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Contribution of photosynthetic rate to growth and reproduction in *Amaranthus hybridus*

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Abstract While it is known that genetic variation for photosynthetic and growth traits exists in natural populations, the functional significance of this variation remains unclear, particularly for photosynthetic traits. To test the hypothesis that photosynthetic rate has direct effects on reproduction as well as contributing indirectly to reproduction through effects on growth, we compared wild-type *Amaranthus hybridus* families to those with a single gene mutation that confers a lower photosynthetic rate. Wild-type and photosynthetic-mutant families were grown in competitive and non-competitive environments and we compared size, biomass allocation, architecture, and reproduction at three developmental stages. To assess the contributions of individual growth traits to reproduction, we calculated covariances between standardized traits and relative fitness (selection differentials), and compared selection between the two biotypes. Finally, we used path analysis to calculate the indirect effects of photosynthetic rate on fitness through growth. The size, allocation, and architecture of photosynthetic mutants did not differ from those of the wild type in either the competitive or non-competitive environment, with the exception that they were taller by the last developmental stage. However, the reproductive biomass of the photosynthetic mutants was significantly reduced compared to the wild type. In the competitive environment, the wild type achieved greater fitness because, while similar in size to the mutants, at any given size it produced more reproductive biomass. This suggests that photosynthetic rate affected the linkage between plant size and reproduction and is evidence of an indirect contribution to fitness. In the non-competitive environment, there were fewer differences in selection differentials between the two plant genotypes, suggesting fewer indirect effects. Path analysis showed that variation in photosynthetic biotype had indirect effects on reproductive biomass, via growth traits, and that there were no direct effects. Photosynthetic rate appears to have fitness consequences primarily through multiple contributions to growth throughout development.

Key words Atrazine · Resistance · Fitness · Path analysis · Photosynthesis · Selection

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Introduction

For ecophysiological traits to evolve there must be heritable variation in these traits and they must contribute to fitness. Genetic variation in photosynthetic traits and biomass allocation patterns has been found among and within natural populations (Teramura and Strain 1979; Kalisz and Teeri 1986; Donovan and Ehleringer 1994; Nienhus et al. 1994; Geber and Dawson 1997). Variation in size and biomass allocation contributes to variation in fitness (Solbrig 1981; Geber 1990; Cheplick 1995). However, the functional significance of photosynthetic variation, especially to reproductive success, is unclear.

Despite the fundamental importance of photosynthesis to plant function, instantaneous photosynthetic rate is not a reliable predictor of plant growth (Lambers 1987; Nelson 1988). However, when photosynthesis is considered together with allocation patterns, growth can be more accurately predicted (Dijkstra and Lambers 1989; van den Boogaard et al. 1996). A similar increase in explanatory power is achieved by integrating the effects of phenology and morphology to explain variation in reproductive success (Mitchell-Olds and Bergelson 1990; Jordan 1991; van Tienderen and van der Toorn

1991; Kelly 1992; Bennington and McGraw 1995). Presumably the incorporation of physiological contributions would further increase this explanatory power (Lechowicz and Blais 1988; Farris and Lechowicz 1990; Dudley 1996); however, the direct and indirect effects of photosynthetic rate on variation in growth and reproduction remain poorly understood.

To determine the contribution of photosynthetic rate to growth and reproduction we compared wild-type (WT) *Amaranthus hybridus* families to those having a single gene mutation that confers resistance to the herbicide atrazine. When applied, atrazine binds to the plastoquinone binding site (Q_b) in photosynthetic electron transport, halts photosynthesis, and kills WT plants. In response to this strong selection, atrazine resistance has evolved in many populations of agricultural weeds (Warwick 1991). Atrazine-resistant (R) plants have a mutation at the *psbA* locus that alters the structure of Q_b enough to greatly reduce atrazine binding (Hirshberg and McIntosh 1983). In the absence of atrazine, photosynthetic electron transport in R plants is reduced compared to the WT (Ort et al. 1983), and the mutation may have a secondary effect of increasing the susceptibility of R plants to photoinhibition (Sundby et al. 1993). Together these effects translate into a 20–35% lower rate of photosynthesis in R plants, depending on temperature and incident light level (Holt et al. 1981; Jursinic and Pearcy 1988; Stowe and Holt 1988; Dekker and Sharkey 1992).

The genetic variation in photosynthetic rate between WT and R plants provides a unique system for assessing its contribution to growth and reproduction. We compared WT and R families with nearly isonuclear genomes as a proxy for natural variation in photosynthetic rate, with the understanding that there may be other pleiotropic effects of the *psbA* mutation (Dekker and Burmester 1992). These families allowed us to control for confounding effects of other loci that are present in other study systems. Isogenic and transgenic plants have been used to assess relationships between photosynthesis and growth (Stitt and Schulze 1994); however, studies do not commonly measure reproduction and have been restricted to greenhouses or growth chambers. Differences in the photosynthetic rates of our WT and R families are typical of intraspecific variation seen in many natural and agricultural systems, and provide a reasonable proxy for measuring the contributions of photosynthesis to growth and reproduction under field conditions.

