MECHANISMS AND CONSEQUENCES OF WATER STRESS–INDUCED PARENTAL EFFECTS IN AN INVASIVE ANNUAL GRASS

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Premise of research.   Tests of the adaptive value of parental effects have generally focused on offspring fitness. However, the evolution of parental effects depends on their consequences for both offspring and parental fitness. Whether parental effects are adaptive can also depend on the mechanism of these effects. Parental effects caused by differences in the quality rather than quantity of resources provisioned to offspring may be more likely to be adaptive because they can persist through the life cycle.

Methodology.   We estimated parental effects in response to water stress in the invasive annual *Avena barbata*. To test whether these effects were adaptive, we reciprocally transplanted offspring of wet- and dry-grown parents into wet and dry environments. We also tested whether seed size and nitrogen content, which represent the quantity and quality of parental investment, were mechanisms of parental effects in *A. barbata*.

Pivotal results.   We found evidence of parental effects in response to water stress in *A. barbata*; dry-grown parents produced offspring with significantly higher germination, longer radicles, and earlier emergence than wet-grown parents. The offspring of dry-grown parents had higher biomass and seed production than the offspring of wet-grown parents. However, when cumulative fitness was calculated across parental and offspring generations, dry-grown parents had significantly lower fitness than wet-grown parents because of trade-offs between seed size and number. Although dry-grown parents provisioned their offspring with more nitrogen than wet-grown parents, offspring performance was primarily explained by variation in seed mass.

Conclusions.   Water stress–induced parental effects were adaptive from the offspring but not the parental perspective, suggesting that the evolution of these effects may be constrained. In addition, water stress–induced parental effects were primarily caused by differences in seed mass, suggesting that the quantity of resources provisioned to offspring is a more important mechanism of parental effects than resource quality.

Keywords: adaptation, *Avena barbata*, parental effects, seed mass, seed nitrogen content, water stress.

Introduction

A parental effect is any offspring phenotype that is a consequence of the parental environment rather than or in addition to the parental genotype (Rossiter 1996; Lacey 1998). These effects are ubiquitous in plants (Roach and Wulff 1987) and animals (Mousseau and Fox 1998). The evolution of parental effects will depend on their consequences for both offspring and parental fitness (Donohue and Schmitt 1998; Marshall and Uller 2007). If parental effects enable parents to increase their own fitness by increasing offspring fitness (Jacobs and Lesmeister 2012), then these effects can be considered adaptive from both the offspring and the parental perspective. Alternatively, parental effects can increase offspring fitness but not parental fitness (reviewed in Uller 2008). For example, a parental effect that increases offspring size can increase offspring fitness but may decrease parental fitness because of a trade-off between offspring size and number (Donohue and

Schmitt 1998). If parental effects are adaptive from the offspring perspective but not from the parental perspective, then the evolution of adaptive parental effects may be constrained. Despite this potential for constraint, tests of whether parental effects are adaptive have generally focused exclusively on offspring fitness (Agrawal 2001; Rotem et al. 2003; Galloway and Etterson 2007; Sultan et al. 2009; Herman et al. 2012). The focus on offspring fitness has made it difficult to predict the conditions under which adaptive parental effects will evolve (Marshall and Uller 2007).

Whether parental effects are adaptive may also depend on the mechanisms of these effects. In particular, parents can differ in both the quantity and the quality of resources provisioned to their offspring (Roach and Wulff 1987). Although parental effects caused by differences in the quantity of resources provisioned are common (Roach and Wulff 1987), they often do not persist through the life cycle (Smart and Moser 1999; Lopez et al. 2003; Rotem et al. 2003; but see Stratton 1989). However, some parental effects increase in magnitude through the life cycle (Alexander and Wulff 1985; Sultan et al. 2009), suggesting that differences in the quality of resources provisioned to offspring may also contribute to the expression of these effects. If parental effects caused by differences in resource quality are persistent, then they may be more likely to

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be adaptive because they can increase both the early survival and the later reproductive output of offspring.

In plants, parental effects caused by differences in seed nitrogen content are particularly likely to increase the early survival of offspring and their later reproductive output. Seed nitrogen content can increase early survival because it is a major component of the enzymes that initiate seedling development (Bewley and Black 1994). Because nitrogen content in developing seedlings influences the synthesis of chlorophyll and Rubisco (Field and Mooney 1986), it could also have persistent effects on leaf photosynthesis (Donovan et al. 2009) and thus reproductive output later in life (Arntz et al. 2000). Although seed nitrogen provisioning is strongly influenced by the parental environment, it has rarely been examined as a mechanism of parental effects (Parrish and Bazzaz 1985; Hrdlickova et al. 2011). If seed nitrogen content and seed mass are positively correlated (Parrish and Bazzaz 1985), then parental effects attributed to differences in seed size may in part reflect differences in seed nitrogen.

We studied parental effects in response to water stress in the annual grass *Avena barbata*, a common invader of Mediterranean climate regions (Corbin and D'Antonio 2004; Standish et al. 2008). In Mediterranean environments, rainfall varies widely from year to year, but water limitation during the growing season is common (Loik et al. 2004; Latta 2009). In *A. barbata*, water limitation can reduce biomass by 69% and seed production by 35% (Sherrard and Maherali 2006). Such negative effects of water stress on performance suggest that parental effects could promote the persistence of *A. barbata* in both established and recently invaded habitats (Dyer et al. 2010). Seed mass and nitrogen content may be particularly important mechanisms of water stress–induced parental effects in *A. barbata* because these traits could allow seedlings to accelerate development and establish before the onset of drought (Wright and Westoby 1999; Padilla and Pugnaire 2007). Although water availability has direct effects on the quantity and quality of resources provisioned to seeds (Smiciklas et al. 1990; Gooding et al. 2003), tests of adaptive parental effects in response to water stress are rare (Sultan et al. 2009; Herman et al. 2012).

