

## Contrasting patterns of transgenerational plasticity in ecologically distinct congeners

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**Abstract.** Stressful parental environments can influence offspring size and development either adaptively or maladaptively, yet little is known about species' differences in this complex aspect of phenotypic plasticity. We performed a reciprocal split-brood experiment to compare transgenerational plasticity in response to drought stress in two closely related annual plant species. We raised inbred replicate parent plants of eight genotypes per species in dry vs. moist soil to generate offspring of each genetic line that differed only in parental environment, then monitored seedling development in both dry and moist conditions. Individuals of the two species expressed contrasting patterns of transgenerational plasticity for traits important to seedling drought tolerance. In *Polygonum persicaria*, a weedy generalist found in moist, dry, and variably dry sites, drought-stressed plants produced offspring with longer and more rapidly extending root systems and greater biomass when growing in dry soil. In contrast, in *P. hydropiper*, a non-weedy congener restricted to moist habitats, the offspring of drought-stressed parents had reduced root system development and seedling biomass in dry soil. In *P. persicaria*, transgenerational and immediate adaptive plasticity combined to produce drought-adapted seedling phenotypes. These results make clear that characteristic patterns of transgenerational plasticity can contribute to ecological diversity among species.

**Key words:** ecological generalist; maternal effects; nongenetic inheritance; phenotypic plasticity; *Polygonum persicaria*; seedling development.

### INTRODUCTION

It is well known that parental (usually maternal) environment can substantially affect offspring development. In many cases, these transgenerational effects simply mirror resource levels, such that parents in stressful environments reduce offspring size or quality (Roach and Wulff 1987, Galloway 1995, Donohue and Schmitt 1998). As a result, parental environmental effects on offspring are generally expected to be negative. However, like other aspects of phenotypic plasticity, transgenerational effects can vary substantially among taxa (Wulff 1995, Weiner et al. 1997, Sultan 2001). Indeed, recent studies have revealed that certain species of both plants and animals express specific changes to offspring traits that enhance offspring success under the stress in question (Lacey 1991, Schmitt et al. 1992, Sultan 1996, Fox et al. 1997, Mousseau and Fox 1998a, Agrawal 2001, Gustafsson et al. 2005, Mondor et al. 2005). When parent and offspring environments are correlated, for

instance in organisms with limited dispersal, this remarkable, transgenerational mode of adaptive plasticity can provide a key advantage to vulnerable early life cycle stages (Agrawal et al. 1999, Rotem et al. 2003, Galloway and Etterson 2007). Conversely, maladaptive transgenerational effects of stress could restrict the range of conditions in which a given species can establish and maintain populations. Accordingly, species-specific patterns of transgenerational plasticity may be a heretofore-unrecognized aspect of ecological diversity.

To date, very little is known about ecologically meaningful differences in transgenerational plasticity among species (Fox et al. 1997, Donohue and Schmitt 1998). In general, appropriately designed transgenerational studies are rare for naturally evolved taxa (Fox et al. 1997, Weiner et al. 1997, Rossiter 1998, Shaw and Byers 1998, Rotem et al. 2003, Lacey and Herr 2005), and comparative experiments testing key environmental factors are lacking. Furthermore, studies of parental environmental effects typically focus on offspring size or mass rather than on the functionally relevant offspring traits that directly influence performance in specific conditions.

Here we present the results of an experiment comparing transgenerational plasticity in response to a critical environmental variable, drought stress, in two ecologically distinct congeners: *Polygonum persicaria* is a widely distributed weed that occurs across much of North America in moist, dry, and variably dry sites; *P.*

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*hydropiper* was introduced concurrently (by European settlers), but is limited to consistently moist sites within the same geographic range. (For distribution data and references, see Sultan et al. [1998].) This species pair affords a robust comparison because they are members of the same monophyletic unit within the genus *Polygonum* (sensu lato) and share similar life histories and morphologies (Sultan 2001). Both species are obligate annuals, so population persistence depends entirely on seedling offspring. Because both individual plasticity and ecological distribution are well documented in these taxa, they provide an excellent model system for studying naturally evolved genotypic norms of reaction.

Phenotypic expression of transgenerational effects is shaped in part by the offspring's immediate environment (Schmitt et al. 1992, Galloway 1995, Rossiter 1998, Sultan 2004), because the growing organism's direct plastic response to its environment can either enhance or counter transgenerational effects (Agrawal et al. 1999). Furthermore, the adaptive or maladaptive consequences of transgenerational effects will differ from one offspring environment to another (e.g., Badyaev et al. 2002). Accordingly, to precisely characterize transgenerational effects and to assess whether they adaptively match parental conditions, their expression must be tested in both similar and contrasting offspring environments (Wulff et al. 1994, Munir et al. 2001, Rotem et al. 2003).

We performed a reciprocal split-brood experiment to compare patterns of transgenerational plasticity in *P. persicaria* and *P. hydropiper* in response to parental drought stress, as expressed in dry vs. moist seedling environments (cf. Schmitt et al. 1992, Wulff et al. 1994, Rossiter 1998, Agrawal 2001). We measured offspring traits critical to drought tolerance (i.e., rate and extent of root system development, which determine the seedling's access to soil moisture and its absorptive surface area) as well as total seedling growth, a robust predictor of individual fitness (Galloway 1995, Wulff 1995). This factorial design allowed us to (1) assess the adaptive match between parental moisture treatment and offspring phenotypes expressed in similar conditions, (2) distinguish the effects of transgenerational plasticity from the seedlings' immediate plastic responses to their soil environment, and (3) examine the interaction between these aspects of phenotypic response. The contrasting results for the two species are discussed in the context of the species' realized differences in field moisture distributions, determined in a previous study (Sultan et al. 1998).