WT and R families were grown in both competitive and non-competitive (open) environments and compared to test the hypothesis that photosynthetic rate has direct effects on reproduction while also indirectly contributing to reproduction through effects on growth. Size, biomass allocation, and reproduction of WT and R families were measured at three developmental stages. From previous work (e.g., Dekker et. al. 1992), we expected that higher irradiance in the open environment would exaggerate photosynthetic differences, and therefore growth and reproductive differences, between the biotypes.

To assess the contributions of individual growth traits to fitness we calculated covariances between each trait and relative fitness (selection differentials). We defined fitness as reproductive biomass at the end of the season, including seeds and support tissue. Reproductive biomass is highly correlated with seed mass in *A. hybridus* ($r = 0.98$; Jordan 1996). Differences in selection differentials between biotypes could indicate that photosynthetic rate affects the linkage between plant growth and reproduction; this would be evidence of an indirect contribution of photosynthetic rate to fitness. Finally, we use path analysis to partition the direct contributions of photosynthetic biotype, leaf area, height, and allocation to fitness, and to determine the indirect effects of photosynthetic rate on fitness via growth throughout development.

Methods

Study species

A. hybridus L. (smooth pigweed) is an annual plant, native to eastern North America and parts of Mexico, Central America, and northern South America (Weaver and McWilliams 1980). It is wind pollinated, monoecious, highly self-pollinating, and has C_4 photosynthetic metabolism.

R and WT *A. hybridus* seeds were collected from agricultural populations in Blacksburg, Virginia (Jordan 1996). The mutation conferring atrazine resistance is in the chloroplast genome and therefore maternally inherited. Because differences in the nuclear genomes can confound comparisons of WT and R biotypes, reciprocal crosses were performed to generate isonuclear families (Jordan 1996). These families have nearly uniform nuclear backgrounds with distinct WT or R cytoplasmic genomes. We used seed from seven isonuclear families of each biotype.

Study site

Plants were grown from June to September 1995 in adjacent 'competitive' and 'open' environments located at the Phillips Tract Research Area, 8 km northeast of Urbana, Ill. (40.06°N, 88.14°W). The competitive environment was a 5-year-old successional field dominated by *Solidago canadensis*. Canopy height in mid-June was approximately 1 m, and the mean leaf area index was 1.56 ± 0.69 ($n = 13$, Licor-2000 Canopy Analyzer, Licor, Lincoln, Neb.). The adjacent open environment was tilled and covered with plastic to exclude competition. On a typical sunny day, instantaneous photosynthetically active photon flux density (PPFD), measured 10 cm above the ground, averaged 255 μmol m^{-2} s^{-1} in the competitive environment, with irradiance levels below 400 μmol m^{-2} s^{-1} for 76% of the day, and above 1000 μmol m^{-2} s^{-1} for 7% of the day. In the open environment, average PPFD was 1073 μmol m^{-2} s^{-1}, with levels below 400 μmol m^{-2} s^{-1} for 22% of the day and above 1000 μmol m^{-2} s^{-1} for 57% of the day. Integrated over the day, this was equivalent to 13.6 mol m^{-2} day^{-1} of photosynthetically active irradiance in the competitive environment compared to 58.0 mol m^{-2} day^{-1} for the open environment. Mean (±SD) soil and air temperatures were, respectively, 20.4 ± 1.0°C and 19.3 ± 5.7°C in the competitive environment and 24.9 ± 2.7°C and 18.5 ± 8.8°C in the open environment. Predawn water potential (Ψ) measured in late June differed between environments; the competitive environment was drier and more variable ($\Psi = -0.11 \pm 0.03$ MPa) than

the open environment ($\Psi = -0.04 \pm 0.01$ MPa) ($P < 0.001$, $n = 10$).

Photosynthetic measurements

To confirm that the altered *psbA* gene in R plants conferred lower photosynthetic rates under field conditions, rates were measured on leaves from eight randomly selected WT and R plants growing in the open environment. Measurements were made on a clear day between 6:00 a.m. and 6:00 p.m. at seven intervals with a portable gas exchange system (Licor-Portable Photosynthesis System). Irradiance was measured inside the chamber with a gallium arsenide-phosphide photodiode (Hamamatsu Photonics K.K., Bridgewater, N.J.).

Experimental design and growth measurements

WT and R seeds were germinated in Cone-tainers (3.5 cm diameter × 14 cm deep; Stuewe, Corvallis, Ore.) with 100 cm^3 of Sunshine Mix (Sun Gro Horticulture, Bellevue, Wash.) in April, in the greenhouse. After 1 month, plants had four to six true leaves, and 200 seedlings of each biotype (approximately equal numbers from seven families per biotype) were transplanted into each environment. To account for environmental heterogeneity, we used a randomized block design with three blocks per environment. Seedlings were planted 1 m apart in two rows and were distributed randomly with respect to biotype.

Growth variables were measured at three developmental stages: (1) 1 month after transplanting (juvenile), (2) 2 months after transplanting but prior to reproduction (pre-reproductive), and (3) 3 months after transplanting, with reproduction virtually complete (late-reproductive). Measurements at each stage included leaf area, vegetative mass, height, percentage of mass in stems, and the distribution of leaf area along the main stem (architecture index). In the competitive environment, surviving plants were censused at each stage. In the open environment, juvenile plants were censused, but a randomly selected subset of plants was measured in the pre-reproductive ($n = 31$) and late-reproductive ($n = 108$) stages. This was necessary to ensure that measurements were made within a given developmental stage (a 1- to 2-week time period). Reproductive biomass was measured at the third stage, and included seeds and support tissue. Reproductive biomass is highly correlated with seed mass ($r = 0.98$; Jordan 1996) and was used as an estimate of fitness.

