

WATER STRESS ALTERS THE GENETIC ARCHITECTURE OF FUNCTIONAL TRAITS ASSOCIATED WITH DROUGHT ADAPTATION IN *AVENA BARBATA*

Mark E. Sherrard,^{1,2} Hafiz Maherali,^{1,3} and Robert G. Latta⁴

¹Department of Integrative Biology, University of Guelph, Guelph, Ontario N1G 2W1, Canada

²E-mail: msherrar@uoguelph.ca

³E-mail: maherali@uoguelph.ca

⁴Department of Biology, Dalhousie University, Halifax, Nova Scotia B3H 4J1, Canada

Received July 24, 2008

Accepted October 23, 2008

Environmental stress can alter genetic variation and covariation underlying functional traits, and thus affect adaptive evolution in response to natural selection. However, the genetic basis of functional traits is rarely examined in contrasting resource environments, and consequently, there is no consensus regarding whether environmental stress constrains or facilitates adaptive evolution. We tested whether resource availability affects genetic variation for and covariation among seven physiological traits and seven morphological/performance traits by growing the annual grass *Avena barbata* in dry and well-watered treatments. We found that differences in the overall genetic variance–covariance (**G**) matrix between environments were driven by physiological traits rather than morphology and performance traits. More physiological traits were heritable in the dry treatment than the well-watered treatment and many of the genetic correlations among physiological traits were environment dependent. In contrast, genetic variation and covariation among the morphological and performance traits did not differ across treatments. Furthermore, genetic correlations between physiology and performance were stronger in the dry treatment, which contributed to differences in the overall **G**-matrix. Our results therefore suggest that physiological adaptation would be constrained by low heritable variation in resource-rich environments, but facilitated by higher heritable variation and stronger genetic correlations with performance traits in resource-poor environments.

KEY WORDS: *Avena barbata*, functional traits, **G**-matrices, physiological adaptation, water stress.

The genetics of functional traits can influence their short-term adaptive evolution. For example, the evolutionary response of a trait to natural selection is directly proportional to the amount of additive genetic variation for that trait (Falconer and Mackay 1996). In addition, genetic covariance among traits can cause correlated responses to natural selection, but whether the response to a specific selection regime is constrained or accelerated depends on the strength and sign of the genetic covariances among traits (Lande 1979; Conner and Via 1992; Caruso 2004). The

additive genetic variation for and covariation among traits is summarized by the genetic variance–covariance (**G**) matrix (Lande 1979). This makes the **G**-matrix an effective tool for predicting the multivariate response to selection (Lande and Arnold 1983) and for comparing the genetic architecture of different populations (Phillips and Arnold 1999; Steppan et al. 2002; Mezey and Houle 2003). When the **G**-matrices of two populations differ, the future evolutionary trajectory of these populations will differ as well.

Populations grown in contrasting environments can also exhibit different **G**-matrices (Begin and Roff 2001; Conner et al. 2003). This is because resource availability and environmental stress, in addition to altering the phenotype, can alter the expression of genetic variation and covariation (Service and Rose 1985; Holloway et al. 1990; Hoffmann and Parsons 1991; Sgró and Hoffmann 2004). For example, environmental stress can decrease the amount of environmental variance for a trait without altering the additive genetic variance. In contrast, environmental stress can increase the additive genetic variation of a trait if phenotypic differences between genetic lines are only expressed when resources are limiting (hypotheses summarized in Hoffmann and Merilä 1999). As a result, the evolutionary potential of populations to respond to natural selection may differ under contrasting environmental conditions, or in years with contrasting resource availability.

Not all functional traits are equally affected by environmental variation, but few studies have assessed how specific classes of traits influence variation in the **G**-matrix (e.g., Sleeman et al. 2002; Brock and Weinig 2007; Steven et al. 2007). Variation in the **G**-matrix may depend on the phenotypic plasticity of the underlying traits. For example, a **G**-matrix for a group of highly canalized traits should be relatively insensitive to environmental variation, whereas a **G**-matrix for highly plastic traits would differ depending on environmental conditions (Brock and Weinig 2007). In addition to the amount of phenotypic plasticity, other aspects of the plastic response could also influence the **G**-matrix. In particular, physiological traits respond rapidly to changes in environmental conditions and can also revert back to the previous phenotype if the environment returns to its initial state (e.g., Fay and Knapp 1993; Piersma and Drent 2003). In contrast, morphological traits and performance measures such as leaf mass per unit area (LMA), growth and fitness respond slowly to changes in environmental conditions (Schlichting and Pigliucci 1998) and this response is often irreversible (e.g., Piersma and Drent 2003; Brakefield et al. 2007). The greater environmental sensitivity of physiological traits compared to morphological and performance traits may make a **G**-matrix for physiology more likely to differ across environments than a **G**-matrix for morphological and performance traits.

Because they are sessile, plants are vulnerable to many forms of abiotic and biotic stress. Of these potential stressors, drought is ubiquitous, highly variable across space and time, and the most limiting to plant growth in many environments (Knapp et al. 2001; Knapp and Smith 2001). Plants respond to water limitation by altering their physiology, morphology, and development. Stomatal closure, which is the most immediate physiological response, minimizes evaporative water loss but simultaneously reduces diffusion of CO₂ to the site of photosynthesis in the chloroplast (Wong et al. 1979). Longer-term physiological responses include changes

in the biochemical regulation of photosynthesis that enhance carbon fixation to compensate for reduced CO₂ concentration in the leaf (Sultan et al. 1998; Lawlor 2002; Farquhar et al. 2002; Flexas et al. 2004; Caruso et al. 2006; Sherrard and Maherali 2006). The slower, nonreversible morphological and developmental responses to water limitation include smaller stomata to restrict water loss (Dunlap and Stettler 2001; Galmes et al. 2007), denser leaves that resist desiccation (higher LMA) (Ackerly 2004), and earlier flowering in annuals to escape water stress (Geber and Dawson 1990; McKay et al. 2003; Heschel and Riginos 2005; Sherrard and Maherali 2006). These functional traits are heritable as well as genetically correlated with each other in natural populations of many species (Geber and Dawson 1990, 1997; Dudley 1996; Ackerly et al. 2000; Arntz and Delph 2001; Caruso et al. 2005). However, genetic variation for and covariation among functional traits is rarely measured in environments with contrasting resource availability or environmental stress. As a result, there is no consensus on whether growth environment constrains or facilitates the evolutionary response of plant functional traits to natural selection.

We examined whether environmental water stress influences genetic variation for and covariation among plant functional traits associated with drought adaptation across genotypes in *Avena barbata* Pott. ex Link. In a companion paper (Sherrard and Maherali 2006), we reported that phenotypic selection on physiology and flowering time was stronger in well-watered versus water-limited environments. We concluded that plant physiology and development could evolve more rapidly in environments or years when water is not limiting; however, this interpretation assumed that the **G**-matrix did not differ across environments. To determine if evolutionary responses to natural selection can differ across moisture environments, we analyzed data from Sherrard and Maherali (2006) and addressed two questions: (1) Does environmental stress through water-limitation affect the amount of heritable genetic variation for and covariation among functional traits associated with drought adaptation? (2) Is the response of the **G**-matrix to the resource environment influenced by functional distinctions among classes of traits? In other words, is a **G**-matrix of physiological traits more likely to differ between environments than a **G**-matrix of morphological and performance traits?

Materials and Methods

STUDY SPECIES AND RECOMBINANT INBRED LINES (RILS)

Avena barbata is a highly selfing (>95%; Clegg and Allard 1973) European annual grass that has invaded the Mediterranean climatic region in the Southwestern United States since its introduction over 200 years ago (Garcia et al. 1989). The study of

adaptation in *A. barbata* to contrasting moisture environments has a long history because of the occurrence of natural ecotypes associated with mesic and xeric habitats in California. These ecotypes differ at five allozyme loci (Clegg and Allard 1972; Hamrick and Allard 1972) as well as numerous AFLP markers (Gardner and Latta 2006) and a suite of quantitative traits including seed size, adult size, below ground allocation, root depth, competitive ability, and fecundity (Hamrick and Allard 1975; Latta et al. 2004; Gardner and Latta 2008). Areas of California receiving less than 500 mm of annual rainfall are monomorphic for one allozyme combination (the “xeric ecotype” Clegg and Allard 1972), whereas regions receiving more rainfall contain a mix of xeric and mesic populations that assort based on local soil and microclimatic conditions (Hamrick and Allard 1972; Hamrick and Holden 1979).

