatments on the line mean for each trait across treatments and a measure of plasticity for that trait (across-environment analysis for adaptive plasticity; van Kleunen and Fischer 2005). We ran separate models for each trait. We relativized each RIL's mean fitness across treatments by dividing by the mean fitness of all RILs across treatments, and we standardized the independent variables to a mean = 0 and variance = 1. Because V_{cmax}, reproductive A, l_g, and stomatal density were higher within the dry treatment, plasticity for these traits was calculated by subtracting the wet-treatment RIL mean from the dry-treatment RIL mean. Plasticity for all other traits was calculated by subtracting the dry-treatment RIL mean from the wet-treatment RIL mean. When plasticity is calculated in this way, a significant positive regression coefficient indicates that plasticity is adaptive. Second, we regressed each individual plant's relativized fitness on its standardized traits (within-environment analysis for adaptive plasticity; van Kleunen and Fischer 2005). We ran separate regression models for each combination of treatment and trait. If trait plasticity between treatments was in the same direction as was predicted by selection on that trait within treatments, then we inferred that plasticity was adaptive. If the direction of plasticity between treatments was in the opposite direction, then we inferred that plasticity was maladaptive. If RILs differed in the direction of response to the treatment, such that some lines responded in the direction predicted by selection and others responded in the opposite direction, then we concluded that plasticity was both adaptive and maladaptive (i.e., mixed; Dorn et al. 2000).

We also used regression to determine whether plasticity and homeostasis were costly (van Kleunen and Fischer 2005). The dependent variable was relativized RIL mean fitness within a treatment. The two independent variables were the standardized RIL mean for each trait within a treatment and a standardized measure of plasticity across treatments for the same trait. We ran separate models for

Table 2: Cross-environment analysis of adaptive plasticity in 10 traits of *Avena barbata* in response to soil water availability

Trait	β	SE	P
Prereproductive A	.068	.068	.329
Prereproductive g_s	.130	.086	.144
Reproductive A	.102	.067	.139
Reproductive g_s	−.135	.075	.084
V_{cmax}	.129	.087	.155
l_g	.250	.071	.002[a]
LMA	.016	.078	.839
Stomatal density	−.012	.065	.860
Stomatal length	−.059	.070	.409
Date of first flower	−.016	.041	.693

Note: The recombinant inbred line (RIL) mean fitness across treatments was regressed on the RIL mean of a trait across treatments and a measure of plasticity of that trait. A significant positive regression coefficient (β) for plasticity indicates that it is adaptive. $N = 26$. A = photosynthetic rate (μmol CO_2 m^{-2} s^{-1}). g_s = stomatal conductance (mol m^{-2} s^{-1}). V_{cmax} = maximum velocity of carboxylation of ribulose 1,5-bisphosphate carboxylase-oxygenase (Rubisco; μmol m^{-2} s^{-1}). l_g = relative stomatal limitation of photosynthesis. LMA = leaf mass per unit area (g cm^{-2}). Stomatal density is number per square millimeter; stomatal length is in micrometers.

[a] Remained significant after sequential Bonferroni correction by the Dunn-Sidak method.

each combination of treatment and trait. We calculated plasticity as described above. Plasticity is inferred to be costly when a genotype that produces a phenotype in a given environment through fixed development has higher fitness than a genotype that produces the same phenotype in the same environment through plastic development. Homeostasis is inferred to be costly when a genotype that produces a phenotype in a given environment through fixed development has lower fitness than another genotype that produces the same phenotype in the same environment through plastic development (reviewed in van Kleunen and Fischer 2005). Consequently, a significant negative regression coefficient for the plasticity term indicates that plasticity is costly within that environment, whereas a significant positive coefficient indicates that homeostasis is costly (van Kleunen and Fischer 2007). If the coefficient is significant within all environments, then the costs of plasticity or homeostasis are global rather than local (see Weinig et al. 2006).

The assumption of normality of residual variance for all regression models was tested using Lilliefors' test (Wilkinson 1997). We tested the assumption of homogeneity of residual variance for these models by calculating the Spearman rank correlation between the residuals and relative fitness (Neter et al. 1989). All regression models met these assumptions.

