

Plasticity to neighbour shade: fitness consequences and allometry

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Summary

1. Phenotypic plasticity may confer a fitness benefit to organisms in a spatially heterogeneous environment. However, the evolution of such adaptive plasticity can be limited by the constraints imposed by interacting environments. Moreover, adaptive plasticity may be masked by reduced growth in a low-quality environment. The allometry between plasticity of morphological traits and overall growth is often overlooked.

2. We addressed these issues by examining the effect of shade produced by neighbouring plants (neighbour shade) on a small annual plant, *Geranium carolinianum*. Plants were grown in two field sites, a wood margin and an old field. A clipping treatment was used to manipulate neighbour shade at both planting sites. We measured petiole length to quantify shade avoidance response. Genotypic selection analysis was used to assess the fitness consequences of petiole length.

3. Petioles were longer in the wood margin than in the old field and were similar between neighbour environments. However, overall plant size differed among the environments. As a result, relative to plant size, petioles were longer when individuals were surrounded by neighbours than when neighbours had been removed, but were similar between sites. After taking the size into account, plants with longer petioles had higher fitness than plants with shorter petioles in all environments.

4. The functional response of petioles to neighbour shade was revealed only when the passive plasticity of plant size was considered. The lack of differential selection across neighbour environments together with active elongation of petioles in response to neighbours suggests that the current shade avoidance response is adaptive.

Key-words: allometry, *Geranium carolinianum*, neighbour shade, petiole, phenotypic plasticity

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Introduction

If there is spatial environmental heterogeneity within a population and patterns of selection vary with the environment, then natural selection will favour a different optimum phenotype in each environment. This selection is expected to result in adaptive phenotypic plasticity; organisms that can change their phenotype in response to environmental variation will have higher fitness than non-plastic organisms. A number of empirical studies have demonstrated that phenotypic plasticity confers a fitness benefit when an individual or population encounters a heterogeneous environment (Kingsolver 1995; Dudley & Schmitt 1996; Van Buskirk, McCollum & Werner 1997). In particular, the plasticity in response to a number of biotic and abiotic environmental variables has been elegantly demonstrated to

confer a fitness advantage for sessile organisms like plants (e.g. Dudley & Schmitt 1996; Weinig 2000; Van Kleunen & Fischer 2003).

However, these and other studies have largely focused on relatively simple environmental settings. In nature, organisms frequently do not experience variation in environmental factors in isolation. Even a single environmental factor like light can have complex effects on the phenotype (Dudley 2004). A reduction in light in a natural plant population can be caused by overhead canopy shade, shade from neighbouring plants or combination of the two. Shade from neighbouring plants results in a gradient of light within an individual where the ratio of red to far-red light (R : FR) decreases (Schmitt & Wulff 1993; Franklin & Whitelam 2005). The shade from a tree canopy decreases the quantity of light as well as reducing R : FR, but does not create gradients within individuals. Therefore, the extent to which a reduction in R : FR indicates the presence of neighbours (e.g. Ballaré,

Scopel & Sánchez 1990; Huber & Wiggerman 1997; Weinig 2000; Schmitt *et al.* 2003) may vary among locations for taxa that occupy habitats that vary in the presence of tree shade. Relatively few studies have addressed whether plastic response depends on context, that is, whether response to neighbour shade varies among habitats with different patterns of light availability (Donohue *et al.* 2000; Griffith & Sultan 2005).

A decrease in light quality or quantity typically results in both active and passive morphological plasticities (Sultan & Bazzaz 1993). Active plasticity is a change in phenotype that mitigates the effect of reduced resources. In contrast, passive plasticity is simply a response to reduced resources, for example, smaller size or growth rate due to lower resource availability. Plants often actively respond to reductions in the R : FR ratio by elongating hypocotyls, petioles and internodes. These phytochrome-mediated changes are termed shade avoidance responses and are often adaptive (Leeflang, Doring & Werger 1998; Donohue *et al.* 2000; Weinig 2000; Tsukaya, Kozuka & Kim 2002; Causin & Wulff 2003). In response to reductions in light quantity, plants reduce branching frequency and increase specific leaf area as well as elongating petioles and internodes (Sultan & Bazzaz 1993; Callaway, Pennings & Richards 2003; Van Kleunen & Fischer 2003). The evolution of active plasticity is expected where a phenotypic response will enhance fitness. Therefore, greater elongation of petioles in response to neighbour shade is predicted in open conditions but not when tree shade is also present. This is because petiole elongation of understorey plants may not result in as great an increase in light capture (see e.g. Leeflang *et al.* 1998).