The recombinant inbred line (RIL) genotypes used in this experiment, were produced by crossing a single individual from the xeric and mesic ecotypes. Plants used for this initial cross were collected in the 1980’s from mesic and xeric sites in Northern California by Dr. Pedro Garcia and their allozyme genotype was verified (Latta et al. 2004). The F_1 offspring from this cross were heterozygous at all loci that differed between the two parents. A single F_1 individual was then allowed to propagate by self-fertilization, producing abundant F_2 seeds. The F_2 seeds were selfed for four generations through single-seed descent to produce an F_6 generation of 188 RILs in which each line is homozygous and fixed for a unique combination of alleles from the parental ecotypes (Lynch and Walsh 1998; for details see Latta et al. 2004; Gardner and Latta 2006).

EXPERIMENTAL DESIGN

To estimate heritable genetic variation and covariation, we selected 26 RILs that were representative of the greenhouse fitness range of all 188 lines. We employed a randomized complete block design, consisting of four temporal blocks of 56 plants ($n = 224$), so that all physiological measurements would be made on plants at the same life stage. Two germinated seeds from the 26 RILs and the two parental genotypes were planted every 12 days. We germinated seeds from each RIL by removing the palea and lemma and placing them on moist filter paper for 96 h at 4°C. After refrigeration, the seeds were returned to room temperature but left in the dark for an additional 24 h. A single seedling from each RIL was planted in a 4.1-L pot with Pro-Mix BX growth medium (Premier Tech, Rivière-du-Loup, Quebec, Canada) and placed on a greenhouse bench. During the experiment, relative humidity was maintained at approximately 50% and temperature fluctuated diurnally from 20°C to 30°C. To ensure that incident irradiance on the bench remained above 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$, we provided the plants with supplemental light (16 h days). We watered the developing seedlings daily for a three-week period.

After the establishment, half the plants from each RIL were assigned to a dry treatment and the other half to a well-watered treatment. The volumetric water content (VWC) of both treatments was monitored using a soil moisture probe (Hydrosense CD620, Campbell Scientific Corp., Edmonton, Alberta, Canada). Well-watered plants were watered daily to saturation (mean VWC = $31.1 \pm 9.9\%$), and plants in the dry treatment received 175 mL of water per week (mean VWC < 5% throughout the treatment). This dry treatment was designed to simulate a constant dry growing season. Although drought can be episodic and unpredictable at the xeric site in California, monthly precipitation averages roughly 120 ± 20.00 mm through the growing season (http://groups.ucanr.org/sierrafoothill/About%5FSierra%5FFoothill%5FREC/Natural_Resources.htm). The dry treatment resulted in plants receiving the equivalent of 132 mm precipitation throughout the experiment, which is comparable to the total rainfall occurring during the driest growing season on record at the xeric site (165 mm; October 1976 to March 1977) and 69 mm less rainfall than any growing season at the mesic site since 1953 (<http://ucce.ucdavis.edu/files/filelibrary/6194/34945.htm>). On days when physiological measurements were made, plants were watered after data collection. All plants were provided with 100 mL of 20-20-20 fertilizer (Plant Products Inc., Brampton, Ontario, Canada) at a concentration of 2.5 g/L every two weeks. To minimize any potential watering treatment \times fertilizer effects, fertilizer was applied after daily watering. However, we cannot rule out the possibility that more fertilizer was washed away in the well-watered than the dry treatment. Physiological measurements began 70 days after planting (49 days after the treatments were initiated), before the plants flowered. Within each block, plants were measured in random order.

TRAIT MEASUREMENTS

Photosynthesis (A) and transpiration (E) were measured on all 224 plants (eight plants from each of 26 RILs and from the mesic and xeric parental lines) in the experiment using an open gas-exchange system (LI-6400, Li-Cor Inc., Lincoln, NE) at 26°C, a vapor pressure deficit of 1.9–2.0 kPa and a saturating irradiance of 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$. We used saturating light for all gas-exchange measurements to ensure they would not be biased by daily light fluctuations and that photosynthetic capacity would not be limited by suboptimal light. Stomatal conductance (g_s) was calculated from transpiration using a boundary layer conductance of 3.54–4.82 $\text{mol m}^{-2} \text{s}^{-1}$, which was determined from fan speed and leaf area using the energy balance algorithms of the LI-6400. Leaf area was calculated from the leaf dimensions.

Gas exchange was initially measured 70 days after germination, before the plants began to flower. At this time, we measured light-saturated instantaneous photosynthetic rate (A) and stomatal conductance to water vapor (g_s), under ambient CO_2

concentration (400 $\mu\text{L/L}$). In addition, we measured apparent chlorophyll concentration (Chl) before the plants flowered, on the three youngest fully expanded leaves of each plant using a portable chlorophyll meter (SPAD 502, Minolta Inc., Ramsey, NJ).

To examine the difference in instantaneous gas exchange between pre- and postreproductive plants, we measured photosynthetic rate and stomatal conductance again, after the plants had flowered. Under ambient CO_2 conditions and 110 days after germination, we measured instantaneous gas exchange on leaves attached to the culm (A_r ; g_{sr}). All pre- and postreproductive measurements were made between 0830 and 1230 EST from 4 May to 27 July 2004.

Photosynthesis is biochemically regulated by two enzymatic processes. When C_i is high, photosynthesis is primarily limited by ribulose 1,5-bisphosphate (RuBP) regeneration and when C_i is low it is primarily limited by ribulose 1,5-bisphosphate (RuBP) carboxylase-oxygenase (Rubisco) activity (Sharkey 1985; Geber and Dawson 1990, 1997). To determine the CO_2 saturated photosynthetic capacity (A_{max} ; which represents the rate of RuBP regeneration) and 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). A/C_i curves were constructed for all 224 plants, by varying the concentration of CO_2 in the LI-6400 cuvette chamber from 50 to 1800 ($\mu\text{L/L}$), at 100–200 ($\mu\text{L/L}$) intervals. Data were fit to the nonlinear model

$$[A = a(1 - \exp(-bCi)) + c], \quad (1)$$

where c is the y-intercept, $1/b$ is the rate constant, and CO_2 saturated A (A_{max}) is calculated as $a + c$ (Jacob et al. 1995; Reid and Fiscus 1998). V_{cmax} was calculated as the linear regression of the A/C_i curve when photosynthesis is CO_2 limited (i.e., at intercellular concentrations of CO_2 lower than 200 $\mu\text{L/L}$) (Wullschlegel 1993; Geber and Dawson 1997). A/C_i curves were constructed for each block over a six-day period, during the first set of gas-exchange measurements (70 days after germination). Because photosynthesis is also dependent on nitrogen concentration, there is typically a positive relationship between leaf nitrogen and photosynthetic capacity (reviewed in Field and Mooney 1986). To correct for variation in A_{max} and V_{cmax} caused by differences in nitrogen concentration (N%), rather than regulation of enzyme activity, we report the biochemical photosynthetic variables as A_{max}/N and V_{cmax}/N . Nitrogen concentration was measured through dry combustion (900°C) using the Variomax CN Elemental Analyzer (Elementar Americas, Inc., Mt Laurel, NJ), on a haphazard sample of nonsenesced vegetative and reproductive leaf tissue from each individual. To avoid destructive sampling

while the experiment was still ongoing, tissue was harvested at the end of the study (165 days after germination). Harvested leaves were of different ages and reported values therefore represent an estimate of whole-plant leaf N.

We also measured three morphological traits associated with drought adaptation (leaf mass per area (LMA), leaf stomatal length, and leaf stomatal density). We measured LMA for each plant as: leaf dry mass (g)/leaf area (cm^2) on a 3-cm portion of leaf tissue, excised from a leaf attached to the culm, after the reproductive gas-exchange measurements were completed. We limited sampling of LMA to the reproductive stage to avoid destructive sampling of plants before all physiological measurements had been completed. Because of this, however, we could not account for potential ontogenetic changes in LMA (e.g., Gunn et al. 1999). To measure stomatal length and density, we made a mould of the adaxial and abaxial surface of a leaf for each individual using polyvinylsiloxane dental impression material ('Extrude' Medium; Kerr Manufacturing Co., Orange, CA) and used the hardened mould as a cast for clear nail polish. 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 a total of eight stomates per leaf side. We measured stomatal density on the nail polish impression as the average number of stomates in two, 1- mm^2 viewing areas per leaf side. The measurements and counts were made using a light microscope interfaced with a Nikon Coolpix4500™ digital camera, and ImageJ (Abramoff et al. 2004, U.S. National Institute of Health; <http://rsb.info.nih.gov/ij/>). The values reported for stomatal length and density are averages of the abaxial and adaxial surfaces.