To examine whether water stress–induced parental effects are adaptive in *A. barbata*, we obtained seeds from a population of recombinant inbred lines (RILs) and their parental genotypes that were grown under contrasting wet and dry conditions (Sherrard and Maherali 2006). We germinated these seeds in a common moisture environment and then reciprocally transplanted them into wet and dry environments. We used our data to answer three questions: (1) Are there parental effects in response to water stress? (2) Do parental effects in response to water stress increase offspring and parental fitness? (3) Are seed mass and seed nitrogen content mechanisms of these parental effects?

Methods

Study Species

Avena barbata (Poaceae) is a winter annual grass that has invaded California's coastal range since its introduction from Europe >200 yr ago (Garcia et al. 1989). In California, *A. barbata* consists primarily of two morphologically distinct genotypes (Latta 2009). These genotypes were historically considered to be adapted to contrasting xeric and mesic soil moisture conditions (Allard et al. 1972; Garcia et al. 1989), but recent reciprocal transplant experiments indicate that they do not represent ecotypes (Latta 2009). Nonetheless, the mesic and xeric genotypes differ in a suite of quantitative traits. For example, the mesic genotype flowers earlier, has higher biomass, produces more seeds, and is shorter in stature than the xeric genotype (Latta et al. 2004; Sherrard and Maherali 2006; Gardner and Latta 2008).

Avena barbata is primarily self-fertilizing (~98%; Gardner and Latta 2008), and genetic variation within mesic and xeric genotypes is negligible (Gardner and Latta 2008). Therefore, we used RILs derived from a cross of the mesic and xeric genotypes for our study. Because these RILs are genetically variable (Latta 2009), they allowed us to identify mechanisms underlying the expression of parental effects in response to water stress. RILs were created from an initial cross between a single individual of each genotype (parental lines; see Latta et al. 2004 for details). This cross produced F_1 progeny that were heterozygous at all loci that differed between the parental genotypes. A single F_1 individual was then self-fertilized to produce 188 F_2 individuals, each with a unique combination of alleles. The parental lines and all F_2 individuals were propagated through single-seed descent for five generations to create RILs that were 96.75% homozygous (Gardner and Latta 2008). *Avena barbata* RILs varied significantly in functional and performance traits, including seed production, biomass, photosynthetic rate, stomatal density, and flowering time (Sherrard et al. 2009). In contrast, there is little evidence of genetic variation among RILs in their response to water stress (nonsignificant line × watering treatment term; Sherrard et al. 2009). Because >90% of *A. barbata* found in California are either the mesic or xeric genotype (Garcia et al. 1989), these RILs capture the standing genetic variation in California populations of this species.

Experimental Design

We used seeds that were the offspring of *A. barbata* from a greenhouse experiment designed to examine the evolution of physiological and morphological traits in response to water limitation (for details, see Sherrard and Maherali 2006; Maherali et al. 2008, 2009, 2010; Sherrard et al. 2009). For this experiment, plants of 26 RILs and the two parental lines were grown in wet and dry soil moisture environments. We selected wet- and dry-grown seeds from 12 of the 26 RILs, plus the two parental lines, for our greenhouse experiment. These 12 RILs were not randomly selected but were instead chosen to capture the range in fitness of all 26 RILs averaged across soil moisture treatments (data not shown).

