

BRIEF COMMUNICATION

SEEDLING EXPRESSION OF  
CROSS-GENERATIONAL PLASTICITY DEPENDS  
ON REPRODUCTIVE ARCHITECTURE<sup>1</sup>

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Through adaptive cross-generational plasticity, stressed plants can alter their offspring in specific ways that promote seedling success. As yet, very little is known about the expression of such plasticity, and whether it varies within a plant due to offspring position. The effects of parental light deprivation on distinct reproductive structures were tested in the annual *Polygonum hydropiper*, which produces both long terminal racemes and inconspicuous axial inflorescences. Inbred replicate parents from four genetic lines were grown in full greenhouse sunlight and simulated shade, and the initial mass, germination rate, and seedling growth traits of their terminal and axial offspring measured under uniform growth chamber conditions. Although parent light environment did not significantly influence seedlings from axial achenes, growth traits of those from terminal achenes were significantly enhanced as a result of parental light deprivation. In shaded conditions where resources are limiting, *P. hydropiper* plants appear to prioritize terminal achenes through increased provisioning as well as specific growth changes. These results show that the expression of cross-generational plasticity may vary depending on architectural position of offspring on the maternal plant.

**Key words:** maternal effects; parental environment; *Polygonum hydropiper*; *Polygonaceae*; seed heteromorphism; seedling development; simulated shade.

Plants have evolved various strategies to reduce the risks to their offspring posed by environmental variation (Venable and Brown, 1988). One such strategy is for maternal plants to modify their offspring in response to their own environmental conditions through adaptive cross-generational effects, a form of phenotypic plasticity (Lacey, 1991; Donohue and Schmitt, 1998). These maternal (or more generally, “parental”) environmental effects can include appropriate changes to traits such as seed structure and provisioning, and consequently seedling germination, development, and even defensive chemistry in response to stresses such as shade, drought, or herbivory (Schmitt et al., 1992; Sultan, 1996; Donahue, 1999; Agrawal, 2001). If the parental environment provides predictive information about the offspring environment, then these cross-generational effects can enhance offspring success (Donohue and Schmitt, 1998). Although numerous studies document parental environment effects on seed size and germination, much remains to be learned about the specific expression of adaptive cross-generational plasticity in seedlings of diverse taxa and environments (Sultan, 2004).

Another way plants accommodate heterogeneous environments is to produce seeds at different architectural positions on the plant, which can lead to diversity in offspring size, germination behavior, and dispersability (Schmitt et al., 1985; Venable and Levin, 1985; Cheplick and Sung, 1998; Donohue, 1999; Imbert, 2002). This architectural heteromorphism may be a form of maternal “bet-hedging” or protection against total offspring mortality: because seeds from different posi-

tions on a plant may be best suited to different environmental conditions, it ensures that some proportion of offspring will survive in heterogeneous or unpredictable environments (Venable, 1985; Wulff, 1995; Gardocki et al., 2000).

Although plant ecologists are aware of both sources of offspring variation, little is known about how cross-generational plasticity and reproductive architecture may interact. Previous studies have shown that parent plant environment can affect the proportions of propagules produced at specific architectural positions (Baker and O’Dowd, 1982; Cheplick and Sung, 1998; Imbert and Ronce, 2001). However, it is not known whether the effects of parental environment on offspring growth traits may differ depending on seed architectural position (see Cheplick and Sung, 1998 for a negative result regarding parent nutrient level). Here we investigate this question using the introduced annual *Polygonum hydropiper* L. (*Polygonaceae*). Plants of this species produce two architecturally distinct reproductive structures: large, indeterminate terminal racemes of approximately 30–50 (usually self-fertilized) flowers, and inconspicuous axial clusters of 2–5 flowers that remain encased in the sheathing ocreae (and are cleistogamous as a result). Both types of inflorescence produce trigonal achenes that are primarily gravity dispersed, or in the case of axials dispersed with the shoot tissue as it decomposes. This species thrives in open, moist habitats and expresses severely reduced growth and reproductive output under low light conditions (Sultan et al., 1998; Sultan, 2001). We compared the effects of parental shade and full sun on timing of production, mass, germination rate, and seedling development of terminal vs. axial achenes, to assess cross-generational plasticity in the two architectural types. We asked, “Do *P. hydropiper* plants grown in low light alter offspring provisioning and seedling traits in ways likely to promote seedling success in shade? If so, do these changes occur in both terminally and axially produced offspring?”