In addition to physiology and morphology, we measured four performance traits (the day of first flower [DFF], vegetative biomass, the proportion of seeds aborted, and seed number). We chose to include the day of first flower as a performance trait because previous research has shown that flowering time is a key determinant of spikelet and seed production in the greenhouse (Gardner and Latta 2008). At the end of the study (165 days after germination), all aboveground biomass was harvested for each individual. This tissue was divided into reproductive (culm and panicle) and vegetative (nonflowering tillers) components, dried to a constant mass (48 h at 65°C) and weighed. Any leaves attached to the culm were included as part of the reproductive biomass. At the time of harvest, all plants had flowered and were still producing culms; however, to represent a realistic growing season length in the field, the study was terminated before the plants were finished flowering. Because environmental stress can influence the amount of seed abortion in plant populations (Volis et al. 2004), we estimated the proportion of seeds aborted for each individual as the number of empty fruit per 100 randomly selected fruit. Because each *A. barbata* spikelet produces two single-seed

florets, we were able to calculate seed number as the product of: total spikelet number $\times 2 \times$ the proportion of aborted seeds. No culms or glumes were lost prior to the final harvest.

STATISTICAL ANALYSES

Within each treatment, we calculated the mean values for all 14 traits using the family line values for the 26 RILs (the parental ecotypes were analyzed separately) in the experiment. To determine whether the traits were phenotypically plastic, and whether the genetic lines differed in plasticity, we used a two-way analysis of variance (ANOVA) with genetic line and treatment as fixed factors. All statistical analyses were done on residuals to account for the block effect on trait values. We also compared the phenotypic values of the two parental ecotypes within each treatment using a paired two-tailed *t*-test. We present *P*-values for these analyses both before and after sequential Bonferroni correction for multiple tests (Sokal and Rohlf 1995).

Within each treatment, we calculated the heritability for each trait as the intraclass correlation ($\tau = (\sigma_g^2)/(\sigma_g^2 + \sigma_e^2)$), where σ_g^2 is the between line variance and σ_e^2 is the within-line environmental variance. Because each RIL is 96.75% homozygous (Gardner and Latta 2006), variation from individual to individual within each line is mostly caused by random environmental effects. Therefore, the intraclass correlation is equivalent to the broad-sense heritability (H^2) (Falconer and McKay 1996), which represents all possible genetic contributions to phenotypic variation (additive genetic variation, dominance effects, epistatic effects, and maternal effects; Lynch and Walsh 1998). However, because the RILs are homozygous at each locus, and have undergone a history of controlled breeding with constant environmental conditions, dominance and maternal environmental effects should be minimal, leaving additive and epistatic variance among lines. Terms for the intraclass correlation were calculated using the mean squares (MS) from one-way analyses of variance (ANOVA). The error mean squares (MSe) term from the ANOVA is considered equivalent to the within-line environmental variance, σ_e^2 . The genetic line mean squares (MSg) term in the ANOVA is equivalent to $\sigma_g^2 + n\sigma_e^2$, where *n* is the number of individuals per genotype (Lynch and Walsh 1998). Therefore, to calculate H^2 using the intraclass correlation, $\sigma_e^2 = \text{MSe}$ and $\sigma_g^2 = [(\text{MSg} - \text{MSe})/n]$. Because most traits were affected by block, the mean squares were calculated on residuals after the block effect was removed.

To make standardized comparisons of the additive and phenotypic variation between traits, we calculated the coefficient of phenotypic variation (CV_P) as $100\sqrt{V_P}/\bar{X}$ and the coefficient of additive variation (CV_A) as $100\sqrt{V_A}/\bar{X}$, where V_P is the total phenotypic variation, V_A is the additive genetic variation, and \bar{X} is the population mean for the trait (Houle 1992). We compared H^2 , CV_P , and CV_A between treatments, and phenotypic differences

between the parental lines, using a two-tailed paired *t*-test. We also compared H^2 , CV_A , and CV_P between classes of traits that differed in the speed and reversibility of their responses to environmental stress (i.e., physiological vs. morphological/performance traits), using the nonparametric Mann–Whitney *U* test (SYSTAT 8.0, Systat Software Inc., San Jose, CA).

In addition to heritabilities and coefficients of variation, we calculated genetic correlations among all traits using Pearson product-moment correlations. Correlations were estimated from the family means of the residuals. Family mean correlations have been commonly used to estimate genetic correlation among traits (e.g., Geber and Dawson 1997; McKay et al. 2003) because they are amenable to standard significance tests and are straightforward to interpret. However, family mean correlations can often be biased because they include a component of environmental variance (Lynch and Walsh 1998). Consequently, we consider a significant family mean correlation between two traits to be representative of significant genetic covariation if both traits also exhibited nonzero heritabilities (Lynch and Walsh 1998; Culley et al. 2006). We present *P*-values for all heritabilities and correlations both before and after sequential Bonferroni correction for multiple tests (Sokal and Rohlf 1995). Statistical analyses for the heritabilities and genetic correlations were performed using JMP (ver. 5.1.2 SAS Institute, Cary, NC), except where indicated.

GENETIC (G) VARIANCE–COVARIANCE MATRICES

We constructed genetic (G) variance–covariance matrices using family means of standardized residuals. Using common principal components (CPC) analysis (Flury 1988; Phillips and Arnold 1999), we compared the G-matrices between the *A. barbata* lines in well-watered and dry treatments. CPC analysis compares the structure of variance–covariance matrices, and tests their relatedness based on the Flury hierarchy of hypotheses (Phillips and Arnold 1999). This method determines the relationship between the matrices based on the number of principal components (PCs) based vectors shared. In the hierarchy, matrices can be equal, proportional, share all or some of the same PCs, or have completely unrelated structures.

Using CPC (Phillips 1998), we employed the Step-up and Jump-up approaches (Phillips and Arnold 1999) to assess which model in the hierarchy best fit the data. In the Step-up approach, the initial assumption is that the matrices are unrelated. Then, in a step-wise fashion, each new model is tested against the previous model using a parametric chi-square test. For example, the model in which the matrices share five PCs is tested against the model in which the matrices share four PCs. The lowest order comparison that is significant ($P < 0.05$) determines the best-fitting model. The Jump-up approach tests each model of the hierarchy against the model in which the matrices have unrelated structure.

Table 1. Mean (SE) of traits measured for *Avena barbata* RILs in the contrasting dry and well-watered treatments. Preflowering measurements of photosynthetic capacity (A_{max}/N), carboxylation efficiency (V_{cmax}/N), chlorophyll concentration, instantaneous photosynthetic rate (A), and stomatal conductance (g_s) were made 70 days after germination. The second set of measurements, photosynthetic rate (A_r) and stomatal conductance (g_{sr}) and leaf mass per area (LMA), were made 110 days after germination (after plants flowered). We compared values across environments and genetic lines using a two-way ANOVA (F -statistics shown and significance is indicated with * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Values that remain significant after sequential Bonferroni correction for multiple tests are indicated in bold. $N=26$ family lines in both environments.

Traits	Trait values		F -statistics		
	Dry treatment	Well-watered treatment	G	E	G×E
A_{max}/N	5.203 (0.223)	8.775 (0.439)	1.067	43.565***	0.532
V_{cmax}/N	36.960 (4.260)	30.496 (1.873)	1.294	2.192	0.886
Chlorophyll concentration	44.702 (0.505)	35.417 (0.600)	2.301**	246.564***	1.093
A	6.575 (0.335)	9.125 (0.361)	1.606*	31.323***	0.614
g_s	0.0627 (0.0043)	0.254 (0.0094)	0.804	271.324***	0.679
A_r	7.959 (0.458)	7.359 (0.417)	1.521	1.995	1.595*
g_{sr}	0.111 (0.0095)	0.358 (0.015)	1.361	252.306***	1.199
LMA	0.00459 (0.00009)	0.00500 (0.00014)	3.538***	16.211***	1.750*
Stomatal length	26.218 (0.333)	28.239 (0.393)	3.640***	33.917***	0.593
Stomatal density	57.978 (1.358)	51.731 (1.268)	3.113***	24.382***	1.034
Day of first flower	92.067 (2.435)	100.000 (2.464)	12.550***	38.104***	1.513
Vegetative biomass	7.455 (0.493)	23.915 (1.680)	3.817***	243.077***	1.480
Proportion of seeds aborted	0.146 (0.012)	0.360 (0.019)	2.449***	194.028***	1.667*
Number of seeds	523.567 (31.865)	806.129 (61.417)	2.310**	24.051***	0.902

Beginning at the bottom of the hierarchy, each hypothesis is tested until a model differs statistically based on a parametric χ^2 test ($P < 0.05$).