To determine whether parental effects were induced by water stress, we grew *A. barbata* seeds from wet and dry parental environments in a randomized complete block design. Fifty seeds from each combination of line and parental environment were germinated over two temporal blocks that were 1 wk apart in October 2009. We removed the palea and lemma and placed the seeds on moist filter paper in complete darkness at 4°C for 96 h. Seeds were then returned to room temperature
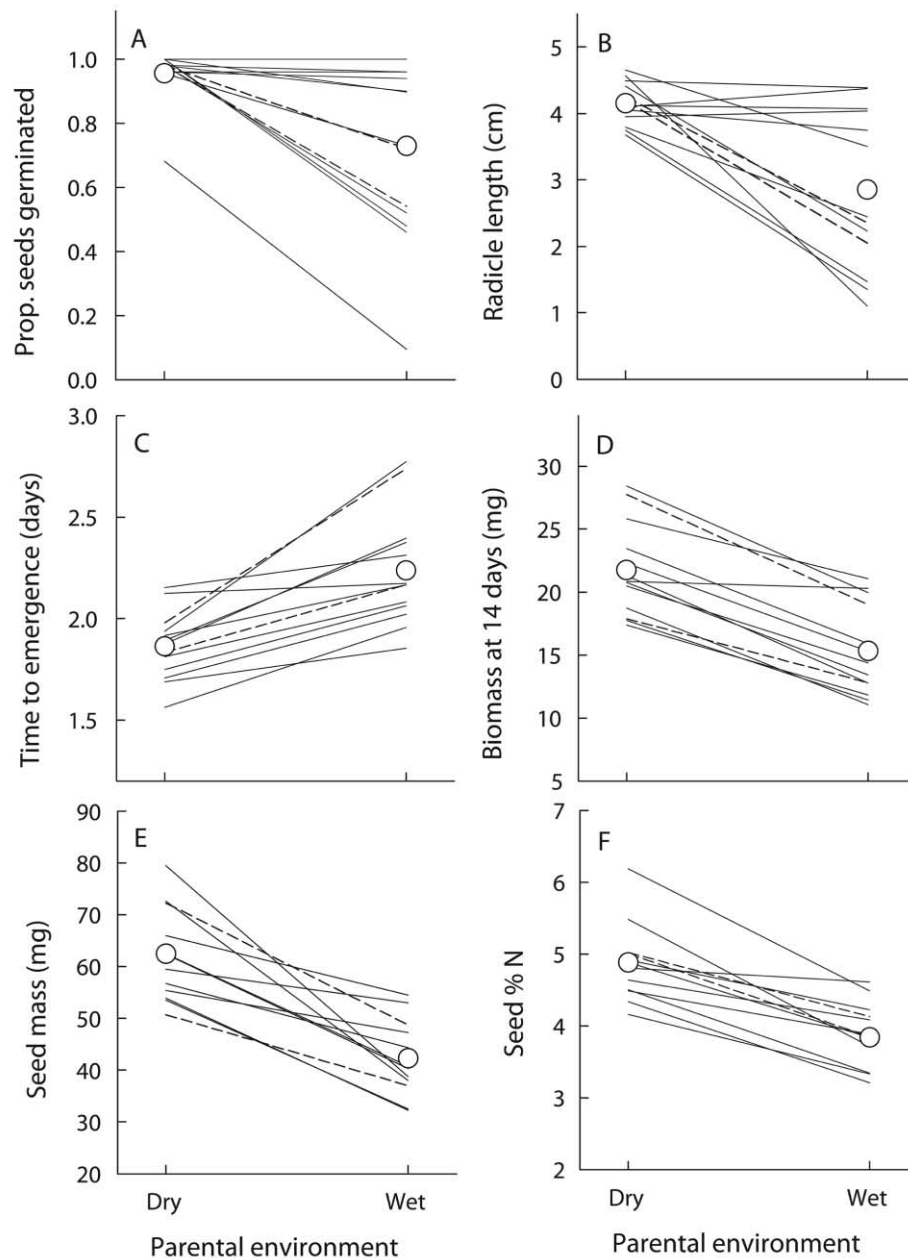
Fig. 1 Germination success (*A*), radicle length (*B*), emergence time (*C*), biomass at 14 d (*D*), seed mass (*E*), and seed % N (nitrogen content expressed as a percentage of seed mass; *F*) for genetic lines of *Avena barbata* from wet and dry parental environments. Reaction norms represent either a recombinant inbred (solid) or parental (dashed) line. Open circles indicate the trait average of each parental environment. $n = 13$–14 lines.

and kept in darkness for an additional 24 h. Because one RIL had poor germination, we transplanted 11 RILs and the two parental lines ($n = 624$). For each block, 12 germinants from each combination of line and parental environment were individually sown into 0.86-L pots filled with a 1 : 1 ratio of Pro-Mix BX and turface (Premier Tech, Rivière-du-Loup, Quebec). Pots were topped with a thin layer of peat moss to protect seedlings from desiccation. Each temporal block was then assigned to a different greenhouse bench in the phytotron at the

University of Guelph, Guelph, Ontario, Canada. Pots within each block were randomly assigned to a position on the bench. Supplemental high-intensity discharge lighting maintained a 16-h photoperiod, with daytime and nighttime temperatures of 23° and 17°C, respectively. Each pot was hand-watered to saturation twice a day.

After a 2-wk establishment period, seedlings from each combination of line and parental environment were randomly assigned to wet or dry offspring environments. By reciprocally

Table 1

Effect of Parental Moisture Environment, Genetic Line, and Their Interaction on Three Early Growth Traits of *Avena barbata*

Source of variation	df	F		
		Radicle length	Emergence time	Biomass at 14 d
Parental environment	1	120.677***	66.840***	111.277***
Genetic line	12	13.445***	5.999***	9.847***
Parental environment × genetic line	12	10.205***	1.984*	.984
Block	1	5.638*	76.717***	30.166***

Note. Traits were analyzed using a two-way ANOVA with parental moisture environment (wet vs. dry) and genetic line as main effects. A spatiotemporal block term was also included. Error df = 304 for radicle length, 589 for emergence time, and 180 for biomass at 14 d.

* $P < 0.05$.
*** $P < 0.001$.

transplanting seedlings into wet and dry environments, we could determine whether water stress–induced parental effects were adaptive in *A. barbata*. Plants in the wet treatment group initially received 100 mL of water twice a day through drip irrigation lines. This was later reduced to 50 mL twice a day to deter algal growth. Plants in the dry treatment group were hand-watered with 100 mL of water once a week. These quantities differed from the treatments that were administered to the parental generation (see Sherrard and Maherali 2006), but the effect of the watering treatment was similar between Sherrard and Maherali (2006) and this study in that water limitation significantly reduced plant biomass and seed production (see "Results"). Over the course of the experiment, we supplied each pot with three 100-mL applications of 50-ppm 18-9-18 NPK fertilizer (Plant Products, Brampton, Ontario). Plants in the dry treatment were not watered during weeks that fertilizer was applied. These watering treatments produced plants whose seed production (mean ± 1 SE = 94.3 ± 3.94 seeds per plant; range = 0–218) was within the range of field-grown *A. barbata* (Latta 2009).