We analyzed the adaptive value and costs of plasticity to soil water availability separately for each of our traits because A and g_s were tightly phenotypically correlated ($r > 0.70$) with each other, which tends to result in unstable multiple regression coefficients (Mitchell-Olds and Shaw 1987). To control Type I error rates, we adjusted α for our analyses of plasticity (table 1), adaptive value (tables 2, 3), and costs (table 4), using the sequential Bonferroni correction by the Dunn-Sidak method (Sokal and Rohlf 1995).

To determine whether selection on plasticity was limited by the opportunity for selection (Arnold and Wade 1984), we estimated variation in seed production among RILs separately within each treatment, using a randomized complete-block ANOVA. If the RIL term was significant, then we inferred that selection was not limited by a lack of variation in fitness.

Results

Reduced soil water availability significantly decreased fitness of *Avena barbata* RILs (table 1). Plants in the dry treatment produced 35% fewer seeds than those in the wet treatment (fig. 1*E*). Although seed production varied significantly among RILs (significant line term; table 1), lines responded similarly to the soil water availability treatment (nonsignificant treatment × line term; table 1). When fitness was analyzed separately within each treatment, there was still significant variation in seed production among

Table 3: Within-environment selection differentials used to infer whether plasticity was adaptive in 10 traits of *Avena barbata* in response to soil water availability

	Wet treatment			Dry treatment		
Trait	S	SE	P	S	SE	P
Prereproductive A	.173	.073	.021	−.050	.042	.245
Prereproductive g_s	−.022	.069	.750	−.047	.043	.281
Reproductive A	.290	.069	<.001[a]	−.005	.048	.919
Reproductive g_s	.003	.072	.972	.031	.045	.493
V_{cmax}	.188	.073	.012	−.041	.045	.363
l_g	.081	.068	.239	.016	.048	.746
LMA	−.062	.070	.376	−.047	.044	.292
Stomatal density	.195	.067	.005[a]	.033	.043	.443
Stomatal length	.006	.069	.931	−.035	.044	.429
Date of first flower	−.439	.055	<.001[a]	−.281	.031	<.001[a]

Note: An individual plant's relative fitness was regressed on standardized trait values within each environment (S). N = 101–104. A = photosynthetic rate (μmol CO_2 m^{-2} s^{-1}). g_s = stomatal conductance (mol m^{-2} s^{-1}). V_{cmax} = maximum velocity of carboxylation of ribulose 1,5-bisphosphate carboxylase-oxygenase (Rubisco; μmol m^{-2} s^{-1}). l_g = relative stomatal limitation of photosynthesis. LMA = leaf mass per unit area (g cm^{-2}). Stomatal density is number per square millimeter; stomatal length is in micrometers.

[a] Remained significant after sequential Bonferroni correction by the Dunn-Sidak method.

Table 4: Analysis of costs of plasticity and homeostasis in 10 traits of *Avena barbata* in response to soil water availability

Trait	Wet treatment			Dry treatment		
	β	SE	P	β	SE	P
Prereproductive A	.082	.106	.445	−.005	.066	.937
Prereproductive g_s	.259	.189	.184	.003	.059	.956
Reproductive A	.137	.101	.185	.025	.078	.747
Reproductive g_s	−.135	.166	.425	−.151	.058	.016
V_{cmax}	.170	.080	.045	.175	.126	.179
l_g	.574	.176	.003[a]	.121	.065	.075
LMA	.076	.142	.600	−.085	.062	.182
Stomatal density	.068	.091	.463	−.051	.072	.487
Stomatal length	−.086	.102	.405	−.036	.062	.571
Date of first flower	.029	.064	.650	−.012	.037	.752

Note: Costs were estimated by regressing the recombinant inbred line (RIL) mean fitness within a treatment (wet vs. dry) on the RIL mean of a trait and a measure of plasticity of that trait. A significant negative regression coefficient (β) for plasticity indicates that it is costly. A significant positive β indicates that homeostasis is costly. $N = 26$. A = photosynthetic rate (μmol CO_2 m^{-2} s^{-1}). g_s = stomatal conductance (mol m^{-2} s^{-1}). V_{cmax} = maximum velocity of carboxylation of ribulose 1,5-bisphosphate carboxylase-oxygenase (Rubisco; μmol m^{-2} s^{-1}). l_g = relative stomatal limitation of photosynthesis. LMA = leaf mass per unit area (g cm^{-2}). Stomatal density is number per square millimeter; stomatal length is in micrometers.

