

Plastic and constant developmental traits contribute to adaptive differences in co-occurring *Polygonum* species

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Adaptive differences among species are often thought to result from developmentally constant trait differences that enhance fitness in alternative environments. Species differences in patterns of individual phenotypic plasticity can also have ecological consequences. Indeed, functionally related constant and plastic traits may interact to determine the phenotype's adaptive value in particular conditions. We compared juvenile shade avoidance traits (height and its components, internode length and node number) across two field density treatments in *Polygonum persicaria* and *P. hydropiper*, annual plant species that co-occur in pastures comprised of a mosaic of plant densities. We used selection analyses to test trait contributions to fitness in alternative density treatments. Seedlings of both species expressed plasticity for internode elongation in response to density; *P. persicaria* plants increased internode length and consequently height significantly more in high density than did those of *P. hydropiper*. As predicted by the shade avoidance hypothesis, increased height was adaptive for both species in high density stands, so *P. persicaria* plants had higher fitness in this environment. By contrast, node numbers were relatively constant across density treatments in both species: *P. hydropiper* seedlings consistently produced more nodes than did those of *P. persicaria*. This constant trait difference contributed to *P. hydropiper*'s greater relative fitness at low density, where more nodes and hence leaves enable plants to better exploit available light. Differences between species in these juvenile shade-avoidance traits did not result from the evolutionary constraints of lack of heritable variation or costs of plasticity. We discuss how these interspecific trait differences may have been generated by divergent selective histories resulting from differences in herbivore resistance. These results illustrate how adaptive differences in both plastic and constantly expressed traits may jointly contribute to ecological distribution, including coexistence in patchy habitats.

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A central focus in ecology is the relationship between phenotypic differences among species and their patterns of ecological distribution. In some cases, ecologically important adaptive differences among species arise from relatively constant traits whose expression is developmentally fixed. For example, species-specific differences in beak size among Galapagos finches (Schluter and Grant 1984, Grant 1986, Grant and Grant 1989), or

among leaf characteristics of Hawaiian silverswords (Robichaux et al. 1990) are thought to determine differences in their resource use and environmental tolerance. Indeed, the view that such species-constant trait differences shape ecological distributions is implicit in much of evolutionary ecology (Schmitz et al. 2003). A second and increasingly recognized source of adaptive diversity among species is individual patterns

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of response to environmental conditions or phenotypic plasticity (Bradshaw 1965, West-Eberhard 1989, Schlichting and Pigliucci 1998, Sultan 2004). For instance, congeneric plant species may differ in their patterns of individual physiological and morphological response to low light or other types of resource limitation (Pigliucci et al. 1999, Sultan 2003). To date, despite elegant demonstrations that particular plasticity patterns can be adaptive in particular species (Dudley and Schmitt 1996, Kingsolver 1996, Donohue et al. 2000, Weinig 2000, Galen et al. 2004, Huber et al. 2004), very little is known about how species differences in patterns of individual plasticity may shape their ecological distribution and coexistence.

Both constant and plastic trait expression may be adaptive, depending upon patterns of environmental variation. Plastic responses to environments are likely to evolve when conditions are reliably predicted by environmental cues, if the evolution of such a response is not constrained by a lack of genotype by environment ($G \times E$) variation or a maintenance cost of plasticity (Via and Lande 1985, Moran 1992, Scheiner 1993, van Tienderen 1997, DeWitt et al. 1998, Tufto 2000, Sultan and Spencer 2002, Berrigan and Scheiner 2004). Species with the ability to adaptively alter phenotypes in response to environmental variation may be able to tolerate, and thus persist in, a wider range of habitats than species with less adaptively plastic phenotypes (Williams et al. 1995, Bazzaz 1996, Parker et al. 2003, Sultan 2003, Voesenek et al. 2004). Conversely, fixed trait expression is favored when a single trait value is optimal in multiple environments or when variation cannot be reliably predicted (Moran 1992, Lively 1999, Tufto 2000, Debat and Patrice 2001). In such cases, ecological distribution of each species is a function of the fitness tradeoffs between environments that are determined by constant traits.