Data Collection

To determine whether there were water stress–induced parental effects in *A. barbata*, we measured four traits during the establishment period: germination success, radicle length, time to emergence, and biomass at 14 d. We estimated early offspring survival as the germination success of samples of 25 seeds from each of the 14 lines (12 RILs plus two parental lines) × two parental environments × two blocks ($n = 56$). Germination success was estimated as the proportion of seeds with a radicle protruding through the seed coat after 24 h at room temperature. We measured radicle length, emergence time, and biomass at 14 d for plants from each of the 13 lines that had adequate germination. We measured radicle length, an early indicator of competitive ability (Harris and Wilson 1970), on 2–7 (mean = 6.0) haphazardly selected 4-d-old germinants per combination of genetic line and block ($n = 331$). For each seed that was planted, we recorded the amount of time that it took for the first leaf to emerge because faster-emerging plants are often better competitors (Howell 1981). Fourteen days after planting, we harvested one-third of the plants in each combination of line and parental environment ($n = 207$) to estimate early performance. These plants were dried to a constant mass at 60°C for 48 h and weighed.

To determine whether water stress–induced parental effects were adaptive in *A. barbata*, we measured three fitness correlates: aboveground biomass at 57 d, final aboveground biomass, and seed production. Fifty-seven days after planting, we harvested the aboveground tissue of half of the plants remaining in each of the 13 lines × two parental environments × two offspring environments ($n = 207$). The remaining plants ($n = 198$) were harvested at senescence or at 186 d after planting, whichever came first. All plants were dried at 60°C for 48 h and weighed. Prior to harvest, we used the number of spikelets to estimate the seed production of plants that had flowered ($n = 203$). Each *A. barbata* spikelet contains two seeds that drop from the plant as it senesces, leaving behind a glume that can be counted (Latta et al. 2004).

We measured seed mass and seed nitrogen content to test whether these traits were mechanisms of water stress–induced parental effects in *A. barbata*. We measured seeds from the same 11 RILs and two parental lines that we grew in the greenhouse. For each of these 13 lines, we haphazardly sampled 10 seeds from all combinations of the two parental environments × four temporal blocks used by Sherrard and Maherali (2006). Each sample of 10 seeds ($n = 99$) was dried to constant mass at 60°C for 48 h and weighed. Weights were divided by 10 to calculate mass per seed for each sample. We combusted seeds in an elemental analyzer (VarioMAX CN Macro Elemental Analyzer; Elementar Analysensysteme, Hanae, Germany) to determine how much nitrogen they contained, expressed as a percentage of seed mass (seed % N).

To test whether any differences in seed % N persisted to later life stages, we measured leaf chlorophyll concentration, a proxy for leaf nitrogen content (Chapman and Barreto 1997; Suwa and Maherali 2008) and a correlate of photosynthetic capacity (Evans 1989). Leaf chlorophyll concentration was measured for all plants that had not been harvested as of 45 d after planting ($n = 414$). We used a portable chlorophyll meter (SPAD 502; Minolta, Ramsey, NJ) to estimate the mean chlorophyll concentration of three haphazardly selected fully expanded leaves per plant.

Statistical Analysis

We used ANOVA and paired *t*-tests to determine whether parental effects were induced by water stress in *A. barbata*. To test for parental effects on radicle length, emergence time, and aboveground biomass at 14 d, we used ANOVA with line,
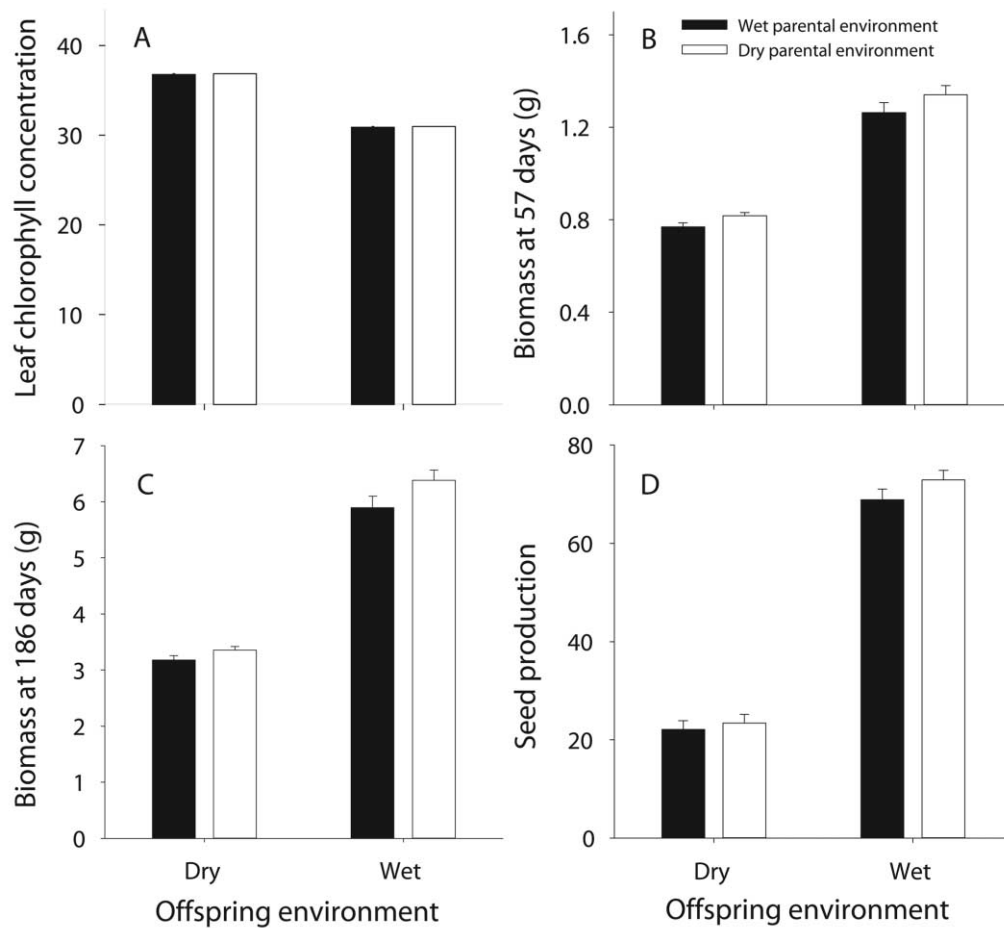
Fig. 2 Mean (± 1 SE) leaf chlorophyll concentration (*A*), biomass at 57 d (*B*), biomass at 186 d (*C*), and seed production (*D*) of *Avena barbata* from four combinations of parental and offspring soil moisture environments.