Individuals growing in different environments may also express passive plasticity. In particular, overall size may differ due to the differences in resource availability among environments (Evans & Hughes 1961; Coleman, McConnaughay & Ackerly 1994), for example, plants in low light conditions often grow more slowly than plants in high light environments. Therefore, if plants are compared at the same age, their phenotypes for traits that enhance light capture (e.g. internode length and petiole length) may differ simply due to differences in overall size. A plastic change in a trait that is independent of plant size is more likely to be the result of an adaptive response to the environment than a passive response (Moriuchi & Winn 2005). Despite a long history of the study of phenotypic plasticity, surprisingly few studies have examined the allometric relationship between plant size and the traits of interest between environments (Coleman *et al.* 1994; but see Moriuchi & Winn 2005; Puijalón & Bornette 2006).

Geranium carolinianum L. is a semi-erect annual plant in which petioles and internodes elongate in response to shade (Bell 2004). It is found in relatively open communities that include patchily distributed

grasses and small herbaceous weeds. In such environments, elongation in horizontal space may allow *G. carolinianum* to extend its leaf blades past local neighbours and into better light environments (Ballaré 1994). An association of increased petiole length with enhanced fitness under low light in *G. carolinianum* (Bell 2004) supports the idea that petioles are horizontal spacers, allowing for increased acquisition of light. *Geranium carolinianum* plants are found in sites without tree shade (old fields) and at wood margins where tree shade is present. Although elongating petioles may allow individuals to escape neighbour shade, regardless of the tree canopy, it does not provide escape from tree shade; therefore, the adaptive value of plasticity may depend on site and neighbour density. Here we examine both active and passive plasticities to neighbour crowding by addressing the following questions: (i) Does petiole length respond plastically to neighbour shade? And does plasticity to neighbour shade change between sites? (ii) Does plasticity of petiole length scale with changes in plant size between light environments? and (iii) Is plasticity of petiole length to neighbour shade adaptive?

Materials and methods

STUDY SYSTEM AND STUDY SITES

Geranium carolinianum L. (Geraniaceae) is a winter annual native to North America and is considered a weed of old fields, disturbed sites and wood margins (Gleason & Cronquist 1991; D.L. Bell, personal observation). The seeds of *G. carolinianum* have non-dormant embryos but hard seed coats and will not germinate unless they experience dry or alternately wet-dry conditions and relatively high temperatures (Baskin & Baskin 1974). In natural populations, germination usually occurs in response to autumnal rains following the summer dormancy period. During the winter, plants grow as rosettes with about five leaves. In the spring, *G. carolinianum* bolts to an erect plant with at least one main stem of several nodes and relatively compact inflorescences. Flowers typically produce five seeds in late May through June (Strausbaugh & Core 1997; D.L. Bell, personal observation). *Geranium carolinianum* largely has a self-fertilizing mating system (Roach 1986). Seed dispersal in the species is explosive; dispersal distances average 1.4 m (± 0.7 SE; Bell 2004).

To test the adaptive value of petiole response to neighbour shade, we used two planting sites that have natural *G. carolinianum* populations (Albemarle County, Virginia, USA, c. 30 km apart). The old field site has a high light environment and lacks tree shade. The wood margin site has scattered trees with average lower light availability and a larger amount of spatial variation for light than the old field site [wood margin mean Photosynthetically Active Radiation (PAR) = 426.92 $\mu\text{mol s}^{-1}\text{m}^{-2}$ (± 44.42 SE), coefficient of variation (CV)

for PAR = $61.7 (\pm 17.3 \text{ SE})$; old field mean PAR = $883.64 \mu\text{mol s}^{-1} \text{m}^{-2} (\pm 4.40 \text{ SE})$, CV for PAR = $7.7 (\pm 2.4 \text{ SE})$]. The measurements of PAR were made over the entire course of *G. carolinianum*'s growing season incorporating autumnal, winter and spring light levels (see Bell 2004). Both sites have grasses and herbaceous weeds, although the old field has a greater density of vegetation (D.L. Bell, personal observation).