Studying plastic and developmentally constant traits as mutually exclusive alternatives can be misleading, because these two types of traits can interact in complex ways to produce adaptive phenotypes. For instance, component organ features such as root length and thickness (Ryser and Eek 2000) or leaf mean and specific area (Griffith and Sultan 2005) may show either plastic or constant expression. Such component traits may be differently constrained genetically in particular taxa, and may affect fitness either directly or indirectly (Kingsolver and Schemske 1991, Scheiner et al. 2000). The integrated phenotype that emerges from these trait interactions ultimately dictates an individual's fitness in its environment (Schlichting 1989, Schlichting and Pigliucci 1998, Pigliucci 2003).

Currently, little is known about how species differences in constant and plastic traits jointly contribute to adaptive diversity and consequent differences in ecological breadth. In this study, we compare phenotypic

differences in adaptively important developmental traits in two closely related but ecologically distinct congeners. *Polygonum persicaria* is a generalist that occurs in a wide range of light, nutrient and moisture habitats, while *P. hydropiper* is a specialist species that occurs in a more narrow range of habitats along these resource axes. Although these species have strikingly different ecological amplitudes, they do co-occur (along with a diverse set of other herbaceous species) in sunny, nutrient rich, moist disturbed habitats such as wet pastures. In such sites, intermittent grazing often creates a mosaic of competitively distinct microsites, including high density patches where neighbor competition is intense and lower density patches where competition from neighbors is much reduced.

Species persistence within this competitive mosaic requires either effectively competing for resources in high density stands or exploiting the greater resource availability of low density stands. Both theoretical and empirical work indicates that alternative plant growth and light foraging strategies expressed early in ontogeny are adaptive in these varying competitive environments. In high density stands where competition for light is strong, the adaptive "shade avoidance" response involves seedlings growing taller than neighboring plants via internode elongation (Schmitt et al. 1999, Donohue et al. 2000, Huber et al. 2004). Conversely, in less dense stands where neighbors do not shade each other, foraging theory suggests that seedlings should allocate resources to producing more nodes (with shorter internodes) and leaves in order to efficiently exploit the available light (Hutchings and de Kroon 1994, Schmitt et al. 2003).

To understand how constant and plastic trait expression jointly influence ecological distribution in these two *Polygonum* species, we focus on these adaptive aspects of seedling development, specifically the interaction between internode elongation and node number in producing seedling height. We document constant and plastic aspects of these traits and use selection analyses to examine their effects on fitness. In discussing the results, we consider the selective factors and potential constraints that favor plastic versus constant trait expression in this system.

Methods

Study system

Polygonum persicaria and *P. hydropiper* L. (Polygonaceae) are closely related taxa within a monophyletic section of the genus (Löve and Löve 1956, Mitchell and Dean 1978, S.-T. Kim and M. J. Donoghue, unpubl.). Both species were introduced to North America where they have similar geographic ranges and often co-occur in sunny, moist, high nutrient sites (Sultan et al. 1998); this co-occurrence is also typical in their European range

(Simmonds 1945, Timson 1966). The species also have similar life histories as obligate annuals with mixed breeding systems that reproduce by numerous small achenes. They grow in highly disturbed areas including grazed or cultivated land (Mitchell and Dean 1978, Gleason and Cronquist 1991, Sultan et al. 1998, T. Griffith, pers. obs.).

Experimental sample

To create a representative sample of individual plasticity patterns for species level comparisons, we collected achenes from two sympatric populations of each species that represent the range of light conditions in which these species co-occur. One set of populations occurred along the edge of a partially shaded grazed pasture (Towle Paddock (TP) population, Dover, MA), while the second set of populations occurred in an open field exposed to full sun (MHF site, Mount Hermon, MA, site details in Sultan et al. 1998). Plants from each population of *P. persicaria* and *P. hydropiper* were grown for two generations in a common glasshouse environment to generate 10–13 inbred lines per population per species (details in Sultan 2001). Achenes from these inbred lines were stratified in distilled water at 4°C for 6 weeks, then sown (12–13 May, 2003) into flats filled with Metro-mix 360™ (Scotts Company, www.scotts.com). Seedlings germinated in the glasshouse under natural day lengths at 22°C day/18°C night and were watered as needed. Once seedlings reached the first true leaf stage, they were transplanted into the field (27–28 May).