For the first and second censuses, we estimated leaf area and leaf and stem mass using multiple regression. On ten randomly chosen individuals of each biotype in each environment, stem diameter, leaf number in three size classes, and the length and width at the largest dimension of the crown were measured (non-destructive measures). Plants were then harvested and brought to the laboratory for determination of leaf area (ΔT Area Meter, Delta-T Devices, Cambridge, UK). Plant tissue was dried at 60°C to a constant mass, separated into leaves and stems, and weighed. Multiple linear regression equations were generated to estimate leaf area and leaf and stem mass from non-destructive measures. We used a stepwise procedure to generate models and used equations only if the adjusted R^2 exceeded 0.90 and the residuals were normally distributed.

Non-destructive measures of aboveground growth were made in the field; plants taller than 30 cm were divided vertically into 10-cm layers and measured within the layers (for architecture index, see below). Leaf area and leaf and stem mass were estimated from regression equations. Vegetative mass was calculated as the sum of leaf and stem mass, and percent stem mass as a proportion of vegetative mass.

At the third census, plants were divided into 20-cm layers, harvested by layer, and dried at 60°C. Leaf, stem, and reproductive parts were separated and weighed. A sample of four to six fresh leaves per plant was taken prior to harvest for calculation of specific leaf area (SLA). The average SLA for each environment was used to calculate leaf area from leaf mass. Vegetative and percent stem mass were calculated as for censuses one and two.

Plant architecture was defined as the distribution of leaf area along the vertical stem axis. To obtain a single number that represents this distribution, we generated a histogram of the leaf area in each layer of a plant. The skew of this histogram was used as an index of plant architecture; a more negative skew indicates that leaf area is distributed at the top of the plant while a positive skew reveals distribution toward the bottom. A value of two was added to the skew for statistical analysis.

Data analyses

Photosynthetic rates were plotted as a function of incident irradiance. The quadratic response of photosynthesis to light (typical for plants with C_4 photosynthetic metabolism) was tested separately for each biotype using polynomial regression (PROC GLM; SAS statistical software, version 6.11, SAS Institute, Cary, N.C.). Both biotypes had significant second-order responses to light ($P < 0.01$). Differences between the biotypes in their photosynthetic responses were tested by the interaction between biotype and a quadratic light response term using analysis of covariance (PROC GLM). Individual nested within biotype was included as a random effect. The 95% confidence intervals of WT and R light response curves were compared to estimate the irradiance at divergence.

Growth characters were analyzed for the main effects of biotype, environment, and their interaction by two-way analysis of variance (ANOVA), with family included as a random effect nested within biotypes (PROC GLM). The block effect was not significant and was not included in the final model. Separate ANOVAs were performed for each census using log-transformed data to minimize heteroscedasticity. We used the Dunn-Sidák method to calculate Bonferroni adjusted αs to control for experiment-wide error rates within each developmental stage (Sokal and Rohlf 1995).

Selection differentials, which measure the sum of direct and indirect selection, were calculated as the covariance between a standardized trait and relative fitness (PROC CORR in SAS, as in Kelly 1992 and Dudley 1996). Values can range from negative to positive; a positive selection differential indicates that an increase in the given trait confers an increase in fitness, and the higher the value, the stronger selection is for that trait. Selection differentials were considered different from zero if the Pearson correlation coefficient between the trait and relative fitness was statistically significant ($P < 0.05$, PROC CORR). Differences in selection differentials between biotypes were analyzed using a test of homogeneity between correlation coefficients (Sokal and Rohlf 1995). Data were standardized within biotypes, environments, and developmental periods.

Selection gradients and path analysis were used to quantify direct and indirect contributions of correlated traits to fitness. Selection gradients are the standardized, partial regression coefficients from a multiple regression of relative fitness on a set of traits. They measure direct selection on a trait and distinguish it from indirect selection via correlated traits. Path analysis links results from several regression analyses and can be used to calculate the indirect contributions of a trait to fitness.

Selection gradients and path analysis were calculated only for the open environment. The recommended sample size for these analyses is 10–20 times as many observations as variables (Mitchell 1993), and plant mortality in the competitive environment produced insufficient sample sizes for this analysis. Herbivory by small mammals living in the protection of the competitive environment was the cause of mortality, and did not differ between WT and R plants. Analysis for the open environment was limited to individuals remaining at the end of the season having complete juvenile and late-reproductive data; pre-reproductive data were eliminated because of reduced sample size.

Standardized, directional selection gradients (βs) were calculated to partition the contributions of photosynthetic biotype and late-reproductive leaf area, percent stem mass, and height to

reproductive success. Photosynthetic biotype was coded such that the estimated gradient corresponds to the difference between R versus WT. To minimize multicollinearity, vegetative mass (highly correlated with leaf area; $r = 0.76$–0.98 depending on census and biotype) was excluded from the analysis. Selection gradients were calculated using multiple linear regression of relative fitness on non-standardized traits (PROC REG in SAS). Collinearity diagnostics revealed that assumptions of multiple regression were upheld.