EXPERIMENTAL DESIGN

Geranium carolinianum seeds were collected from six naturally occurring populations (Bell 2004). Seeds used in this experiment were the result of three generations of self-propagation under uniform conditions in the University of Virginia, Department of Biology greenhouse. We combined the seeds of several maternal plants belonging to the same family. As a consequence, seeds from a 'family' shared the same grandmother. To include possible genetic differentiation within and among populations in response to shade, we selected families that demonstrated a range of response to greenhouse shade environments (Bell 2004). Each population contributed between four and eight families to 36 self-sibling families used in this study. Because populations were not represented by a random sample of families but rather families with broad responses to shade, any difference between populations likely reflects our sampling. Therefore, we focus analyses at the level of family.

In early October, seeds were individually scarified with a wood file to promote temporally homogeneous germination across families. Scarified seeds were individually placed in plug trays filled with Pro-Mix (Premier Horticulture, Dorval, Canada). Trays were placed on a misting bench in the greenhouse for 6 days through cotyledon emergence, followed by 2 days on a regular greenhouse bench. Seedlings were then moved outside to acclimate to autumnal temperatures and watered as necessary.

Plants were subjected to experimental variation in neighbour shade and to natural variation in tree shade. In late October, 30 seedlings at the first or second true leaf stage from each family were transplanted into the wood margin and the old field planting sites. Both sites were prepared for transplanting by mowing to initially standardize the local neighbour environments. Individual seedlings were randomly assigned to a position in a 36×30 (rows \times columns) grid with 0.5 m between each experimental plant. The grids were each 18×15 m, and represent a random sample of the light environments that occur in each planting site. Within each site, 15 individuals from each family were randomly assigned to the clipped neighbour environment while the other 15 individuals of the family were assigned to the unclipped neighbour environment. In total, 2160 individuals were used in the study (36 families \times 2 planting sites \times 2 neighbour environments \times 15 individuals/family/neighbour

environment/planting site). At transplanting, the clipped neighbour environment was created by clipping all vegetation to the ground within 15 cm of the plant. The plants in the unclipped neighbour environment were left surrounded by the native vegetation. Neighbour environments were maintained throughout the life of the experimental plants by repeated clipping.

In early June, after the end of fruiting, all surviving experimental plants were harvested and dried at 72°C for at least 48 h. Petiole length at node 1 (first node up the bolted stem from plant base) was measured on each individual to determine plastic response to light, and all leaves on a plant were counted as an estimate of plant size. Survivorship was 60.0% at the wood margin site and 83.9% at the old field site and did not vary among families (analyses not shown). Fitness was estimated by fecundity (number of fruits); almost all mortality was prior to bolting and therefore the expression of characters reported here.

STATISTICAL ANALYSIS

Analysis of variance (ANOVA) was used to examine the effects of planting site and neighbour environment on petiole length and leaf number. Mixed model ANOVA included planting site and neighbour environment as fixed effects, family as a random effect, and the interaction between planting site and neighbour environment (PROC MIXED, SAS Institute Inc., Cary, NC, USA). We initially tested for interactions between the family and each of the fixed effects (family \times neighbour environment, family \times planting site, family \times neighbour environment \times planting site). All these interactions were non-significant ($P > 0.20$) and therefore were not retained in the model. However, we tested the fixed effects with family as the error term, which provides a test that is as conservative as if the family interactions were maintained in the model. Petiole length was square-root transformed and number of leaves was log transformed to meet the assumptions of normality and heteroscedasticity. In *G. carolinianum*, number of leaves is highly correlated with total dry plant biomass ($r = 0.96$, D.L. Bell, unpublished data) and therefore is used as an estimate of plant size. Number of leaves was then included as a covariate in petiole length analysis to assess whether differences detected in petiole length were simply due to differences in plant size. Petiole length is correlated with leaf number within each treatment combination ($r = 0.66\text{--}0.74$), and 96% of the leaf number values are common to all treatments, indicating that it is appropriate to use as a covariate although it does vary among treatments.

We then directly tested the allometric relationship between petiole length and overall size in the two neighbour environments by performing a mixed model ANCOVA. The ANCOVA included petiole length as the dependent variable; neighbour environment, planting site, family, neighbour environment \times planting site as the independent variables and leaf number as the

covariate. The interaction leaf number \times neighbour environment tested for differences in the relationship between leaf number and petiole length across neighbour environments.

