Fitness effects of a photosynthetic mutation across contrasting environments

A. M. ARNTZ,* E. H. DELUCIA† & N. JORDAN‡

*Department of Biology, Indiana University, Bloomington, Indiana, USA
†Department of Plant Biology, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA
‡Department of Agronomy and Plant Genetics, University of Minnesota, St Paul, Minnesota, USA

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Abstract

To test the hypothesis that variation in photosynthesis can cause differences in fitness, we compared wild-type (WT) *Amaranthus hybridus* genotypes to those having a single-gene mutation (R) that affects photosynthetic rate. By using light and water treatments, we generated a range of differences between WT and R genotypes in photosynthetic rate, growth and reproduction at three developmental stages. In two cases photosynthetic differences were in the expected direction (WT > R), they did not differ in others, and in one case the R genotype had a higher rate than the WT. Across light and water treatments, higher rates of photosynthesis were related to increases in specific leaf area, leaf nitrogen content and stomatal conductance relative to the other genotype. Differences between genotypes in growth and allocation paralleled those in photosynthesis; in treatments where photosynthetic rate differed between the genotypes (high light), growth and reproduction did as well. In high light, the effects of genotype on fitness were indirect with high-water availability, but were direct with low-water availability. When photosynthetic rate did not differ between genotypes (low light), neither did growth and reproduction. These results demonstrate that variation in photosynthesis can cause differences in growth and reproduction. Furthermore, resource availability can moderate the ways in which selection operates on photosynthetic traits.

Introduction

Genetic variation for photosynthetic traits within plant species has been documented for nearly four decades (Mooney & Billings, 1961) and the potential contributions of this variation to evolutionary population biology has been appreciated (Mooney, 1976; McGraw & Wulff, 1983). Ecotypic variation (among populations) in photosynthetic physiology is perhaps the most commonly documented type of variation (Teeri, 1978; Teramura & Strain, 1979; Kalisz, 1986; Winn & Evans, 1991; Monson *et al.*, 1992; Nienhuis *et al.*, 1994; Dudley, 1996b; Sandquist & Ehleringer, 1997; Jonas & Geber, 1999), and is often used as evidence for local adaptation.

The evolution of physiological adaptations requires genetic variation within populations (among families), and for photosynthetic traits this type of variation also has been well documented (Zangerl & Bazzaz, 1983; Scheiner *et al.*, 1984; Geber & Dawson, 1990; Schuster *et al.*, 1992; Ehleringer, 1993; Donovan & Ehleringer, 1994; Teese, 1995; Geber & Dawson, 1997; Case *et al.*, 1998). Evidence for the evolution of physiological adaptations also includes linking physiological variation within populations to variation in plant performance, that is, some measure of phenotypic selection. Because photosynthesis is the fundamental process for acquiring carbon, variation in photosynthetic traits is predicted to affect growth and reproduction in natural populations (Mooney & Chiariello, 1984; Bloom *et al.*, 1985; Lambers & Poorter, 1992). However, studies of selection in natural

Correspondence: A. M. Arntz, Department of Biology, Indiana University, Bloomington, Illinois, USA.
Fax: +1 812 855 6705; e-mail: arntz@indiana.edu

populations are far less common than those documenting genetic variation.

Identifying the contributions of specific photosynthetic traits to reproduction can be complicated when photosynthetic traits that may affect fitness are correlated with other traits. Across and within species, phenotypic and genetic correlations among physiological, morphological and other traits do exist, and are thought to be associated with integrative plant function (Geber & Dawson, 1990; Dudley, 1996b; Ackerly & Reich, 1999; Reich *et al*., 1999). Resolving the contributions of individual traits from a set of correlated characters requires the control of multivariate statistical approaches.

One example is multivariate selection analysis, which determines whether selection is acting directly on trait, or indirectly via a correlated trait. This analysis partitions the relative contribution of each trait in a set of correlated traits to fitness. It provides a selection gradient which measures direct selection on each trait, and separates it from indirect selection via traits to which it is correlated (Lande & Arnold, 1983). Indirect effects can be estimated from selection gradients and correlation coefficients among traits using path analysis, another multivariate statistical technique (Arnold, 1983; Crespi & Bookstein, 1989; Kingsolver & Schemske, 1991). Farris & Lechowicz (1990) used path analysis to show that growth and photosynthetic traits primarily affect fitness indirectly through their influence on plant size during earlier stages of development. Variation in photosynthetic rate may also affect reproduction directly if photosynthate is partitioned from leaves immediately to seeds during the reproductive phase. This direct effect would exist regardless of variation in other traits, and can occur along with indirect effects. Multivariate analyses have been effective in demonstrating direct and indirect selection on photosynthetic traits in the few other cases where they have been used (Dudley, 1996a; Arntz *et al*., 1998).

Although multivariate statistical methods are powerful for identifying the traits that cause fitness differences, Wade & Kalisz (1990) argued that selection be studied with a reciprocity between the observational approach of multivariate selection analyses and a manipulative approach using experimentation. Dudley (1996a) used this approach to demonstrate selection for intermediate leaf size and higher water-use efficiency in dry but not in wet environments, supporting the hypothesis that efficient water use is adaptive in dry environments. In another example, Schmitt *et al*. (1995) used transgenic and mutant plants with disabled phytochrome responses to demonstrate the adaptive value of plasticity in response to shading. Genetic manipulations have the advantage of isolating effects of specific genes, and are increasingly being used to test evolutionary questions [see Schmitt (1999) and references therein].