Path analysis was used to determine the relative magnitude of direct versus indirect contributions of photosynthetic biotype to relative fitness. The paths analyzed in the model include the selection gradients described above, as well as the effects of photosynthetic biotype on juvenile and late-reproductive leaf area, percent stem mass, and height. The contributions of juvenile traits to late-reproductive growth were incorporated through leaf area because it was predicted to have a large contribution to fitness based on the established relationship between leaf area and reproductive success (Solbrig 1981; Maddox and Antonovics 1983; Bennington and McGraw 1995). The cumulative indirect effect of biotype on fitness, via direct effects on growth, was calculated using PROC CALIS (in SAS, as in Mitchell 1993). Three outliers were eliminated to reduce Mardia's multivariate normality index from 10.10 to –0.16, resulting in a total sample size of 105. Individual path coefficients were tested for difference from zero using linear regression (PROC REG).

Results

Photosynthesis

A significant interaction between the quadratic light response term and biotype ($P < 0.05$) demonstrated that WT and R plants have divergent photosynthetic responses to irradiance (Fig. 1). Comparison of 95% confidence intervals (not shown) for these light responses revealed that under field conditions, R plants have lower photosynthetic rates at irradiances above approximately 600 μmol m^{-2} s^{-1}. The decrease in photosynthetic rates of R plants compared to the WT ranged from 15 to 27%, and the reduction increased with irradiance (Fig. 1, inset).

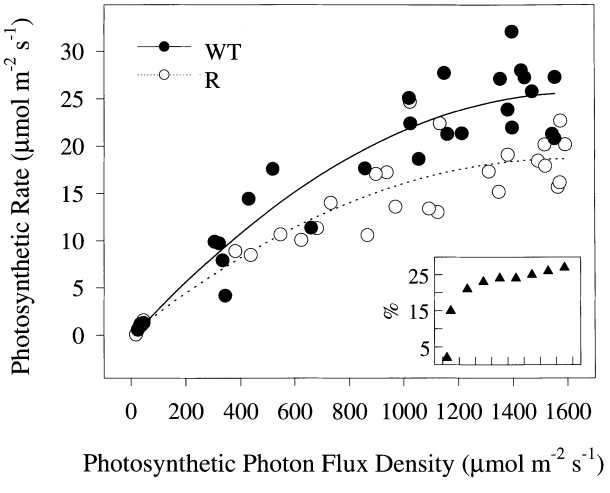

Fig. 1 The composite response of net photosynthetic rate to incident irradiance for atrazine-resistant (*R*) and wild-type (*WT*) *Amaranthus hybridus*. Four R and four WT plants were measured repeatedly on a clear day between 6:00 a.m. and 6:00 p.m. The relationships between net photosynthetic rate and incident irradiance were fit by second-order polynomial regression and diverge between 500–700 PPFD. The *inset graph* shows the percent reduction in R photosynthetic rate from the WT as a function of irradiance. The units and scale for irradiance are identical to those for the composite response of net photosynthetic rate to incident irradiance

Biotype and environment effects on trait means

There were no detectable differences in growth of R and WT plants in either environment, with the exception that late-reproductive R plants were taller (Fig. 2, Table 1). However, R plants produced less reproductive mass (Fig. 2, Table 1).

Plants in the open environment consistently had greater leaf area, vegetative mass, height, and reproductive biomass compared to those in the competitive environment (Fig. 2, Table 1). Allocation to stem mass was greater in the open environment for juvenile and late-reproductive plants but was lower at the pre-reproductive stage. Plant architecture did not differ between environments.

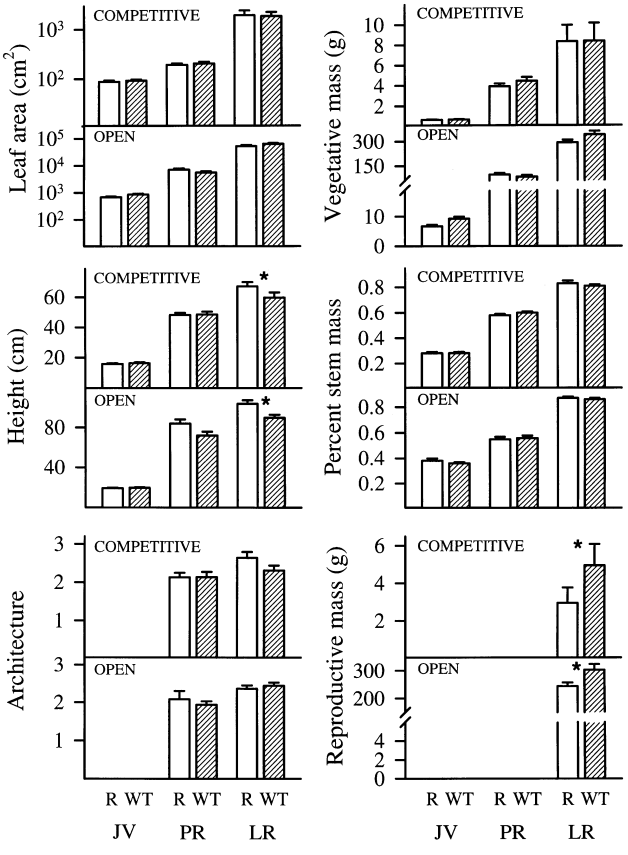

Fig. 2 Mean values for leaf area, vegetative mass, height, percent stem mass, architecture, and reproductive mass of R (*open bars*) and WT (*hatched bars*) *A. hybridus* grown in competitive and open environments. Data are presented for juvenile (*JV*), pre-reproductive (*PR*), and late-reproductive (*LR*) censuses separately. *Error bars* are ±1 SE. Sample sizes are given in Table 1. *Asterisks* indicate differences significant at $P < 0.05$

