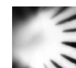

The adaptive significance of ontogenetic changes in physiology: a test in *Avena barbata*

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Summary

- Physiological changes with ontogeny are common in plants. Although ontogenetic changes are hypothesized to improve plant function, their adaptive significance has rarely been tested.
- Here, we estimated phenotypic selection on ontogenetic change in photosynthesis (*A*) and stomatal conductance (g_s) of *Avena barbata*. We tested whether ontogenetic changes in *A* and g_s increased fitness in wet and dry soil environments. To determine whether evolution in response to this selection would be constrained, we estimated the heritability of ontogenetic change in physiology, as well as cross-environment genetic correlations.
- Ontogenetic change in *A*, but not g_s, was adaptive in the wet soil environment; plants that maintained or increased *A* from the prereproductive to the reproductive phase had higher fitness. In the dry soil environment, ontogenetic change in *A* and g_s was adaptively neutral. We detected significant genetic variation for ontogenetic change in *A* and g_s, but no cross-environment genetic correlations, suggesting that the evolution of these traits would not be genetically constrained.
- We demonstrate that ontogenetic changes in physiological traits can increase fitness but the adaptive value of these changes varies among traits and environments.

Introduction

Physiological and morphological changes during growth and development occur in all organisms but are especially common in plants because their growth is modular (Goebel, 1900; Allsopp, 1967; Jones, 1999). Although most studies have focused on morphological changes during development (Goebel, 1900; Ashby, 1948; Allsopp, 1967; Jones, 1999), physiology can also change with ontogeny (Jones, 2001; Kubien *et al.*, 2007). For example, a common pattern in angiosperms is for photosynthetic rate to increase with age, size or with the transition from the prereproductive to reproductive phase of the life cycle (Bauer & Bauer, 1980; Knapp & Fahnestock, 1990; Donovan & Ehleringer, 1991; Maherali *et al.*, 1997; Cavender-Bares & Bazzaz, 2000; Bond, 2000; Zotz *et al.*, 2001; Thomas & Winner, 2002). These temporal shifts in morphology and physiology within a single individual can be caused by programmed development (Allsopp, 1967), plasticity in response to a spatially or temporally dynamic environment (Bell & Sultan, 1999) or a combination of development and environment (Pigliucci, 1998). Within-individual variation in traits primarily caused by programmed development has been referred to as heteroblasty (Poethig, 1990). By contrast, within-individual variation in traits primarily caused by responses to a dynamic environment has been referred to as phenotypic plasticity (Winn, 1999). Regardless of the mechanism, within-individual temporal variation (hereafter ontogenetic change) in traits has been hypothesized to improve plant function and thus be adaptive (Jones, 1999). Here we focus on testing whether programmed ontogenetic change is adaptive.

The adaptive significance of ontogenetic change has rarely been tested by using phenotypic selection analysis (Lande & Arnold, 1983) to link variation in the magnitude and direction of ontogenetic change with variation in fitness (Winn, 1999). The three studies that have measured phenotypic selection (Winn, 1999; van Kleunen *et al.*, 2007; Picotte *et al.*, 2007) suggest that ontogenetic change in most traits and environments is adaptively neutral. However, van Kleunen *et al.* (2007)

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found that an ontogenetic decrease in internode length when plants were moved from flooded to nonflooded conditions increased fitness. In addition, Picotte *et al.* (2007) found that an ontogenetic decrease in leaf size and increase in trichome density as soil moisture declined increased plant fitness. In all of these studies, ontogenetic changes were attributed primarily to plasticity in response to a temporally dynamic environment. The adaptive value of ontogenetic change that is caused primarily by programmed development has not been tested. In addition, previous tests of the adaptive value of ontogenetic change have focused on morphological rather than physiological traits. Although phenotypic selection has been estimated for physiological traits (Dudley, 1996a; Caruso *et al.*, 2006; Sherrard & Maherali, 2006; Donovan *et al.*, 2007; Saldaña *et al.*, 2007), approx. 80% of selection studies in plants have focused on morphological, vegetative and life-history traits (Geber & Griffen, 2003).

If ontogenetic changes in physiology or morphology are adaptive, then evolution in response to selection may be genetically constrained in at least two ways. First, evolution can be constrained by a lack of genetic variation. Although significant genetic variation has been detected for ontogenetic changes in leaf morphology (Winn, 1996a), there are no estimates of heritable variation for changes in physiology with ontogeny. Because heritabilities for gas exchange physiology are generally lower than those for morphological traits (Geber & Griffen, 2003), the evolution of ontogenetic change in physiology may be more likely to be genetically constrained. Second, evolution in response to selection can be constrained if the same genes contribute to ontogenetic change in different environments (cross-environment genetic correlation; Via, 1984). Significant cross-environment genetic correlations would cause ontogenetic changes in one environment to evolve indirectly in response to selection in the other environment. If selection differs between environments, then this indirect response could result in adaptation to one environment constraining adaptation to another environment. Cross-environment genetic correlations for ontogenetic changes in morphology are weak (Winn, 1996a), but these correlations have not been estimated for ontogenetic changes in physiology.