## MATERIAL AND METHODS

### *Split-brood experiment*

In the parental generation, two replicate plants from each of eight inbred genetic lines per species (representing four natural field populations spanning each species' ecological range; Sultan et al. 1998) were randomly

assigned to controlled dry and moist greenhouse soil treatments ( $13.2\% \pm 5.8\%$  vs.  $26.6\% \pm 4.1\%$  soil moisture by mass [mean  $\pm$  SD], respectively) and raised to maturity in a fertilized 1:1:1 mixture of sterilized topsoil, horticultural sand, and fritted clay under full summer sun (photosynthetically active radiation [PAR],  $1239 \pm 108 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  [1  $\mu\text{Einstein} = 1 \mu\text{mol}$  of photons]; details in Sultan 2001). Dry and moist treatments were equivalent respectively to  $\sim 50\%$  and  $100\%$  of field capacity for this soil mix; mean conditions for field populations of *P. persicaria* are close to  $50\%$  of field capacity, while the mean for *P. hydropiper* is  $\sim 75\%$ , and both species inhabit sites at or above  $100\%$  field capacity (Sultan et al. 1998). The dry- and moist-grown parental plants of each genetic line self-fertilized to produce inbred achenes (one-seeded fruits) that were harvested, stratified in deionized water at  $4^\circ\text{C}$  for five weeks, and germinated on moist filter paper in petri plates on a greenhouse bench ( $22^\circ/18^\circ\text{C}$  day/night temperature regime). (Both species are primarily selfing and show no inbreeding depression; we refer to "parental effects" since each parent plant was both the maternal and paternal parent; Sultan 1996, Rotem et al. 2003.) Parental moisture treatment did not affect the percentage of viable offspring in either species, and there was no confounding species  $\times$  treatment interaction effect on germination rate or percentage.

Forty-eight hours after their germination, 16 randomly selected seedlings from dry- and moist-grown parents of each line were individually transplanted into flat plexiglass rhizotrons fashioned from 245-mm square bioassay dishes that had been split into two 400-mL growth compartments by vertical plastic dividers bonded with inert acrylic aquarium resin. The lid of each dish was attached with silicone caulk, the top removed with a table saw, and four 0.5-cm drainage holes were drilled along the bottom surface. The rhizotrons were filled with a naturalistic 2:2:1 sterilized topsoil:horticultural sand:fritted clay mix moistened with 40 mL of distilled water per liter of soil mix, and placed in a dual Conviron growth chamber (Controlled Environments, Winnipeg, Manitoba, Canada) set at  $23^\circ/18^\circ\text{C}$  for a ramped 14-h daylength with  $700 \mu\text{E}$  PAR. Rhizotrons were set in stainless steel racks at a  $50^\circ$  angle to maximize gravitropic root growth against the transparent front surfaces (Gross et al. 1992), which were covered with moist chamois cloths to maintain cool and dark conditions for root growth.

Seedlings were randomly assigned to chamber positions and compartments and to either dry or moist offspring soil treatments. The experimental sample consisted of 16 replicate seedlings of each inbred, grandparental line from each parental moisture treatment, eight in each offspring moisture treatment (dry vs. moist). Treatments were maintained on a plant-by-plant basis to effect a randomized complete block design. Dry treatment containers were hand-watered sparingly ( $\sim 10$  mL of distilled water two to four times daily) to

maintain ~13% soil moisture by mass; moist treatment containers were continually bottom-watered to keep soil moisture consistently at 100% field capacity. All seedlings were grown for 21 d in the rhizotron treatments. After losses due to one missing parental line, treatment and measurement error (15 seedlings), and failure to thrive in two seedlings, the total sample consisted of 415 seedlings. The experiment was carried out in two temporal blocks.

#### *Data collection*

Offspring root extension over time was nondestructively measured by manually tracing the visible portion of each seedling's root system onto an acetate sheet on days 5, 10, 15, and (for a subsample) 20 of its growth in the rhizotron treatments. Traced root systems were scanned on a flatbed scanner and digitized as Adobe Photoshop 5.5 images from which total traced root length was determined (RootLength+ software; Berntson 1997). The acetate tracing method captured 70% of the variation in total root growth, based on a regression of root lengths determined from scanned manual tracings against root lengths directly measured from harvested root systems on a Comair optical root length scanner (Hawker de Havilland, Melbourne, Victoria, Australia) for a subsample of four to seven randomly chosen seedlings from each of the four parent treatment  $\times$  offspring treatment combinations for each species (total subsample  $N = 42$  plants,  $R^2 = 0.703$ ,  $P \leq 0.0001$ ). Maximum depth of any root ("deepest root") was determined from tracings for each seedling on each measurement day.

Seedlings were harvested on day 21 and separated into shoot and root tissues, and the shoots were oven-dried at 100°C for 1 h and then at 65°C for >48 h. Washed root systems were stored in 40% isopropanol before measuring total (fresh) root length (in meters) with the Comair optical scanner (total root length is a robust way to indirectly quantify a plant's absorptive surface area for water uptake; Fitter and Hay 2002). After oven-drying, roots were weighed on a Cahn microbalance (Cahn Instruments, Cerritos, California, USA). The following growth traits were calculated for each seedling on day 21: total biomass (shoot + root dry mass, in grams); root:shoot biomass ratio (in grams per gram); and specific root length (SRL; total root length [in meters]/root mass [in grams]). Root:shoot biomass ratio and SRL (root length per gram of tissue) are functionally important aspects of developmental plasticity that measure, respectively, (1) the amount of root tissue for water uptake relative to the shoot system being supplied and (2) the surface area produced per unit of root tissue to absorb moisture and other soil resources (Fitter 1994, Bell and Sultan 1999, Ryser and Eek 2000, Sultan 2003).