We used these statistical approaches to compare three types of \mathbf{G} -matrices across the two treatments. The first \mathbf{G} -matrix contained all 14 measured traits. We then divided the overall \mathbf{G} -matrix into two classes of traits to identify which portion of the matrix contributed more to the environmental differences. The second \mathbf{G} -matrix was composed of just the seven physiological traits (A_{max}/N , V_{cmax}/N , A , g_s , chlorophyll concentration, A_r , and g_{sr}). The third \mathbf{G} -matrix consisted of the remaining seven morphological and performance traits (LMA, leaf stomatal length, leaf stomatal density, day of first flower, vegetative biomass, proportion of seeds aborted, and seed number). All covariances were included in the CPC analyses regardless of whether the traits were heritable. We note that the outcome of the CPC analysis can depend on the traits included in each matrix. For this reason, we used CPC analysis on the physiological and morphological/performance \mathbf{G} -matrices only to summarize comparisons across environments for specific classes of traits for which we had an a priori hypothesis. Our interpretations of environmentally caused differences in the entire \mathbf{G} -matrix are based on examining environment-specific differences in pairwise genetic correlations, rather than trait groupings.

Results

The dry treatment represented an effective abiotic stress, as plant performance was significantly reduced under water limitation (Table 1). Genetic lines in the well-watered treatment had 221% more vegetative biomass and produced 54% more seeds. Increased seed production was achieved in the well-watered treatment despite an eight-day delay in flowering time, and 146% higher seed abortion compared to the dry treatment (Table 1). For the morphological traits, leaf mass per area (LMA) was 9% higher and stomata were 8% longer in the well-watered treatment. In contrast, stomatal density was 12% higher in the dry treatment (Table 1), which is a common response to moderate drought stress (e.g., Yang et al. 2004; Xu and Zhou 2008) because of reductions in leaf size. Seventy days after germination, the well-watered genetic lines had 39% higher photosynthetic rate (A), 303% higher stomatal conductance (g_s), and 69% higher photosynthetic capacity (A_{max}/N). In contrast, chlorophyll concentration was 26% higher in the dry treatment and V_{cmax}/N did not differ significantly between treatments (Table 1). One hundred and ten days after germination, the well-watered genetic lines had 215% higher stomatal conductance (g_{sr}). Although photosynthetic rate (A_r) did not differ significantly between the two treatments (Table 1), there was genetic variation for phenotypic plasticity in this trait (significant $G \times E$; Table 1), which could be responsible for the

Table 2. Mean (SE) of traits measured for the parental mesic and xeric lines of *Avena barbata* in the contrasting dry and well-watered treatments. See Table 1 for abbreviations of the physiological traits. Statistical differences of traits between environments were determined using paired two-tailed *t*-tests (*t*-ratios are shown and significance is indicated with **P*<0.05, ***P*<0.01, ****P*<0.001). Values that remain significant after sequential Bonferroni correction for multiple tests are indicated in bold.

	Dry treatment			Well-watered Treatment		
	Mesic line	Xeric line	<i>t</i> -stat	Mesic line	Xeric line	<i>t</i> -stat
A_{max}/N	5.120 (0.934)	5.349 (1.076)	-0.174	6.562 (1.646)	9.897 (3.037)	-1.109
V_{cmax}/N	31.349 (4.646)	34.450 (1.637)	-0.618	19.425 (3.685)	36.333 (14.078)	-1.452
Chlorophyll conc.	44.108 (2.441)	47.742 (1.573)	-2.045	35.442 (3.232)	38.350 (2.556)	-2.405
<i>A</i>	7.655 (0.926)	6.400 (0.972)	1.168	6.463 (0.600)	10.163 (2.188)	-1.685
g_s	0.0781 (0.019)	0.0565 (0.006)	1.828	0.205 (0.0704)	0.254 (0.0647)	-2.311
A_r	6.518 (2.159)	6.953 (1.213)	-0.152	5.388 (1.173)	7.783 (2.283)	-1.043
g_{sr}	0.0977 (0.051)	0.0736 (0.007)	0.601	0.238 (0.0628)	0.300 (0.0570)	-0.740
LMA	0.00456 (0.00045)	0.00477 (0.00064)	-0.441	0.00457 (0.00021)	0.00580 (0.00023)	-3.717*
Stomatal length	28.964 (0.615)	24.110 (1.286)	9.587**	27.278 (1.286)	25.917 (0.639)	1.381
Stomatal density	49.750 (3.066)	59.563 (4.701)	-2.264	49.313 (4.701)	51.750 (4.077)	-0.701
Day of first flower	87.250 (4.975)	103.500 (3.448)	-4.679*	105.667 (3.528)	123.250 (8.477)	-1.126
Vegetative biomass	8.178 (1.276)	8.570 (1.640)	-0.337	20.453 (1.080)	34.680 (7.192)	-2.021
Seeds aborted	0.105 (0.0401)	0.1525 (0.0251)	-1.656	0.488 (0.199)	0.532 (0.0742)	-2.667
Seed number	605.240 (65.400)	338.82 (44.881)	5.085*	409.370 (177.493)	312.077 (209.539)	0.496

nonsignificant treatment effect. Therefore, 13 of 14 traits were phenotypically plastic, with A_r , LMA, and the proportion of aborted seeds exhibiting genetic variation for phenotypic plasticity (Table 1).

Several phenotypic traits also differed between the two parental ecotypes within each treatment. In the dry treatment, seed production and stomatal length were both significantly higher and flowering time was significantly earlier in the mesic parental line than the xeric (Table 2). In the well-watered treatment, LMA was significantly higher in the xeric parental line than the mesic (Table 2).

GENETIC VARIATION

Heritable variation for the physiological traits was higher in the dry treatment (average $H^2 = 0.180$) than the well-watered treatment (average $H^2 = 0.059$; Tables 3 and 4). In the dry treatment, there was significant heritable variation for A_{max}/N , A_r , and g_{sr} . In addition, there was marginally significant heritable variation for chlorophyll concentration, *A*, and g_s . In the well-watered treatment there was significant heritable variation for chlorophyll concentration and A_r , but no heritable variation for A_{max}/N , V_{cmax}/N , *A*, g_s , and g_{sr} (Table 3). For the coefficients of variation (CV_A and CV_P), additive variation was higher for the physiological traits in the dry treatment (average $CV_A = 19.835$) than the well-watered treatment (average $CV_A = 5.301$), whereas phenotypic variation did not differ between treatments (Table 4).

Heritable variation for the morphological and performance traits did not differ between the dry (average $H^2 = 0.378$) and the well-watered treatment (average $H^2 = 0.326$; Table 4).

All seven morphological and performance traits were heritable in both treatments (Table 3) and there was no difference between treatments for the coefficients of variation (CV_A and CV_P ; Table 4).

GENETIC CORRELATIONS

There were several genetic correlations among traits that differed across environments (Table 5). In no case did the sign of these correlations change, but in many cases genetic correlations were statistically significant in one environment and not in the other.

Of the 21 pairwise correlations between physiological traits, there were five significant genetic correlations in the dry treatment and three in the well-watered treatment (Table 5). In the dry treatment, g_s was positively correlated with *A* and A_{max}/N (Table 5), indicating that genetic lines with high rates of gas exchange early in development also had high biochemical capacity for photosynthesis. In the well-watered treatment, both *A* and A_r were positively correlated with chlorophyll concentration (Table 5). A_r was correlated with g_{sr} in both treatments, indicating that genetic lines with high transpiration rate also had high carbon assimilation.

Most of the significant genetic correlations among pairs of morphological and performance traits were common to both treatments. Genetic lines that flowered earlier produced more seeds and less vegetative biomass in both treatments (Table 5). Similarly, genetic lines with more vegetative biomass produced fewer seeds in both treatments. There was also a strong negative correlation between leaf stomatal density and leaf stomatal length in both treatments.

Table 3. Broad-sense heritabilities (H^2) of physiological, performance, and morphological traits for *Avena barbata* in contrasting dry end well-watered treatments. Preflowering measurements of photosynthetic capacity (A_{max}/N), carboxylation efficiency (V_{cmax}/N), chlorophyll concentration, instantaneous photosynthetic rate (A), and stomatal conductance (g_s) were made 70 days after germination. The second set of measurements, photosynthetic rate (A_r) and stomatal conductance (g_{sr}) and leaf mass per area (LMA), were made 110 days after germination (after plants flowered). H^2 that were significantly different from 0 in a one-way ANOVA are denoted with *s or °s ($^{\circ}P < 0.10$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Values that remain significant after sequential Bonferroni correction for multiple tests are indicated in bold. $N=26$ family lines in both treatments. Also included are the coefficients of additive (CV_A) and phenotypic variation (CV_P).