We used recombinant inbred lines (RILs) of the Mediterranean annual grass *Avena barbata* to determine the adaptive significance of, and genetic variation for, programmed ontogenetic change in physiological traits. These RILs were derived from a cross of parental mesic and xeric ecotypes from California, which differ in quantitative traits associated with drought adaptation (Hamrick & Allard, 1975; Latta *et al.*, 2004). Because of this evolutionary history, we examined ontogenetic change in physiology in both wet and dry soil environments. Unlike other studies that have measured selection on ontogenetic change (Winn, 1999; van Kleunen *et al.*, 2007; Picotte *et al.*, 2007), we used constant watering treatments. This ensured that any changes in physiology from the prereproductive to reproductive phase were primarily caused by programmed development, rather by plasticity in response to a temporally dynamic environment (Winn, 1996b). We predicted that photosynthesis and stomatal opening would increase as individuals transitioned from the prereproductive to reproductive phase of the life cycle and that this ontogenetic pattern would be adaptive in both wet and dry soil environments. To test these predictions, we estimated the strength of phenotypic selection on ontogenetic changes in photosynthesis and stomatal opening of *A. barbata* growing in wet and dry soil environments. To determine whether evolution in response to this selection would be genetically constrained, we also estimated heritabilities and cross-environment genetic correlations.

Materials and Methods

Study species

Avena barbata Pott. ex. Link is a European annual grass that has invaded the Mediterranean region in the southwestern USA since its accidental introduction over 200 yr ago (Garcia *et al.*, 1989). Because *A. barbata* is highly selfing (> 95%), each parental ecotype is a genetically homogeneous monomorphic lineage (Johansen-Morris & Latta, 2006; Latta *et al.*, 2007). The RILs used in this study were created by crossing the xeric and mesic ecotypes, followed by one generation of selfing that produced 188 F_2 families. The F_2 families were then selfed for four generations through single-seed descent, producing true breeding hybrids with different combinations of the parental alleles. These RILs mimic a population of progeny from a hybridization event between the mesic and xeric ecotypes in regions where they overlap (Gardner & Latta, 2006). Because there is little quantitative genetic variation within each ecotype (Gardner & Latta, 2008), these RILs should capture the range of genetic variation found in *A. barbata*.

Experimental design and data collection

As part of a larger study of the evolution of physiological traits (Sherrard & Maherali, 2006; Sherrard *et al.*, 2009), we grew 26 RILs that were selected to match the range and frequency distribution of glasshouse fitness of all 188 lines (Gardner & Latta, 2006). The mean fitness of this subsample was similar to the mean fitness of all 188 lines and included RILs in both the 5th and 95th percentiles of the glasshouse fitness distribution. To allow sufficient time for physiological measurements at each life stage, we grew plants in a randomized complete block design with four temporal blocks of 52 plants (eight plants in each of 26 RILs; n = 208). To create the temporal blocks, four groups of seeds were germinated 12 d 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

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(Premier Tech, Riviere de Loupe, PQ, Canada) and placed on a glasshouse bench.

After 21 d, when seedlings had their first true leaves, four plants from each RIL were randomly assigned to a wet or dry soil environment. Plants in the wet soil environment were watered daily to saturation. Plants in the dry soil environment were provided with 50 ml of water every 2 d, which is the equivalent of 132 mm of precipitation for the duration of the treatment (5.5 mm wk^{-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 69 mm less than any growing season at the mesic site since 1953 (Sherrard & Maherali, 2006). Volumetric water content (VWC) of the soil was measured using a moisture probe (Hydrosense CD620; Campbell Scientific, Edmonton, AB, Canada). When we began gas exchange measurements on day 70, mean VWC (1 SD) was 31.1 (9.9)% for the wet soil environment and 3.17 (0.62)% for the dry soil environment. We did not measure VWC of soils in either treatment after plants were 70 d old, but VWC in the dry soil environment likely declined as plants grew. By contrast, we expect that VWC remained constant in the wet soil environment because soils were watered to saturation each day.

Plants in a dry soil environment can be nutrient stressed relative to plants in a wet soil environment because of limits on the soluble uptake of minerals (Marschner, 1995; Donovan *et al.*, 2007). As a result, we fertilized all plants to ensure that the watering treatment did not cause differences in nutrient limitation. Every 2 wk, plants were fertilized with 100 ml of 20%–20%–20% NPK fertilizer (Plant Products Inc., Brampton, ON, Canada) at a concentration of 2.5 g l^{-1}. The fertilizer also contained the micronutrients boron (B) (0.02%), copper (Cu) (0.05%), iron (Fe) (0.1%), manganese (Mn) (0.05%), molybdenum (Mo) (0.0005%) and zinc (Zn) (0.05%). Providing the same level of nutrients to all plants regardless of size could have caused large plants to have lower nutrient concentrations than small plants, potentially affecting covariances between physiological traits and fitness. However, although there was significant variation in plant biomass and fitness among RILs, biomass and fitness were not correlated with leaf nutrient concentration in either soil environment (Sherrard *et al.*, 2009). Plants were provided with supplemental light from high-intensity discharge lamps to maintain the photoperiod at 16 h of light per day.