Selection on petiole length was measured using genotypic selection analysis (Lande & Arnold 1983; Rausher 1992). All traits except fitness were standardized (mean of 0 and standard deviation of 1), but were untransformed to retain biological meaning (Mitchell-Olds & Shaw 1987). Relative fitness was calculated by dividing the fitness of each individual by the mean fitness within each combination of planting site and neighbour environments; beyond this calculation, fitness was not transformed (Lande & Arnold 1983). Genotypic selection analysis uses the mean of the 15 members of each family within a neighbour environment and site as the unit of observation to avoid bias resulting from local environmentally induced covariance between phenotypes and fitness (Rausher 1992).

Directional and quadratic genotypic selection gradients (β and γ , respectively) were estimated. Selection gradients indicate the strength and direction of direct selection on a trait while holding all other traits in the analysis constant (Lande & Arnold 1983). Directional selection gradients (β) were estimated as the partial regression coefficients from an analysis of covariance (ANCOVA) including neighbour environment and site as fixed effects, and petiole length and leaf number as covariates. Directional selection gradients indicate whether there is a linear relationship between a trait and fitness. Quadratic (γ) gradients estimate nonlinear relationships between traits and fitness, and were similarly estimated, but in a model that included both directional and quadratic terms. Quadratic selection gradients were estimated separately from directional selection gradients because the directional and quadratic variables may be correlated, biasing directional estimates from a model that includes both (Wade & Kalisz 1990). Partial regression coefficients were estimated using ANCOVA to evaluate selection on petiole length and leaf number while accounting for differences in phenotype due to neighbour environment,

planting site and their interaction. The selection differentials were estimated by conducting ANCOVA as above, but including only petiole length or leaf number, such that the relationship between each trait and fitness is assessed without the other trait in the model. This is analogous to the more typical formulation of the selection differential as the covariance of each trait and fitness, but it allows for the inclusion of fixed effects in the model. The residuals from the selection analyses were not normally distributed. The selection gradients were therefore bootstrapped to assess the accuracy of the parametric tests (Mitchell-Olds & Shaw 1987). The results from the original selection analyses are reported because the patterns of significance from the bootstrapped analyses did not deviate from the parametric analyses (cf. Weinig 2000).

We performed ANCOVA to determine if selection differed in strength or direction between environments. The ANCOVA included relative fitness as the dependent variable and planting site, neighbour environment, planting site \times neighbour environment, number of leaves, petiole length, and interactions between the covariates and fixed effects. A significant interaction with petiole length or leaf number indicates that the pattern of selection on these traits differs between the experimental treatments.

Results

Petiole length responded differently to neighbour shade and planting site. Longer petioles were found on *G. carolinianum* growing in the site at the wood margin than in the site at old field, whereas neighbour presence did not influence petiole length (Table 1; Fig 1a). However, plants produced more leaves and therefore were larger at the wood margin than at the old field, and in the clipped environment than in the unclipped one (Table 1; Fig. 1b). After accounting for differences in size, plants in the two planting sites had very similar petiole lengths (Table 1; Fig. 1c). However, longer petioles were produced in the unclipped neighbour environment than in the clipped neighbour environment for a given plant size (Table 1; Fig. 1c). The allometric relationship between petiole length and leaf number differed significantly between the neighbour environments (leaf number \times neighbour environment $F_{1,1227} = 9.90$, $P = 0.002$). There was a greater increase in petiole length with plant size in the unclipped neighbour environment (slope = 2.70) than in the clipped neighbour environment (slope = 2.22). The interaction of planting site and neighbour environment did not significantly affect petiole length or leaf number (Table 1), indicating that the environmental treatments had independent effects on the two traits.

Selection consistently favoured larger plants and plants with longer petioles (Table 2A). The pattern of selection on petiole length did not differ between neighbour environments or planting sites (Table 2A). The selection differential for petiole length is greater

Table 1. Analyses of variance and covariance for the effects of planting site, neighbour environment and family on petiole length and leaf number of *Geranium carolinianum*

Source of variation	df	Petiole length		Leaf number ANOVA
		ANOVA	ANCOVA	
Planting site	1	86.77***	0.00	124.55***
Neighbour environment	1	0.13	20.17***	21.71***
Family	35	1.64*	1.89**	2.46***
Planting site \times neighbour environment	1	0.12	0.00	0.03
Leaf number	1	–	1151.54***	–

The ANCOVA permits separating the effect of plant size and environment on petiole length. F values are shown; df for the denominator: petiole length = 35, leaf number = 38.