Table 1 *F*-ratios from two-way analyses of variance comparing size, allocation, architecture, and fitness traits. Differences between biotypes and sites and the interaction between biotype and site were tested separately for each census. Family lines nested within biotypes were tested as a random effect. All data were log transformed prior to analysis. Sample sizes in the competitive and open environments, respectively, are juvenile 156/176, pre-reproductive 108/31, and late-reproductive 28/108. Within each census and environment, biotypes are represented in approximately equal numbers

Effect	Leaf area	Vegetative mass	Height	Percent stem mass	Architecture	Reproductive mass
Juvenile						
Biotype	2.49	3.12	0.01	0.44	–	–
Family	0.91	1.24	1.84*	1.32	–	–
Environment	494.64***	396.82***	18.70***	91.49***	–	–
B × E	1.35	2.59	0.00	0.04	–	–
Pre-reproductive						
Biotype	0.78	0.16	2.78	0.17	0.03	–
Family	0.78	0.97	0.75	4.66***	0.90	–
Environment	1141.77***	809.91***	69.32***	9.00**	0.02	–
B × E	1.88	1.85	2.68	0.91	0.04	–
Late-reproductive						
Biotype	2.28	0.92	11.30**	3.02	0.02	8.20**
Family	1.10	1.42	4.38***	1.33	1.22	0.60
Environment	390.32***	1168.16***	77.05***	12.92***	0.44	1039.41***
B × E	0.04	0.82	0.42	0.89	1.39	1.73

*$P < 0.05$; **$P < 0.01$; ***$P < 0.0001$

Relationships of individual growth traits to reproductive biomass: selection differentials

We compared selection differentials to determine if photosynthetic biotype affected the strength of selection for individual growth traits. In the competitive environment, selection was consistently strong for increased leaf area and vegetative biomass (Table 2). For juveniles, increased leaf area, vegetative mass, height, and allocation to stems contributed to WT fitness, whereas none of these traits increased fitness in R plants. By the pre-reproductive stage, selection for increased size was significant in both biotypes, but was stronger in the WT for vegetative mass and height. At the late-reproductive stage selection was only significant for leaf area and vegetative mass and did not differ between biotypes.

In the open environment, selection for increased leaf area and vegetative biomass was consistent throughout development. Selection differentials did not differ in magnitude between biotypes with the exception of significant selection for increased pre-reproductive leaf area in WT but not R plants (Table 2). In juvenile plants, selection was positive for all traits except percent stem mass, which had a negative relationship with fitness. For pre-reproductive traits, selection was only significant for leaf area and vegetative mass in WT plants. By the late-reproductive stage, selection was significant for leaf area and vegetative mass in both biotypes and for height in the R biotype.

Table 2 Selection differentials for size, biomass allocation, and architecture traits at each census. For comparison of biotypes, data are given for each environment with biotypes presented separately (*Competitive* and *Open*, *R*/*WT*). Coefficients significantly different from zero are *italicized* ($P < 0.05$) and marginally significant coefficients are *underlined* ($P < 0.10$). Differences in selection differentials between biotypes or environments are indicated by *asterisks* ($P < 0.05$)

Trait	Juvenile	Pre-reproductive	Late-reproductive
Competitive			
Sample size	10/18	10/18	10/18
Leaf area	0.21/*0.89**	*0.77*/*0.90*	*0.64*/*0.67*
Vegetative mass	0.28/*0.78**	*0.74*/*0.96**	*0.63*/*0.83*
Height	0.01/*0.76**	*0.56*/*0.93**	−0.04/0.36
Percent stem mass	0.27/*0.56*	−0.35/0.23	−0.39/0.20
Architecture		−0.18/–0.07	−0.50/–0.09
Open			
Sample size	58/51	15/15	56/50
Leaf area	0.27/0.33	0.10/*0.48**	*0.12*/*0.17*
Vegetative mass	*0.25*/*0.30*	0.10/*0.36*	*0.28*/*0.36*
Height	*0.28*/*0.32*	0.16/0.16	*0.12*/0.03
Percent stem mass	*−0.24*/*−0.18*	−0.03/−0.06	0.05/0.13
Architecture		−0.13/−*0.25*	0.04/−0.01

Direct and indirect effects of photosynthetic biotype: selection gradients and path analyses

Variation in biotype and late-reproductive leaf area, percent stem mass, and height explained 52% of the variation in relative fitness in the open environment ($P < 0.001$). Direct selection was strongest for increased leaf area ($\beta = 1.13$). Increased allocation to stems had the second largest effect on fitness ($\beta = 0.95$) and height was negatively related to fitness (−0.32). The direct effect of photosynthetic biotype on fitness was not significant (Fig. 3).