Trait and fitness measurements

Photosynthetic rate (A) and transpiration rate (E) were measured using an open gas-exchange system (LI-6400; Li-Cor Inc.). Steady-state leaf gas exchange was measured under approximately ambient CO_2 concentration (400 µl l^{-1}) between 08:30 and 12:30 h EST. On days when physiology was measured, watering was delayed until after measurements were completed. Plants within each block were measured in random order each day. During measurements, incident irradiance was provided by red-blue light-emitting diodes and cuvette leaf-to-air vapor pressure deficit (D) was maintained at 1.9–2.0 kPa to reflect prevailing ambient conditions. Leaf temperature was held at near-ambient conditions (*c.* 26°C) with a Peltier cooling module. We used saturating light (1500 µmol m^{-2} s^{-1}) for all gas exchange measurements to ensure they would not be biased by daily light fluctuations, and that measurements of photosynthetic capacity would not be limited by suboptimal light. The saturating light levels used were determined from the light response curves of experimental plants (M. E. Sherrard & H. Maherali, unpublished) and were similar to values experienced by *A. barbata* plants growing in the field (e.g. 1000–1500 µmol m^{-2} s^{-1}; Jackson *et al.*, 1995). 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 based on fan speed and leaf area using the energy balance algorithms of the LI-6400. The first set of gas exchange measurements was made on the youngest fully expanded leaf blade on the vegetative tiller 70 d after germination (49 d after the treatments were initiated), just before flowering. We made a second set of instantaneous gas exchange measurements 110 d after germination, when plants had flowered. To test whether ontogenetic changes in gas exchange differed between tissue types, we made the second set of measurements on leaf blades attached to both vegetative tillers and the flowering culm. By always measuring the youngest fully expanded leaf we ensured that gas exchange measurements were made on the same age of leaf on vegetative and reproductive tissue.

We ended the experiment 165 d 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. We then estimated total seed number as total spikelet number × 2 × proportion of unaborted seeds. In addition, all aboveground biomass was harvested for each individual. The vegetative tissue (nonflowering tillers) was dried to a constant mass (48 h at 65°C) and weighed to estimate vegetative biomass. However, total seed number was used as the estimate of fitness for all selection analyses.

Statistical analysis

A repeated-measures ANOVA was used to test whether there were significant ontogenetic changes in A and g_s. Because these two traits were measured over time (before and at reproduction) and on two tissue types at reproduction (vegetative tillers and flowering culms), we analysed four trajectories of ontogenetic change in each watering treatment. Photosynthesis or g_s before and at reproduction (hereafter ontogenetic change, OC) was the within-subjects factor. Recombinant inbred line (L) and block (B) were the between-subjects factors. A significant OC or OC × L term indicated that A or g_s changed

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with ontogeny. We ran separate ANOVA models for plants in the wet and dry soil environments. We present test statistics and *P*-values for the within-subjects term and for within-subject × between-subject terms from a multivariate repeated measures analysis. The multivariate approach was used because it makes fewer assumptions about data distributions than the univariate repeated measures analysis (Stevens, 1992).

Directional phenotypic selection gradients on the magnitude of ontogenetic changes in physiology were estimated separately within each watering treatment. Because ontogenetic change is a property of the individual, it can be described by the slope of the within-individual reaction norm across the time interval between measurements (Winn, 1996a). We regressed fitness on the initial (prereproductive) estimate of A or g_s (as in Picotte *et al.*, 2007) and on an estimate of the magnitude of the ontogenetic change for each plant (n = 101–104). By including the initial estimate of A or g_s in the model, we could separate the adaptive value of the initial value of each trait from the adaptive value of ontogenetic change in that trait (Picotte *et al.*, 2007). We estimated the magnitude of ontogenetic change by subtracting A or g_s before reproduction from A or g_s at reproduction. Because measurements were made at the same two ontogenetic time-points for all plants, this value represents the slope of the temporal reaction norm. When calculated in this way, a significant positive regression coefficient indicates that plants which maintained or increased A or g_s over time had higher fitness. For all analyses, we relativized fitness by dividing by mean fitness (Lande & Arnold, 1983) and standardized each trait to mean = 0 and variance = 1 (Sokal & Rohlf, 1995). Block was included as a fixed factor. We also estimated nonlinear selection on ontogenetic changes in physiology, but these gradients were not significant for any trait and thus are not presented.

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 by calculating the Spearman rank correlation between the residuals and relative fitness (Neter *et al.*, 1989). All models met these assumptions. Using Cook's D (Neter *et al.*, 1989), we confirmed that none of the significant selection gradients detected (see Table 3) were driven by a few outliers. To control for multiple tests, the α of all regressions was adjusted within each treatment using the sequential Bonferroni correction by the Dunn–Sidak method (Sokal & Rohlf, 1995). Because our sample size was *c.* 20% lower than the mean for phenotypic selection studies (Kingsolver *et al.*, 2001), we also performed a *post hoc* power (1-β) analysis for the selection gradients using SPSS. A statistical test for which 1-β is ≥ 0.80 is considered to have adequate power to reject the null hypothesis (Whitlock & Schluter, 2009).