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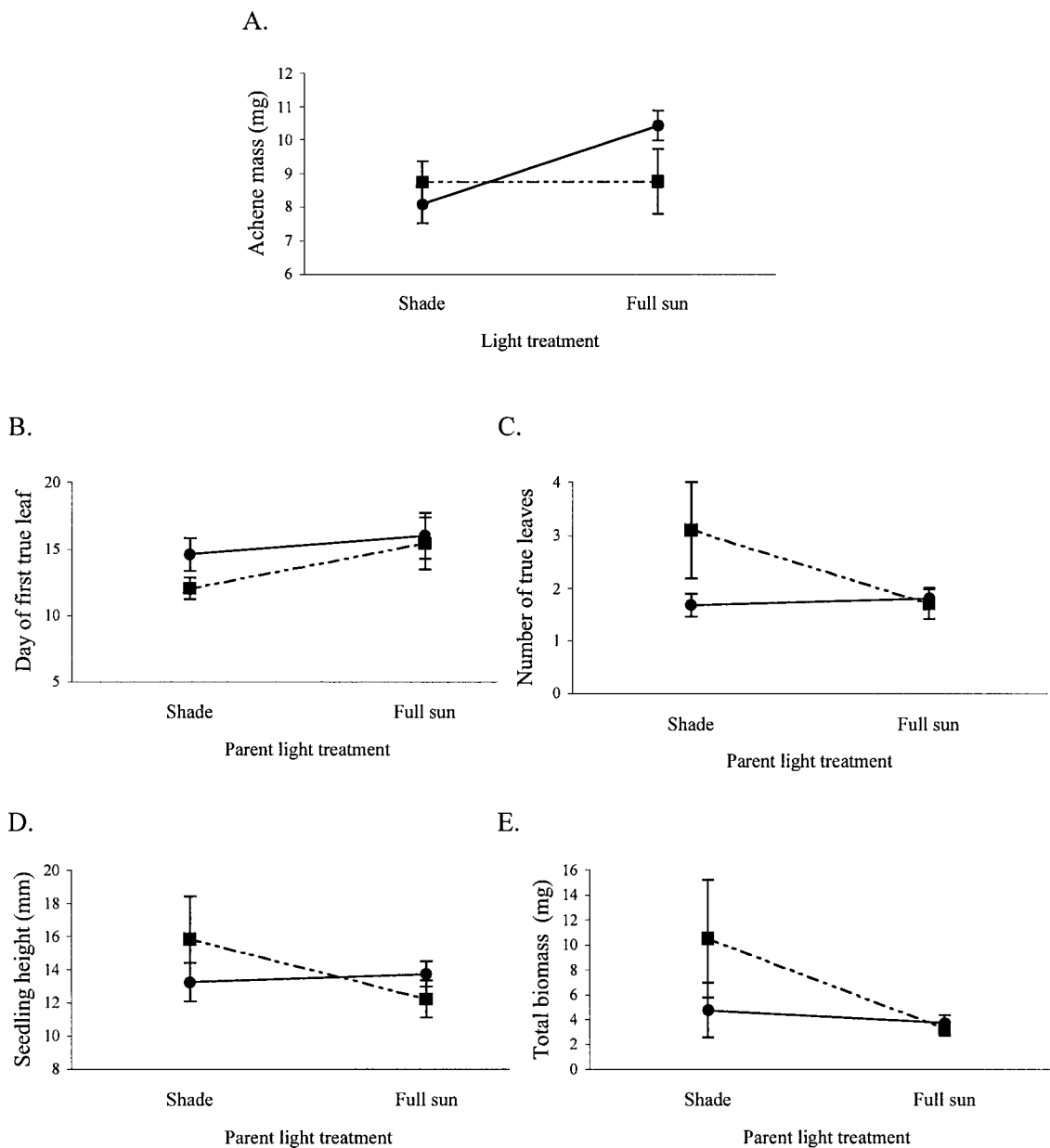