The indirect effect of photosynthetic biotype conferred a 0.17 standardized unit increase in fitness. Therefore, assuming that the photosynthetic rates of the biotypes differed by approximately 25% (Fig. 1), variation in photosynthesis effected about a 17% difference in fitness. The indirect effect can be primarily attributed to the effect of photosynthetic biotype on late-reproductive height (Fig. 3: $\beta = -0.30$, $P < 0.002$). Paths from photosynthetic biotype to juvenile leaf area

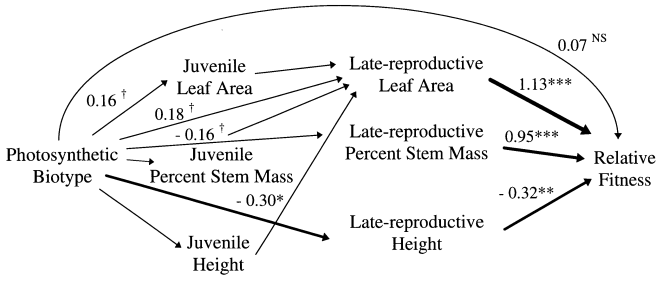

Fig. 3 Path diagram for R and WT *A. hybridus* grown in the open environment. *Line thickness* indicates the strength of the relationship (β), which is given above the line. Differences of β from zero are indicated as $^{\dagger}P < 0.15$; $^{*}P < 0.01$; $^{**}P < 0.001$; $^{***}P < 0.0001$. βs for paths with $P > 0.15$ are not given

and late-reproductive leaf area and percent stem mass were marginally significant ($P < 0.15$), and may reflect additional indirect effects of photosynthetic biotype on fitness. All other paths were non-significant with $\beta < 0.10$ and $P > 0.15$.

Discussion

Biotype and environment effects on photosynthesis, growth, and reproduction

Contrary to our expectations, the lower observed photosynthetic rates of the R biotype were not associated with reduced growth or altered allocation and architecture in plants growing in either competitive or non-competitive environments. In greenhouse studies, nearly isonuclear R plants had reduced total vegetative biomass compared to WT plants (*S. vulgaris*, McCloskey and Holt 1990, 1991; *B. napus*, Hart et al. 1992; *A. hybridus*, Jordan 1996). The lack of significant growth differences between biotypes in this study may result from plastic responses to environmental heterogeneity, which led to large variances in trait means. Such responses are common (Bell et al. 1991), and an increase in environmental variances would reduce the power to detect subtle growth differences between biotypes. The lack of biotype by environment interactions indicates that biotypes had similar responses to environmental differences. Because we did not measure belowground growth, it remains possible that R and WT plants differed in allocation to roots. An increase in WT root-shoot ratios has been observed in *B. napus* (Hart et al. 1992).

Although reduced photosynthetic rate did not have detectable effects on growth, R plants produced significantly less reproductive biomass than WT plants in both environments. This 'fitness cost' reveals selection against the R biotype that is consistent across environments. Comparable results for *S. vulgaris* show greenhouse-grown R plants to have lower reproductive mass as a percent of shoot dry weight than WT plants (McCloskey and Holt 1990). With the system used in this study, field experiments also revealed decreased fitness of the R biotype, although the magnitude of the fitness reduction varied with the year of study and population of origin (Jordan 1996). Genotype by environment interactions for fitness show that selection can be spatially heterogeneous (Bazzaz and Sultan 1987; Stratton 1995). However, consistent selection for the WT across environments in this study suggests that R plants may eventually be excluded, due to fitness costs associated, at least in part, with reduced photosynthetic rates. Large fitness reductions despite similarities in growth suggest that lower photosynthetic rates have small effects on growth that accumulate throughout development.

Based on the photosynthetic responses to irradiance of the two biotypes, we expected that the R biotype would have reduced reproductive success when grown under high irradiances, but not in the lower light levels of the competitive environment. The finding that fitness costs were similar across environments suggests that the R biotype was not at a greater disadvantage when grown at higher irradiances. High growth temperatures in 1995 may have influenced the relative fitness of the biotypes in both environments; higher growth temperatures increased differences in shoot dry weight between R and WT biotypes of *S. vulgaris* (McCloskey and Holt 1991). Alternatively, different environmental effects may have operated at different developmental stages in the two environments, yet produced a similar fitness reduction in R plants. For example, increased belowground competition for water and nutrients in the competitive site may have differentially affected R and WT root growth, which could potentially affect reproduction.

Contributions of individual traits to reproductive biomass

In both biotypes and environments, leaf area, vegetative mass, and height were positively correlated with reproductive output. Positive relationships between plant size and reproduction are well established (Samson and Werk 1986; Thompson et al. 1991). Architecture did not significantly contribute to fitness. Our measure of architecture, skew in the vertical distribution of foliage along the main axis, may not have accurately reflected all of the factors that influence light interception. For example, there was no provision for variation in leaf angle or clumping in our index. Studies of plant form show that the number of branches (Lechowicz and Blais 1988; Pigliucci et al. 1995) and branch growth (Farris and Lechowicz 1990) contribute to reproduction, but branching does not necessarily capture the spatial display of leaves to light and may be more indicative of plant size.