Site and density treatments

The experimental field site was located in an open pasture with moist, nutrient rich soil in Mount Hermon, Massachusetts (42.7° N, 72.4° W) where both species naturally co-occur (Sultan et al. 1998). Perennial grasses were the predominant vegetation at the site, along with other forbs including *Trifolium pratense*, *Ranunculus acris*, *R. repens*, *Plantago major* and *Rumex crispus* (S. Sultan, pers. obs.). Prior to transplanting, the site was grazed by sheep to create the disturbed conditions in which these species are typically found. Seedlings were transplanted into a low density (30 cm apart) and high density (5 cm apart) treatment in each block. These treatments were comparable to the range of densities in naturally occurring populations of both species at this and other sites (S. Sultan and T. Griffith, pers. obs.) and thus comprised realistic competitive microsites. In the low density treatment, grass was clipped by hand once per week to simulate continued grazing during the summer and to reduce variation in non-*Polygonum* competition among blocks. Competition from other species within high density treatments was negligible.

To minimize edge effects, each treatment plot was surrounded by a border row. One seedling per line was randomly assigned to each of the two density treatments in eight replicate blocks, for an experimental sample of 8 seedlings per line per treatment (8 plants per line × 46 lines (24 *P. persicaria*, 22 *P. hydropiper*) × 2 treatments; total N = 736).

Data collection

Four weeks after transplanting, we measured the height of each seedling and counted the number of nodes on the main stem. These values were used to compute the average internode length for each plant. Seedlings that had begun to flower produced a greatly extended penultimate internode and so were not included in these measurements; seedlings in which the primary meristem was damaged were also removed from the data set (final N = 608). Every experimental plant was allowed to grow until it senesced naturally (>50% of leaves senescent), then harvested (26 August–18 September). Total dry above ground mass was measured for all plants, while total achene mass was measured for plants in two blocks (1 seedling per line per population per species per treatment in each block). Total achene mass included both achene mass at harvest and previous bi-weekly achene collections that prevented the loss of mature achenes over the course of the growing season. Separate correlations between plant mass and achene mass were then computed for each population in each treatment, and used to estimate total achene mass (lifetime fitness) for all experimental plants.

Statistical analyses

We performed an ANOVA to compare the species' responses to high and low density with respect to height, its component traits (average internode length and node number), and total achene mass. Achene mass was $[\log(x+1)]$ transformed to reduce heterogeneity of variances. Treatment, species, population nested within species, and line nested within population and species were included as main effects. Line within population was considered a random factor, while population was treated as a fixed effect because the sample populations were specifically selected to represent a range of light habitats (Sultan 2001). In this mixed model, main effects were tested over their interaction with line (Sokal and Rohlf 1995). Block was also included in the model since it was significant for all traits ($P < 0.0001$), but was not reported in the ANOVA table. Because there were significant species and/or species × treatment effects on all four traits, a Tukey's HSD test was performed on the least square means for each trait to identify significant differences

between species within each density treatment (JMP v. 5.0.1).

The global ANOVA showed significant line and line \times treatment effects for several traits, so we also performed single-species ANOVAs to assess the presence of variation among lines for height and its components within each species. The presence of significant line and/or line by treatment variation indicates that genetic variation exists on which selection can potentially act (Via and Lande 1985, Donohue et al. 2000). Each 1-species ANOVA included treatment, population, line within population, and block as main effects (block was not reported); these effects were again tested over the line \times treatment interaction term.

We performed genotypic selection analyses to measure the adaptive value of trait variation within each density treatment (Lande and Arnold 1983, Rausher 1992). Genotype (line) means were computed for each trait to reduce the impact of microsite variation on the correlation between trait expression and fitness (Stinchcombe 2002). Lines from both populations within a species were pooled to increase the range of trait variation and thus better detect the strength and direction of selection (Wade and Kalisz 1990, Weinig 2000, Etterson 2004). Linear selection differentials (S) were computed for each trait in each species as the regression coefficient of the standardized trait values on relative fitness (Donohue et al. 2000, Heschel and Riginos 2005). Pooling populations within species was justified since selection differentials within individual populations were similar to the overall differentials for the pooled populations: only 2 of the 24 within-population differentials for traits (2 populations \times 2 species \times 2 sites \times 3 traits) fell outside of the 95% confidence interval for the overall differential for that trait. Relative fitness was computed for each line within each species and density treatment as the ratio of mean total achene mass for that line to the mean total achene mass of each species \times density combination. Linear selection gradients (β) were computed for the height components, mean internode length and node number, to determine whether selection acted on each trait independent of the other. Selection gradients could not be computed for height and its components together because height is the computational product of node number and average internode length and so is collinear with these traits. Selection gradients were computed as the partial regression coefficients of the standardized trait values on relative fitness and were computed individually for each species and trait combination. Again, this pooled approach was valid since only 1 of the 16 within population gradients (2 populations \times 2 species \times 2 densities \times 2 traits) fell outside of the 95% confidence interval for the overall trait gradient. Genetic correlations were computed to measure the indirect effect of traits on fitness via the intermediate trait, height (Arnold 1983, Scheiner et al. 2000, Conner and

Hartl 2004). Genetic correlations were computed as the standardized correlation coefficients of line means for each trait pair in each species and density combination.