	Dry treatment			Well-watered treatment		
	H^2	CV_A	CV_P	H^2	CV_A	CV_P
A_{max}/N	0.218**	15.702	33.621	0	0	53.468
V_{cmax}/N	0.076	28.649	103.870	0	0	63.962
Chlorophyll concentration	0.119°	3.366	9.737	0.206**	6.043	13.318
A	0.122°	15.131	43.297	0.024	5.898	38.237
g_s	0.131°	21.010	58.080	0	0	42.972
A_r	0.289***	22.139	41.165	0.148*	18.045	46.845
g_{sr}	0.307***	32.850	59.255	0.033	7.121	39.300
Leaf mass per area	0.290***	7.622	14.138	0.331***	11.461	19.917
Leaf stomatal length	0.140*	3.988	10.661	0.363***	5.799	9.621
Leaf stomatal density	0.198**	8.259	18.552	0.297***	9.711	17.827
Day of first flower	0.688***	12.614	15.207	0.564***	11.278	15.018
Vegetative biomass	0.612***	30.952	39.577	0.314***	28.255	50.413
Proportion of seeds aborted	0.361***	35.127	58.486	0.224**	19.194	40.554
Number of seeds	0.355***	25.024	42.016	0.187*	28.962	66.915

The main difference in genetic architecture between the dry and well-watered treatment was associated with correlations between a physiological trait and a morphological/performance trait. There were nine significant correlations among these classes of traits in the dry treatment and only three in the well-watered treatment (Table 5). In the dry treatment, genetic lines with high gas exchange early in development (A and g_s) produced more

vegetative biomass and flowered later, whereas lines with high gas exchange after flowering (g_{sr}) produced less vegetative biomass (Table 5). In addition, genetic lines with high photosynthetic rate at flowering time (A_r) had smaller stomata in the dry treatment. In the well-watered treatment, genetic lines with high photosynthetic rate early in development (A) produced more vegetative biomass and had larger stomata (Table 5).

Table 4. Mean (SE) values for broad-sense heritabilities (H^2), the coefficients of additive (CV_A) and phenotypic variation (CV_P) across the two treatments. Values were compared using paired two-tailed t -tests and significant differences are indicated in bold. For the comparison across all traits, physiological traits and morphological/performance traits, $N=14$, 7, and 7 respectively. * $P < 0.05$, ** $P < 0.01$.

Functional group	Dry treatment	Well-watered treatment	t -stat
All traits			
H^2	0.279 (0.051)	0.192 (0.047)	2.176*
CV_A	18.745 (3.000)	10.840 (2.659)	2.584*
CV_P	39.119 (7.169)	37.026 (5.261)	0.463
Physiological traits			
H^2	0.180 (0.037)	0.059 (0.034)	2.802*
CV_A	19.835 (3.959)	5.301 (2.640)	3.328*
CV_P	49.861 (11.862)	42.586 (6.412)	0.980
Morphology/performance traits			
H^2	0.378 (0.083)	0.326 (0.047)	0.766
CV_A	17.655 (5.103)	16.380 (3.778)	0.488
CV_P	28.377 (7.462)	31.466 (8.764)	-0.625

Table 5. Genetic correlations among traits in the well-watered (upper, off-diagonal) and dry (lower, off-diagonal) treatments in *Avena barbata*. Genetic correlations were tested against the null hypothesis of $r=0$ using two-tailed, one sample t -tests and all statistically significant values are indicated *** $P<0.001$, ** $P<0.01$, * $P<0.05$. Values that remained significant after sequential Bonferroni correction for multiple tests are indicated in bold. Genetic correlations between pairs of traits, in which at least one trait exhibited heritable genetic variation, are indicated with N/A. Preflowering measurements of photosynthetic capacity (A_{max}/N), carboxylation efficiency (V_{cmax}/N), chlorophyll concentration (Chl), instantaneous photosynthetic rate (A) and stomatal conductance (g_s) were made 70 days after germination. The second set of measurements, photosynthetic rate (A_r) and stomatal conductance (g_{sr}) and leaf mass per area (LMA), were made 110 days after germination (after plants flowered). $N=26$ family lines in each environment.

	A_{max}/N	V_{cmax}/N	Chl	A	g_s	A_r	g_{sr}	LMA	Stomatal density	Stomatal length	DFP	veg. biomass	Aborted seeds	Seeds number
A_{max}/N	—	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
V_{cmax}/N	0.3261	—	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Chl	0.0137	0.0561	—	0.6177***	N/A	0.4129*	0.3058	0.2612	0.3907*	0.0542	0.0297	0.0483	-0.3252	0.3026
A	0.6977***	0.1937	0.2833	—	N/A	0.3803	0.0886	0.1353	0.0689	0.4760*	0.1651	0.4208*	-0.1720	0.0867
g_s	0.7096***	0.0479	0.2265	0.8931***	—	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
A_r	-0.4699*	-0.1219	-0.0939	-0.1444	-0.1694	—	0.5457**	0.2769	-0.1245	0.3516	0.0963	0.2002	0.0463	0.2590
g_{sr}	-0.3108	0.0367	-0.1164	-0.2470	-0.2977	0.6815***	—	-0.0412	-0.2054	0.0368	0.2495	0.1193	-0.1304	-0.0701
LMA	0.2498	-0.0746	0.3624	0.4645*	0.3397	-0.1451	-0.0618	—	-0.1566	0.0706	0.3223	0.3689	-0.0305	-0.0210
Stomatal density	-0.3758	-0.1578	0.0973	-0.2420	-0.3692	0.2558	0.3694	0.2073	—	-0.4331*	-0.2538	-0.3697	0.0666	0.3250
Stomatal length	0.3800	0.1780	0.0383	0.2008	0.2330	-0.3933*	-0.4869*	-0.1403	-0.6673***	—	0.2236	0.6009**	-0.0162	-0.1449
DFP	0.1584	-0.1749	0.1608	0.4017*	0.5059**	0.0009	-0.3630	0.3499	-0.2133	0.2044	—	0.6085***	0.2293	-0.7244***
Veg. biomass	0.2826	-0.1119	0.1352	0.4728*	0.5752**	-0.0883	-0.4692*	0.3509	-0.3458	0.3562	0.9112***	—	0.0596	-0.5358**
Aborted seeds	0.0999	-0.0757	-0.1677	0.2172	0.1308	0.3022	0.3817	-0.0726	0.3076	-0.2005	-0.2817	-0.2449	—	-0.2917
Seed number	-0.2372	-0.0283	0.1560	-0.3588	-0.4364*	-0.0390	0.3162	-0.1858	0.2449	-0.1871	-0.8265***	-0.7880***	0.0426	—

Table 6. Across environment comparison of the overall, physiological and morphological/performance G-matrices using CPC. Results of both the step-up and jump-up approach for statistically comparing the two models are presented.

	Functional group of traits		
	All traits	Physiology	Morphology/ performance
Step – up approach	Two common PCs	One common PC	Equality
Jump – Up Approach	Five common PCs	One common PC	Equality

CPC ANALYSES

The G-matrices from the dry and well-watered treatment with all 14 traits shared some PCs but were not equal to one another (Table 6). The Step-up approach suggested that these matrices shared two PCs and the Jump-up approach suggested they shared five PCs. In addition, the G-matrices consisting of only physiological traits shared some principal components but were not equal to one another (Table 6). Both the Step-up approach and Jump-up approach suggested that these physiological trait matrices shared one PC. In contrast, both the Step-up approach and Jump-up approach suggested that the G-matrices consisting only of morphological and performance traits were equal to one another (Table 6).

Discussion

G-MATRIX COMPARISONS ACROSS RESOURCE ENVIRONMENTS

Physiological traits were more important for driving environment-dependent differences in genetic architecture of *A. barbata* than morphological and performance traits. We found that the G-matrices consisting of physiological traits differed between the two treatments, but the matrices composed of morphological and performance traits did not (Table 6). This result is similar to studies in *Silene latifolia* and *Arabidopsis thaliana*, where differences in the G-matrix were primarily driven by one functional group of traits (vegetative) over another (floral) (Brock and Weinig 2007; Steven et al. 2007).

Although the comparisons indicated that the environmental-sensitivity of the physiological G-matrix differed from the morphological/performance G-matrix, this pattern was not associated with contrasting plastic responses between classes of traits because almost all traits were phenotypically plastic (Table 1). These results differ from those of Brock and Weinig (2007), who found that trait plasticity was a key determinant of differences in the G-matrices of *A. thaliana* raised in contrasting light quality environments. Our results suggest that phenotypic plasticity for individual traits is not necessarily responsible for variation in the G-matrix across environments.