[a] Remained significant after sequential Bonferroni correction by the Dunn-Sidak method.

RILs (wet treatment: $F_{25,75} = 1.848$, $P = .022$; dry treatment: $F_{25,75} = 3.076$, $P < .001$).

Nine of 10 traits responded to soil water availability (significant treatment term; table 1). Plants in the wet treatment had a 39% higher photosynthetic rate (A) before reproduction (fig. 2*A*), but A did not differ between treatments during reproduction (fig. 2*C*). Plants in the wet treatment also had higher stomatal conductance (g_s) than those in the dry treatment, but the magnitude of this plasticity varied between time points. Stomatal conductance was three times higher in the wet treatment than in the dry treatment before reproduction (fig. 2*B*) but only two times higher during reproduction (fig. 2*D*). *Avena barbata* RILs in the dry treatment had a maximum rate of carboxylation (V_{cmax}) that was 2.2 times as high (fig. 2*E*) and a 78% higher relative stomatal limitation of photosynthesis (l_g; fig. 2*F*), relative to wet-treatment RILs. Plants in the wet treatment had 11% lower stomatal density (fig. 1*A*) but 8% longer stomates (fig. 1*B*) than plants in the dry treatment. In addition, wet-treatment plants had 9% higher leaf mass per unit area (LMA; fig. 1*C*) and flowered 8 days later (fig. 1*D*) than dry-treatment plants.

For two of 10 traits, RILs differed in their response to the watering treatment (significant treatment × line interaction; table 1). Plasticity in photosynthesis at reproduction (fig. 2*C*) and in LMA (fig. 1*C*) varied among lines, in terms of both the magnitude and direction of the response. The rank order of lines growing in the wet treatment was not correlated with the rank order of those in the dry treatment for either LMA ($r_s = 0.322$, df = 24, $P = .108$) or A at reproduction ($r_s = 0.014$, df = 24, $P = .946$). This suggests that the significant treatment × line interaction for these traits was caused by changes in rank order of lines between the wet and dry treatments. Heritabilities for plasticity were all <0.1 (table 5). Six traits differed among RILs (significant line term; table 1), including photosynthetic rate before reproduction (fig. 2*A*), V_{cmax} (fig. 2*E*), stomatal density (fig. 1*A*), stomatal length (fig. 1*B*), LMA (fig. 1*C*), and date of first flower (fig. 1*D*).

Three traits of the parental ecotypes exhibited significant plastic responses to soil water availability (table A1 in the online edition of the *American Naturalist*). The values of l_g of both mesic- and xeric-ecotype plants were ~70% higher in the dry treatment than in the wet treatment. V_{cmax} of mesic-ecotype plants was also three times higher in the dry treatment than in the wet treatment. In contrast, g_s at reproduction of xeric-ecotype plants was four times higher in the wet treatment than in the dry treatment.

Although all traits were plastic, as indicated by a significant treatment or treatment × line term, this plasticity was adaptive for only two traits (tables 2, 3). In the cross-environment genotypic selection analysis, lines with higher plasticity for l_g had higher fitness across treatments (table 2; fig. A1 in the online edition of the *American Naturalist*). The within-environment phenotypic selection analysis indicated that plasticity in prereproductive A was also adaptive. Selection on prereproductive A was significant and positive within the wet treatment, but it was not significant within the dry treatment (table 3). Given that a majority of RILs had higher prereproductive A in the wet treatment than in the dry treatment (fig. 2*A*), our data suggest that plasticity in this trait increased fitness.