#### *Statistical analysis*

We used ANOVA to test for differences between the two *Polygonum* species in the effects of dry vs. moist

parent environment on trait expression of seedlings in dry vs. moist soil (interaction effect of species  $\times$  parental environment  $\times$  offspring environment; Schmitt et al. 1992, Shaw and Byers 1998). Variation in seedling traits due to species, parent treatment, offspring treatment, factor interactions, and block was tested by ANOVA using type III sums of squares (JMP version 4.02, SAS Institute, Cary, North Carolina, USA). To avoid pseudoreplication and to remove the effects of parental treatment imbalance from the species term (one missing dry parent in *P. persicaria*), we included in our model population effects nested within species; including the population term and its interactions did not change significance test results but improved resolution (model multiple  $R^2$ ) by ~5%. Population was treated as fixed because the populations used in this experiment were selected to represent the species' entire habitat ranges (Sultan et al. 1998). Treating population as a random factor, which implies that the effect level for each population is drawn from a probability distribution (Littell et al. 2002:92), was therefore not appropriate for this study. (Nonetheless, when performed these analyses showed nearly identical significance test results for model factors and their interactions, although species' main effects were occasionally affected.)

The inclusion of distinct lines from each of several populations of each species provided robust samples for the species-level comparison of interest (cf. Sultan 2001), however we did not separately test the main effect of genetic line within populations, because this effect would have been confounded with treatment (due to the missing cell). Instead, variation due to line within population remained within the error term, providing for a conservative test of the species main and interaction effects. We treated block as a random factor, conservatively allowing the two compartment and two temporal blocks to vary independently; all other factors were treated as fixed. Data were transformed to meet the assumptions of ANOVA as necessary: seedling biomass was Box-Cox-transformed, total root length was  $(\ln + 1)$ -transformed, and root length data from tracings were  $(\text{square root} + 1)$ -transformed; root:shoot ratio, specific root length, and deepest root were untransformed. Covariate analysis was performed to identify an effect of total root length on seedling biomass.

We analyzed root system length and deepest root extension over time by multivariate repeated-measures ANOVA (Von Ende 2001) using the full traced data sets available from measurement days 5, 10, and 15. We employed the relatively conservative Greenhouse-Geisser corrected  $F$  values to assess significance of comparisons between treatments within dates (Von Ende 2001); in all cases significance at  $P \leq 0.05$  was also true for Wilks' lambda or Pillai's trace, indicating these analyses were robust to choice of test statistic.

Both univariate and multivariate analyses were followed by a set of six planned post hoc comparisons

TABLE 1. Effects of species, parental moisture environment, offspring moisture environment, and their interactions on seedling growth traits.

Growth trait	Source of variation					
	Species	PE	OE	Species × PE	Species × OE	Species × PE × OE
Seedling traits at harvest (univariate ANOVA)						
Biomass	0.0058	NS	≤0.001	0.010	0.0137	0.0103
Total root length	0.0084	NS	≤0.001	NS	≤0.001	0.0021
Root:shoot ratio	0.0290	NS	≤0.001	NS	NS	NS
Specific root length	0.0020	NS	≤0.001	NS	0.0088	NS
Root extension over time (repeated-measures MANOVA)						
Root length	0.0153	NS	≤0.001	0.0107	NS (0.09)	0.0130
Deepest root	≤0.001	NS	0.0019	NS	NS (0.08)	0.0337

Notes: Probability levels are shown from *F* tests for effects of species, parental moisture environment (PE), offspring moisture environment (OE), and their interactions; see *Material and methods* for model details. A significant species × PE × OE term indicates that species differ in transgenerational plasticity patterns. The abbreviation NS indicates  $P \geq 0.05$ ; NS probability levels < 0.10 are given. Effects shown for root extension traits over time are all interaction terms with the between-date (time) factor; e.g., species effect = species × time, etc., indicating that the effect of species varies over time. Biomass data were Box-Cox-transformed; total root length data were (ln + 1)-transformed; and root length tracing data were (square root + 1)-transformed.

(pairwise *t* tests), with alpha levels verified for multiple tests using a Dunn-Sidak sequential procedure.

## RESULTS

Individuals of the two species expressed strikingly different patterns of transgenerational plasticity for total seedling growth in response to dry vs. moist conditions (species × parent treatment × offspring treatment; Table 1). In *P. persicaria*, offspring grew more in soil environments that matched the parental conditions: in dry soil, seedling offspring of drought-stressed *P. persicaria* plants parents produced 36.2% greater biomass than did offspring of genetically identical, well-watered parents (difference significant at  $P \leq 0.0002$ ), and offspring of parents given ample moisture grew (nonsignificantly) more in moist soil than did offspring of drought-stressed parents (Fig. 1a). In contrast, offspring of drought-stressed *P. hydropiper* plants produced less biomass in both dry and moist seedling environments compared with offspring of well-watered parents (15.2% and 10.0% less biomass, respectively; Fig. 1b). These seedling biomass differences largely reflected shoot mass, which comprised up to 81% of total seedling biomass; there was no effect of parental treatment or its interaction effects on biomass allocation (root:shoot ratio; Table 1).