The genetic variation for the magnitude of ontogenetic change in A and g_s was estimated by calculating 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 *A. barbata* inbred lines are 96.75% homozygous (Latta *et al.*, 2007), variation among individuals within each line is caused almost entirely by random environmental effects and the intraclass correlation is equivalent to the broad-sense heritability (H^2) (Falconer & McKay, 1996). The H^2 estimates all possible genetic contributions to phenotypic variation in a trait, including additive genetic, dominance, epistatic, and maternal effects (Lynch & Walsh, 1998). However, dominance effects can be discounted because RILs are homozygous at each locus. In addition, RILs were grown in a common environment in the generation before our experiment, which should minimize variation in maternal effects across lines. Terms for the intraclass correlation were calculated using the mean squares from an ANOVA with genetic line (RIL) and block as terms. The mean squares error (MSe) term is equivalent to the within-line environmental variance (σ_e^2). The mean squares for genetic line (MSg) is equivalent to $\sigma_e^2 + n\sigma_g^2$, where n is the number of individuals per genotype (Lynch & Walsh, 1998). Thus, to calculate H^2 using the intraclass correlation, σ_e^2 = MSe and $\sigma_g^2 = ((\text{MSg} - \text{MSe})/n)$. We used the *P*-value for the RIL term in the ANOVA to determine if H^2 for a trait was significant. If σ_g^2 for a trait was < 0, then we set H^2 for that trait to zero.

Cross-environment genetic correlations for ontogenetic change were estimated using the Pearson correlation coefficient among family means for each trait (Winn, 1996a). In this analysis, a significant positive correlation indicates that the genetic basis of ontogenetic change in A or g_s is the same in the wet and dry soil environments. These family mean correlations can be biased estimates of the genetic correlation because they include a component of environmental variance (Lynch & Walsh, 1998). However, family mean correlations have commonly been used to estimate genetic correlations (Geber & Dawson, 1997; Maherali *et al.*, 2008) because they are amenable to standard significance testing and straightforward to interpret.

Results

Changes in A and g_s with ontogeny

We detected significant ontogenetic changes in A and g_s within the wet soil environment (Table 1, Fig. 1). Photosynthesis measured on vegetative tillers at reproduction was 23% lower than A measured on the same tissue type before reproduction (Fig. 1a). Similarly, A measured on the flowering culm was 19% lower than A measured before reproduction (Fig. 1c). In contrast to A, g_s changed significantly with ontogeny only when comparing the prereproductive value with that measured on the flowering culm. Stomatal conductance of leaves on the flowering culm was 41% higher than g_s of leaves on prereproductive tillers (Fig. 1g). Mean (1 SE) vegetative biomass for plants in the wet soil environment was 23.92 g (1.7) and mean (1 SE) seed production of these plants was 806.13 (61.4).

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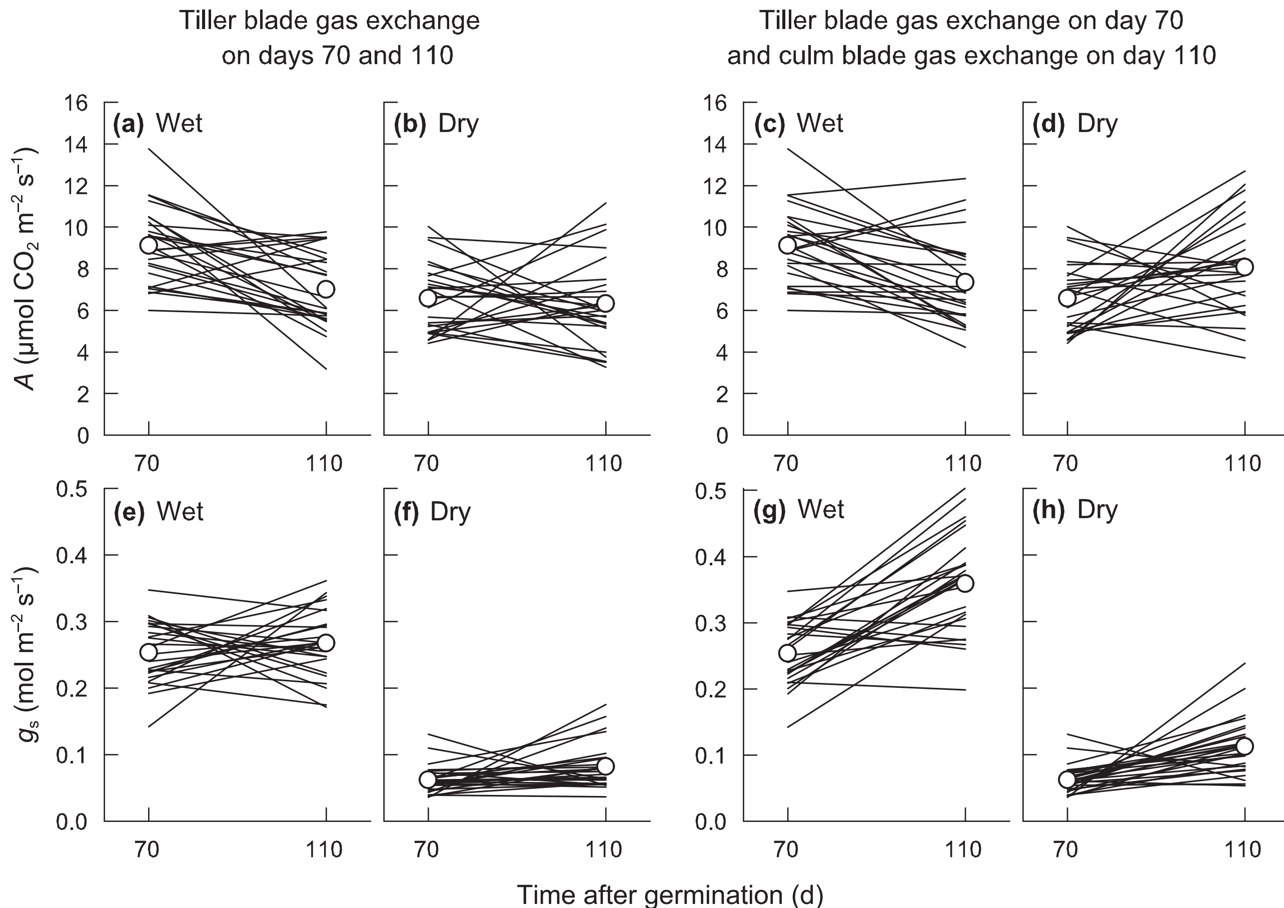