We used multiple regression to measure the costs of plasticity and homeostasis for height and its components (Donohue et al. 2000, Dorn et al. 2000, Poulton and Winn 2002). For each species in each density treatment, the trait value in that treatment and the trait's plasticity across density treatments were regressed against fitness (total achene mass), using line (genotype) means for the trait and fitness values. Plasticity for each line was measured as the difference between mean trait values in the high and low density treatments. A significant negative partial regression coefficient for trait plasticity indicates that plasticity has a negative effect on fitness independent of the trait value per se in that treatment. Such an effect is generally interpreted as a maintenance cost of plasticity (DeWitt et al. 1998, Scheiner and Berrigan 1998). A significant positive partial regression for trait plasticity indicates that trait constancy has a negative effect on fitness independent of the trait value in that treatment, and has therefore been interpreted as a "cost of homeostasis" (Dorn et al. 2000, Poulton and Winn 2002).

Results

Trait differences between species

Individuals of both species responded to greater competition for light in the high density treatment by increasing internode length, but the species differed significantly in the extent of this elongation plasticity (species and species \times treatment effects, Table 1; Fig. 1a). In the low density treatment, both species produced short internodes of virtually identical length; in the high density treatment, *P. persicaria* lines elongated internodes by an average of 67.6% relative to low density plants, compared to an increase of only 42.1% in *P. hydropiper* lines (Fig. 1a).

In contrast to this plasticity for internode length, plants of each species produced a constant number of nodes across density treatments (NS treatment and species \times treatment effects, Table 1). *P. hydropiper* seedlings produced significantly more nodes than those of *P. persicaria* in both density treatments (species effect, Table 1; Fig. 1b). However, this fixed species difference was relatively small compared with the pronounced difference due to contrasting levels of internode plasticity: *P. hydropiper* seedlings produced 10.6% more nodes than *P. persicaria* seedlings in both treatments, while at high density *P. persicaria* seedlings produced 19.6% longer internodes than *P. hydropiper* plants.

Internode elongation at high density resulted in seedlings of both species being taller in this treatment than at low density (treatment effect, Table 1; Fig. 1c).

Table 1. ANOVA for effects of density treatment (TRT), species (SP), population (POP), and line (LN) on height components and achene mass.

	Mean internode length	Node no.	Height	Achene mass
TRT	590.0*** 1, 34	0.1 1, 34	552.7*** 1, 34	2094.7*** 1, 42
SP	20.4*** 1, 34	62.3*** 1, 34	1.0 1, 34	29.6*** 1, 42
SP × TRT	30.1*** 1, 34	2.2 1, 34	34.9*** 1, 34	195.2*** 1, 42
POP [SP]	1.1 2, 34	6.4** 2, 34	2.1 2, 34	28.8*** 2, 42
POP [SP] × TRT	0.2 2, 34	2.6 2, 34	1.5 2, 34	7.0** 2, 42
LN[POP, SP]	1.0 34, 34	2.2* 34, 34	1.4 34, 34	1.9* 42, 42
LN[POP, SP] × TRT	2.0** 34, 364	1.9** 34, 364	1.6* 34, 361	1.0 42, 517

Top number F; lower numbers numerator, denominator df. P < 0.05; **P < 0.01; ***P < 0.0001. Bold values are significant at $\alpha = 0.05$.

Because the much longer internodes produced by *P. persicaria* plants at high density more than offset the species difference in node number, *P. persicaria* seedlings in this treatment were significantly taller than those of *P. hydropiper* (species × treatment effect, Table 1; Tukey's HSD test, Fig. 1c). This height difference was reversed at low densities, where internode lengths were equivalent and *P. hydropiper* seedlings produced more nodes than *P. persicaria* seedlings (Fig. 1a–c).