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

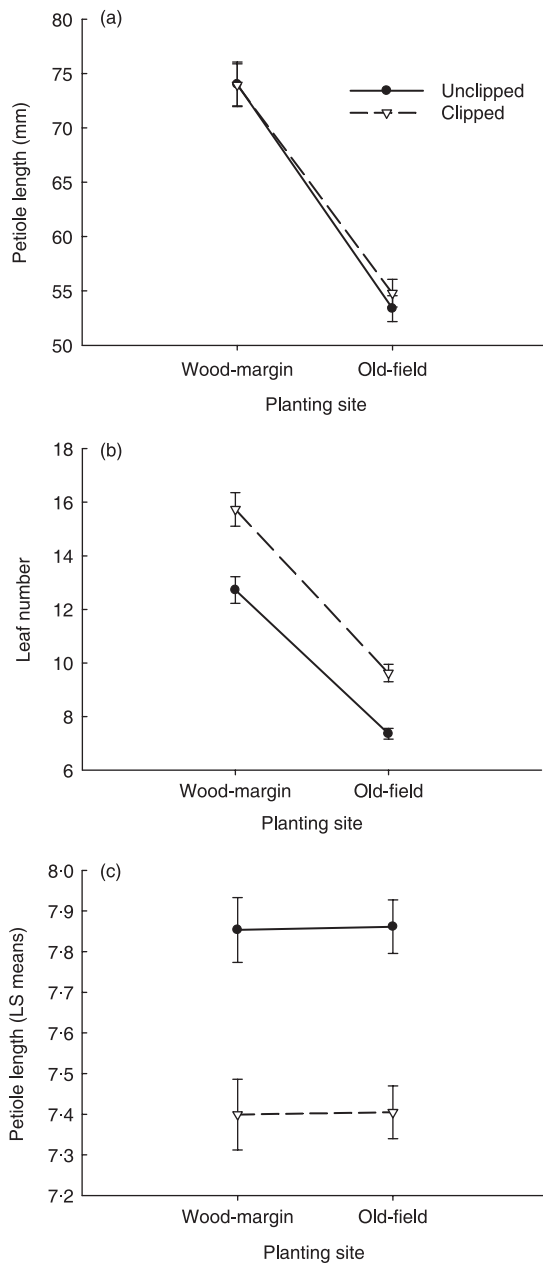


Fig. 1. Mean (\pm SE) petiole length (a) and leaf number (b), and least-square means of petiole length after accounting for plant size (estimated by leaf number, c) for *Geranium carolinianum* growing with shade from neighbours or where neighbours had been clipped at two sites.

than the selection gradient. This indicates that much of the genotypic selection on petiole length is indirect due to a positive correlation between petiole length and plant size. In contrast, the selection differential and gradient for leaf number was similar (Table 2A), reflecting little indirect selection on plant size due to correlations with petiole length. The positive quadratic selection on petiole length indicates that the relationship between petiole length and fitness is accelerating, such that a given increase in petiole length will enhance fitness more in genotypes with longer petioles (Table 2B). In contrast, although there was selection

for plants with more leaves, this selection was decelerating, resulting in selection for a more modest increase in plant size for larger individuals than for smaller plants (Table 2B).

Discussion

Overall size was greater for *G. carolinianum* plants grown without neighbours in both sites, but petiole lengths were similar regardless of whether plants were grown with or without neighbours. If the differences in plant size induced by the neighbour environment are taken into account, relatively longer petioles were produced on plants growing in crowded conditions. This increase in relative petiole length in the presence of neighbours is a characteristic of active developmental plasticity because it is independent from the response of plant size and because it potentially ameliorates the impact of low light availability (Sultan 1995; Dorn, Pyle & Schmitt 2000). Although the scale of environmental variation is not known, the fact that increased petiole length is associated with enhanced fitness suggests that petiole elongation results in increased light interception. The elongation of petioles is likely to be mediated by phytochrome photoreceptor molecules to both R : FR and blue light (Smith 1995; Leeftang *et al.* 1998). Other studies have also found elongation in petioles in response to simulated or actual neighbour shade (Stuefer & Huber 1998; Tsukaya *et al.* 2002). The elongation of the internodes in response to neighbour shade is another commonly observed shade avoidance response (Dudley & Schmitt 1996; Dorn *et al.* 2000; Weinig 2000; Bell 2004; Franklin & Whitlam 2005; McGuire & Agrawal 2005). Shade avoidance response to neighbour shade, whether via petiole or internode elongation, may result in increased light interception by leaf blades.