In this study we test the hypothesis that variation in photosynthetic rate is responsible for differences in fitness and use an approach similar to those described above. We use wild-type (WT) and photosynthetic mutants (R) of *Amaranthus hybridus* L. (smooth pigweed) to generate variance in photosynthetic phenotype. These photosynthetic genotypes are well-characterized (see description in methods) and in our study system can produce up to a 30% lower photosynthetic rate in the R genotype compared with the WT. However, growth and reproductive differences between genotypes vary considerably with the year of study, population of origin, and environment (Jordan, 1996; Arntz *et al*., 1998; Jordan *et al*., 1999; Arntz *et al*., 2000). One explanation for this variation is heterogeneity in light availability; in this and other species WT and R genotypes in low light typically do not differ in photosynthetic rate. As irradiance increases, the WT has an increasingly greater photosynthetic rate than the R genotype (e.g. Dekker & Burmester, 1992). However, we found that the fitness reduction of the R genotype compared with the WT in a low light, competitive field environment was twice that in a high light, noncompetitive field environment (Arntz *et al*., 1998). This result suggests that differences in growth and reproduction may result from differences in photosynthesis caused by competition for other resources in addition to light.

Water availability may modify fitness differences between the genotypes because it differentially affects water-use efficiency of the WT and R genotype. In *Brassica napus* (Dekker & Burmester, 1992; A. Arntz, unpublished data) and *A. hybridus* (Arntz *et al*., 2000) (the same photosynthetic mutation occurs in both species), the lower photosynthetic rate of the R genotype is not accompanied by a reduction in stomatal conductance, and thus results in reduced water-use efficiency. Exaggerated reductions in growth and reproductive mass of R genotypes at increased planting densities or levels of neighbour interference (low-light and -water availability) in the greenhouse also provide indirect evidence that water availability may differentially affect the R genotype (Jordan, 1996).

To test the hypothesis that variation in photosynthesis produces variation in growth and reproduction, we created several levels of photosynthetic variation between the genotypes by manipulating light and water availability. We measured photosynthetic and growth traits of the two genotypes at two developmental stages (juvenile and pre-reproductive), and growth and reproductive mass (fitness) at the late-reproductive stage. We then determined if differences in growth and reproduction between genotypes scaled according to differences in photosynthetic rate. We used multivariate selection analysis to account for correlations among growth traits in order to determine whether photosynthetic genotype (a proxy for rate) directly increases reproductive success.

The R genotype was predicted to have a lower photosynthetic rate than the WT, and differences in growth and reproductive mass were expected to be in the same direction. Furthermore, we expected differences in photosynthetic rate to increase with higher light and lower water availability, and that environments producing the largest difference in photosynthesis between genotypes would show the largest differences in growth and reproductive mass.

We found that photosynthetic rates differed between the genotypes in the expected direction (WT > R) in two cases, but did not differ in others, and in one case the R genotype actually had a higher rate than the WT. Although photosynthetic differences between the genotypes were not always in the expected direction, they generally produced the predicted effects on growth and reproduction. In high-light treatments where photosynthetic rate differed between genotypes, growth and reproduction did as well. Photosynthetic rate did not differ between genotypes in low light, and neither did growth or reproduction.

Materials and methods

Study species

Amaranthus hybridus is an annual with C_4 photosynthetic metabolism (Weaver & McWilliams, 1980). The mutant genotype occurs naturally in agricultural populations and has a mutation, in the chloroplast genome, that alters a photosystem II electron transport protein that binds the electron carrier plastoquinone (Hirshberg & McIntosh, 1983). The mutation confers resistance to the herbicide atrazine (a plastoquinone analogue) by preventing its binding, and has evolved in many weedy species (Warwick, 1991). In the absence of atrazine, plastoquinone binds with reduced affinity, and rates of electron transport are reduced in R compared with WT genotypes (Ort *et al.*, 1983).

Seed was collected from 25 WT and 50 R individuals (representing families of siblings sharing the female parent) in separate populations in Blacksburg, Virginia. For R seed, a single agricultural field was sampled at 3.5 m intervals on transects laid every 20 m. Vegetable gardens within 3 km of this field were sampled for S seed. Eight to ten WT and R individuals were chosen randomly, paired and crossed to produce reciprocal F_1s. The F_2 families were produced by selfing the F_1s and F_3s families by selfing the F_2s. These crosses produced 8–10 reciprocal family lines with nearly uniform nuclear backgrounds but distinct WT or R chloroplast genomes (Jordan, 1996). We used seed from seven lines of each genotype. To the extent that the chloroplast genomes used in the initial reciprocal crosses did not have other mutations with major and consistent fitness effects, these families should accurately assess the fitness effects of the photosynthetic mutation. In this study we define fitness as total reproductive mass, which is highly correlated with total seed mass in this species ($r = 0.98$; Jordan, 1996).

Experimental design

A randomized complete block design with two levels of genotype, light and water availability was established in a greenhouse. Blocks were located in two adjacent greenhouse rooms. Within each block there were 25 plants of each genotype per treatment combination; the total sample size was 400 plants.

On a typical sunny day, instantaneous photosynthetically active photon flux density, measured on the greenhouse bench at mid-day, averaged 1085 ± 438 μmol m^{-2} s^{-1}. This irradiance was designated as the high-light treatment, and provided lower irradiances than full sun in the field (up to 2000 μmol m^{-2} s^{-1}) with a greater variance resulting from uneven shading by the structure of greenhouse. Shade cloth was used to provide an average irradiance of 503 ± 290 μmol m^{-2} s^{-1} for the low-light treatment. This treatment is approximately 25% of full sun in the field, is roughly equivalent to the leaf-area index of broad-leaved dicotyledonous community (Larcher, 1995) and approximates the leaf-area index measured for a 5-year-old field in a previous study (Arntz *et al.*, 1998). Seeds were germinated in these respective light treatments. At the two-leaf stage, seedlings (approximately equal numbers from each of seven families per genotype) were transplanted into 1.4-L pots with an equal mixture of soil and Strong-lite® Germination mix (Horticultural Products, Seneca, IL). Plants were rotated weekly within light treatments to achieve randomization. Two levels of water availability were provided by watering daily or on alternate days to field capacity, and were initiated following transplanting. Six weeks after transplanting, fertilizer was applied at half strength once per week (Peter's 20:20:20). Mid-day leaf water potentials (Ψ) measured with a pressure chamber (Soil Moisture Equipment Corporation, Santa Barbara, CA) were not different between light treatments but were significantly higher in the high-water treatment ($\Psi = -0.21 \pm 0.07$ MPa) than in the low-water treatment ($\Psi = -0.71 \pm 0.36$ MPa; two-way ANOVA, $P < 0.01$, $n = 10$). Assuming that the water potential of dry soil is –2.5 MPa, and a wilting point of –1.5 MPa (that of agricultural plants) (Larcher, 1995), plants in the high-water treatment would have experienced little or no water stress. The low-water treatment would have more closely approximated field conditions of moderate water stress.