Trait effects on fitness at high and low density

Species differences in seedling height corresponded to differences in fitness (total achene mass) in both density treatments. However, the component of height asso-

ciated with greater reproductive output depended on density. At high density, the taller species, *P. persicaria*, had significantly greater reproductive output (Tukey's HSD test, Fig. 1d). In this treatment, greater height was achieved through significantly greater internode elongation (Fig. 1a–c). In the low-density treatment, *P. hydropiper* plants were taller (Tukey's HSD test, Fig. 1c) and had greater reproductive output (Tukey's HSD test, Fig. 1d), but their greater height was achieved through the production of more nodes (Fig. 1b–c). Interestingly, these fitness difference between species in the two environments were equally great: at high density the reproductive output of *P. persicaria* was 2.1 times that of *P. hydropiper*, and at low density the reproductive output of *P. hydropiper* was 2.0 times that of *P. persicaria*.

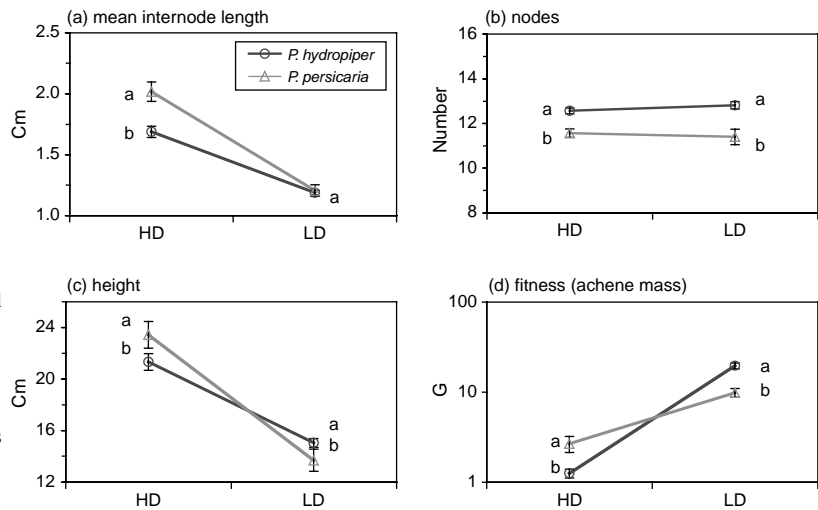


Fig. 1. Mean internode length (a), node number (b), height (c) and total lifetime reproductive output (achene mass) (d) of *P. persicaria* and *P. hydropiper* seedlings at high (HD) and low (LD) densities four weeks following transplantation. Within each density treatment, species means with the same letter do not differ significantly according to Tukey's HSD test. Error bars ± 2 SE.

The strength of selection on height and its components also differed in the two density treatments. As expected, in both species there was significant selection (S) for greater height in the high density treatment, but not at low density (Table 2). Genetic correlations between internode length and seedling height were highly significant for both species at both densities (Table 3a–b). Although no direct selection was detected for longer internodes at either density (β , Table 2), this strong genetic correlation coupled with selection on height would result in indirect selection for longer internodes in the high density treatment. To confirm that selection in the high density treatment was acting primarily on height independent of node number, we calculated selection gradients that measured the direct effect of height and node number on fitness. For both species, selection on height (β) was marginally significant ($p \leq 0.09$) while selection on node number was non-significant ($p \leq 0.46$).

In contrast, at low density both total and direct selection (S and β , respectively) for greater node number was significant in both species (Table 2). Significant direct selection for node number at low density indicates that the adaptive advantage of this trait in this environment did not depend on a related effect on internode length or stem height. At high density, node number contributed to fitness both directly and indirectly (Table 2). The indirect effect of node number arose from its influence on height, with which it is strongly genetically correlated (Table 3a–b).

Genetic variance

The study revealed genetic variation for all traits (Fig. 2). There was significant genotype (line) and/or $G \times E$ (line by treatment) variation in internode length and node number within populations of both species; *P. persicaria* also had significant $G \times E$ variation in height (Table 4). In both species, genetic variation for node number also occurred at the population level (Table 4).

Table 3. Genetic correlation coefficients between seedling height and height components (mean internode length and node number) at high density (HD) below the diagonal and low density (LD) above the diagonal.