Plasticity in A at reproduction was both adaptive and maladaptive. Selection on reproductive A was significant and positive within the wet treatment but not significant within the dry treatment (table 3). However, the response of reproductive A to the watering treatment varied among RILs, with some lines increasing A in the wet treatment and others decreasing A (fig. 2*C*; table 1). Together, these results suggest that plasticity in reproductive A increased fitness of some but not all lines (mixed-direction plastic responses; see Dorn et al. 2000).

Plasticity in three of 10 traits was maladaptive (table 3). There was significant selection for early flowering within both the wet and dry treatments (table 3), and selection was stronger in the wet treatment. However, most RILs flowered earlier in the dry treatment than in the wet treatment (fig. 1*D*), suggesting that plasticity in flowering time was maladaptive. There was selection for higher stomatal density and V_{cmax} within the wet treatment (table 3), but both traits were higher in the dry treatment than in the

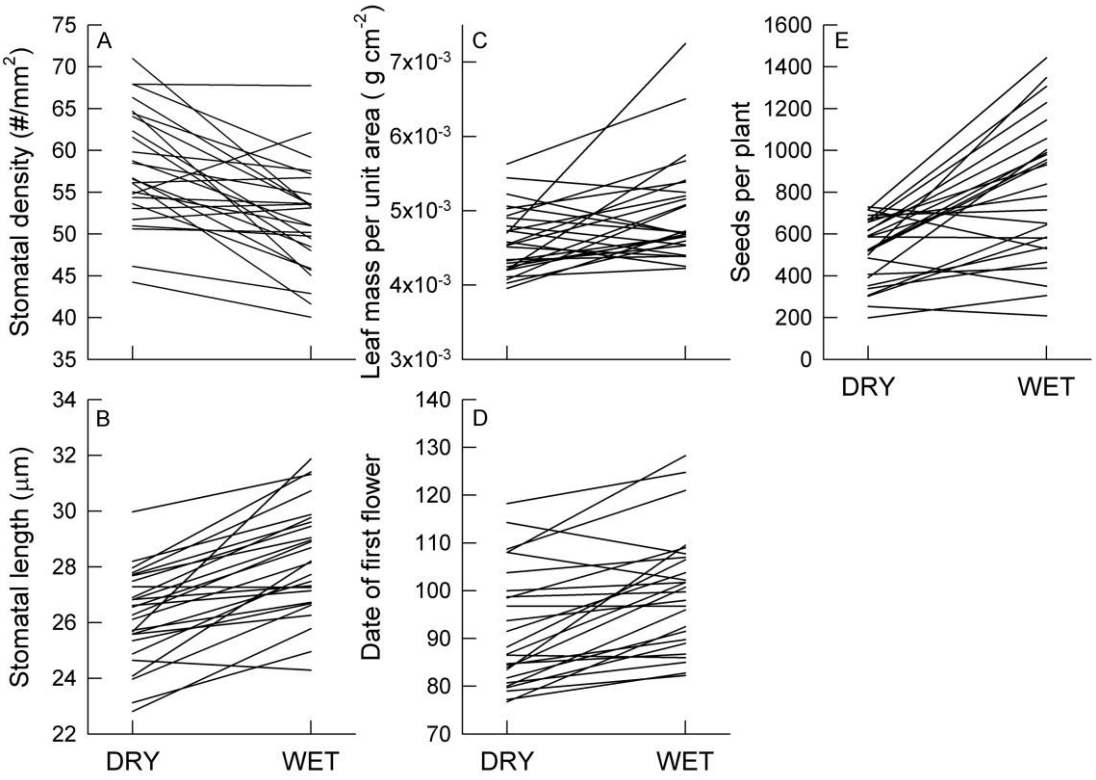

Figure 1: Response of four morphological traits and seed production of 26 *Avena barbata* recombinant inbred lines to dry and wet treatments. *A*, Stomatal density. *B*, Stomatal length. *C*, Leaf mass per unit area. *D*, Date of first flower. *E*, Seeds per plant.

wet treatment (figs. 1*A*, 2*E*). This suggests that plasticity in stomatal density and V_{cmax} was also maladaptive.