Fig. 1 Ontogenetic changes in leaf blade photosynthesis (*A*) and stomatal conductance (g_s) of 26 *Avena barbata* recombinant inbred lines grown in wet (a,c,e,g) and dry (b,d,f,h) soil environments. (a,b) Change in *A* between leaves on vegetative tillers produced at prereproductive (Day 70) and reproductive (Day 110) life history stages. (c,d) Change in *A* between leaves on vegetative tillers produced at the prereproductive life-history stage and leaves on the flowering culm. (e,f) Change in g_s between leaves on vegetative tillers produced at prereproductive and reproductive life-history stages. (g,h) Change in g_s between leaves on vegetative tillers produced at the prereproductive life history stage and leaves on the flowering culm. Open circles are means for each trait at each time point.

Table 1 Ontogenetic changes in photosynthesis (*A*; μmol CO_2 m^{-2} s^{-1}) and stomatal conductance (g_s; mol m^{-2} s^{-1}) of *Avena barbata* recombinant inbred lines grown in a wet soil environment

	Trait							
	Day 70 tiller *A* to Day 110 tiller *A*		Day 70 tiller *A* to Day 110 flowering culm *A*		Day 70 tiller g_s to Day 110 tiller g_s		Day 70 tiller g_s to Day 110 flowering culm g_s	
Term	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
OC	28.761	< 0.001	31.754	< 0.001	0.779	0.380	39.194	< 0.001
OC × L	1.551	0.075	1.755	0.033	0.930	0.565	0.941	0.551
L	1.386	0.141	1.159	0.306	0.626	0.905	0.980	0.503
B	13.835	< 0.001	12.050	< 0.001	2.155	0.100	2.069	0.112

Data were analysed using repeated-measures ANOVA. Ontogenetic change (OC; prereproductive (day 70) vs reproductive (day 110)) was the within-subjects factor. Genetic line (L) was the between-subjects factors. An additional between-subjects factor (B) was included to account for variation among temporal blocks. A significant ($P < 0.05$) effect of OC and/or OC × L indicates that there was significant ontogenetic change in *A* or g_s. $df_{numerator}$, $df_{demoninator}$ for OC = 1, 151–152. $df_{numerator}$, $df_{demoninator}$ for OC × L and L = 25, 151–152. $df_{numerator}$, $df_{demoninator}$ for *B* = 3, 151–152.

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Table 2 Ontogenetic changes in photosynthesis (A; µmol CO_2 m^{-2} s^{-1}) and stomatal conductance (g_s; mol m^{-2} s^{-1}) of *Avena barbata* recombinant inbred lines grown in a dry soil environment

	Trait							
	Day 70 tiller A to Day 110 tiller A		Day 70 tiller A to Day 110 flowering culm A		Day 70 tiller g_s to Day 110 tiller g_s		Day 70 tiller g_s to Day 110 flowering culm g_s	
Term	F	P	F	P	F	P	F	P
OC	0.389	0.535	18.337	< 0.001	10.773	0.002	52.798	< 0.001
OC × L	1.753	0.034	2.951	< 0.001	2.080	0.008	2.292	0.003
L	1.425	0.124	1.553	0.075	1.631	0.056	1.450	0.112
B	0.896	0.447	3.134	0.031	3.292	0.025	4.346	0.007

Data were analysed using repeated-measures ANOVA. Ontogenetic change (OC; prereproductive (day 70) vs. reproductive (day 110)) was the within-subjects factor. Genetic line (L) was the between-subjects factors. An additional between-subjects factor (B) was included to account for variation among temporal blocks. A significant ($P < 0.05$) effect of OC and/or OC × L indicates that there was significant ontogenetic change in A or g_s. $df_{numerator}$, $df_{demoninator}$ for OC = 1, 151–152. $df_{numerator}$, $df_{demoninator}$ for OC × L and L = 25, 151–152. $df_{numerator}$, $df_{demoninator}$ for B = 3, 151–152.