HD\LD	height	mean internode length	node number
a) <i>P. persicaria</i>			
height	–	0.709**	0.367
mean internode length	0.852***	–	–0.276
node number	0.704**	0.367	–
b) <i>P. hydropiper</i>			
height	–	0.622**	0.521*
mean internode length	0.778***	–	–0.103
node number	0.746***	0.267	–

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Costs of adaptive responses

A significant cost of plasticity was detected in *P. persicaria* only for height in high density stands ($r = -0.509$, $P = 0.0293$). No significant costs of plasticity were detected in *P. hydropiper*: the 4 negative partial regression coefficients of plasticity on fitness for this species were not significant ($0.19 \leq P \leq 0.70$). A significant “cost of homeostasis” (i.e. a positive partial regression coefficient of trait plasticity on fitness) was also detected in *P. persicaria* for height in low density stands ($r = 0.969$, $P = 0.0217$) but not for any other trait in either species.

Discussion

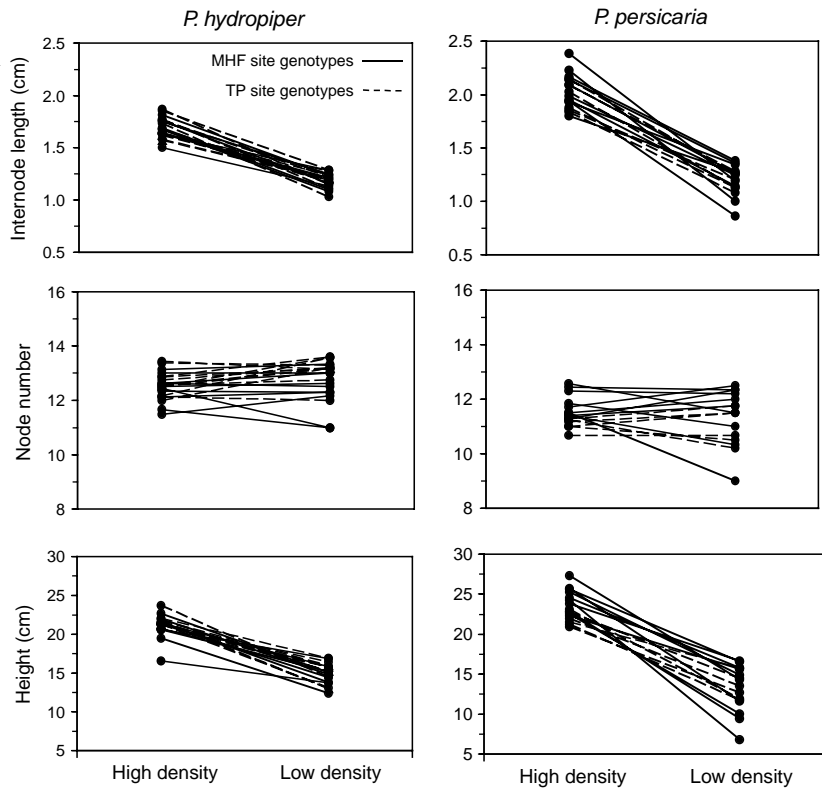
The results of this study reveal ecologically important differences between the *Polygonum* species in both trait plasticity (i.e. stem elongation) and the mean value of a constantly expressed trait (i.e. node number). The data are among the first to examine both aspects of adaptive trait expression in individuals of closely related congeners (for other examples see Morey and Reznick 2000, Voesenek et al. 2004, Brock et al. 2005). These comparisons of shade-avoidance component traits in *Polygonum persicaria* and *P. hydropiper* show how interacting

Table 2. Linear selection differentials (S) and selection gradients (β) for seedling height and height components in high (HD) and low (LD) density treatments. Height was not included in the measures of selection gradients (see Methods for details).

	<i>P. persicaria</i>		<i>P. hydropiper</i>	
	HD	LD	HD	LD
Height	S = 0.351*	S = 0.139	S = 0.150*	S = 0.065
Mean internode length	S = 0.039 $\beta = 0.137$	S = 0.058 $\beta = 0.057$	S = 0.052 $\beta = 0.064$	S = 0.041 $\beta = 0.032$
Node number	S = 0.318* $\beta = 0.356^*$	S = 0.233* $\beta = 0.232^*$	S = 0.117† $\beta = 0.124^†$	S = 0.087* $\beta = 0.081^*$

† $P < 0.1$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Fig. 2. Individual reaction norms for height and its component traits for *P. persicaria* and *P. hydropiper* in high and low density treatments. Each line represents the response mean of a single inbred genotype.



differences in trait expression can influence species' fitness in alternative environments and hence their ecological distributions, including their potential for coexistence in heterogeneous sites.