Effect of photosynthetic biotype on selection for growth traits

The effect that photosynthetic rate has on the relationship between growth and fitness can be inferred from

differences in selection between the biotypes. In the competitive environment, these differences were most apparent early in development and diminished over time. For juvenile plants, there was significant selection for increased leaf area, vegetative mass, height, and allocation to stems in the WT but not the R biotype. At the pre-reproductive stage, selection for increased vegetative mass and height was significant in both biotypes, but was greater in the WT. Thus, at an equivalent size, R plants had lower fitness than WT plants. These differences in selection between biotypes suggest that a lower photosynthetic rate affected the linkage between plant size and fitness in the competitive environment by uncoupling early growth from reproduction later in life. Because these differences are seen early in development, the influence of photosynthetic rate on fitness is not solely through direct contributions during seed production. Rather, lower photosynthetic rates seem to affect storage and allocation of photosynthate, possibly belowground, that may later be remobilized for seed production. These effects would therefore be indirect.

In the open environment, a lower photosynthetic rate generally did not affect selection on growth traits. This suggests that there are fewer indirect effects of photosynthetic rate on fitness when plants are grown without competition. The direct and indirect effects of photosynthetic biotype on fitness in the open environment were assessed using path analysis. After controlling for the effects of leaf area, stem allocation, and height, photosynthetic biotype did not have a significant direct effect on fitness. Rather, the effect of photosynthetic rate on fitness operated primarily indirectly, via direct effects on juvenile leaf area and late-reproductive leaf area, stem allocation, and height. Even though these effects were small, when coupled to the direct contributions of late-reproductive traits to fitness, photosynthetic rate did contribute indirectly to reproduction.

Although there were no significant interactions between the effects of photosynthetic biotype and the environment, the photosynthetic mutation tended to have a greater effect on fitness when plants were grown in competition; the relative fitness of the R biotype was 0.59 in the competitive environment and 0.80 in the open environment. In the competitive environment, the differences between biotypes in selection on growth traits suggest that photosynthetic rate had large indirect effects via growth, but this could not be formally tested with path analysis due to limited sample sizes. In the open environment, photosynthetic rate affected fitness indirectly through several small direct effects on growth. Environmental conditions may therefore substantially alter the direct and indirect ways in which photosynthetic rate influences reproductive success.

Our experimental system provides well-characterized differences in photosynthetic rate. Although other differences associated with the chloroplast genome may exist in the family lines used, the photosynthetic differences have a known genetic basis, with effects of other nuclear-encoded genes nearly randomized. This system has enabled us to show that variation in photosynthetic rate contributes to fitness indirectly through relatively small effects on growth throughout development. Indirect selection may therefore be a primary mechanism enabling the evolution of photosynthetic traits.

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References

Bazzaz FA, Sultan SE (1987) Ecological variation and the maintenance of plant diversity. In: Urbanska KM (eds) Differentiation patterns in higher plants. Academic Press, London, pp 69–93

Bell G, Lechowicz MJ, Schoen DJ (1991) The ecology and genetics of fitness in forest plants. III. Environmental variance in natural populations of *Impatiens pallida*. J Ecol 79:697–713

Bennington CC, McGraw JB (1995) Natural selection and ecotypic differentiation in *Impatiens pallida*. Ecol Monogr 65:303–323

Boogaard R van den, Goubitz S, Veneklaas EJ, Lambers H (1996) Carbon and nitrogen economy of four *Triticum aestivum* cultivars differing in relative growth rate and water use efficiency. Plant Cell Environ 19:998–1004

Cheplick GP (1995) Life history trade-offs in *Amphibromus scabrivalvis* (Poaceace): allocation to clonal growth, storage, and cleistogamous reproduction. Am J Bot 82:621–629

Dekker JH, Burmester RG (1992) Pleiotropy in triazine-resistant *Brassica napus*. 1. Ontogenetic and diurnal influences on phoftosynthesis. Plant Physiol 100:2052–2058

Dekker JH, Sharkey TD (1992) Regulation of photosynthesis in triazine-resistant and -susceptible *Brassica napus*. Plant Physiol 98:1069–1073

Dijkstra P, Lambers H (1989) A physiological analysis of genetic variation in relative growth rate within *Plantago major* L. Funct Ecol 3:577–587

Donovan LA, Ehleringer JR (1994) Potential for selection on plants for water-use. Am J Bot 81:927–935

Dudley SA (1996) Differing selection on plant physiological traits in response to environmental water availability: a test of adaptive hypotheses. Evolution 50:92–102

Farris MA, Lechowicz MJ (1990) Functional interactions among traits that determine reproductive success in a native annual plant. Ecology 71:548–557

Geber MA (1990) The cost of meristem limitation in *Polygonum arenastrum*: negative genetic correlations between fecundity and growth. Evolution 44:799–819

Geber MA, Dawson TE (1997) Genetic variation in stomatal and biochemical limitations to photosynthesis in the annual plant, *Polygonum arenastrum*. Oecologia 109:535–546

Hart JJ, Radosevich SR, Stemler A (1992) Influence of light intensity on growth of triazine-resistant rapeseed (*Brassica napus*). Weed Res 32:349–356

Hirshberg J, McIntosh L (1983) Molecular basis of herbicide resistance in *Amaranthus hybridus*. Science 222:1346–1349