Gas-exchange measurements

To determine the magnitude of differences in gas exchange between WT and R plants in the four treatments, photosynthetic and respiration rates were measured 1–2 weeks after the juvenile (5–6 weeks after

transplanting) and pre-reproductive (9–10 weeks after transplanting) stages. Measurements were made on the most recently expanded leaf of four to six plants per genotype using a portable gas-exchange system (LI-6400, LI-COR, Lincoln, NE). All plants were watered to field capacity at the time of measurement.

For photosynthetic measurements, light was supplied by an internal light-emitting diode at an irradiance of 450 μmol m^{-2} s^{-1} for the low-light treatments and 900 μmol m^{-2} s^{-1} for high-light treatments. These irradiances were chosen to best reflect the average ambient irradiances within the treatments. Measurements of photosynthetic response to a broader range of irradiances (0, 450, 900, 1350 and 1800 μmol m^{-2} s^{-1} for high- and low-light treatments at the juvenile stage and for high light at the pre-reproductive stage and 0, 225, 450, 675 and 900 μmol m^{-2} s^{-1} for low light at the pre-reproductive stage) showed that in most cases the selected irradiances were near maximal within each treatment (data not shown). Respiration was measured on the same leaf used for photosynthetic measurements, after reducing the irradiance to 0 for 5 min.

To interpret differences in photosynthetic rate between genotypes and treatments we measured attributes of leaves that affect photosynthetic physiology. Specific leaf area (SLA: leaf area per leaf mass) was measured immediately following juvenile and pre-reproductive photosynthetic measurements. Leaf thickness was measured following pre-reproductive measurements by examining leaf cross sections at 100× magnification. Leaves were sampled at both stages, dried at 60 °C and analysed for total Kjeldahl nitrogen content using an autoanalyser (Traacs 800, Bran and Leubbe, Buffalo Grove, IL) following acid digestion (Lowther, 1980). Nitrogen content was expressed on leaf-area basis using calculations of SLA.

Growth measurements

Growth variables were measured at the juvenile, pre-reproductive and late-reproductive (3 months after transplanting with reproduction near completion) stages. At the juvenile and pre-reproductive stages, height, stem diameter and number of leaves and branches were measured. At the late-reproductive stage height, branch number and dry masses of leaf, stem, root and reproductive parts were measured. Reproductive mass included seeds and support tissue, and was used as an estimate of fitness.

If parents with lower photosynthetic rates have less available photosynthate, it is possible that they could allocate less to each seed. In a previous study, the average mass of an R seed was 0.365 and 0.388 mg for the WT (unpublished data; $n = 10$, $P < 0.001$). If there is a tradeoff between seed number and size, then it is also possible that more seed could be produced for a fixed amount of photosynthate. A tradeoff of this type might enable the R genotype to compensate for the effect of the mutation, by producing smaller seeds but a larger number than could be made with a fixed amount of available photosynthate. If a tradeoff occurred, the R genotype would not necessarily be able to produce more seeds than the WT, just more than it would if individual seeds were larger. In the field, the R genotype (with a lower photosynthetic rate) produced fewer seeds than the WT (Arntz *et al.*, 2000). In this study, our measure of reproductive mass is proportional to total seed mass, and we did not measure individual seed masses of the genotypes or count the total number of seed produced.

Data analyses

The effects of genotype, light, water and their interactions on gas-exchange and leaf properties were tested for each stage using analysis of variance (ANOVA). Because interactions between light and water were prevalent for most variables, separate two-way ANOVAS were performed for each light treatment (PROC GLM; SAS statistical software, version 6.12, SAS Institute Inc., Cary, NC).

For growth and allocation variables, the effects of block, genotype, light, water and their interactions were tested using a multivariate analysis of variance (MANOVA), with family line included as a random effect nested within genotype (PROC GLM). Interactions between main effects were prevalent, so separate MANOVAS (12) were performed for light and water treatments to test for overall differences in growth between the genotypes at each stage. The block effect was not included in these models because it was not significant in the overall model. Reducing general linear models is valid when the variance components are small and have a large *P*-value (Neter *et al.*, 1990); any variation owing to block was therefore incorporated into the error term.

For significant MANOVAS ($P < 0.004$, α corrected for 12 tests), subsequent univariate ANOVAS were performed to identify the variables contributing to overall differences between the genotypes. The use of MANOVA reduces the probability of rejecting true null hypotheses (type 1 error) and accounts for correlations among variables (Stevens, 1996), eliminating the need to correct for multiple ANOVA tests with each MANOVA. Differences in reproductive mass were tested using ANOVA with family included as a random effect nested within genotype (PROC GLM).