Table 3 Directional selection gradients on ontogenetic changes in photosynthesis (A; µmol CO_2 m^{-2} s^{-1}) and stomatal conductance (g_s; mol m^{-2} s^{-1}) of *Avena barbata* exposed to wet and dry soil environments

	Wet soil environment				Dry soil environment			
Trait	β	SE	P	Power	β	SE	P	Power
Day 110 tiller A – day 70 tiller A	0.313	0.100	0.002†	0.872	–0.033	0.060	0.587	0.084
Day 110 tiller A – day 70 flowering culm A	0.259	0.073	0.001†	0.942	–0.005	0.062	0.938	0.051
Day 110 tiller g_s – day 70 tiller g_s	0.115	0.105	0.276	0.192	–0.027	0.057	0.635	0.076
Day 110 tiller g_s – day 70 flowering culm g_s	0.010	0.085	0.905	0.052	0.031	0.055	0.576	0.086

Fitness was regressed on initial A or g_s and the magnitude of the ontogenetic change in that trait. An additional term was included to control for variation among temporal blocks. A significant positive regression coefficient (β) indicates that individuals which maintained or increased A or g_s with age had higher fitness, and thus that ontogenetic change was adaptive. Power (1-β) was estimated *post hoc*. n = 101–104. †Remained significant after sequential Bonferroni correction by the Dunn–Sidak method.

We also detected significant ontogenetic changes in A and g_s within the dry soil environment (Table 2, Fig. 1). Photosynthesis measured on the flowering culm was 22% higher than A measured before reproduction (Fig. 1d). However, A measured on vegetative tillers at reproduction did not differ from A measured on the same tissue type before reproduction (Fig. 1b). In contrast to A, ontogenetic changes in g_s were significant for both vegetative tillers and the flowering culm. Stomatal conductance measured on vegetative tillers at reproduction was 30% higher than g_s measured on the same tissue type before reproduction (Fig. 1f), whereas g_s measured on the flowering culm was 78% higher than before reproduction (Fig. 1 h). Mean (1 SE) vegetative biomass for plants in the dry soil environment was 7.46 g (0.49) and mean (1 SE) seed production for these plants was 523.57 (31.9).

The magnitude and or/direction of ontogenetic changes in A and g_s varied among RILs more frequently within the dry than the wet soil environment (significant OC × L term; Tables 1, 2). Within the wet soil environment, only ontogenetic change in A varied among lines (Table 1, Fig. 1a,c). By contrast, we detected significant OC × L terms for all four ontogenetic changes within the dry soil environment (Table 2, Fig. 1b,d,f,h).

Adaptive value of ontogenetic changes in physiology

Ontogenetic changes in physiology were adaptive in *A. barbata*, but only within the wet soil environment (Table 3). In this treatment, plants that increased or maintained A from the prereproductive to the reproductive phase had higher fitness, regardless of whether the transition was measured on vegetative tillers or on the flowering culm. By contrast, ontogenetic changes in g_s were adaptively neutral within the wet soil environment. Within the dry soil environment, ontogenetic changes in both A and g_s were adaptively neutral. Power (1-β) for the strong selection gradients (> 0.25) on ontogenetic

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Table 4 Broad-sense heritabilities (H^2) and cross-environment genetic correlations for ontogenetic changes in photosynthesis (A; μmol CO_2 m^{-2} s^{-1}) and stomatal conductance (g_s; mol m^{-2} s^{-1}) of *Avena barbata* exposed to wet and dry soil environments

	Wet		Dry			
Trait	H^2	P	H^2	P	Cross-environment r	P
Day 110 tiller A – day 70 tiller A	0.121	0.075	0.158	0.034	0.132	0.522
Day 110 tiller A – day 70 flowering culm A	0.159	0.033	0.328	< 0.001	0.017	0.935
Day 110 tiller g_s – day 70 tiller g_s	0	> 0.05	0.213	0.008	–0.033	0.871
Day 110 tiller g_s – day 70 flowering culm g_s	0	> 0.05	0.244	0.003	–0.022	0.991

Broad-sense heritabilities were estimated from mean squares of ANOVA models with line and block terms included (see text for details). Cross-environment genetic correlations were estimated as the Pearson correlation coefficient between family mean ontogenetic changes for the same trait in wet and dry soil environments. n = 101–104 for H^2 and 26 for r.

change in A within the wet soil environment was > 0.80. However, power for the weak selection gradients (*c.* 0) within the dry soil environment was < 0.10.