GENETIC VARIATION

Variation in the physiological G-matrix may instead be caused by differences in broad-sense heritability (H^2) across environments. We found that heritable variation for physiology was higher in the dry than the well-watered treatment (Tables 3 and 4). Higher H^2 was caused by increased expression of additive genetic variation in the dry treatment (CV_A , Table 4) because phenotypic variation (CV_P) did not differ between treatments (Table 4). Our results are therefore consistent with the hypothesis that environmental stress increases heritable variation for physiology because the phenotypic differences between the genetic lines only became apparent when resources were limiting (Hoffmann and Merilä 1999). The effects of water-limitation on heritable variation for physiology have been considered in a small number of other studies. In contrast to our results, previous studies found that heritable variation for plant physiological traits either decreases (e.g., Johnson et al. 1990) or remains constant under water limitation (e.g., Donovan and Ehleringer 1994). These opposing empirical results may suggest that, like other types of traits, heritable variation for physiology does not consistently increase or decrease under unfavorable conditions, but rather, responds variably (Hoffmann and Parsons 1991; Ceccarelli 1994; Hoffmann and Merilä 1999; but see Charmantier and Garant 2005).

The observation that H^2 for the morphological and performance traits did not differ between treatments (Table 4) is consistent with the similarity in the morphological and performance G-matrix across environments. Our results differ from those of some other plant and animal studies, which found that heritable variation for morphological and performance traits was reduced under stressful conditions (e.g., Bennington and McGraw 1996; Merilä 1997; Conner et al. 2003; Gardner and Latta 2008). However, other studies have reported no change in heritable variation for morphological and performance traits under stress (e.g., Merilä and Fry 1998; reviewed in Ceccarelli 1994 and Charmantier and Garant 2005), which is consistent with our results.

Variation in the G-matrices across environments may also have been influenced by differences in the amount of heritable variation for the two groups of traits. We found that H^2 was lower for the physiological traits than the morphological and

performance traits across both treatments ($U_{0.05(2),14,14} = 179$, $P < 0.001$; Table 3), which was due to marginally higher phenotypic variation ($U_{0.05(2),14,14} = 134$, $P = 0.098$) rather than lower additive genetic variation ($U_{0.05(2),14,14} = 124$, $P = 0.232$). Heritable variation for physiology may have been lower because physiological measurements respond rapidly to environmental change (Piersma and Drent 2003), which makes them more sensitive to daily environmental fluctuations than morphological and performance measurements. This leads to increased estimates of environmental variation and underestimation of H^2 (Ackerly et al. 2000; Arntz and Delph 2001). Because heritable variation for physiology was low (Table 3), the genetic covariance among physiological traits was often low as well, which could have caused differences in the physiological **G**-matrix. In contrast, all seven morphological and performance traits exhibited high heritable variation in both treatments, producing strong and similar genetic covariances among these traits.

GENETIC CORRELATIONS

Variation in the physiological **G**-matrix was also caused by environment-dependent expression of many of the pairwise genetic correlations. For example, preflowering stomatal conductance (g_s) was only correlated with photosynthesis (A_{max}/N and A) in the dry treatment, and chlorophyll concentration was only correlated with photosynthesis (A and A_r) in the well-watered treatment (Table 5). Because photosynthesis is regulated both by stomatal opening (Wong et al. 1979) and leaf biochemical capacity (Sharkey 1985; Field and Mooney 1986; Geber and Dawson 1997), these correlations should reflect contrasting limitations on photosynthesis between the two watering treatments. In support of this prediction, we found that genetic lines with higher stomatal conductance had higher photosynthesis in the dry treatment, where photosynthesis should be primarily limited by stomatal closure. In the well-watered environment where photosynthesis would be more limited by biochemical photosynthetic capacity associated with nitrogen availability, genetic lines with higher chlorophyll concentration (and thus higher N; Chapman and Barreto 1997) had higher photosynthesis.

In contrast to physiology, most of the genetic correlations among morphological and performance traits were common to both treatments. For example, we found that stomatal density was negatively correlated with stomatal length in both environments (Table 5), which is consistent with previous observations across plant taxa (e.g., Grubb et al. 1975; Bongers and Popma 1980). We also found that genetic lines that flowered earlier produced less vegetative biomass and more seeds in both environments (Table 5), indicating that an earlier developmental switch from vegetative to reproductive tissue allocation led to greater seed production (e.g., Gardner and Latta 2008). This suggests that similarity of the morphological/performance **G**-matrix across treat-

ments was driven by a shared pattern of genetic correlation in the underlying traits.

In addition to genetic correlations among physiological traits, differences in the overall **G**-matrix between environments were also caused by stronger genetic correlations between physiology and plant performance traits in the dry versus well-watered treatment. Plant physiology and development are hypothesized to be correlated in a way that reflects drought adaptation (Grime 1977; Ludlow 1989; Geber and Dawson 1997; McKay et al. 2003). For example, plants that escape drought complete their life cycle before the onset of water limitation by having high carbon assimilation and high water use to facilitate accelerated development. Preflowering genetic correlations between gas exchange and development in the dry treatment did not support this hypothesis because genetic lines with high stomatal conductance early in development (g_s) produced more vegetative biomass, fewer seeds and flowered later (DFF). At flowering however, when drought was more intense and heritable variation for stomatal conductance was higher, high stomatal conductance was correlated with lower vegetative biomass and earlier flowering, although this second relationship was marginally significant (Table 5). This result suggests that physiological mechanisms that confer drought escape are not uniform throughout development. The lack of correlation between stomatal conductance and plant development in the well-watered treatment, regardless of direction or time point (Table 5), suggests that the expression of genetic correlations between physiology and development are weaker when water is not limiting.

CONSEQUENCES FOR PHYSIOLOGICAL EVOLUTION

Our work indicates that the potential for physiological traits to evolve in response to natural selection is highly environment-dependent. Because heritable variation was higher in the dry treatment (Table 4), physiological evolution in response to natural selection should be faster in dry than well-watered environments or years (Falconer and MacKay 1996). This is particularly true for physiological traits like A_{max}/N , g_s , and g_{sr} , which were only heritable in the dry treatment (Table 3), and would therefore evolve slowly in resource-rich environments. However, the rate of evolutionary change for a phenotypic trait in a given environment also depends on the strength of selection on that trait. In a companion paper, we reported that selection on physiology was much stronger in the well-watered than the dry treatment (Sherrard and Maherali 2006). For example, there were significant linear selection differentials for five of seven of these physiological traits in the well-watered treatment, but only one in the dry treatment. As a result, evolutionary change for physiological traits in *A. barbata* could be slow regardless of resource environment. Adaptive physiological evolution would be slow in resource-poor environments because of weak selection, and constrained in resource-rich

environments because of low genetic variation. Previous studies have also reported a pattern of opposing evolutionary constraints depending on environment quality. In a population of wild sheep, for example, evolutionary change in birth-weight is impeded by low heritable variation in poor environments and weak selection in good environments (Wilson et al. 2006).

Drought escape in winter annuals is characterized by early flowering and high leaf gas exchange (Ludlow 1989; Chapin et al. 1993; McKay et al. 2003; Heschel and Riginos 2005). Although the higher number of genetic correlations in the dry environment could constrain adaptation (Caruso 2004), these correlations may facilitate the correlated evolution of mechanisms of drought escape in *A. barbata*. We found that covariation between physiology and development time was stronger in the dry treatment than the well-watered treatment (Table 5). For example, genetic lines with high stomatal conductance at flowering time ($g_{s,r}$) flowered earlier and produced significantly less vegetative biomass (Table 5). Consequently, selection for reduced vegetative biomass and early flowering would result in indirect selection for increased stomatal conductance at flowering time. Thus early flowering and high gas exchange could both evolve in dry environments even if only early flowering is under selection in *A. barbata* (Sherrard and Maherali 2006).

DIFFERENTIAL FITNESS BETWEEN PARENTAL ECOTYPES

The two distinct genotypes of *A. barbata* that occur in California are differentially associated with contrasting moisture environments (Clegg and Allard 1972; Hamrick and Holden 1979), suggesting that these genotypes are locally adapted. In contrast to this hypothesis, we found that the mesic ecotype produced more seeds than the xeric ecotype in both the well-watered and the dry treatment (Table 2). Although our results conflict with the genotype-habitat association observed in California, it is consistent with other field and greenhouse studies in *A. barbata* that have shown that the mesic ecotype outperforms the xeric ecotype across a wide variety of field and experimentally manipulated environments (Latta et al. 2007; Johnsen-Morris and Latta 2008). Overall, our results are consistent with the hypothesis that the distribution of *A. barbata* genotypes in California has yet to reach equilibrium (Latta et al. 2007; Johnsen-Morris and Latta 2008).

CONCLUSIONS

Although our results are consistent with theoretical and empirical studies (Hoffmann and Merilä 1999; Conner et al. 2003; Sgró and Hoffmann 2004; Charmantier and Garant 2005) which suggest that the evolutionary potential for quantitative traits will differ depending on growth environment, we show that the sensitivity of the **G**-matrix to the environment depends on the functional class of traits examined. The environmental sensitivity of the **G**-matrix

could not be predicted from differences in phenotypic plasticity or **G** × **E** interactions because traits did not differ systematically in these attributes. Variation in the **G**-matrix was instead associated with differences in heritable variation for physiology between environments and many environment-specific genetic correlations between physiological traits and performance traits. Our results therefore highlight the value of trait-based perspectives (e.g., Roff 1996) in the search for a consensus about the effects of environmental variation on the **G**-matrix.