The direct contribution of photosynthetic genotype to reproduction was determined with multivariate selection analysis. Separate analyses were performed for each treatment, using multiple linear regression of fitness on photosynthetic genotype and late-reproductive leaf, stem and root mass (PROC GLM). The standardized multiple regression coefficients produced by this analysis are analogous to directional selection gradients (β). Selection gradients measure the direct contribution of each trait to

fitness, while accounting for correlations with other traits included in the analysis. Selection gradients therefore measure direct selection and distinguish it from indirect selection via correlated traits. In the models, photosynthetic genotype was coded as a nominal variable (WT = 1, R = 0) such that a positive β indicates that being the WT has a positive effect on fitness. Including nominal, independent variables in regression analyses is common and is statistically valid (Kendall & Stuart, 1977). Analysis of collinearity diagnostics and residual plots showed that the assumptions of multiple regression were upheld in all cases. Variance inflation factors for the variables ranged from 1.3 to 4.1 (values less than 10 indicate low collinearity) and first order autocorrelations from 0.02 to 0.28 (not significant in any case as tested by Durbin–Watson D, $N = 96$).

Results

The light and water treatments created a range of differences between genotypes in photosynthetic, leaf, growth and reproductive traits. We focus on the genotypic differences, and interpret them separately for each treatment.

Photosynthesis and leaf properties

Across genotypes and water treatments, high-light leaves had a lower SLA and less nitrogen on a mass and area basis than low-light leaves at a given stage (Tables 1 and 2). In all but the high-light and high-water treatment, photosynthetic rate decreased in both genotypes from the juvenile to the pre-reproductive stage (Fig. 1). This decrease was accompanied by decreases in stomatal conductance, SLA and nitrogen content on a mass basis (and increases on an area basis) (Tables 1 and 2). All significant differences between the genotypes in photosynthetic rate (three of eight cases) were seen in the high-light treatments (Fig. 1).

For the high-light and high-water treatment (900 μmol m^{-2} s^{-1}), photosynthetic rate of the R genotype was 17.3% lower than the WT at the juvenile stage, but higher by 30.2% at the pre-reproductive stage. This result occurred because photosynthetic rate of the WT was similar at the juvenile and pre-reproductive stages (not statistically tested), but for the R genotype it actually increased (Fig. 1). This same result was seen for stomatal conductance (Table 1). Genotypes did not differ at the juvenile stage in other leaf properties, but by the pre-reproductive stage the higher R photosynthetic rate was

Table 1 Gas-exchange and leaf properties for WT and R *Amaranthus hybridus* grown in high light with high- or low-water availability. Values are mean values of 4–6 plants with standard errors given in parentheses. Letters indicate differences significant at $P < 0.05$ from analysis of variance performed separately for each developmental stage. $g_{s\ ambient}$: Somatal conductance at 900 μmol m^{-2} s^{-1} (the growth irradiance representative for these treatments); SLA: specific leaf area, or area of leaf tissue per gram of leaf tissue.

	Juvenile				Pre-reproductive			
	High water		Low water		High water		Low water	
Variables	WT	R	WT	R	WT	R	WT	R
Respiration rate (μmol m^{-2} s^{-1})	−3.1 (0.13)^a	−2.6 (0.3)^a	−3.8 (0.4)^b	−3.6 (0.6)^b	−2.3 (0.4)^a	−2.9 (0.5)^a	−2.4 (0.2)^b	−2.2 (0.1)^b
$g_{s\ ambient}$ (mol m^{-2} s^{-1})	0.18 (0.01)^a	0.19 (0.01)^a	0.28 (0.01)^b	0.29 (0.01)^b	0.19 (0.01)^a	0.29 (0.01)^b	0.18 (0.01)^c	0.15 (0.01)^c
SLA (cm^2 g^{-1})	268 (18)^a	300 (18)^a	274 (20)^a	273 (14)^a	195 (5)^a	240 (26)^b	161 (7)^c	186 (7)^d
%N	4.2 (0.1)^a	4.6 (0.2)^a	4.7 (0.6)^a	4.8 (0.3)^a	2.4 (0.1)^a	2.9 (0.2)^b	3.0 (0.1)^c	3.3 (0.1)^c
mmol N m^{-2}	111 (2.5)^a	111 (4.3)^a	121 (8.1)^b	126 (4.1)^b	214 (4.8)^a	207 (3.7)^a	206 (7.3)^a	204 (4.8)^a
Leaf thickness (μm)	–	–	–	–	82 (3.4)^a	93 (5.2)^a	125 (2.4)^b	122 (4.1)^b

Table 2 Gas-exchange and leaf properties for WT and R *Amaranthus hybridus* grown in low light with high- or low-water availability. Values are mean values of 4–6 plants with standard errors given in parentheses. Letters indicate differences significant at $P < 0.05$ from analysis of variance performed separately for each developmental stage. $g_{s\ ambient}$: Stomatal conductance at 450 μmol m^{-2} s^{-1} (the growth irradiance representative for these treatments); SLA: specific leaf area, or area of leaf tissue per gram of leaf tissue.

	Juvenile				Pre-reproductive			
	High water		Low water		High water		Low water	
Variables	WT	R	WT	R	WT	R	WT	R
Respiration rate (μmol m^{-2} s^{-1})	−3.3 (0.4)^a	−3.5 (0.2)^a	−3.1 (0.3)^a	−3.0 (0.2)^a	−1.8 (0.1)^a	−1.9 (0.1)^a	−1.2 (0.1)^a	−1.4 (0.1)^a
$g_{s\ ambient}$ (mol m^{-2} s^{-1})	0.24 (0.01)^a	0.27 (0.01)^b	0.23 (0.01)^a	0.28 (0.01)^b	0.10 (0.01)^a	0.13 (0.01)^a	0.10 (0.01)^a	0.09 (0.01)^a
SLA (cm^2 g^{-1})	597 (37)^a	647 (29)^a	562 (52)^a	621 (49)^a	266 (10)^a	317 (8)^b	297 (20)^c	343 (48)^d
%N	6.2 (0.4)^a	6.5 (0.4)^a	5.7 (0.3)^a	6.1 (0.1)^a	3.6 (0.2)^a	4.0 (0.2)^a	3.8 (0.2)^b	4.2 (0.1)^b
mmol N m^{-2}	74 (1.8)^a	72 (4.5)^a	74 (5.3)^a	71 (3.4)^a	88 (4.2)^a	93 (4.0)^a	104 (5.0)^b	115 (3.4)^b
Leaf thickness (μm)	–	–	–	–	182 (5.7)^a	188 (6.6)^a	176 (4.1)^a	183 (2.9)^a