Transgenerational plasticity for root developmental traits of offspring also differed significantly between the species. Differences in total seedling root length mirrored those described above for seedling biomass (species × parent treatment × offspring treatment; Table 1, Fig. 1c, d): in *P. persicaria*, offspring of drought-stressed parents produced root systems in dry soil that were 25% longer than those produced in the same treatment by seedling offspring of genetically identical, well-watered parents (Fig. 1c; difference significant at  $P \leq 0.0005$ ). In moist soil, offspring of well-watered parents produced 9% longer root systems than those of droughted parents (difference not significant). In *P.*

*hydropiper*, offspring of droughted parents produced slightly shorter root systems in both seedling environments (Fig. 1d). The effect of seedling root length tested as a covariate on biomass was highly significant ( $F = 893.8$ ,  $df = 1, 276$ ;  $P \leq 0.0001$ ). This effect on seedling growth differed significantly in moist vs. dry soil (effect of root length × offspring treatment interaction on biomass,  $F = 152.0$ ,  $df = 1, 276$ ;  $P \leq 0.0001$ ). The difference in the species' transgenerational effects on seedling biomass (species × parent treatment × offspring treatment; Table 1) became nonsignificant when corrected for differences in total root length ( $P = 0.262$ ).

The effects of parental moisture environment on seedlings' patterns of root extension over time (based on manual root tracings) also differed significantly in the two species (repeated-measures MANOVA; Table 1). In *P. persicaria*, offspring of drought-stressed parents grew larger root systems faster in dry soil, compared with offspring of well-watered parents growing in the same treatment (total root length contrast significant at  $P \leq 0.036$ ; Fig. 2a); there was no significant difference in moist soil (Fig. 2b). In *P. hydropiper*, offspring of drought-stressed parents extended root systems more slowly in both dry and moist soil than offspring of well-watered parent plants (Fig. 2c, d). The *P. persicaria* seedlings whose parents had experienced drought also extended deep roots into dry soil more quickly than offspring of well-watered parents (Fig. 3a): by day 15, their deepest root was 15% longer (deepest root contrast significant at  $P < 0.032$ ). In moist soil, offspring of droughted and well-watered *P. persicaria* plants achieved similar root depths over time (Fig. 3b). In *P. hydropiper*, offspring of drought-stressed parents reached deep soil slightly more slowly than offspring of well-watered parents in both dry and moist seedling environments (nonsignificant deepest root differences of 3.8–5.4% at day 15; Fig. 3c, d). Both the positive effect of parental drought on *P. persicaria* root extension in dry soil and the negative effect of parental drought on *P.*

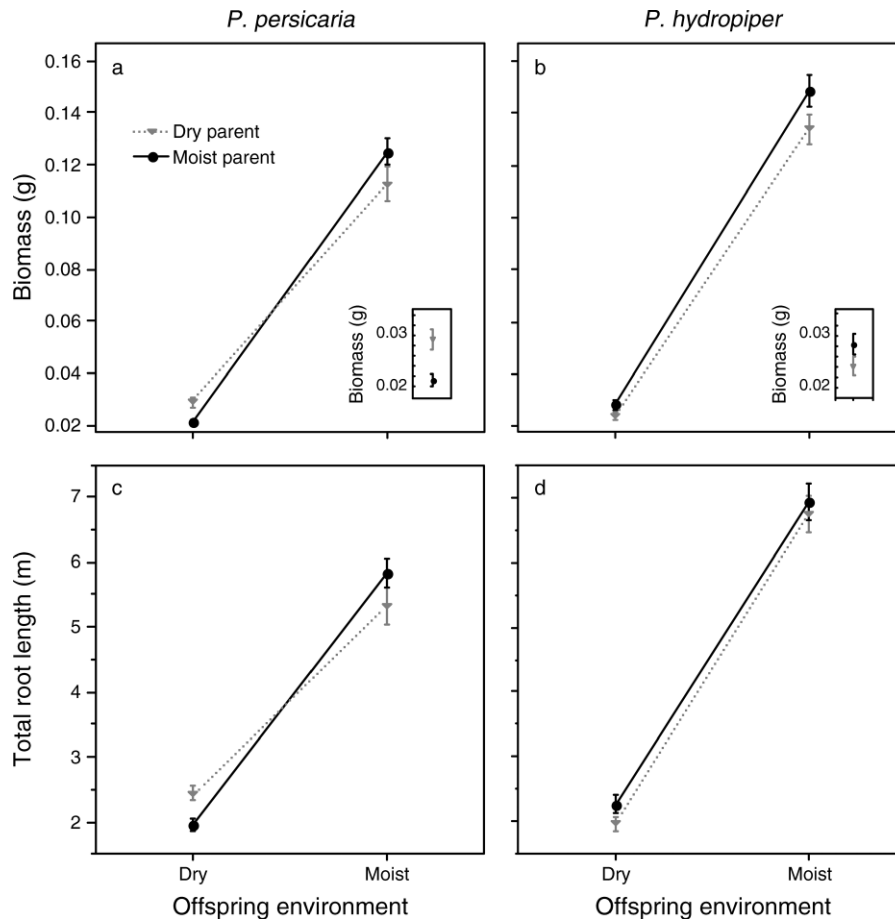


FIG. 1. Transgenerational plasticity for total seedling growth: total seedling biomass (mean  $\pm$  SE) on day 21 of (a) *Polygonum persicaria* and (b) *P. hydropiper* offspring of dry- vs. moist-grown (genetically uniform) parents in dry and moist offspring environments; and total length of seedling root systems on day 21 in (c) *P. persicaria* and (d) *P. hydropiper* offspring of dry- vs. moist-grown parents in dry and moist offspring environments. Insets show details of results in the dry offspring environment for seedlings of each species.

*hydropiper* seedlings in moist soil became more pronounced over time (Fig. 2a, d).