parental environment, and block as fixed factors. We assumed that there were no interactions between block and the other main effects. We treated lines as fixed effects because the RILs were not a random sample of the 26 RILs used by Sherrard and Maherali (2006). A significant parental environment effect indicates that there were water stress–induced parental effects for that trait. We used paired *t*-tests to determine whether there were significant parental effects on germination success. We paired by genetic line and pooled across the two temporal blocks.

We also used ANOVA to test whether water stress–induced parental effects were adaptive in the offspring of *A. barbata*. To test for adaptation, we analyzed three fitness correlates: aboveground biomass at 57 d, aboveground biomass at final harvest, and seed production. We analyzed biomass at both 57 d and final harvest because aboveground biomass prior to reproduction is correlated with survival in *A. barbata*, whereas final aboveground biomass is correlated with seed production (Latta and McCain 2009). The ANOVA model included line, parental environment, offspring environment, and block as fixed factors. We assumed that there were no interactions between block and the other main effects.

To test whether water stress–induced parental effects in *A. barbata* were adaptive from the parental perspective, we calculated the cumulative fitness of each RIL as the product of parental and offspring seed production (Donohue and Schmitt 1998; Marshall and Uller 2007). Parental seed production was estimated from Sherrard and Maherali (2006), and offspring seed production was estimated from our current greenhouse experiment. Because parental and offspring fitness were measured in different experiments, we relativized fitness by dividing by mean fitness within each experiment. We then used these estimates to calculate cumulative fitness in each of the four combinations of parental and offspring environments (wet parent/wet offspring, wet parent/dry offspring, dry parent/wet offspring, and dry parent/dry offspring). We analyzed the cumulative fitness data with an ANOVA model that included parental environment, offspring environment, their interaction, and genetic line as fixed factors.

Two different results could indicate that water stress–induced parental effects were adaptive in *A. barbata*. First, offspring of dry-grown *A. barbata* parents could have high relative fitness only when grown in a dry environment (adaptive matching; Moran 1992), resulting in a significant parental
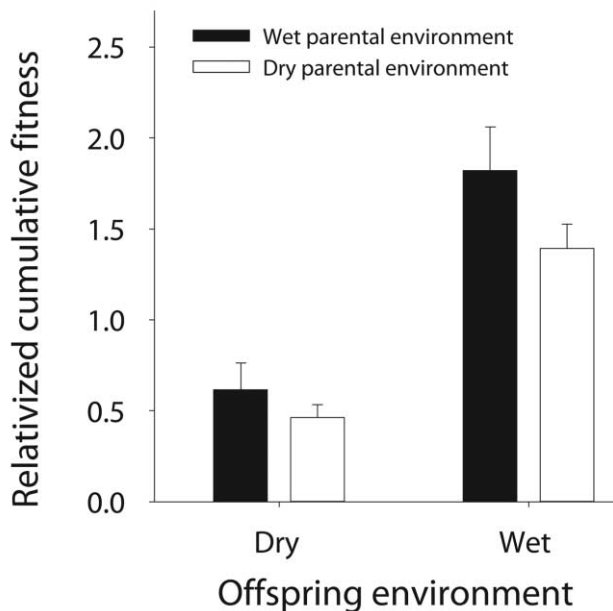
Fig. 3 Mean (± 1 SE) cumulative fitness in *Avena barbata* from four combinations of parental and offspring soil moisture environments.

environment × offspring environment effect. Second, offspring of dry-grown *A. barbata* parents could have high relative fitness when grown in both wet and dry environments, resulting in a significant parental environment effect (see Donohue and Schmitt 1998 for a similar interpretation). The difference between these two results is whether adaptation to one environment reduces relative fitness in an alternative environment (i.e., a fitness trade-off; Hereford 2009). Although studies often assume that maternal effects are adaptive only when parents and offspring are grown in the same environment (Marshall and Uller 2007; Burgess and Marshall 2011), a re-

cent review of the local adaptation literature suggests that such trade-offs are not ubiquitous (Hereford 2009).