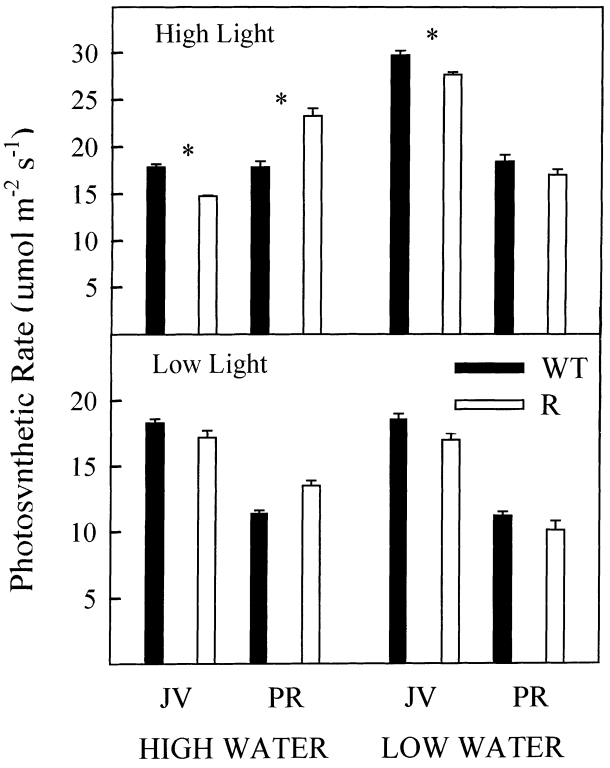

Fig. 1 Photosynthetic rate of WT and R *Amaranthus hybridus* grown in four light and water treatments. Data are mean values (+standard errors) of 4–6 plants from two developmental stages: juvenile (JV) and pre-reproductive (PR). Asterisks indicate a significant difference between the genotypes from two-way ANOVAS performed separately for each light treatment and developmental stage ($P < 0.05$). In the high-light treatment, the interaction between water and genotype was significant for the pre-reproductive stage and differences were tested with a one-way ANOVA.

also accompanied by a relatively higher SLA and more leaf nitrogen on a mass basis compared with the WT. Leaves of both genotypes decreased in SLA and leaf nitrogen content on a mass basis over time (Table 1).

In the high-light and low-water treatment (900 μmol m^{-2} s^{-1}), the R genotype had a 7.0% lower photosynthetic rate than the WT at the juvenile stage, but genotypes did not differ at the pre-reproductive stage (Fig. 1). There were no differences between genotypes in other leaf properties at either stage, except that pre-reproductive R plants had a higher SLA than the WT (though similar thickness), suggesting that R leaves were less dense than the WT (Table 1).

In the high-light treatment it is notable that, at the juvenile stage, photosynthetic rates were lower in the high-water treatment compared with the low-water treatment for both genotypes (Fig. 1). Respiration rate, stomatal conductance and leaf nitrogen content on an area basis were also lower in the high-water treatment (Table 1). These differences between water treatments were likely the result of a slightly faster growth rate in the high-water treatment that depleted soil nitrogen. Plants were fertilized shortly after the juvenile stage and a large difference in photosynthetic rate between water treatments was not seen at the pre-reproductive stage (Fig. 1). However, for both genotypes at the pre-reproductive stage, leaves had a higher SLA, were thinner and had less nitrogen on a mass basis in the high-water compared with low-water treatment (Table 1).

In the low-light treatment (450 μmol m^{-2} s^{-1}), the only difference between genotypes in photosynthesis and other leaf properties was that the R genotype had higher stomatal conductance than the WT at the juvenile stage and higher SLA at the pre-reproductive stage (Table 2). This was true for both water treatments (Table 2). Note, however, that across water treatments and stages, the pattern of photosynthetic differences between genotypes in low light parallels that seen in high light.

Growth and reproduction

The light and water availability treatments resulted in a range of growth and reproductive differences between genotypes. Differences between genotypes in reproduction are interpreted in the context of differences in photosynthetic rate and growth within each treatment, and the results from multivariate selection analyses.

High light with high-water availability

At the juvenile stage, genotypes were similar in all aspects of growth (Table 3) and the WT had a higher photosynthetic rate (Fig. 1). Therefore, it could be expected that at the pre-reproductive stage the WT would have more mass. However, at the pre-reproductive stage genotypes maintained similar growth (Table 3) and the R genotype had a higher photosynthetic rate than the WT (Fig. 1). By the late-reproductive stage, the R genotype had more leaf mass than the WT, and as a result had more mass allocated to leaves vs. stems and less to roots vs. shoots (leaves and stems) (Table 3).

Reproductive mass of the R genotype was greater than the WT in this treatment (Fig. 2), and was not the result of direct effects of photosynthetic genotype on fitness (Table 4). Rather, leaf mass and root mass had large, positive selection gradients and stem mass had a large, negative selection gradient (Table 4). Therefore, higher fitness of the R genotype must have resulted from indirect influences of genotype on fitness, via growth differences. Such indirect effects could have arisen through the higher leaf mass of the R genotype and the direct effect of late-reproductive leaf mass on fitness. Higher R reproductive mass would then be the result of the R genotype having more leaf mass than the WT, possibly as a result of similar pre-reproductive size with higher photosynthetic rates than the WT.