We detected costs of plasticity or homeostasis for three of 20 trait-environment combinations (table 4). Plasticity in g_s at reproduction was costly in the dry treatment, but this regression coefficient did not remain significant after Bonferroni correction. Homeostasis in V_{cmax} and l_g was costly in the wet treatment (fig. A2 in the online edition of the *American Naturalist*), but only the coefficient for l_g remained significant after Bonferroni correction.

Discussion

We found that costs of plasticity in physiological traits of *Avena barbata* RILs were infrequent and small relative to costs detected in previous studies that also used recombinant progeny (Callahan et al. 2005; Weinig et al. 2006; Dechaine et al. 2007). Although all 10 traits responded plastically to soil water availability (table 1), we found evidence for a significant cost of plasticity in only one trait. Although plasticity in g_s at reproduction was not adaptive (tables 2, 3), it was costly in the dry treatment, at least before Bonferroni correction (table 4). This result contrasts with studies of *Arabidopsis thaliana* (Callahan et al. 2005; Weinig et al. 2006) and *Brassica rapa* (Dechaine et al. 2007) RILs, which found that 50% of measured traits exhibited significant local or global costs. In addition, the mean cost of plasticity, whether statistically significant or not, for traits of *A. barbata* RILs was −0.070 (calculated from all negative β values in table 4). This is smaller than the mean cost of plasticity detected using RILs of *A. thaliana* ($\beta = -0.315$ [Callahan et al. 2005]; $\beta = -0.148$ [Weinig et al. 2006]) and *B. rapa* ($\beta = -0.140$ [Dechaine et al. 2007]).

The modest costs of plasticity detected in *A. barbata* RILs could represent a more realistic estimate of the magnitude of these costs in natural populations. The *A. thaliana* and *B. rapa* RILs used in previous studies represent crosses that are unlikely to occur in the field, either because one parent is a laboratory genotype (Dechaine et al. 2007) or because the parental genotypes are from populations located on different continents (Callahan et al. 2005). In contrast, the *A. barbata* RILs mimic rare but recurrent outcrossing between mesic and xeric genotypes in the field in California (Latta et al. 2007). RILs derived from geographically distant parental populations are valuable for