Fig. 1. Norms of reaction for seedlings from terminal (dashed) and axial (solid line) achenes to shade vs. full sun. (A) achene mass; (B) day of first true leaf; (C) number of true leaves; (D) seedling height; (E) total biomass. Bars represent  $\pm 2$  SE.

## MATERIALS AND METHODS

**Experimental material**—Achenes were collected from four field plants from two Massachusetts populations of *Polygonum hydropiper*. Plants grown from these achenes were inbred under uniform greenhouse conditions for two generations, and replicate parent plants of these four inbred lines were grown in two greenhouse treatments (16 June 2002–22 September 2002): full sun [(full available photosynthetically active radiation (PAR), treatment mean  $946 \pm 128 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  with red : far-red ratio of  $1.08 \pm 0.023$ ), and shade (21% PAR, treatment mean  $202 \pm 36 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  with red : far-red ratio of  $0.70 \pm 0.020$ , produced using a tent of 60% neutral-density shade cloth (DeWitt Co., Sikeston, Missouri, USA) lined with a plastic filter (#138 pale green, Lee Filters, Burbank, California, USA) perforated with 0.635-cm holes to insure air circulation). Treatment plots consisted of adjacent greenhouse benches and were not replicated. Relative humidity (40–50%) and temperature (22–40°C day, 18°C night) did not differ significantly between the light treat-

ments (D. Sloan, Wesleyan University, unpublished data). Five terminal and five axial achenes were randomly collected from the same shoot axes of each replicate parent plant in each light treatment (4 parental lines  $\times$  2 treatments  $\times$  10 achenes per parent individual;  $N = 80$  experimental offspring).

**Data collection**—First flowering date was recorded separately for terminal and axial inflorescences on each parent plant. The five replicate achenes collected from each inflorescence type of a given parent plant were collectively weighed. Achenes were cold stratified at 4°C for 47 days, and sown into vermiculite-filled flats. Seedlings were germinated and grown for 21 days under uniform growth chamber conditions (Conviroon E7/2, Winnipeg, Manitoba, Canada) with a 14-h day at 24/18°C and 500  $\mu\text{E}$  fluorescent light. Germination was monitored and the following seedling traits measured: seedling height (on days 4, 14, 21); day of first true leaf; final leaf number; and final biomass (oven-dry mass after  $\geq 24$  h at 64°C). Biomass was determined from separately weighed shoot and root tissues.

TABLE 1. Three-way ANOVA for the effects of parent light treatment (PLT), terminal vs. axial achene type (AT), line, and their interactions for seedling traits. *P* values in bold represent significance after sequential Bonferroni adjustment, while (\*) denotes *P* values significant at *P* < 0.05 without sequential Bonferroni adjustment. \*Seedling germination day.

Source	df	Germination Day <sup>a</sup>		Day First Leaf		Leaf Number		Final Height		Final Biomass	
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
PLT	1	74.3	≤ <b>0.0001</b>	45.9	≤ <b>0.0001</b>	12.2	< <b>0.001</b>	5.00	0.029*	23.9	≤ <b>0.0001</b>
AT	1	3.72	0.059	9.83	<b>0.003</b>	5.90	0.018*	0.00	0.959	4.29	0.043*
Line	3	11.2	≤ <b>0.0001</b>	34.2	≤ <b>0.0001</b>	7.43	< <b>0.001</b>	8.01	< <b>0.001</b>	7.54	< <b>0.001</b>
PLT × AT	1	1.86	0.178	9.74	<b>0.003</b>	15.0	< <b>0.001</b>	9.68	<b>0.003</b>	10.8	<b>0.002</b>
Line × PLT	3	0.43	0.732	6.89	< <b>0.001</b>	7.18	< <b>0.001</b>	3.75	<b>0.016</b>	6.63	< <b>0.001</b>
Line × AT	3	4.12	0.010*	1.21	0.315	1.97	0.129	1.16	0.334	1.43	0.244
PLT × AT × Line	3	0.64	0.591	2.08	0.114	3.92	0.013*	1.83	0.152	1.27	0.293