Table 3 Differences in growth and allocation between WT and R *Amaranthus hybridus* [(WT–R)/WT × 100] at three developmental stages: juvenile (JV), pre-reproductive (PR) and late-reproductive (LR). A positive value indicates that the WT had a larger trait mean value, whereas a negative value indicates the R mean was greater. *P*-values for separate multivariate analyses of variance (MANOVA) for each treatment and developmental stage are shown in bold type above the values for each treatment combination. For significant MANOVAS ($P < 0.004$, α corrected for 12 tests), the traits shown by univariate ANOVAS to be different between the genotypes are in bold ($P < 0.05$). For marginally significant MANOVAS ($0.10 > P > 0.01$), traits contributing to the genotype difference ($P < 0.05$ from univariate ANOVAS) are in italics; NI indicates the variable was not measured and not included in the model.

	High water			Low water		
Variable	JV	PR	LR	JV	PR	LR
High light	**0.4883**	**0.2709**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
Height	2.1	2.3	0.1	–3.8	**4.5**	0.3
Stem diameter	3.3	3.9	NI	**8.3**	**8.3**	NI
Branch number	0.1	–1.3	–1.9	**–14.0**	**9.8**	2.1
Leaf number	–1.3	4.1	NI	**8.0**	**8.6**	NI
Leaf mass	NI	NI	**–15.2**	NI	NI	**–8.7**
Stem mass	NI	NI	1.3	NI	NI	–3.9
Root mass	NI	NI	1.3	NI	NI	4.1
Leaf/stem mass	NI	NI	**–18.8**	NI	NI	**–10.5**
Root/shoot mass	NI	NI	**6.0**	NI	NI	**9.7**
Low light	**0.6869**	**0.6428**	**0.3380**	**0.0194**	**0.0506**	**0.0716**
Height	2.0	1.6	–3.9	2.0	*4.4*	2.7
Stem diameter	1.9	–0.5	NI	2.1	0.8	NI
Branch number	3.1	2.0	–5.1	0.5	*6.3*	2.0
Leaf number	–10.1	–7.9	NI	*9.1*	3.6	NI
Leaf mass	NI	NI	–15.7	NI	NI	–7.6
Stem mass	NI	NI	–6.3	NI	NI	–6.2
Root mass	NI	NI	–6.8	NI	NI	–6.2
Leaf/stem mass	NI	NI	–9.7	NI	NI	–4.1
Root/shoot mass	NI	NI	4.9	NI	NI	0.7

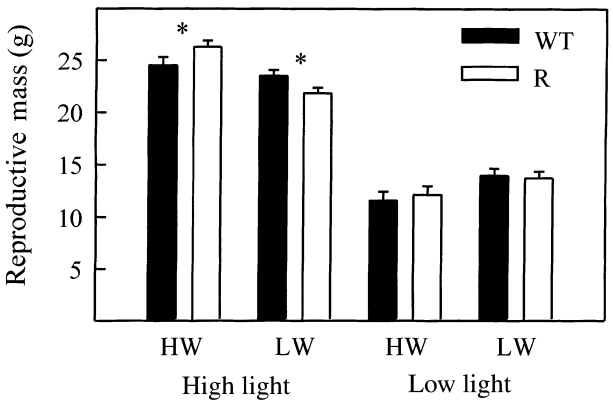

Fig. 2 Reproductive mass (+standard error) of WT and R *Amaranthus hybridus* grown in two light treatments with high-water (HW) or low-water (LW) availability. Differences between the genotypes were tested separately for each light treatment with a two-way ANOVA. In high light, the interaction between water and genotype was significant and differences were tested with a one-way ANOVA. Asterisks indicate a significant difference between the genotypes ($P < 0.05$).

High light with low-water availability

At the juvenile stage, the R genotype had a thinner main stem and more branches with fewer leaves than the WT (Table 3), as well as a lower rate of photosynthesis (Fig. 1). The R genotype remained smaller than the WT at the pre-reproductive stage; R plants were shorter, with a thinner main stem, and fewer branches and leaves (Table 3). At this stage the genotypes no longer differed in photosynthetic rate (Fig. 1). By the late-reproductive stage, the genotypes did not differ in height or branch number, but the R genotype had more leaf mass and as a result had more mass allocated to leaves vs. stems and less to roots vs. shoots than the WT (Table 3).

Table 4 Selection gradients (βs) for photosynthetic genotype and late-reproductive growth traits for *Amaranthus hybridus* in four light and water treatments. Values in bold are significant at $P < 0.02$ and values in italics at $0.10 > P > 0.05$. Positive values indicate a trait had a positive relationship with fitness; for photosynthetic genotype they indicate a positive, direct fitness effect of being the WT.

	High light		Low light	
Variable	High water	Low water	High water	Low water
Photosynthetic genotype	–0.026	**0.240**	0.015	0.102
Leaf mass	**0.552**	**0.507**	–0.069	–0.124
Stem mass	**–0.553**	**–0.371**	*0.285*	**0.378**
Root mass	**0.549**	**0.331**	**0.347**	**0.451**

In this treatment the R genotype produced less reproductive mass than the WT (Fig. 2). Greater WT fitness was directly attributable to genotype after controlling for the effects of other growth variables. Leaf mass and root mass had large, direct selection gradients and stem mass had a large, negative selection gradient (Table 4). The direct genotype effect implies that photosynthesis during later development must have been an important factor determining fitness. WT photosynthetic rates were higher at the juvenile stage and, although not significant, tended to be higher at the pre-reproductive stage. Although photosynthetic rates were not measured at the late-reproductive stage, the WT must have had higher rates than the R genotype to produce a direct effect. Although the WT had less leaf mass than the R genotype – and more leaf mass increased fitness – any negative indirect effects of photosynthetic genotype on fitness as a result of this difference must have been small or cancelled out by the large, positive direct effect.