We used ANOVA to test whether two traits that were potential mechanisms of water stress–induced parental effects responded to the parental moisture environment. To test whether seed mass and seed % N differed between offspring of wet- and dry-grown parents, we used ANOVA with line, parental environment, and parental block as fixed factors. To test whether any differences in seed nitrogen persisted to later stages of the life cycle, we analyzed leaf chlorophyll concentration using an ANOVA model that also included the offspring environment as a fixed factor. For all analyses, we assumed that there were no interactions between block and the other main effects. If these traits are mechanisms of water stress–induced parental effects, then we predict that seed mass and seed % N will be higher in offspring of dry-grown parents relative to that in offspring of wet-grown parents. If effects of seed nitrogen on performance persist to later stages of the life cycle, then we predict that leaf chlorophyll concentration will also be higher in offspring of dry-grown parents.

We used multiple regression to test whether seed mass and seed % N were mechanisms of water stress–induced parental effects in *A. barbata*. We tested whether seed mass and seed % N influenced four seedling traits: germination success, radicle length, emergence time, and aboveground biomass at 14 d. These analyses were performed on the genotypic means from each parental environment. We pooled across parental environments because there was no interaction between the effects of seed mass or seed % N and the parental environment for any seedling trait (ANCOVA, all $P > 0.10$). Multicollinearity was low, as indicated by variance inflation factors <10.0 (Neter et al. 1989). We initially hypothesized that leaf chlorophyll concentration would be correlated with seed % N, but we did not analyze this trait because the parental environment term from the ANOVA was not significant (table 2).

We used Levene's test and visually inspected residual plots to determine whether the data met the homogeneity of error variance and normality assumptions required for analysis

Table 2

Effects of Parental Moisture Environment, Offspring Moisture Environment (for Leaf Chlorophyll Only), Genetic Line, and Their Interaction on the Seed Mass, Seed % N (Nitrogen Content Expressed as a Percentage of Seed Mass), and Leaf Chlorophyll Concentration of *Avena barbata*

	Seed mass		Seed % N		Leaf chlorophyll	
Source of variation	df	F	df	F	df	F
Parental environment	1	122.877***	1	22.476***	1	.138
Offspring environment	...	...	...	...	1	323.044***
Genetic line	12	3.468***	12	3.378***	12	8.662***
Parental environment × genetic line	12	.701	12	.668	12	1.112
Offspring environment × genetic line	...	...	...	...	1	1.018
Parental environment × offspring environment	...	...	...	...	12	.001
Parental environment × offspring environment × genetic line	...	...	...	...	12	.316
Block	3	2.989*	3	3.033*	1	73.819***

Note.   Traits were analyzed using two- and three-way ANOVAs with parental moisture environment (wet vs. dry), offspring moisture environment (wet vs. dry), and genetic line as main effects. A spatiotemporal block term was also included. Ellipses indicate that traits were measured prior to the initiation of the offspring moisture environment. Error df = 70 for seed mass and seed % N and 414 for leaf chlorophyll concentration.

   * $P < 0.05$.
   *** $P < 0.001$.

Table 3

Effect of Seed Mass and Seed % N (Nitrogen Content Expressed as a Percentage of Seed Mass) on Four Early Growth Traits of *Avena barbata*

Response variable	Seed mass ($\beta \pm 1$ SE)	Seed % N ($\beta \pm 1$ SE)	R^2
Germination success	$-6.84 \times 10^{-4} \pm 3.29 \times 10^{-3}$	$9.09 \times 10^{-3} \pm 0.07$	.01
Radicle length	$.04 \pm .02*$	$.63 \pm .29*$	.57
Emergence date	$-.02 \pm 4.52 \times 10^{-3}**$	$-.03 \pm .09$	.50
Biomass at 14 d	$.26 \pm .07**$	$.26 \pm 1.29$	.50

Note. Relationships were analyzed using multiple regression analyses with seed mass and seed % N included as predictor variables for each seedling trait, pooled across parental moisture treatments. $n = 24$–25.

* $P < 0.05$.

** $P < 0.01$.

(Kuehl 2000). Data that failed Levene's test ($P < 0.05$; radicle length, emergence date, aboveground biomass at 57 and 186 d, and cumulative fitness) were $\log_{10} x + 1$ transformed and reanalyzed. With one exception, the transformed data still failed Levene's test. Consequently, we used nonparametric Mann-Whitney U-tests to confirm the ANOVAs. We focused on checking the offspring environment terms because there was more variation in the wet than the dry offspring environment. The nonparametric tests (data not shown) confirmed the results of the ANOVAs on the untransformed data, and thus we report these ANOVAs. All analyses were done using PASW (ver. 17.0; SPSS, Chicago, IL).

Results

We detected significant water stress–induced parental effects on all four early growth traits of *Avena barbata*. Germination success of seeds produced by dry-grown parents was 35.3% greater after 24 h ($t_{12} = 3.90$, $P = 0.002$; fig. 1*A*) and 20.5% greater after 48 h ($t_{12} = 2.99$, $P = 0.011$) than that of seeds produced by wet-grown parents. Parents grown in dry environments produced offspring with 39.5% longer radicles than offspring of parents grown in wet environments (table 1; fig. 1*B*). Offspring of parents grown in dry environments emerged from the soil 16.6% (or 0.37 d) earlier (fig. 1*C*) and had 42.2%

higher biomass after 14 d (fig. 1*D*) than offspring of parents grown in wet environments (table 1). In addition to parental environment effects, we detected significant variation among *A. barbata* lines in radicle length, emergence time, and biomass at 14 d (table 1). We also detected significant line × parental environment effects for radicle length and emergence time (table 1).