ACKNOWLEDGMENTS

We thank C. M. Caruso, B. C. Husband, and R. L. Peterson for helpful discussions and/or comments on earlier versions of this manuscript. The assistance of M. Clifford, T. Suwa, and J. Thompson with data collection was also greatly appreciated. This work was supported by the Ontario Graduate Scholarship Program, a Discovery grant from the Natural Sciences and Engineering Research Council and grants from the Canada Foundation for Innovation and the Ontario Innovation Trust.

LITERATURE CITED

- Abramoff, M. D., P. J. Magelhaes, and S. J. Ram. 2004. Image processing with ImageJ. *Biophotonics Intl.* 11:36–42.
- Ackerly, D. D. 2004. Adaptation, niche conservatism, and convergence: comparative studies of leaf evolution in the California chaparral. *Am. Nat.* 163:654–671.
- Ackerly, D. D., S. A. Dudley, S. E. Sultan, J. Schmitt, J. S. Coleman, C. R. Linder, D. R. Sandquist, M. A. Geber, A. S. Evans, T. E. Dawson, et al. 2000. The evolution of plant ecophysiological traits: recent advances and future directions. *Bioscience* 50:979–995.
- Arntz, A. M., and L. F. Delph. 2001. Pattern and process: evidence for the evolution of photosynthetic traits in natural populations. *Oecologia* 127:455–467.
- Begin, M., and D. A. Roff. 2001. An analysis of G matrix variation in two closely related cricket species, *Gryllus firmus* and *G. pennsylvanicus*. *J. Evol. Biol.* 14:1–13.
- Bennington, C. C., and J. B. McGraw. 1996. Environment-dependence of quantitative genetic parameters in *Impatiens pallida*. *Evolution* 50:1083–1097.
- Bongers, F., and J. Popma. 1980. Leaf characteristics of the tropical, rain forest flora of Los Tuxtlas, Mexico. *Bot. Gaz.* 151:354–365.
- Brakefield, P. M., J. Pijpe, and B. J. Zwaan. 2007. Developmental plasticity and acclimation both contribute to adaptive responses to alternating seasons of plenty and of stress in *Bicyclus* butterflies. *J. Biosci.* 32:465–475.
- Brock, M. T., and C. Weinig. 2007. Plasticity and environment-specific covariances: an investigation of floral-vegetative and within flower correlations. *Evolution* 61:2913–2924.
- Caruso, C. M. 2004. The quantitative genetics of floral trait variation in *Lobelia*: potential constraints on adaptive evolution. *Evolution* 58:732–740.
- Caruso, C. M., H. Maherali, A. Mikulyuk, K. Carlson, and R. B. Jackson. 2005. Genetic variance and covariance for physiological traits in *Lobelia*: are there constraints on adaptive evolution? *Evolution* 59:826–837.
- Caruso, C. M., H. Maherali, and M. Sherrard. 2006. Plasticity of physiology in *Lobelia*: testing for adaptation and constraint. *Evolution* 60:980–990.
- Ceccarelli, S. 1994. Specific adaptations and breeding for marginal conditions. *Euphytica* 77:205–219.
- Chapin III, F. S., K. Autumn, and F. Pugnaire. 1993. Evolution of suites of traits in response to environmental stress. *Am. Nat.* 142(Suppl.):78–92.

- Chapman, S. C., and H. J. Barreto. 1997. Using a chlorophyll meter to estimate specific leaf nitrogen of tropical maize during vegetative growth. *Agron. J.* 89:557–562.
- Charmantier, A., and D. Garant. 2005. Environmental quality and evolutionary potential: lessons from wild populations. *Proc. R. Soc. Lond. B.* 272:1415–1425.
- Clegg, M. T., and R. W. Allard. 1972. Patterns of genetic differentiation in the slender wild oat species *Avena barbata*. *Proc. Natl. Acad. Sci. USA* 69:2100–2104.
- . 1973. Viability vs fecundity selection in the slender wild oat, *Avena barbata*. *Science* 181:667–668.
- Conner, J., and S. Via. 1992. Natural selection on body size in *Tribolium*: possible genetic constraints on adaptive evolution. *Heredity* 69:73–83.
- Conner, J. K., R. Franks, and C. Stewart. 2003. Expression of additive genetic variances and covariances for wild radish floral traits: comparison between field and greenhouse environments. *Evolution* 57:487–495.
- Culley, T. M., A. K. Dunbar-Wallis, A. K. Sakai, S. G. Weller, M. Mishio, D. R. Campbell, M. Herzenach. 2006. Genetic variation of ecophysiological traits in two gynodioecious species of *Schiedea* (Caryophyllaceae). *New Phytol.* 169:589–601.
- Donovan, L. A., and J. R. Ehleringer. 1994. Potential for selection on plants for water-use efficiency as estimated by carbon isotope discrimination. *Am. J. Bot.* 81:927–935.
- Dudley, S. A. 1996. The response to differing selection on plant physiological traits: evidence for local adaptation. *Evolution* 50:103–110.
- Dunlap, J. M., and R. F. Stettler. 2001. Variation in leaf epidermal and stomatal traits of *Populus trichocarpa* from two transects across the Washington Cascades. *Can. J. Bot.* 79:528–536.
- Falconer, D. S., and T. F. C. MacKay. 1996. Introduction to quantitative genetics. 4th ed. Longman, New York.
- Farquhar, G. D., T. N. Buckley, and J. M. Miller. 2002. Optimal stomatal control in relation to leaf area and nitrogen content. *Silva. Fenn.* 36:625–637.
- Fay, P. A., and A. K. Knapp. 1993. Photosynthetic and stomatal responses of *Avena sativa* (Poaceae) to a variable light environment. *Am. J. Bot.* 80:1369–1373.
- Field, C., and H. A. Mooney. 1986. The photosynthesis-nitrogen relationship in wild plants. Pp. 25–49 in T. Givnish, ed. On the economy of plant form and function. Cambridge Univ. Press, New York.
- Flexas, J., J. Bota, J. Cifre, J. M. Escalona, J. Galmes, J. Gulias, E. K. Lefi, S. F. Martinez-Canellas, M. T. Moreno, M. Ribas-Carbo, et al. 2004. Understanding down regulation of photosynthesis under water stress: future prospects and searching for physiological tools for irrigation management. *Ann. Appl. Biol.* 144:273–283.
- Flury, B. 1988. Common principal components and related multivariate models. Wiley, New York.
- Galmes, J., J. Flexas, R. Save, and H. Medrano. 2007. Water relations and stomatal characteristics of Mediterranean plants with different growth forms and leaf habits: responses to water stress and recovery. *Plant and Soil* 290:139–155.
- García, P., F. J. Vences, M. P. de la Varga, and R. W. Allard. 1989. Allelic and genotypic composition of ancestral Spanish and Colonial Californian gene pools of *Avena barbata*: evolutionary implications. *Genetics* 122:687–694.
- Gardner, K. M., and R. G. Latta. 2006. Identifying the targets of selection across contrasting environments in *Avena barbata* using quantitative trait locus mapping. *Mol. Ecol.* 15:1321–1333.
- . 2008. Heritable variation and genetic correlation of quantitative traits within and between ecotypes of *Avena barbata*. *J. Evol. Biol.* 21:737–748.
- Geber, M. A., and T. E. Dawson. 1990. Genetic variation in and covariation between leaf gas exchange, morphology, and development in *Polygonum arenastrum*, an annual plant. *Oecologia* 85:153–158.
- . 1997. Genetic variation in stomatal and biochemical limitations to photosynthesis in the annual plant, *Polygonum arenastrum*. *Oecologia* 109:535–546.
- Grime, J. P. 1977. Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *Am. Nat.* 111:1169–1194.
- Grubb, P. J., E. A. A. Grubb, and I. Miyata. 1975. Leaf structure and function in evergreen trees and shrubs of Japanese warm temperate rain forest. I. The structure of the lamina. *Bot. Mag. Tokyo* 88:197–211.
- Gunn, S., J. F. Farrar, B. E. Collis, and M. Nason. 1999. Specific leaf area in barley: individual leaves versus whole plants. *New Phytol.* 143:45–51.
- Hamrick, J. L., and R. W. Allard. 1972. Microgeographical variation in allozyme frequencies in *Avena barbata*. *Proc. Natl. Acad. Sci. USA* 69:2100–2104.
- . 1975. Correlations between quantitative characteristics and allozyme genotypes in *Avena barbata*. *Evolution* 29:438–442.
- Hamrick, J. L., and L. R. Holden. 1979. Influence of microhabitat heterogeneity on gene frequency distribution and gametic phase disequilibrium in *Avena barbata*. *Evolution* 33:521–533.
- Heschel, M. S., and C. Riginos. 2005. Mechanisms of selection for drought stress tolerance and avoidance in *Impatiens capensis* (Balsaminaceae). *Am. J. Bot.* 92:37–44.
- Hoffmann, A. A., and J. Merilä. 1999. Heritable variation and evolution under favourable and unfavourable conditions. *Trends Ecol. Evol.* 14:96–101.
- Hoffmann, A. A. and P. A. Parsons. 1991. Evolutionary genetics and environmental stress. 1st ed. Oxford Univ. Press, Oxford, U.K.
- Holloway, G. J., S. R. Povey, and R. M. Sibly. 1990. The affect of new environment on adapted genetic architecture. *Heredity* 64:323–330.
- Houle, D. 1992. Comparing evolvability and variability of quantitative traits. *Genetics* 130:195–204.
- Jacob, J., C. Greitner, and B. G. Drake. 1995. Acclimation of photosynthesis in relation to Rubisco and non structural carbohydrate contents and in situ carboxylase activity in *Scirpus olnayi* at elevated CO₂ in the field. *Plant Cell Environ.* 18:875–884.
- Johansen-Morris, A. D. and R. G. Latta. 2008. Genotype by environment interactions for fitness in hybrid genotypes of *Avena barbata*. *Evolution* 62:573–585.
- Johnson, D. A., K. H. Asay, L. L. Tieszen, J. R. Ehleringer, and P. G. Jefferson. 1990. Carbon isotope discrimination: potential in screening cool-season grasses for water-limited environments. *Crop Sci.* 30:338–343.
- Knapp, A. K., and M. D. Smith. 2001. Variation among biomes in temporal dynamics of aboveground primary production. *Science* 291:481–484.
- Knapp, A. K., J. M. Briggs, and J. K. Koelliker. 2001. Frequency and extent of water limitation to primary production in a mesic temperate grassland. *Ecosystems* 4:19–28.
- Lande, R. 1979. Quantitative genetic analysis of multivariate evolution applied to brain: body size allometry. *Evolution* 33:402–416.
- Lande, R., and S. J. Arnold. 1983. The measurement of selection on correlated characters. *Evolution* 37:1210–1226.
- Latta, R. G., J. L. MacKenzie, A. Vats, and D. J. Schoen. 2004. Divergence and variation of quantitative traits between allozyme genotypes of *Avena barbata* from contrasting habitats. *J. Ecol.* 92:57–71.
- Latta, R. G., A. D. Johansen, and K. M. Gardner. 2007. Hybridization, recombination, and the genetic basis of fitness variation across environments in *Avena barbata*. *Genetica* 129:167–177.
- Lawlor, D. W. 2002. Limitation to photosynthesis in water-stressed leaves: stomata vs. metabolism and the role of ATP. *Ann. Bot.* 89:871–885.