Seedlings also expressed considerable plasticity in response to their immediate moisture conditions (significant offspring treatment effects; Table 1). In both species, the impact of reduced seedling biomass on total root length in dry soil was partially offset by plastic increases in both biomass allocation to roots and specific root length (compare Fig. 1a, b with c, d). Seedlings of both species increased root:shoot biomass allocation by 74.4% on average in dry compared with moist soil. Seedlings of both species also made longer, thinner roots in dry soil, but *P. persicaria* seedlings expressed greater plasticity for this trait (24.6% increased SRL compared with 14.7% in *P. hydropiper* seedlings; significant species  $\times$  offspring treatment effect on this trait and consequently on total root length; Table 1).

In *P. persicaria*, these immediate responses interacted with positive effects of parental drought to maximize the extent of offspring root systems in dry soil (Figs. 1c and

2a): offspring of droughted *P. persicaria* parents produced 10.2% deeper roots in dry soil than they did in moist soil (cf. Fig. 3a, b). Conversely, in *P. hydropiper*, immediate seedling plasticity did not offset the negative transgenerational effects of drought on seedling growth (Figs. 1d and 2c). As a result of these differences in both transgenerational and immediate plasticity, seedlings of the two species performed significantly differently in dry conditions after one generation of parental drought stress: in dry soil, *P. persicaria* offspring of drought-stressed parents made longer root systems ( $P \leq 0.021$ ) and reached deeper soil sooner ( $P \leq 0.007$ ) than did similar *P. hydropiper* seedlings and produced greater total biomass ( $P \leq 0.0001$ ; significant effects of species  $\times$  parent treatment  $\times$  offspring treatment; Table 1).

#### DISCUSSION

The *Polygonum* species expressed contrasting patterns of transgenerational plasticity in response to parental

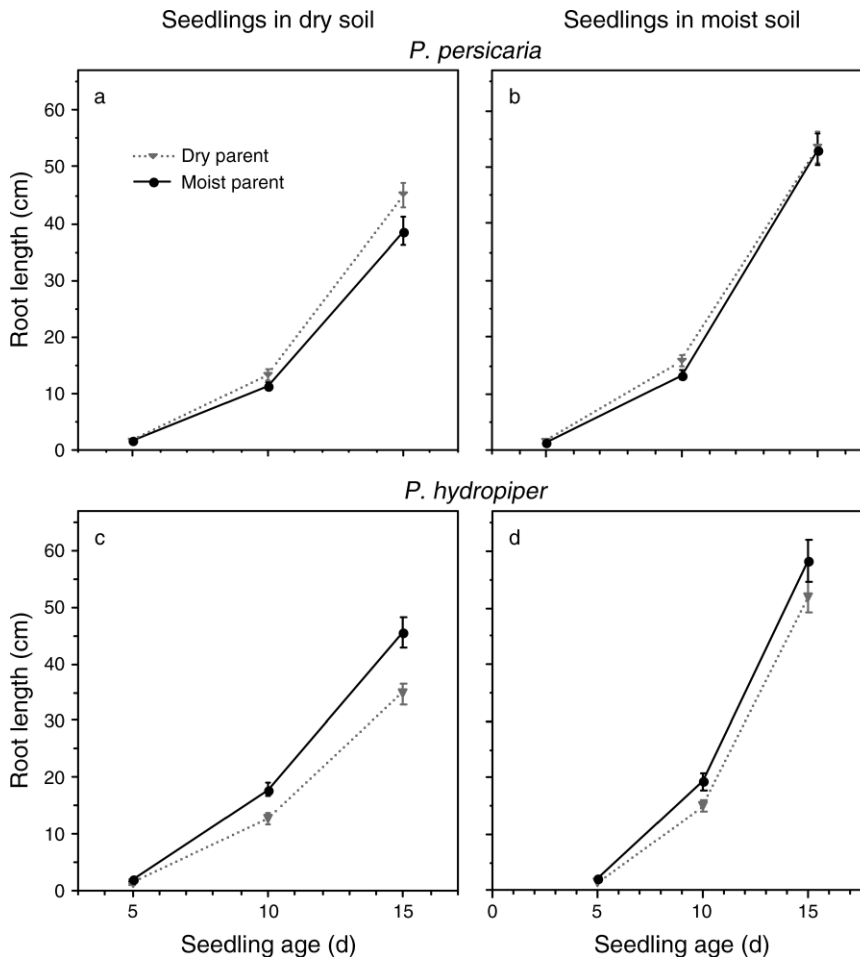


FIG. 2. Transgenerational plasticity for root elongation over time: total lengths (mean  $\pm$  SE) of manually traced individual root systems at days 5, 10, and 15 for *Polygonum persicaria* offspring of dry- vs. moist-grown (genetically uniform) parents, grown in (a) dry and (b) moist offspring environments, and for *P. hydropiper* offspring of dry- vs. moist-grown (genetically uniform) parents, grown in (c) dry and (d) moist offspring environments.

drought stress for specific, functionally important aspects of offspring development. Chief among these traits is root extension, since rapid and efficient access to soil resources is essential to seedling survival and competitive success. It is particularly critical for seedlings growing in dry soil to quickly produce extensive, deep root systems to maximize access to soil moisture (Mazer 1989, Hofmann and Isselstein 2004, Moles and Westoby 2006). (The significantly different impact in our data of root length on seedling biomass in moist vs. dry soil reflects this greater adaptive value of long root systems in dry conditions.) The enhanced extension rate, soil depth, and total length of root systems produced in dry soil by *P. persicaria* offspring of drought-stressed parents are important functional adaptations to this environment, as confirmed by the significantly greater total growth of these seedlings in dry soil compared with offspring of well-watered replicate parents of the same inbred genetic lines. The *P. persicaria* offspring of well-watered parents also

showed slightly enhanced root and total growth in the moist seedling environment, compared with offspring of drought-stressed parents.