Species differences in internode plasticity had the greatest fitness impact in highly competitive conditions, where plant height (the product of internode length and number) is of primary adaptive value (reviewed by Schmitt et al. 2003, Dudley 2004). Although *P. persicaria* seedlings produced consistently fewer nodes than *P. hydropiper* seedlings, their pronounced internode

elongation response to high density more than offset this lower node number, allowing them to overtop the less plastic *P. hydropiper* plants in this treatment. As predicted by the shade avoidance hypothesis (Morgan and Smith 1979, Smith 1982, Schmitt 1997), taller plants achieved greater fitness in this competitive environment. These results are consistent with other studies that have shown an adaptive value for increased internode length and height in high density stands (Dudley and Schmitt 1996, Donohue et al. 2000, Weinig 2000, Callahan and Pigliucci 2002, Huber et al. 2004).

Table 4. Separate 1-species ANOVA for effects of density treatment (TRT), population (POP), and line (LN) on height and component traits in *P. persicaria* and *P. hydropiper*.

	Internode length		Node no.		Height	
	<i>P. persicaria</i>	<i>P. hydropiper</i>	<i>P. persicaria</i>	<i>P. hydropiper</i>	<i>P. persicaria</i>	<i>P. hydropiper</i>
TRT	230.0*** 1, 14	351.6*** 1, 20	0.2 1, 14	2.9 1, 20	205.3*** 1, 14	380.1*** 1, 20
POP [SP]	1.9 1,14	0.0 1, 20	5.0* 1, 14	7.4* 1, 20	2.3 1, 14	1.6 1, 20
POP × TRT	0.0 1, 14	0.3 1, 20	0.2 1, 14	6.0* 1, 20	1.0 1, 14	0.8 1, 20
LN[POP]	0.9 14, 14	1.0 20, 20	1.6 14, 14	2.9* 20, 20	1.3 14, 14	1.5 20, 20
LN[POP] × TRT	2.8** 14, 117	1.8* 20, 240	2.1* 14, 115	1.9* 20, 242	3.2*** 14, 112	1.1 20, 242

Top number F; lower numbers numerator, denominator df. P < 0.05; **P < 0.01; ***P < 0.001. Bold values are significant at $\alpha = 0.05$.

In contrast, each species expressed a constant node number across environments, with *P. hydropiper* seedlings producing more nodes in both density treatments. Greater node number was favored in both high and low density treatments, although the precise adaptive value of this trait differed between treatments. In high density plots, some of the fitness contribution of greater node number derived from its effect on plant height, with which it was strongly genetically correlated. In low density plots, however, both species showed strong genotypic selection for greater node number in the absence of corresponding selection for greater height: here, the adaptive value of more nodes was independent of the effect of node number on height. In these non-competitive conditions, seedlings with more nodes and therefore more leaves may be better able to exploit the ample light to enhance growth and reproduction (Hutchings and de Kroon 1994). Accordingly, the consistently higher node numbers of *P. hydropiper* seedlings contributed to their greater fitness in low density stands compared with *P. persicaria* plants. These results suggest an intriguing selective explanation for constant trait expression: a particular trait value (such as maximum node number) may be adaptive across diverse environments – and hence selectively maintained – when it contributes differently to fitness in each environment. Investigating both functional trait interactions and trait genetic correlations with fitness is critical to understanding how patterns of trait expression are related to adaptation within and across environments (Rausher 1992, Scheiner et al. 2000, Schlichting and Smith 2002, Pigliucci 2003, Schmitt et al. 2003).

These comparative *Polygonum* results also provide insight into how species' respective genetic architectures and selective histories may have shaped adaptive differences in plastic and constant trait expression. The question of differential trait evolution is particularly intriguing in this species pair since they co-occur in the same open, nutrient-rich habitat. One potential explanation for the trait differences (i.e. the lack of elongation plasticity in *P. hydropiper* and the constant lower node number in *P. persicaria*) would be evolutionary constraints, i.e. a lack of appropriate genetic variation or a cost of plasticity for the trait in question (DeWitt et al. 1998, Scheiner and Berrigan 1998, Schlichting and Pigliucci 1998, Dorn et al. 2000).