Genetic correlations and broad-sense heritabilities

Ontogenetic changes in A and g_s of *A. barbata* were heritable, but not to the same degree in both soil environments (Table 4). In the dry soil environment, H^2 for all traits were > 0 and statistically significant. In the wet soil environment, by contrast, we detected significant H^2 only for ontogenetic change in A. Ontogenetic changes in physiology were not genetically correlated across wet and dry soil environments for any trait (Table 4).

Discussion

We found that increasing or maintaining A with ontogeny was adaptive for *A. barbata* growing in a wet soil environment, regardless of the tissue type measured (Table 3). Although phenotypic changes with ontogeny are common in plants (Jones, 2001), the few studies that have directly tested whether they are adaptive have had conflicting results (Winn, 1999; Picotte *et al.*, 2007; van Kleunen *et al.*, 2007). Unlike these previous studies, we maintained plants in constant moisture environments to isolate ontogenetic change caused by programmed development. We also measured physiological rather than morphological traits and focused on an annual rather than a perennial plant. Nevertheless, our results complement those of Picotte *et al.* (2007) and van Kleunen *et al.* (2007) by suggesting that ontogenetic changes caused by programmed development, in addition to those caused by phenotypic plasticity, are adaptive.

There are two potential explanations for why programmed ontogenetic change in A and seed production covary in annual plants such as *A. barbata*. First, because annual plants allocate 15–30% of their lifetime net carbon gain to reproduction (Mooney, 1972), the increased carbon sink demands associated with reproduction could cause an increase in A from the pre-reproductive to reproductive phases (Hirose *et al.*, 2005). Second, the biochemical and photochemical upregulation of photosynthesis at reproduction could cause seed production to increase (Arntz *et al.*, 2000), suggesting that ontogenetic change in A is adaptive. Our previous work with *A. barbata* RILs indicates that increasing carbon sink demand from seed production is unlikely to be responsible for the ontogenetic changes in A that we observed. Specifically, experimental defoliation reduced seed production of *A. barbata* by about 50%, but had no effect on maximum photosynthetic light-use efficiency (Suwa & Maherali, 2008). Consequently, ontogenetic change in A in the wet soil environment likely caused increased seed production in *A. barbata* and thus is adaptive.

Because ontogenetic change in A is adaptive only for *A. barbata* RILs growing in a wet soil environment, we would predict that the mesic ecotype is more likely than xeric ecotype to maintain or increase A with ontogeny in a mesic environment. Although we have not measured A for field-grown mesic and xeric ecotypes, we have measured gas exchange on prereproductive and reproductive leaf blades on these ecotypes in a wet soil environment in the glasshouse. For both ecotypes ontogenetic change in A was negative, but the decline was less pronounced in the mesic (–16%) than the xeric (–23%) ecotype (Sherrard *et al.*, 2009). This result is consistent with selection to increase A with ontogeny in mesic field environments.

Three possible mechanisms could explain how some individuals were able to increase A as they shifted from the prereproductive to reproductive phase of the life cycle. First, leaves produced at reproduction may be denser and thus have more photosynthetic machinery per unit surface area (higher leaf mass per unit area (LMA); Ellsworth & Reich, 1992; Thomas & Winner, 2002). Second, leaves produced at reproduction could have higher ribulose 1,5-bisphosphate (RuBP) carboxylase-oxygenase (rubisco) activity and nitrogen concentration (Field & Mooney, 1986). Third, leaves produced at reproduction could have higher g_s, which would reduce both resistance to CO_2 diffusion into the leaf and CO_2 limitations on photosynthesis (Wong *et al.*, 1979). Of these

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three possibilities, we have direct evidence only for increased g_s on *A. barbata* leaves produced at reproduction (Fig. 1 g). However, ontogenetic change in g_s was not adaptive in *A. barbata*, suggesting that it is unlikely to explain the adaptive ontogenetic change in A that we observed in the wet treatment. Data from other species indicate that LMA, rubisco activity, and leaf N are higher in leaves produced later in the life cycle (Bauer & Bauer, 1980; Carey *et al.*, 1998; Gunn *et al.*, 1999; Thomas & Winner, 2002; Juárez-López *et al.*, 2008). Consequently, ontogenetic changes in leaf density, leaf N and photosynthetic biochemistry may be the most likely explanations for ontogenetic increases in A in some *A. barbata* individuals.