The parental environment had significant effects on both offspring and cumulative (e.g., parental × offspring) fitness, but the direction of these effects differed (tables 4, 5). Offspring of dry-grown parents had higher biomass and marginally higher seed set than offspring of wet-grown parents, regardless of the offspring growth environment (significant parental environment term and nonsignificant parental environment × offspring environment term; table 4). Within the dry offspring environment, offspring of dry-grown parents had 6.1% greater biomass at 57 d, had 5.4% greater biomass at 186 d, and produced 5.9% more seeds than offspring of wet-grown parents (fig. 2*B*–2*D*). Within the wet offspring environment, offspring of dry-grown parents had 6.2% greater biomass at 57 d, had 8.4% greater biomass at 186 d, and produced 5.7% more seeds than offspring of wet-grown parents (fig. 2*B*–2*D*). In contrast to offspring fitness, wet-grown parents had higher cumulative fitness than dry-grown parents, regardless of offspring growth environment (ta-

Table 4

Effects of the Parental Moisture Environment, Offspring Moisture Environment, Genetic Line, and Their Interaction on Three Fitness Correlates of *Avena barbata*

Source of variation	df	Biomass at 57 d	Biomass at 186 d	Seed production
Parental environment	1	4.903*	9.484**	2.848
Offspring environment	1	334.771***	813.503***	1084.074***
Genetic line	12	2.928**	4.551***	8.822***
Parental environment × genetic line	12	.773	.857	1.111
Offspring environment × genetic line	12	1.279	4.337***	5.466***
Parental environment × offspring environment	1	.279	2.593	.950
Parental environment × offspring environment × genetic line	12	.616	.874	1.169
Block	1	38.376***	108.714***	1.044***

Note. Traits were analyzed using a three-way ANOVA with parental moisture environment (wet vs. dry), offspring moisture environment (wet vs. dry), and genetic line as main effects. A spatiotemporal block term was also included. Error df = 154 for biomass at 57 d, 145 for biomass at 186 d, and 150 for seed production.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

Table 5

Effects of the Parental Moisture Environment, Offspring Moisture Environment, Genetic Line, and Their Interaction on the Cumulative Fitness of *Avena barbata*

Source of variation	df	F
Parental environment	1	6.299*
Offspring environment	1	85.436***
Parental environment × offspring environment	1	1.423
Genetic line	12	4.740***

Note.   Cumulative fitness was analyzed using a two-way ANOVA with parental moisture environment (wet vs. dry) and offspring moisture environment (wet vs. dry) as main effects. Genetic line was also included as a covariate. Error df = 36.

* $P < 0.05$.

*** $P < 0.001$.

ble 5). Specifically, wet-grown parents produced 39.5% more seeds than dry-grown parents (fig. 3).

The offspring environment also had significant effects on both offspring and cumulative fitness (tables 4, 5). Offspring grown in the dry environment had 46.9% less biomass at 186 d (fig. 2C), produced 67.9% fewer seeds (fig. 2D), and had 66.5% lower cumulative fitness (fig. 3) than offspring grown in the wet environment. In addition to the offspring environment effects, we detected significant variation among *A. barbata* lines in biomass at 57 d, biomass at 186 d, seed production, and cumulative fitness (tables 4, 5). We also detected significant line × offspring environment effects for biomass at 186 d and seed production (table 4).

The parental moisture environment influenced both seed mass and seed % N of *A. barbata* but not leaf chlorophyll concentration (table 2). Seeds of dry-grown parents were 47.4% heavier and contained 10.2% more nitrogen than seeds of parents grown in wet environments (fig. 1E, 1F). Despite the effect of the parental growth environment on seed % N, there was no difference in chlorophyll concentration in the leaves of plants from contrasting parental moisture environments in either offspring moisture environment (nonsignificant parental environment and parental × offspring environment terms; table 2). However, chlorophyll concentration was significantly higher in the dry offspring environment than in the wet offspring environment (table 2; fig. 2A). Seed mass, seed % N, and leaf chlorophyll concentration all varied significantly among lines, but none of the interactions between line and environment were significant (table 2).

Seed mass and seed % N were significant predictors of parental effects on early growth traits of *A. barbata* (table 3). Heavier seeds produced seedlings that emerged earlier, had longer radicles, and had higher biomass at 14 d. In contrast, seeds with higher % N produced seedlings with longer radicles, but % N did not have a significant effect on emergence time or biomass at 14 d. Neither seed mass nor seed % N had a significant effect on germination success.

Discussion

Our results suggest that parental effects in response to water stress occur in *Avena barbata* but that their adaptive value is not consistent between the offspring and parental generations.