The transgenerational plasticity expressed by individuals of *P. persicaria* thus results in adaptive matching between alternative parental moisture conditions and successful offspring phenotypes in like conditions. Adaptive transgenerational plasticity was also identified by Galloway and Etterson (2007), who found that seedling offspring of American bellflower (*Campanulastrium americanum*) had higher early survival when planted into a light environment that matched that of their mother plants. In such taxa, traits that are adaptive to the offspring's environment can be inherited as a result of the parent plant having experienced a similar environment, i.e., by means of individual plasticity expressed across generations.

In contrast, in *P. hydropiper* the offspring of drought-stressed parents produced reduced root systems as well as lower total growth in both dry and moist soils. This

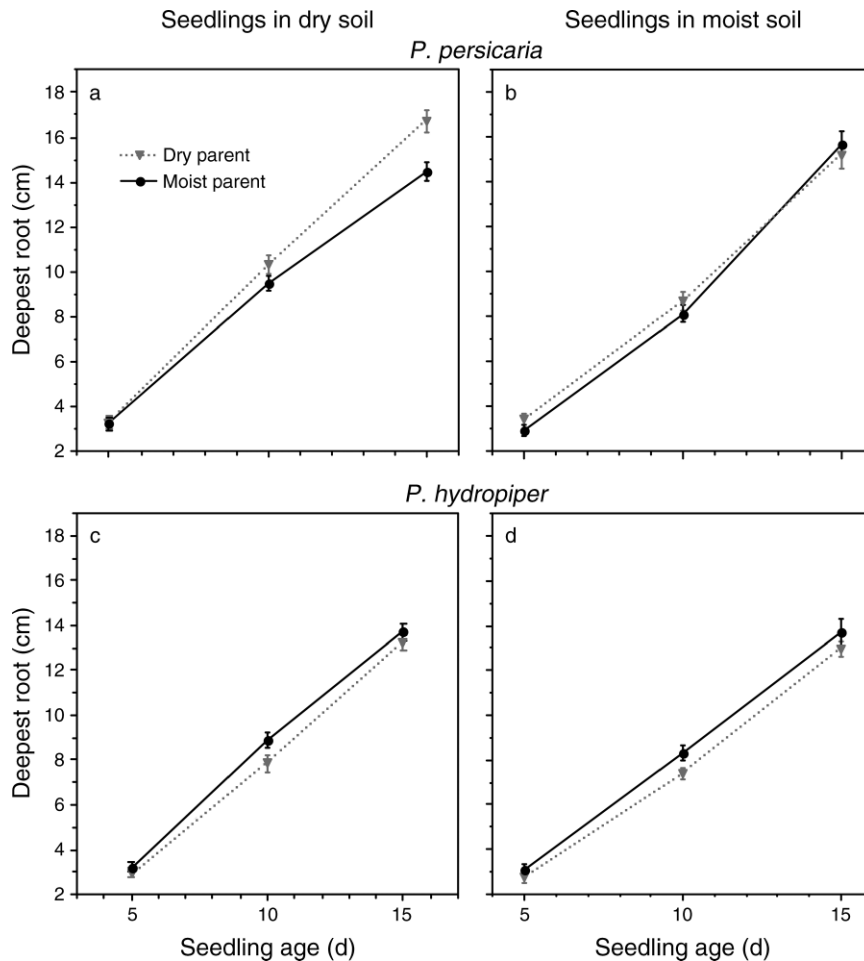


FIG. 3. Transgenerational plasticity for root depth extension over time: deepest root of manually traced individual root systems (mean  $\pm$  SE) at days 5, 10, and 15 for (a,b) *Polygonum persicaria* and (c,d) *P. hydrophiper* offspring of dry- vs. moist-grown (genetically uniform) parents, grown in (a, c) dry and (b, d) moist offspring environments.

negative transgenerational effect of dry parental conditions would be particularly detrimental to the competitive success and/or survival of seedlings encountering a dry microsite or season, where as noted above an extensive root system is critical. Note that seedling biomass differences are not only the result of differences in functional traits, but, as the basis of future root and shoot growth, are themselves a key predictor of both survival and adult reproductive output (Galloway 1995, Wulff 1995). These early biomass differences have a particularly strong fitness impact in stressful conditions such as dry soil (Mazer 1989, Sultan 1996, Mousseau and Fox 1998a), but are expected to be less crucial in favorable, moist environments (see also Mazer 1989, Sultan 1996, Mousseau and Fox 1998a). Accordingly, the plastic response to parental drought stress expressed by *P. hydrophiper* offspring is maladaptive to both moist and dry seedling conditions, but is likely to most strongly affect seedling establishment in dry soil.

These results suggest the intriguing possibility that adaptive vs. negative transgenerational effects of paren-

tal drought on *P. persicaria* and *P. hydrophiper* offspring in dry soil might influence the species' respective abilities to establish populations in consistently or variably dry habitats. The realized field distributions of the two species within their common, introduced New England range are consistent with this interpretation. As determined in a previous study (Sultan et al. 1998), *P. hydrophiper* is restricted to consistently moist habitats (i.e., sites where all soil microsites contain at least 30% of the soil's field capacity for moisture at both shallow and deep strata throughout the growth season and with mean site moisture levels both early and late in the season above 42% at each soil depth). In contrast, *P. persicaria* populations occur in both drier and more heterogeneous sites (those with as little as 1% of field capacity moisture at some microsites and mean site moisture levels as low as 7.5% of that soil's field capacity; Sultan et al. 1998). A comparison of adult developmental traits that contribute to function in dry conditions, such as root biomass allocation and ratio of root length to leaf surface area, has not shown any clear

differences between the two species that might explain this difference in their distributions, suggesting that seedling traits that affect establishment might be an important factor in this system.