**Statistical analysis**—Three-way analysis of variance (ANOVA; JMP 5.0.1a, SAS Institute, 2001) was used to test the fixed effects of parent light treatment, terminal vs. axial achene type, inbred line, and their interactions on measured parental and offspring traits. The line term reflects both population and line identity (cf. Lechowicz and Blais, 1988); line was considered fixed rather than random because our non-random choice of populations and our sample of only 2 lines per population did not represent a random sample of lines for the species. Because mean achene mass and flowering date were not replicated within the parents, the line term was excluded from these ANOVA models. To meet normality assumptions, all data were log transformed, except for germination day, which was inverse transformed (Zar, 1984). Residuals were inspected to confirm homogeneity across treatments. A sequential Bonferroni test was applied to ensure tablewide significance levels for tests of seedling traits (Rice, 1989). Post hoc contrast tests were performed for shaded terminal seedlings against shaded axial, full sun terminal and full sun axial seedlings for all traits.

## RESULTS

Parent plants in both light treatments produced axial inflorescences significantly earlier than terminals. Parental light deprivation delayed both axial and terminal flowering, but the delay was greater for axials (effects of achene type, parent light treatment, and their interaction were significant at  $P \leq 0.0001$ ). The effects of parental light treatment on offspring provisioning also differed for axial vs. terminal infructescences: mean mass of terminal achenes remained constant across treatments, while axial achene mass was significantly reduced by light-stressed parent plants (effect of parental light treatment × achene type significant at  $P \leq 0.0009$ ;  $F = 12.07$ ; Fig. 1A). Because axial achenes were either heavier or lighter than terminals depending on light treatment, there was no main effect of achene type on achene mass ( $P \leq 0.1432$ ).

Parental light treatment had a highly significant effect on offspring germination timing (Table 1): regardless of achene type, achenes from shaded parents germinated on average 1.2 days earlier than those produced by parents given full sun; genotype effects on germination timing were also significant (Table 1).

When parent plants were given full sun, their terminal and axial offspring developed similarly (Fig. 1B–E). However, parental shade had significantly different effects on terminal compared with axial offspring (significant interactions of parent light treatment × achene type for all seedling traits, Table 1). Furthermore, seedlings from shaded terminal achenes were significantly different than shaded axials and both full sun achene types in post hoc contrasts. For instance, when parents were shaded, they produced terminal seedlings with 220% greater biomass, while biomass of axial offspring did not vary

due to parent light treatment (Fig. 1E). Seedlings from terminal achenes of shaded parents produced leaves earlier and in greater numbers, grew taller, and produced more biomass, compared with either terminal achenes from full sun parents or axially produced offspring from either parental light treatment (Table 1, Fig. 1B–E). Although parental shade did not alter the timing or number of leaves produced by axial offspring, terminally produced seedlings from shaded parents had true leaves more than 3 days earlier and added 1.4 of them on average, compared with terminal offspring of high light parents (Fig. 1B, C). Although seedling height did not differ significantly on days 4 and 14, by day 21, terminal offspring of shaded parents were significantly taller, while parental light treatment did not affect the seedling height of axially produced offspring (Fig. 1D; Table 2). Similarly, seedlings from shaded terminal achenes were significantly different than shaded axials and both full sun achene types in post hoc contrasts for height at day 21, but not days 4 and 14.