Offspring of dry-grown parents outperformed offspring of wet-grown parents in both wet and dry environments (fig. 2), suggesting that water stress–induced parental effects were adaptive from the offspring perspective. However, dry-grown parents had lower cumulative fitness than wet-grown parents in both wet and dry offspring environments (fig. 3), suggesting that water stress–induced parental effects were not adaptive from the parental perspective. Although dry-grown parents produced 53.3% heavier seeds (fig. 1E), they had lower cumulative fitness because they produced 32.9% fewer seeds than wet-grown parents ($F_{1,165} = 22.21$, $P < 0.001$). This trade-off between seed size and number eliminated the fitness advantage of water stress–induced parental effects that we observed in the offspring generation. Consequently, our results support the hypothesis that a trade-off between offspring size and number reduces the adaptive value of parental effects (Donohue and Schmitt 1998; Uller 2008). Our results also suggest that the adaptive evolution of parental effects is particularly likely to be constrained in species where there is a trade-off between offspring size and number.

Our results support the hypothesis that parental effects, like other forms of plasticity, are not a negative consequence of resource limitation (Roach and Wulff 1987; Donohue and Schmitt 1998; Caruso et al. 2006; Sultan et al. 2009; Dyer et al. 2010; Herman et al. 2012). If resource limitation was the primary cause of water stress–induced parental effects in *A. barbata*, then offspring of dry-grown parents should have performed more poorly than offspring of wet-grown parents. Instead, we found that offspring of dry-grown parents consistently outperformed offspring of wet-grown parents (figs. 1D and 2B–2D). Our experiment adds to a growing body of evidence that stressful parental growth conditions can have beneficial effects on offspring performance (Sultan et al. 2009; Dyer et al. 2010; Latzel et al. 2010; Herman et al. 2012). However, more studies are needed to determine whether these parental effects are consistent across species that differ in their ecological breadth (Sultan et al. 2009) and reproductive mode (Latzel and Klimesova 2010).

Contrary to our hypothesis, we found that the quantity of resources provisioned contributed more to water stress–induced parental effects in *A. barbata* than the quality of resources provisioned. Dry-grown parents produced heavier seeds (fig. 1E) that had longer radicles, emerged earlier, and accumulated greater seedling biomass after 14 d of growth (table 3). Although seed % N also increased in response to water stress (fig. 1F), it explained less variation in offspring traits and had no effect on biomass after 14 d of growth (table 3). Seed mass could have an even stronger effect on seedling performance in field environments, because intra- and interspecific competition increases the severity of resource limitation (Stratton 1989). Consequently, our estimate of the importance of seed mass as a mechanism of parental effects is likely to be conservative relative to field-grown *A. barbata*.

Although parental effects caused by propagule size are often considered a short-term strategy to offset environmental stress during early development (Smart and Moser 1999; Lopez et al. 2003), water stress–induced parental effects caused by seed size were persistent in *A. barbata*. The offspring of dry-grown parents had significantly higher biomass at maturity and produced marginally more seeds than offspring of wet-grown par-

ents (fig. 2*B*–2*D*). Because offspring with higher seed nitrogen content did not have higher leaf chlorophyll concentration, this result is best explained by the early growth advantage provided by increased seed mass in the offspring of dry-grown parents (table 3). Such fitness benefits likely accrue from the influence of seed mass on resource acquisition traits, such as root length (table 3; Sultan et al. 2009). Persistent parental effects in response to water stress are significant because they allow offspring to elicit a greater phenotypic response than would be possible through plasticity alone (Sultan et al. 2009). This strategy is particularly likely to be adaptive in Mediterranean climates because water becomes more limiting in late spring, when annual plants such as *A. barbata* mature seed (Hamrick and Allard 1975; Sherrard and Maherali 2006).

One limitation of our experiment was that it took place in a greenhouse environment, and thus we cannot rule out the possibility that the adaptive value of water stress–induced parental effects differs for field-grown *A. barbata*. However, the fitness of greenhouse-grown plants in this experiment (fig. 2*D*) was within the range of that observed in the field (Latta 2009). In addition, seedling growth is a primary determinant of variation in fitness among field-grown *A. barbata* (Latta and McCain 2009). As such, the water stress–induced parental effects that we observed on seedling growth traits (table 1) could have even greater effects on the fitness of *A. barbata* in the field, where drought can occur shortly after germination.

More generally, our results have implications for the role of parental effects in the success of biological invasions. If we had focused solely on the offspring generation, then we would have concluded that the enhanced performance of seeds from dry-grown *A. barbata* can preadapt progeny to conditions en-countered following dispersal (Fox and Savalli 2000; Dyer et al. 2010) and thus contribute to the successful invasion of *A. barbata* in Mediterranean climates (Corbin and D'Antonio 2004; Standish et al. 2008). However, the trade-off between offspring size and number resulted in water stress–induced parental effects that were not adaptive from the parental perspective, suggesting that these parental effects are unlikely to increase invasion success in *A. barbata*. Consequently, future studies of parental effects in invasive species should focus on evaluating whether parental effects that increase offspring fitness also increase parental fitness. If our findings are generalizable to other invasive species that share life history characteristics with *A. barbata*, then local adaptation and plasticity are likely to be more important determinants of successful invasion (reviewed in Sakai et al. 2001; Lee 2002) than adaptive parental effects.

Acknowledgments

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QUERIES TO THE AUTHOR

q1. Au: Is "turface" a generic term (and so should be lowercase), or is it a trademarked name (and so should be capitalized)?

q2. Au: In table 3, correct to edit the P value criterion for two asterisks to read "$P < 0.01$" (not $P < 0.05$)?

q3. Au: Okay to edit to read "higher seed nitrogen content"?

q4. Au: Is the title of Latta 2009 okay as edited?