Parental environment can influence offspring phenotype through changes in the quantity of egg or seed provisioning (Roach and Wulff 1987, Mousseau and Fox 1998b, Fox and Savalli 2000) as well as hormone and mRNA changes that alter specific developmental traits (Arnold et al. 1991, Sultan 1996). In the *Polygonum* species, the effects of parental drought on offspring root extension primarily reflected changes in seed provisioning. Previous studies confirmed that, compared with genetically identical well-watered parents, drought-stressed *P. persicaria* plants increased provisioning to individual offspring by 13.3% while *P. hydropiper* parents decreased provisioning by 14.5% (Sultan 2001).

Increased seed provisioning in response to drought can promote offspring success in two ways. First, transgenerational induction of functional phenotypes such as longer and deeper root systems can allow offspring to avoid the initial lag time required for immediate plastic response to an environmental challenge. This "head start" can be crucial to survival in early life stages, when most mortality occurs (Agrawal et al. 1999, Moles and Westoby 2006). In addition, greater initial provisioning or other cross-generational adjustments by the parent may allow offspring to express a more extreme developmental response than would be possible through immediate offspring plasticity alone (Agrawal et al. 1999). This was clearly the case in *P. persicaria*, in which greater provisioning due to parental drought interacted with immediate seedling increases to root biomass allocation and specific length in dry soil, to maximize offspring adaptation by producing extensive, deep systems of fine roots. These root systems were significantly longer than those produced by these same offspring in moist soil. Conversely, in *P. hydropiper*, the seedlings' direct plastic responses to dry soil did not offset the negative plastic response to parental drought, resulting in offspring that were poorly adapted to survive and grow in dry soil. Interestingly, although transgenerational effects are sometimes limited to very early in the life cycle (Agrawal et al. 1999, Rotem et al. 2003), both the positive effect of parental drought on *P. persicaria* root extension in dry soil and the negative effect of parental drought on *P. hydropiper* seedlings in moist soil generally became more pronounced over time.

The *Polygonum* species' responses to parental drought stress revealed a dramatic contrast between adaptive transgenerational plasticity (and its interaction with immediate offspring plasticity) in a widespread generalist and maladaptive, resource-based effects in an ecologically restricted congener. Such species differences in cross-generational effects raise important though heretofore overlooked ecological implications that are particularly intriguing for introduced species. The ability

to maximize offspring survival in diverse conditions through adaptive transgenerational plasticity may contribute to a generalist distribution (Fox et al. 1997) and to rapid spread of a nonnative species to contrasting and variable habitats. By contrast, negative parental effects on offspring quality (such as the effects of drought stress in *P. hydropiper*) are likely to create both evolutionary and ecological constraints, by promoting directional gene flow from individuals in favorable sites, which impedes selective adaptation to marginal environments (Stanton and Galen 1997), as well as by restricting establishment to such sites.

We conclude that even closely related taxa may express substantially different repertoires of transgenerational plasticity in response to an environmental stress such as drought. When they affect offspring functional traits such as root extension, such characteristic patterns of transgenerational plasticity may potentially contribute to ecological diversity among species. Further comparative studies are required to assess whether species differences in this complex aspect of phenotypic plasticity can contribute to different patterns of spread in nonnative species or affect species' relative abilities to maintain populations in the face of rapid environmental change (Fox and Savalli 2000, Sultan 2004).

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#### LITERATURE CITED

- Agrawal, A. A. 2001. Transgenerational consequences of plant responses to herbivory: An adaptive maternal effect? *American Naturalist* 157:555–569.
- Agrawal, A. A., C. Laforsch, and R. Tollrian. 1999. Transgenerational induction of defenses in animals and plants. *Nature* 401:60–63.
- Arnold, R. L. B., M. Fenner, and P. J. Edwards. 1991. Changes in germinability, ABA content and ABA embryonic sensitivity in developing seeds of *Sorghum bicolor* induced by water stress during grain filling. *New Phytologist* 118:339–347.
- Badyaev, A. V., G. E. Hill, M. L. Beck, A. A. Dervan, R. A. Duckworth, K. J. McGraw, P. M. Nolan, and L. A. Whittingham. 2002. Sex-biased hatching order and adaptive population divergence in a passerine bird. *Science* 295:316–318.
- Bell, D. L., and S. E. Sultan. 1999. Dynamic phenotypic plasticity for root growth in *Polygonum*: a comparative study. *American Journal of Botany* 86:807–819.
- Berntson, G. M. 1997. Root Length+. Version 1.1 e. (<http://plantecohost.harvard.edu/pub/RL+/RootLengthPlus.html>)
- Donohue, K., and J. Schmitt. 1998. Maternal environmental effects in plants. Pages 137–158 in T. A. Mousseau and C. W.