Holt JS, Stemler AJ, Radesevich SR (1981) Differential light responses of photosynthesis by triazine-resistant and triazine-susceptible *Senecio vulgaris* biotypes. Plant Physiol 67:744–748

Jordan N (1991) Multivariate analysis of selection in experimental populations derived from hybridization of two ecotypes of the annual plant *Diodia teres* W. (Rubiaceae). Evolution 45:1760–1772

Jordan N (1996) Effects of the triazine-resistance mutation on fitness in *Amaranthus hybridus* (smooth pigweed). J Appl Ecol 33:141–150

Jursinic PA, Pearcy RW (1988) Determination of the rate limiting step for photosynthesis in a nearly isonuclear rapeseed (*Brassica napus* L.) biotype resistant to atrazine. Plant Physiol 88:1195–1200

Kalisz S, Teeri JA (1986) Population-level variation in photosynthetic metabolism and growth in *Sedum wrightii*. Ecology 67:20–26

Kelly CA (1992) Spatial and temporal variation in selection on correlated life-history traits and plant size in *Chamaecrista fasciculata*. Evolution 46:1658–1673

Lambers H (1987) Does variation in photosynthetic rate explain variation in growth rate and yield? Neth J Agric Sci 35:505–519

Lechowicz MJ, Blais PA (1988) Assessing the contributions of multiple interacting traits to plant reproductive success: environmental dependence. J Evol Biol 1:255–273

Maddox GD, Antonovics J (1983) Experimental ecological genetics in *Plantago*: a structural equation approach to fitness components in *P. aristata* and *P. patagonica*. Ecology 64:1092–1099

McCloskey WB, Holt JS (1990) Triazine resistance in *Senecio vulgaris* parental and nearly isonuclear backcrossed biotypes is correlated with reduced productivity. Plant Physiol 92:954–962

McCloskey WB, Holt JS (1991) Effect of growth temperature on biomass production of nearly isonuclear triazine-resistant and -susceptible common groundsel (*Senecio vulgaris* L.). Plant Cell Environ 14:699–705

Mitchell RJ (1993) Path analysis: pollination. In: Scheiner SM, Gurevitch J, (eds) Design and analysis of ecological experiments. Chapman & Hall, New York, pp 211–231

Mitchell-Olds T, Bergelson J (1990) Statistical genetics of an annual plant, *Impatiens capensis*. II. Natural selection. Genetics 124:417–421

Nelson CJ (1988) Genetic associations between photosynthetic characteristics and yield: review of the evidence. Plant Physiol Biochem 26:543–554

Nienhus J, Sills GR, Martin B, King G (1994) Variance for water-use efficiency among ecotypes and recombinant inbred lines of *Arabidopsis thaliana* (Brassicaceae). Am J Bot 81:943–947

Ort DR, Ahrens WH, Martin B, Stoller EW (1983) Comparison of photosynthetic performance in triazine-resistant and susceptible biotypes of *Amaranthus hybridus*. Plant Physiol 72:925–930

Pigliucci M, Whitton J, Schlichting CD (1995) Reaction norms of *Arabidopsis*. I. Plasticity of characters and correlations across water, nutrient and light gradients. J Evol Biol 8:421–438

Samson DA, Werk KS (1986) Size-dependent effects in the analysis of reproductive effort in plants. Am Nat 127:670–679

Sokal RR, Rohlf FJ (1995). Biometry. W.H Freeman and company, New York

Solbrig OT (1981) Studies on the population biology of the genus *Viola*. II. The effect of plant size on fitness in *Viola sororia*. Evolution 35:1080–1093

Stitt M, Schulze D (1994) Does Rubisco control the rate of photosynthesis and plant growth? An exercise in molecular ecophysiology. Plant Cell Environ 17:465–487

Stowe AE, Holt JS (1988) Comparison of triazine-resistant and -susceptible biotypes of *Senecio vulgaris* and their F_1 hybrids. Plant Physiol 87:183–189

Stratton DA (1995) Spatial scale of variation in fitness of *Erigeron annuus*. Am Nat 146:608–624

Sundby C, Chow WS, Anderson JM (1993) Effects on photosystem II function, photoinhibition, and plant performance of the spontaneous mutation of serine-264 in the photosystem II reaction center D1 protein in triazine-resistant *Brassica napus* L. Plant Physiol 103:105–113

Teramura AH, Strain BR (1979) Localized populational differences in the photosynthetic response to temperature and irradiance in *Plantago lanceolata*. Can J Bot 57:2559–2563

Thompson BK, Weiner J, Warwick SI (1991) Size-dependent reproductive output in agricultural weeds. Can J Bot 69:442–446

van Tienderen PH, van der Toorn J (1991) Genetic differentiation between populations of *Plantago lanceolata*. II. Phenotypic selection in a transplant experiment in three contrasting habitats. J Ecol 79:43–59

Warwick SI (1991) Herbicide resistance in weedy plants: physiology and population biology. Annu Rev Ecol Syst 22:95–114

Weaver SE, McWilliams EL (1980) The biology of Canadian weeds. 44. *Amaranthus retroflexus* L., *A. powellii* S. Wats. and *A. hybridus* L. Plant Sci 60:1215–1234