Surprisingly, we found no evidence for either of these putative constraints on the evolution of either node number or adaptive elongation plasticity. Even within our relatively modest genotypic sample, both species showed significant genetic variation for these traits. Furthermore, costs of plasticity (i.e. a negative partial regression coefficient between trait plasticity and fitness) associated with the ability to adaptively alter a phenotype across density environments did not appear to have constrained trait evolution in these species. No costs of

plasticity for height or its components were found in *P. hydropiper*, the species with limited plasticity. Indeed, it was the highly plastic *P. persicaria* in which a significant cost was found for height plasticity, and only in the high density treatment, indicating that such a cost may not in fact prevent the evolution of plasticity that is associated with increased fitness. We also found no significant fitness costs in either species of maintaining a constant node number, i.e. a “cost of homeostasis” (Donohue et al. 2000, Dorn et al. 2000). These results indicate that while statistically demonstrable plasticity or homeostasis costs may exist, (a) they are likely to be both species- and environment- specific, and (b) they may not necessarily prevent the evolution of adaptive plasticity. These results confirm other recent studies showing that global or maintenance costs of plasticity may be less common and less critical as an evolutionary constraint than initially believed (Donohue et al. 2000, Dorn et al. 2000, reviewed by Schmitt et al. 2003, Sultan 2004).

Constraints on evolution may also arise through functional tradeoffs between pleiotropically linked traits (Antonovics 1976, Schlichting and Pigliucci 1998, Pigliucci and Schmitt 1999, Barton and Partridge 2000). The fact that *P. persicaria* has not evolved a higher node number in spite of the availability of genetic variation for this trait may result from such a tradeoff. For instance, plants of this species produce an extremely long peduncle that apparently enhances achene dispersal; this trait may be developmentally incompatible with the production of numerous short internodes.

In addition to genetic constraints and costs, selective history may influence the evolution of phenotypic expression. Although the two *Polygonum* species considered in this study co-occur in a common habitat, they actually encounter dramatically different herbivory pressures and therefore selective regimes within these common sites. *P. persicaria* and other species in these disturbed herbaceous communities suffer considerable tissue damage from both ungulates and chewing insects, while *P. hydropiper* is almost completely avoided by these herbivores (T. Griffith, M. Bogonovich and S. E. Sultan, unpubl.). As a result, *P. hydropiper* is more likely to experience reduced interspecific competition and lower-density conditions as neighboring species around it are consumed over time. As competition is reduced over the course of the growing season, *P. hydropiper* seedlings that expressed strong shade avoidance morphology early in their growth (internode elongation and greater height) would actually be at a selective disadvantage once the surrounding vegetation that provided structural support was removed: in the absence of neighbors, elongation has been shown to be maladaptive in several species (reviewed by Schlichting and Smith 2002, Schmitt et al. 2003, Dudley 2004). Moreover, because plants in low density stands grow larger and have a much higher absolute fitness than plants in high density stands, the

fitness benefit experienced by seedlings able to survive in the absence of competition would far exceed the fitness benefit of being slightly taller in high density stands (M. Turelli in Stanton et al. 1997). Thus, reduced plasticity at the seedling stage in *P. hydropiper* may have evolved as an adaptive growth strategy in habitats where interspecific competition at the seedling stage is an unreliable predictor of competition during later growth (for models testing the importance of cue reliability on the evolution of adaptive plasticity, see Moran 1992, Lively 1999, Tufto 2000, Sultan and Spencer 2002).

Interestingly, the different trait combinations expressed in high versus low density conditions by *P. persicaria* and *P. hydropiper* seedlings may contribute to their coexistence in open, disturbed sites. As in many grazed pastures, the locations of grazed and ungrazed microsites can change within or between years, thus generating a temporal and spatial mosaic of high and low density patches. Because *P. persicaria* seedlings can elongate internodes to overtop interspecific competitors, this species will persist in this habitat if there is a sufficient frequency of dense, ungrazed microsites. Conversely, because *P. hydropiper* seedlings can exploit high light availability via the consistently greater production of more nodes and leaves, this species will persist if there is a sufficient frequency of effectively low density patches in which interspecific neighbors suffer herbivory. Thus differences between these species in both constant and plastic juvenile traits contribute to their ability to succeed in alternative competitive microsites and hence to coexist in a patchy habitat.

This comparative study provides evidence that the construction of adaptive phenotypes in alternative environments involves the integration of multiple traits, both plastic and constant. Fully understanding the adaptive effect of species differences entails studying the functional interactions and fitness effects of such traits in a real world setting.

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