## DISCUSSION

Our results showed (1) dramatic cross-generational effects of parent plant environment on seedling traits, and (2) these effects differed depending on seed architectural position. *Polygonum hydropiper* plants raised in shade rather than full sun altered axial and terminal offspring differently: in response to shade, parent plants produced slightly smaller, later-developing axial offspring and dramatically different terminal offspring. The rapid development and greater height, leafiness, and total biomass of these terminal offspring of shaded parents may be important components of seedling establishment and competitive success in shaded or other resource-limited environments (Wulff, 1995; additional references in Sultan, 1996). Interestingly, achene mass did not change in these terminal progeny; shaded parents may economize on carbon-rich pericarp tissue to maximize provisioning to nutritive and embryonic tissues (as occurs in the closely related *Polygonum persicaria*; Sultan, 1996), resulting in equivalent achene mass.

Evidently, *P. hydropiper* plants express adaptive cross-generational plasticity, but only in terminally produced offspring. This architectural difference in expression of plasticity likely reflects a combination of physiological constraints and selection due to different contributions to fitness by the two achene types. Developmental constraints on axial vs. terminal achenes may include differential nutrient supply to distinct architectural locations on the parent plant (Venable, 1985; Imbert, 2002). Nutrient flow to developing seeds may be influenced by seed location relative to plant vasculature (Diggle, 1995)

TABLE 2. Three-way ANOVA for the effects of parent light treatment (PLT), terminal vs. axial achene type (AT), line, and their interactions on seedling height at day 4, 14, and 21. *P* values in bold represent significance after sequential Bonferroni adjustment, while (\*) denotes *P* values significant at *P* < 0.05 without sequential Bonferroni adjustment.

Source	df	Day 4		Day 14		Day 21	
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
PLT	1	0.07	0.798	0.56	0.457	5.00	0.029*
AT	1	0.60	0.442	0.36	0.552	0.01	0.959
Line	3	1.98	0.128	1.93	0.135	8.01	<0.001
PLT × AT	1	1.37	0.247	1.02	0.318	9.68	0.003
Line × PLT	3	2.36	0.082	1.47	0.234	3.75	0.016
Line × AT	3	0.48	0.695	0.37	0.775	1.16	0.334
PLT × AT × Line	3	1.23	0.308	1.24	0.304	1.83	0.152

and may decrease with distance from the main plant axis (Susko and Lovett-Doust, 2000). This may explain the large mass of axial achenes produced by resource-rich *P. hydropiper* plants. However, in this species the greater strength of the large terminal inflorescences as a nutrient sink may direct limited resources to these achenes (Lee, 1988 and references therein). In addition, the location of axial flowers within the sheathing ocrea may restrict gas exchange or water transport (Cheplick, 1996; Cheplick and Sung, 1998); when plants are light-deprived this constraint may be sufficient to reduce achene mass.

The differential expression of cross-generational plasticity may also reflect an evolved response in *P. hydropiper* plants to “prioritize” terminal rather than axial achenes. One possible selective factor is the greater dispersal ability of terminal offspring. The terminally produced achenes are located higher on the shoot and at branch ends and are likely to disperse farther from the parent than the axial achenes, which are bound to the stem by the sheathing ocrea. Seed types associated with distinct architectural and positional locations or other types of within-parent heteromorphism often have significantly different dispersal capacities (Rocha, 1996; Imbert, 1999; Imbert and Ronce, 2001).

A second difference between terminal and axial offspring is that axial inflorescences are obligately cleistogamous, while terminal inflorescences may be outcrossed and/or selfed. Similarly, the chasmogamous and cleistogamous flowers produced at different shoot positions on *Impatiens capensis* are associated with dispersal and seedling fitness differences: chasmogamous flowers at upper nodes and branch tips produce seeds that can disperse farther and give rise to more vigorous seedlings than do cleistogamous flowers produced at lower nodes close to the main stem (Waller, 1979; Schmitt et al., 1985). Finally, because terminal achenes are exposed to predation by waterfowl (S. Sultan, personal observation), the inconspicuous, sheathed axial achenes may be evolutionarily maintained as a bet-hedging or “insurance” cache of offspring despite the apparent constraints on provisioning to these offspring under conditions of low light availability.

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