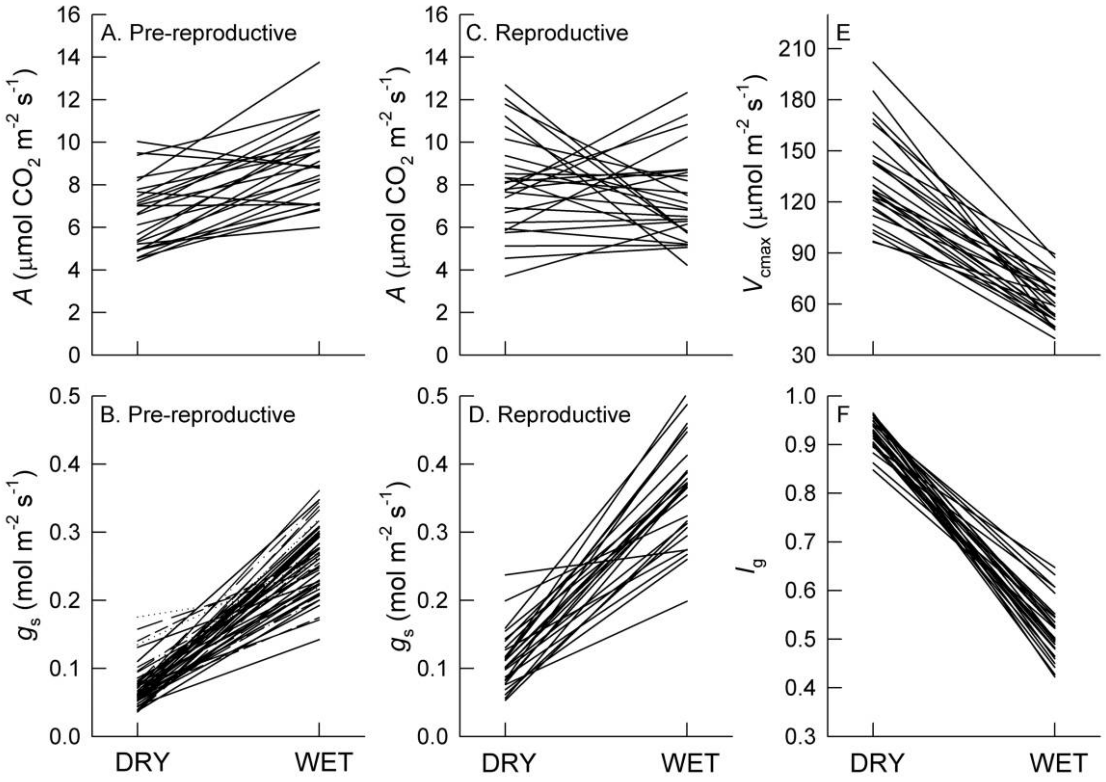

Figure 2: Response of six physiological traits of 26 *Avena barbata* recombinant inbred lines to dry and wet treatments. *A*, Prereproductive photosynthetic rate. *B*, Prereproductive stomatal conductance. *C*, Reproductive photosynthetic rate. *D*, Reproductive stomatal conductance. *E*, Maximum velocity of carboxylation of ribulose 1,5-bisphosphate carboxylase-oxygenase (Rubisco; V_{cmax}). *F*, Relative stomatal limitation of photosynthesis (l_g).

determining the genetic basis of costs of plasticity (e.g., Callahan et al. 2005). However, crosses between geographically distant populations are also more likely to be genetically distant, and they can exhibit stronger epistatic interactions than can crosses between nearby populations (e.g., Edmands 1999). If epistasis is an important source of genetic costs of plasticity, then previous studies that have used RILs may have overestimated the magnitude of such costs in natural populations.

We cannot rule out three other possible explanations for why costs of plasticity in *A. barbata* RILs were small relative to those in other studies that also used RILs. First, plasticity in physiological traits may be less costly than plasticity in other types of traits. Consistent with this explanation, the only other study of costs of plasticity in physiological traits also found that these costs were small in magnitude (mean $\beta = -0.097$; Caruso et al. 2006). Second, costs of plasticity may be smaller for plants raised in greenhouse conditions (van Kleunen and Fischer 2005, 2007). Although the dry treatment simulated a very low rainfall year and significantly reduced fitness (table 1; fig. 1*E*), *A. barbata* RILs were not exposed to the competition and herbivory that they experience in the field in California (Johansen-Morris and Latta 2006). We cannot exclude the possibility that *A. barbata* RILs growing in the field express larger costs of plasticity than we detected in the greenhouse. Third, plasticity in unmeasured traits such as root : shoot ratio may have been costly.

One limitation of our study is that we used fewer RILs ($N = 26$) than did other studies of costs of plasticity in recombinant populations (Callahan et al. 2005; Weinig et al. 2006; Dechaine et al. 2007). The time required to measure physiological traits limited the number of lines and individuals within lines that we could include. However, as we noted above, the mean cost of plasticity for traits of *A. barbata* RILs was smaller than the mean cost detected using RILs of *A. thaliana* (Callahan et al. 2005; Weinig et al. 2006) and *B. rapa* (Dechaine et al. 2007). Our smaller sample size would reduce the power to detect costs of plasticity, but it should not bias our point estimates of these costs. Consequently, our data suggest that we found less evidence for costs of plasticity than did other studies, because these costs are smaller in *A. barbata*.

Our results provide mixed support for predictions of

Table 5: Broad-sense heritability (H^2) for plasticity in 10 traits of *Avena barbata* in response to soil water availability

Trait	H^2
Prereproductive A	0
Prereproductive g_s	0
Reproductive A	.081*
Reproductive g_s	.007
V_{cmax}	0
l_g	.019
LMA	.086*
Stomatal density	.004
Stomatal length	0
Date of first flower	.060

Note: We used the P value for the treatment × line term in table 1 to determine whether H^2 for plasticity in a trait was significant. A = photosynthetic rate (μmol CO_2 m^{-2} s^{-1}). g_s = stomatal conductance (mol m^{-2} s^{-1}). V_{cmax} = maximum velocity of carboxylation of ribulose 1,5-bisphosphate carboxylase-oxygenase (Rubisco; μmol m^{-2} s^{-1}). l_g = relative stomatal limitation of photosynthesis. LMA = leaf mass per unit area (g cm^{-2}). Stomatal density is number per square millimeter; stomatal length is in micrometers. No H^2 remained significant after sequential Bonferroni correction by the Dunn-Sidak method.