- Fox, editors. Maternal effects as adaptations. Oxford University Press, New York, New York, USA.
- Fitter, A. H. 1994. Architecture and biomass allocation as components of the plastic response of root systems to soil heterogeneity. Pages 305–323 in M. M. Caldwell and R. W. Pearcy, editors. Exploitation of environmental heterogeneity by plants. Academic Press, New York, New York, USA.
- Fitter, A. H., and R. K. M. Hay. 2002. Environmental physiology of plants. Third edition. Academic Press, New York, New York, USA.
- Fox, C. W., and U. M. Savalli. 2000. Maternal effects mediate host expansion in a seed-feeding beetle. *Ecology* 81:3–7.
- Fox, C. W., M. S. Thakar, and T. A. Mousseau. 1997. Egg size plasticity in a seed beetle: an adaptive maternal effect. *American Naturalist* 149:149–163.
- Galloway, L. F. 1995. Response to natural environmental heterogeneity: maternal effects and selection on life-history characters and plasticities in *Mimulus guttatus*. *Evolution* 49:1095–1107.
- Galloway, L. F., and J. R. Etterson. 2007. Transgenerational plasticity is adaptive in the wild. *Science* 318:1134–1136.
- Gross, K. L., D. Maruca, and K. S. Pregitzer. 1992. Seedling growth and root morphology of plants with different life-histories. *New Phytologist* 120:535–542.
- Gustafsson, S., K. Rengefors, and L. Hansson. 2005. Increased consumer fitness following transfer of toxin tolerance to offspring via maternal effects. *Ecology* 86:2561–2567.
- Hofmann, M., and J. Isselstein. 2004. Effects of drought and competition by a ryegrass sward on the seedling growth of a range of grassland species. *Journal of Agronomy and Crop Science* 190:277–286.
- Lacey, E. 1991. Parental effects on life-history traits in plants. Pages 735–744 in E. C. Dudley, editor. The unity of evolutionary biology. Volume II. Dioscorides Press, Portland, Oregon, USA.
- Lacey, E. P., and D. Herr. 2005. Phenotypic plasticity, parental effects, and parental care in plants? I. An examination of spike reflectance in *Plantago lanceolata*. *American Journal of Botany* 92:920–930.
- Littell, R. C., W. W. Stroup, and R. J. Freund. 2002. SAS for linear models. Fourth edition. SAS Institute, Cary, North Carolina, USA.
- Mazer, S. J. 1989. Ecological, taxonomic, and life-history correlates of seed mass among Indiana dune angiosperms. *Ecological Monographs* 59:153–175.
- Moles, A. T., and M. Westoby. 2006. Seed size and plant strategy across the whole life cycle. *Oikos* 113:91–105.
- Mondor, E. B., J. A. Rosenheim, and J. F. Addicott. 2005. Predator-induced trans-generational phenotypic plasticity in the cotton aphid. *Oecologia* 142:104–108.
- Mousseau, T. A., and C. W. Fox. 1998a. Maternal effects as adaptations. Oxford University Press, New York, New York, USA.
- Mousseau, T. A., and C. W. Fox. 1998b. The adaptive significance of maternal effects. *Trends in Ecology and Evolution* 13:403–407.
- Munir, J., L. A. Dorn, K. Donohue, and J. Schmitt. 2001. The effect of maternal photoperiod on seasonal dormancy in *Arabidopsis thaliana*. *American Journal of Botany* 88:1240–1249.
- Roach, D. A., and R. D. Wulff. 1987. Maternal effects in plants. *Annual Review of Ecology and Systematics* 18:209–235.
- Rossiter, M. C. 1998. The role of environmental variation in parental effects expression. Pages 112–134 in T. A. Mousseau and C. W. Fox, editors. Maternal effects as adaptations. Oxford University Press, New York, New York, USA.
- Rotem, K., A. A. Agrawal, and L. Kott. 2003. Parental effects in *Pieris rapae* in response to variation in food quality: Adaptive plasticity across generations? *Ecological Entomology* 28:211–218.
- Ryser, P., and L. Eek. 2000. Consequences of phenotypic plasticity vs. interspecific differences in leaf and root traits for acquisition of aboveground and belowground resources. *American Journal of Botany* 87:402–411.
- Schmitt, J., J. Niles, and R. D. Wulff. 1992. Norms of reaction of seed traits to maternal environments in *Plantago lanceolata*. *American Naturalist* 139:451–466.
- Shaw, R. G., and D. L. Byers. 1998. Genetics of maternal and paternal effects. Pages 97–111 in T. A. Mousseau and C. W. Fox, editors. Maternal effects as adaptations. Oxford University Press, New York, New York, USA.
- Stanton, M. L., and C. Galen. 1997. Life on the edge: adaptation versus environmentally mediated gene flow in the snow buttercup, *R. adoneus*. *American Naturalist* 150:143–178.
- Sultan, S. E. 1996. Phenotypic plasticity for offspring traits in *Polygonum persicaria*. *Ecology* 77:1791–1807.
- Sultan, S. E. 2001. Phenotypic plasticity for fitness components in *Polygonum* species of contrasting ecological breadth. *Ecology* 82:328–343.
- Sultan, S. E. 2003. Phenotypic plasticity in plants: a case study in ecological development. *Evolution and Development* 5:25–33.
- Sultan, S. E. 2004. Promising directions in plant phenotypic plasticity. *Perspectives in Plant Ecology, Evolution, and Systematics* 6:227–233.
- Sultan, S. E., A. M. Wilczek, S. D. Hann, and B. J. Brosi. 1998. Contrasting ecological breadth of co-occurring annual *Polygonum* species. *Journal of Ecology* 86:363–383.
- Von Ende, C. N. 2001. Repeated-measures analysis: growth and other time-dependent measures. Pages 134–157 in S. M. Scheiner and J. Gurevitch, editors. Design and analysis of ecological experiments. Second edition. Oxford University Press, New York, New York, USA.
- Weiner, J., S. Martinez, H. Muller-Scharer, P. Stoll, and B. Schmid. 1997. How important are environmental maternal effects in plants? A study with *Centaurea maculosa*. *Journal of Ecology* 85:133–142.
- Wulff, R. 1995. Environmental maternal effects on seed quality and germination. Pages 491–505 in J. Kigel and G. Galili, editors. Seed development and germination. Marcel Dekker, New York, New York, USA.
- Wulff, R. D., A. Caceres, and J. Schmitt. 1994. Seed and seedling responses to maternal and offspring environments in *Plantago lanceolata*. *Functional Ecology* 8:763–769.