Low-light and high- and low-water availability

Genotypes did not differ in photosynthetic rate (Fig. 1), growth or allocation (Table 3) or reproductive mass (Fig. 2) at any stage in either low-light water treatment. Therefore, there was no effect of photosynthetic genotype on fitness in either treatment (Table 4). In the high-water treatment, root mass had a positive selection gradient. In the low-water treatment stem and root mass had positive selection gradients.

Discussion

To estimate the effects of variation in photosynthetic rate on growth and reproduction we used nearly isonuclear genotypes known to generate up to a 30% difference in photosynthetic rate. We grew them in environments hypothesized to generate a range of genotypic differences in photosynthetic rate, and characterized photosynthetic and growth differences between genotypes at several developmental stages. Across the four combinations of light and water treatments we found a range of genotypic differences in photosynthetic rate, with the R genotype having higher rates than the WT in one case. When relating photosynthetic differences to those in growth and reproduction, we assume that the observed photosynthetic differences can be extrapolated to other times of the day or different days during the experiment to at least some extent.

For juvenile plants in the high light and high- and low-water treatments, the WT had higher photosynthetic rates than the R genotype (17%). These results are consistent with expectations based on the function of the mutation conferring atrazine resistance. For many species, the mutation typically lowers R photosynthetic rates compared with the WT in high- but not in low-light environments and, when measured, differences in growth and reproduction are generally seen only at higher irradiances (Ort *et al.*, 1983; Jursinic & Pearcy, 1988; Hart *et al.*, 1992; Sundby *et al.*, 1993; Dominguez *et al.*, 1994; Arntz *et al.*, 2000). This pattern may result from increased susceptibility of the R genotype to inhibition of photosynthesis by high light (photoinhibition; Hart & Stemler, 1990; Sundby *et al.*, 1993).

In contrast, with high-light and high-water availability at the pre-reproductive stage, the WT had a lower rate than the R genotype by 30%. The lower photosynthetic rate of the WT in this case is likely the result of slight differences in growth rates between genotypes. Although the WT had a higher photosynthetic rate at the juvenile stage, by the pre-reproductive stage its leaves were 12% thinner (not significant), had a 23% lower SLA and 21% less nitrogen on a mass basis (3% higher N on area basis) and stomatal conductance was 53% lower. These patterns of nitrogen translocation were not detectable by external observation of the whole-plant, but they do suggest that the R genotype may have developed more slowly than the WT (McCloskey & Holt, 1990; Dekker & Burmester, 1992). A slower growth rate could account for the higher R photosynthetic rates, as suggested by Dekker & Burmester (1992) who reported lower photosynthetic rates in *B. napus* R genotypes earlier in development, but higher rates during the final developmental stages.

The effects of photosynthetic genotype on growth, allocation and reproduction in the high-light treatment depended on water availability. With high-water availability the differences in photosynthetic rate between genotypes (WT greater at juvenile stage, R greater at pre-reproductive stage) were accompanied by only a few changes in growth and allocation (3 of 15 comparisons). Reproductive mass of the R genotype was 7% higher than the WT in accordance with its higher pre-reproductive photosynthetic rate, and the effects of photosynthetic genotype on fitness were not direct. However, with low-water availability, differences in photosynthetic rate between genotypes (WT greater at juvenile stage, no difference at prereproductive stage) were accompanied by numerous differences in growth and allocation (10 of 15 comparisons). In this treatment, the WT had a 7% higher reproductive mass than the R genotype and photosynthetic genotype had a direct effect on fitness.

Water availability can alter the ways in which photosynthesis affects reproduction in two ways. First, photoinhibitory damage increases with water deficits (Havaux, 1989). The R genotype is inherently sensitive to photoinhibition (Hart & Stemler, 1990), and this sensitivity may be further exacerbated by water deficits (J. Whitmarsh, personal communication). Although we did not measure photoinhibition, it could probably have affected photosynthetic differences between genotypes in the high-light treatment. In addition, water availability can differentially affect size and allocation traits and alter the correlation structure among traits (Pigliucci *et al.*, 1995). Changes in the correlations among photosynthesis

and growth traits would alter the direct and indirect effects of photosynthesis on fitness. Water availability has been shown to affect the magnitude and direction of selection on water-use efficiency (carbon gained by photosynthesis per water used) for a number of desert species (Schuster *et al.*, 1992; Ehleringer, 1993; Donovan & Ehleringer, 1994a). In this study it affected the direction and mode of the effects of photosynthetic genotype on fitness. This effect of water availability may account for decreases in R fitness in low-light, competitive field environments (Arntz *et al.*, 1998; Arntz *et al.*, 2000).

Differences between genotypes in photosynthetic rate, growth, allocation and reproductive mass were not significant in either low-light treatment. However, it should be noted that for photosynthetic rate and reproductive mass, the pattern of differences between genotypes, across water treatments, paralleled those seen in high light. This pattern also lends support to the conclusion that water availability is important in determining the contributions of photosynthesis to reproduction.

Assuming that other genetic differences between genotypes had no other consistent and major effects on fitness, the low-light treatment served as a standard of comparison, similar to a control, in our study of the effects of photosynthesis on fitness. Because there were no significant differences in photosynthesis between the genotypes in the low-light treatment, differences in growth or reproduction should not be expected. This is the result we found. In contrast, differences in photosynthetic rate between the genotypes were found in the high-light treatment, and so were differences in growth, allocation and reproduction. These results support the idea that photosynthetic differences caused the observed differences in growth and reproduction between genotypes.