* $P < .05$.

how a drought-escaping annual plant should respond to reduced soil moisture (Mooney et al. 1976; Bazzaz 1979; Geber and Dawson 1997). Our prediction that *A. barbata* plants would flower earlier in the dry treatment (table 1; fig. 1*D*) was supported. However, contrary to expectations, we found that selection for early flowering was stronger in the wet treatment, suggesting that plasticity for earlier flowering in the dry treatment is maladaptive (table 3). In addition, two lines of evidence contradicted the prediction that earlier flowering and increased photosynthetic capacity would be adaptive in the dry treatment. First, although both V_{cmax} and stomatal density were higher for RILs growing in the dry treatment, which would support higher photosynthetic rates (Sharkey 1985; Xu and Zhou 2008), selection favored increased V_{cmax} and stomatal density only in the wet treatment (table 3). Second, a number of RILs had higher A at reproduction when growing in the dry treatment, but this response was also maladaptive because selection favored higher A at reproduction only in the wet treatment (table 3).

Despite weak selection for physiological plasticity that facilitates drought escape, the plastic response of the parental ecotypes to the watering treatment reflected some degree of local adaptation to differences in moisture availability (but see Johansen-Morris and Latta 2008). In particular, the mesic ecotype—but not the xeric ecotype—had significantly higher V_{cmax} when growing in the dry treatment relative to the wet treatment (table A1). Selection for higher V_{cmax} in the wet treatment but not in the dry treatment suggests that the response of the mesic ecotype to the severe drought we imposed was maladaptive.

Rather than plasticity to facilitate drought escape, we found that a plastic increase in water conservation through stomatal closure was adaptive in *A. barbata*. Lines with the largest plastic increase in l_g in the dry treatment relative to the wet treatment had the highest fitness across treatments (table 2). This selection suggests that reducing the water cost of carbon gain in the dry environment through stomatal closure was adaptive in *A. barbata*, even though it reduces total carbon gain. Although Mediterranean annuals such as *A. barbata* are thought to adapt to dry soil environments primarily through drought escape (Stanton et al. 2000; Volis et al. 2002, 2004; Sherrard and Maherali 2006), our results suggest that plasticity in traits that promote water conservation may also be under selection because they increase global fitness across wet and dry soil environments. This interpretation is consistent with the plastic responses of the xeric and mesic ecotypes, both of which had significantly higher l_g in the dry treatment than in the wet treatment (table A1).

There are two possible explanations for why we did not detect selection for physiological plasticity associated with drought escape in *A. barbata*. First, the *A. barbata* plants in our experiment received rainfall that was representative of an extreme drought at the xeric site. Second, we imposed a constant watering treatment, which differs from the high variability in the timing and amount of rainfall that *A. barbata* would experience in the field. If our dry treatment was less extreme and more variable, there may have been stronger selection for physiological responses that promote drought escape (Dudley 1996; Heschel and Riginos 2005; Picotte et al. 2007; Donovan et al. 2009).

We detected significant costs of homeostasis for l_g and V_{cmax}. *Avena barbata* RILs with higher plasticity in l_g and V_{cmax} had higher fitness within the wet treatment (fig. A2), although only selection on l_g remained significant after Bonferroni correction (table 4). These selection gradients can be unambiguously interpreted as costs of homeostasis because plasticity values for l_g and V_{cmax} were positive (i.e., all RILs responded to soil moisture in the same way (figs. 2*E*, 2*F*; van Kleunen and Fischer 2007). Our results support recent reviews suggesting that costs of plasticity and of homeostasis are detected with equal frequency (van Kleunen and Fischer 2007; van Buskirk and Steiner 2009). Although costs of homeostasis have received less attention than have costs of plasticity (Dorn et al. 2000), our results suggest that costs of homeostasis could facilitate the evolution of adaptive plasticity in l_g because less plastic lines would be selected against when *A. barbata* grows in well-watered environments. However, these costs of homeostasis are local because less plastic lines would not be se-