- Ludlow, M. M. 1989. Strategies of response to water stress. Pp. 269–281 in K. H. Kreeb, H. Richter, and T. M. Hinckley, eds., *Structural and functional responses to environmental stresses: water Shortage*. SPB Academic Publishing, The Hague, The Netherlands.
- Lynch, M., and B. Walsh. 1998. *Genetics and analysis of quantitative traits*. Sinauer, Sunderland, MA.
- Maherali, H., C. D. Reid, H. B. Johnson, H. W. Polley, and R. B. Jackson. 2002. Stomatal acclimation over a subambient to elevated CO₂ gradient in a C₃/C₄ grassland. *Plant Cell Environ.* 25:557–566.
- Malone, S. R., H. S. Mayeux, H. B. Johnson, and H. W. Polley 1993. Stomatal density and aperture length in four plant species grown across a subambient CO₂ gradient. *Am. J. Bot.* 80:1413–1418.
- McKay, J. K., J. H. Richards, and T. Mitchell-Olds. 2003. Genetics of drought adaptation in *Arabidopsis thaliana*: I. Pleiotropy contributes to genetic correlations among ecological traits. *Mol. Ecol.* 12:1137–1151.
- Merilä, J. 1997. Expression of genetic variation in body size of the collared flycatcher under different environmental conditions. *Evolution* 51:526–536.
- Merilä, J., and J. D. Fry. 1998. Genetic variation and causes of genotype-environment interaction in the body size of blue tit (*Parus caeruleus*). *Genetics* 148:1233–1244.
- Mezey, J. G., and D. Houle. 2003. Comparing G matrices: are common principal components informative? *Genetics* 165:411–425.
- Phillips, P. C. 1998. CPC: common principal components analysis program. Available at <http://www.uoregon.edu/~pphil/programs/cpc/cpc.htm>. Accessed May 1, 2005.
- Phillips, P. C., and S. J. Arnold. 1999. Hierarchical comparison of genetic variance-covariance matrices. I. Using the Flury hierarchy. *Evolution* 53:1506–1515.
- Piersma, T., and J. Drent. 2003. Phenotypic flexibility and the evolution of organismal design. *Trends Ecol. Evol.* 18:228–233.
- Reid, C. D., and E. L. Fiscus. 1998. Effects of elevated [CO₂] and/or ozone on limitations to CO₂ assimilation in soybean (*Glycine max*). *J. Exp. Bot.* 49:885–895.
- Roff, D. A. 1996. The evolution of genetic correlations: an analysis of patterns. *Evolution* 50:1392–1403.
- Schlichting, C. D., and M. Pigliucci. 1998. *Phenotypic evolution*. Sinauer, Sunderland, MA.
- Service, P. M., and M. R. Rose. 1985. Genetic covariation among life-history components: the effect of novel environments. *Evolution* 39:943–945.
- Sgró, C. M., and A. A. Hoffman. 2004. Genetic correlations, tradeoffs and environmental variation. *Heredity* 93:241–248.
- Sharkey, T. D. 1985. Photosynthesis in intact leaves of C₃ plants: physics physiology and rate limitation. *Bot. Rev.* 51:53–105.
- Sherrard, M. E., and H. Maherali. 2006. The adaptive significance of drought escape in *Avena barbata*, an annual grass. *Evolution* 60:2478–2489.
- Sleeman, J. D., S. A. Dudley, J. R. Pannell, and S. C. H. Barrett. 2002. Responses of carbon acquisition traits to irradiance and light quality in *Mercurialis annua* (Euphorbiaceae): evidence for weak integration of plastic responses. *Am. J. Bot.* 89:1388–1400.
- Sokal, R. R., and F. J. Rohlf. 1995. *Biometry*. 3rd ed., Freeman, New York.
- Steppan, S. J., P. C. Phillips, and D. Houle. 2002. Comparative quantitative genetics: evolution of the G matrix. *Trends Ecol. Evol.* 17:320–327.
- Steven, J. C., L. F. Delph, and E. D. Brodie III. 2007. Sexual dimorphism in the quantitative-genetic architecture of floral, leaf and allocation traits in *Silene latifolia*. *Evolution* 61:42–57.
- Sultan, S. E., A. M. Wilzek, D. L. Bell, and G. Hand. 1998. Physiological response to complex environments in annual *Polygonum* species of contrasting ecological breadth. *Oecologia* 115:564–578.
- Volis, S., K. J. F. Verhoeven, S. Mendlinger, and D. Ward. 2004. Phenotypic selection and regulation of reproduction in different environments in wild barley. *J. Exp. Bot.* 17:1121–1131.
- Wilson, A. J., J. M. Pemberton, J. G. Pilkington, D. W. Coltman, D. V. Mifsud, T. H. Cutton-Brock, and L. E. B. Kruuk. 2006. Environmental coupling of selection and heritability limits evolution. *PLOS Biol.* 4:1270–1275.
- Wong, S. C., I. R. Cowan, and G. D. Farquhar. 1979. Stomatal conductance correlates with photosynthetic capacity. *Nature* 282:424–426.
- Wullschlegel, S. D. 1993. Biochemical limitations to carbon assimilation in C₃ plants—A retrospective analysis of the A/Ci curves from 109 species. *J. Exp. Bot.* 44:907–920.
- Xu, Z., and G. Zhou. 2008. Responses of leaf stomatal density to water status and its relationship with photosynthesis in a grass. *J. Exp. Bot.* 59:3317–3325.
- Yang, H. M., X. Y. Zhang, and G. X. Wang. 2004. Relationships between stomatal character, photosynthetic character and seed chemical composition in grass pea at different water availabilities. *J. Agric. Sci.* 142:675–681.

Associate Editor: J. Kohn