The effects we found across treatments are not particularly large. However, they are remarkable because the ability to detect a contribution of photosynthetic rate to reproductive success can be restricted by at least three factors. First, if variation is small, effects on fitness might be subtle and more difficult to detect statistically. Genetically based variation within natural populations may account for differences in photosynthetic rate up to 30%, but differences are often smaller (Zangerl & Bazzaz, 1983; Geber & Dawson, 1990; Gehring & Monson, 1994; Laport & Delph, 1996). Second, differences among individuals in photosynthesis may not be the factor limiting growth in all environments or developmental stages. Photosynthetic differences may affect small changes in growth that may or may not result in indirect effects on reproduction, depending on the availability of biotic (e.g. pollinator limitation) and other resources. Direct effects of variation in photosynthetic rate on seed provisioning may also occur, but their detection is also limited by statistical power and the operation of other resource constraints. Third, as seen in this study, the pattern of variation in photosynthesis may change during development. Differences at one time point cannot necessarily be assumed to operate consistently throughout development, and a lack of effect of photosynthetic variation on growth or reproduction may result because variation is not adequately quantified. Changes in the temporal pattern of variation could even cancel opposing fitness effects. Because of these considerations, variation in photosynthetic rate should not necessarily be expected to translate into growth or reproductive differences of the same direction or magnitude. By minimizing some of these issues in this study we support the hypothesis that variation in photosynthetic rate can cause differences in fitness.

Physiological mutants and multivariate analysis have been used separately to explore subsets of the relationships between photosynthesis, growth and reproduction. For photosynthetic traits, most studies with mutants have used agricultural species and focus on the effects of mutations, or transgenes, on physiological performance and to some degree on growth and allocation (Stitt & Schulze, 1994). Multivariate selection analysis is commonly used to measure phenotypic selection in natural plant populations, but mostly the focus has been on morphological and life-history traits (e.g. Maddox & Antonovics, 1983; Kalisz, 1986; Kelly, 1992; Bennington & McGraw, 1995; Winn, 1999). Photosynthetic traits have been included in few studies. These studies are discussed briefly below, not only because they are rare examples, but also because they make similar conclusions and point to directions for future studies.

In one example Farris & Lechowicz (1990) used crosses from 12 *Xanthium strumarium* populations to show that the relative importance of morphological, photosynthetic and phenological traits to reproductive success were all approximately equal. These traits primarily contributed indirectly to fitness through their influence on plant size. In another experiment with *X. strumarium*, the relative contributions of structural, photosynthetic and phenological traits varied with resource availability (Lechowicz & Blais, 1988). Rapid relative growth rates at emergence, higher stomatal conductances and greater allocation to stems vs. leaves were most important in determining reproductive success in a resource-poor environment. In a resource-rich environment, relatively slow growth rates and lower stomatal conductances conferred higher reproductive success.

Work by Geber & Dawson (1990, 1997) and Geber (1990) with *Polygonum arenastrum* also indicates that selection favours contrasting suites of morphological and physiological traits in an environment-dependent manner. Their work suggests that short growing seasons or frequent disturbances select for early flowering, small leaves, high rates of gas exchange and low water-use efficiencies. In contrast, longer growing seasons or less-frequent disturbances favour selection for slower rates of

development, and larger leaves with lower photosynthetic rates and higher water-use efficiencies. However, they did not use multivariate selection analysis to determine which traits contributed most to fitness.

Multivariate selection analysis was used in the first rigorous test of the hypothesis that smaller leaves and higher water-use efficiency are adaptive in drier environments (Dudley, 1996a). Populations of *Cakile edentula* grown in dry and wet dune field sites showed that selection in the dry site favoured higher water-use efficiencies and small, intermediate leaf sizes, whereas these traits had no linear effects on fitness in the wet site. Correlational selection was also found in the dry site; plants with high water-use efficiencies were more fit if they had large leaves, and those with lower efficiencies had higher fitness if they had small leaves. These relationships were either weaker or absent in the wet site, providing evidence that the patterns were adaptive in dry environments.

Together these studies demonstrate that selection on ecophysiological traits differ across environments and that it may operate on suites of traits. Because organisms co-ordinate processes across multiple levels of organization and are integrated, trait correlations within suites of leaf traits or across suites of physiological, morphological, and life-history traits are very prevalent. In order to more fully appreciate how selection operates, we need more studies that simultaneously measure a variety of physiological, morphological and life-history traits, and match variation and covariation in these traits to variation in fitness. However, the use of mutants has a benefit over such strictly correlational approaches because they provide some control over the genotype and phenotype.

There are limitations to using mutants, and they should be used with these in mind. First, control over the phenotype is not complete because of pleiotropic effects. In this study, we compared nearly isonuclear genotypes that differ at a single locus in the chloroplast genome that codes for a major photosynthetic protein. In environments where this genetic difference generated phenotypic differences in photosynthetic rate, there were also differences in a variety of leaf properties. Photosynthetic rate was not the only physiological trait affected by the mutation. Therefore, although we could more definitively assess the effect of variation in photosynthetic rate on reproduction by using mutants in this experiment, some covariation with other traits still exists in our analyses. Second, the effects of any mutation can depend on genetic background. In this study, we used seven family lines of WT and R genotypes, each having different, random sets of nuclear genes. In our analyses the variation among families was significant in many cases, and family differences were factored out statistically. Therefore, the effects of a given mutation may not exist or be strong in some genetic backgrounds, and one should be aware of and control for potential genetic interactions when possible.

We found that differences in photosynthesis between WT and R genotypes, generated by manipulating light and water availability, corresponded with observed changes between these genotypes in growth and reproduction. Our results strongly support the hypothesis that photosynthetic rate is under selection in annual plants. Moreover, the sensitivity of relationships among traits to environmental factors may influence whether selection on photosynthetic traits is direct or indirect, and explain how the contribution of photosynthesis to growth and reproduction will vary during development.

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