lected against when *A. barbata* grows in dry environments. Because global costs have a much stronger effect on the evolution of plasticity than local costs (Sultan and Spencer 2002), any positive effect of the cost of homeostasis on the evolution of plasticity in l_g of *A. barbata* may be modest.

Our data suggest that there is little genetic variation for plasticity in physiological traits of *A. barbata*. Although H^2 values for many physiological traits of *A. barbata* within each soil water environment were significant (Sherrard et al. 2009), we detected significant genetic variation only for plasticity in *A* at reproduction and LMA (table 1), and H^2 values for plasticity in all traits were low (<0.10; table 5). This result is not consistent with a recent review of phenotypic plasticity in plants (van Kleunen and Fischer 2005), which concluded that there is often significant genetic variation for plasticity. However, most published estimates of genetic variation for plasticity are for morphological traits. Of the three studies (Sultan and Bazzaz 1993; Heschel et al. 2004*a*, 2004*b*) that have estimated genotype × environment interactions for plasticity in physiological traits, two studies (Sultan and Bazzaz 1993; Heschel et al. 2004*a*) had results that were were consistent with our finding that there was little genetic variation for plasticity in physiology. In addition, heritabilities for physiological traits of both plants (Geber and Griffen 2003) and animals (Mousseau and Roff 1987) tend to be modest relative to heritabilities for morphological traits. More generally, our results indicate that there could be genetic constraints on the evolution of plasticity in physiological traits of *A. barbata*. If so, we would predict that there would be little response to artificial selection for increased plasticity in physiological traits of *A. barbata*, relative to selection on morphological traits.

Our results are consistent with those of other studies that have found significant costs of plasticity or homeostasis for traits where significant genetic variation for plasticity was not detected (e.g., Dorn et al. 2000; Agrawal et al. 2002). Although we detected costs of plasticity or homeostasis for three traits (table 4), treatment × line terms were not significant for plasticity in any of these traits (table 1). However, the reaction norms were divergent (fig. 1), suggesting that our ability to detect significant genotype × environment interactions was likely limited by the relatively low replication ($N = 4$) within each treatment × line combination. More generally, the infrequent detection of costs of plasticity and homeostasis in the literature (van Kleunen and Fischer 2005; van Buskirk and Steiner 2009) could in part reflect the reluctance to test for costs in the absence of significant genotype × environment interactions (e.g., Dechaine et al. 2007). If significant genotype × environment interactions are particularly difficult to detect for traits, such as physiology, that are time-consuming to measure (Ackerly et al. 2000), then this reluctance could result in costs being underreported for certain trait classes. This bias could explain why a recent meta-analysis failed to detect any influence of trait class on the magnitude of costs of plasticity or homeostasis (van Buskirk and Steiner 2009). Consequently, we suggest that testing for costs of plasticity and homeostasis, even in the absence of significant genotype × environment interactions, would be useful for future meta-analyses of these costs.

In conclusion, we found limited evidence that the evolution of plasticity in a recombinant population was constrained by costs. There was a significant cost of plasticity for only one trait of *A. barbata* RILs, and that cost was local rather than global (table 4). Instead, our results suggest that the evolution of adaptive plasticity may be constrained more by a lack of genetic variation than by costs. We detected significant genotype × environment interactions in only one of the three traits in which plasticity was adaptive, even though recombination between mesic and xeric ecotypes releases genetic variation in *A. barbata* (Latta et al. 2004; Gardner and Latta 2008; Sherrard et al. 2009). Although models of plasticity generally focus on costs (e.g., van Tienderen 1991; Sultan and Spencer 2002), our results suggest that costs are less likely to constrain the evolution of adaptive plasticity in *A. barbata* than genetic variation for plasticity.

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