There are three possible explanations for why ontogenetic changes in gas exchange physiology were not adaptive in the dry soil environment. First, the opportunity for selection (Arnold & Wade, 1984) could have been lower in the dry soil environment. However, our previous studies indicate that the opportunity for selection on physiology is similar for *A. barbata* growing in wet and dry soil environments (Sherrard & Maherali, 2006), suggesting that this explanation is unlikely. Second, the absence of selection in the dry soil environment could be a consequence of the life history of Mediterranean annuals such as *A. barbata*. Because these plants complete their life cycle before the onset of the summer drought (Volis *et al.*, 2002), flowering phenology may be a more important adaptation to drought than ontogenetic changes in physiology. Consistent with this explanation, there is strong selection for early flowering of *A. barbata* growing in dry soil conditions, but no selection on gas exchange (Sherrard & Maherali, 2006). Third, we exposed plants to constant, sustained drought, which represented an extreme dry year that *A. barbata* could experience in the field. However, plants at xeric sites in California typically experience a period of relatively high soil moisture during winter, followed by an intense spring drought that coincides with the timing of reproduction (Hamrick & Allard, 1975). It is possible that increased A with ontogeny would be adaptive in an environment with fine-grained temporal variation in soil water availability (Picotte *et al.*, 2007).

Ontogenetic changes in both A and g_s were heritable within the dry soil environment, but only changes in A were heritable in the wet soil environment (Table 4). This difference in the number of traits for which we detected significant heritability supports the hypothesis that environmental stress increases the expression of additive genetic variation for physiology. Specifically, our results suggest that phenotypic differences between genetic lines become more apparent when resources, such as soil water, are limiting (Hoffmann & Merilä, 1999). Given that estimates of both phenotypic selection (Table 3) and heritability (Table 4) for changes in A within the wet soil environment were significant, the adaptive evolution of ontogenetic change in leaf physiology of *A. barbata* is unlikely to be constrained by a lack of genetic variation. However, we cannot rule out the possibility that the evolution of ontogenetic change in A is constrained by genetic correlations with other traits (Sherrard *et al.*, 2009). Because there are no other published estimates of genetic variation for ontogenetic change in physiology, we cannot determine whether the heritabilities we report are representative. However, the H^2 values that we present are similar to those estimated for gas exchange traits in other species such as *Brassica campestris*, (Evans, 1991), *Cakile edentula* (Dudley, 1996b) and *Polygonum arenastrum* (Geber & Dawson, 1997). Our results thus support recent reviews suggesting that heritabilities for plant physiological traits are low to moderate (Arntz & Delph, 2001; Geber & Griffen, 2003).

Like Winn (1996a), we found that cross-environment genetic correlations for ontogenetic changes in leaf traits were not significant (Table 4). The lack of significant correlations suggests that the adaptive evolution of ontogenetic changes in physiology in a wet soil environment is unlikely to constrain adaptation to a dry soil environment and vice versa. In addition, the nonsignificant cross-environment genetic correlations imply that the mechanisms responsible for ontogenetic changes in leaf physiology of *A. barbata* differ between wet and dry soil environments. Ontogenetic shifts in A in the wet soil environment may be caused primarily by changes in rubisco biochemistry and leaf N (Field & Mooney, 1986), whereas shifts in A in the dry soil environment could be caused primarily by changes in stomatal behavior (Wong *et al.*, 1979; Cavender-Bares & Bazzaz, 2000). In support of this hypothesis, we found that A and g_s of *A. barbata* were genetically correlated only when growing in a dry soil environment, whereas A and leaf N were genetically correlated only when plants were growing in a wet soil environment (Sherrard *et al.*, 2009).

Using RILs to study the evolution of ontogenetic changes in physiology has advantages and disadvantages. One advantage is that hybridization and recombination recovers variation that was purged by selection (reviewed in Arnold, 1997). If past selection has eliminated genotypes that express maladaptive phenotypes, then it will be difficult to detect significant selection on a trait even if it does have adaptive significance (Schluter, 1988). In *A. barbata*, the range of phenotypic variation within the experimental population of RILs is greater than within either the mesic or xeric ecotype (Latta *et al.*, 2007; Maherali *et al.*, 2008), increasing statistical power to test whether ontogenetic changes in traits are adaptive. In support of this expectation, one of the few other studies that detected significant selection on the magnitude of ontogenetic change in leaf traits also used recombinant progeny (Picotte *et al.*, 2007). One disadvantage of using RILs is that they do not necessarily reflect patterns of phenotypic or genetic variation in natural populations. Because *A. barbata* is a highly selfing species and mesic and xeric ecotypes are often spatially segregated, hybridization between the ecotypes is rare (Johansen-Morris & Latta, 2006). However, our results suggest that when hybridization events do occur in regions where

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the ecotypes overlap (Latta *et al*., 2007), ontogenetic change in physiology may contribute to the success of recombinant *A. barbata* within more mesic environments.

We demonstrated that ontogenetic change in physiology can be adaptive in *A. barbata*. Individuals that maintained or increased *A* from the prereproductive to reproductive phase of the life cycle had higher fitness when growing in a wet soil environment. Ontogenetic changes in physiology and morphology, whether caused by programmed development, plasticity in response to a temporally dynamic environment or their interaction, are both common and often assumed to be adaptive (Winn, 1999; Pigliucci, 2001; Picotte *et al*., 2007). Contrary to this assumption, we did not find that ontogenetic change in g_s was adaptive in *A. barbata*. Consequently, our work highlights the importance of testing adaptive hypotheses about ontogenetic change in physiological and morphological traits in plants.

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