After accounting for differences in plant size within and between environments, planting site had little effect on petiole length in *G. carolinianum*. Plants in the wood margin were larger than plants in the old field. The plants at the old field received greater levels of radiation than those in the wood margin, and their smaller size suggests that old field plants may have been limited by another environmental factor (e.g. herbivory, Cipollini 2004; water availability, Huber *et al.* 2004). Petiole length scaled with plant size, suggesting that the longer petioles in the wood margin are a generalized plastic response due to changes in plant size rather than a shade avoidance response to the reduced light conditions found in the wooded areas. In contrast to the active plasticity of petioles to neighbour environments, this response to planting site exemplifies passive plasticity since it is entirely dependent on plant size.

Studies examining phenotypic plasticity often fail to consider the effects of size on the trait of interest (Coleman *et al.* 1994). Not doing so would severely affect the interpretation of the results in this case.

Table 2. Genotypic selection analysis on petiole length and leaf number

(A) Directional selection differentials (S) and gradients (β)					
Trait	S	β	$\beta \times \text{site}, F$	$\beta \times \text{neighbour}, F$	$\beta \times \text{site} \times \text{neighbour}, F$
Petiole length	0.38***	0.08**	0.02	0.13	0.09
Leaf number	1.04***	0.94***	32.85***	9.27**	0.55
(B) Quadratic selection gradients (γ)					
Trait	γ	$\gamma \times \text{site}, F$	$\gamma \times \text{neighbour}, F$	$\gamma \times \text{site} \times \text{neighbour}, F$	
Petiole length	0.08*	0.08	0.25	0.00	
Leaf number	-0.17	0.24	0.01	0.11	

F values test whether directional and quadratic genotypic selections on petiole length differed between planting sites, neighbour environments or the interaction between the two. df: directional = 1,132, quadratic = 1,124.

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

When comparing plants of a similar size, plants in the more crowded environment elongated their petioles. But if plants are simply compared between the neighbour environments without accounting for plant size, we would conclude that there was no shade avoidance response. Passive plasticity to an environmental factor for traits like size may mask active responses for other traits. Therefore, size-related traits or the ontogenetic trajectory should be considered when evaluating plasticity to the environment (Coleman *et al.* 1994). Both size and ontogenetic stage have influenced the understanding of plastic responses in other systems (Bell & Sultan 1999; Moriuchi & Winn 2005; reviewed in McCoy *et al.* 2006). For example, in *Viola septumbula*, differences in specific leaf area between low- and high-quality environments were twice as large when corrected for plant size (Moriuchi & Winn 2005).

We found selection for longer petioles across both neighbour and planting site environments. There was no evidence that selection varied among environments or depended on the interaction of canopy and neighbour environments, suggesting that current levels of plasticity are adaptive. In contrast, studies under controlled conditions have found that interactions between environments can affect plant fitness and that these interactions may depend on the precise level of the interacting environmental factors (Sultan *et al.* 1998; Sultan 2001). However, we have much less information about whether environmental factors have interacting effects on fitness in natural environments (but see Huber *et al.* 2004). Here, under field conditions, selection always favoured longer petioles regardless of whether neighbour shade was present or absent. This similarity in selection between environments indicates that selection did not favour greater differences in petiole length, that is plasticity, between the two neighbour environments.

The selection analysis, which used genotypic means within environment, revealed that the magnitude of selection on petiole length was reduced when the correlation between petiole length and leaf number

was taken into account (S vs. β). This indicates that the selection on petiole length is largely indirect, with selection favouring larger plants (Lande & Arnold 1983). Genotypic selection analysis is usually preferred over phenotypic selection analysis because it decreases the possibility that covariance between the trait of interest and the environment leads to misleading conclusions about whether selection is acting on the trait (Rausher 1992; van Tienderen & de Jong 1994; Stinchcombe *et al.* 2002). Given that we corrected for environmentally induced biases by conducting an analysis of family means, the difference between S and β is likely to reflect the genetic correlation between these traits rather than environmentally determined phenotypic correlations. As such, selection is expected to increase petiole length both through changes in plant size as well as changes in allometry, given sufficient genetic variation.

In summary, both passive and active plasticities influence the expression of petiole length. Consistent selection across environments suggests that longer petioles are favoured, but the relative magnitude of the current response to neighbour environments and planting site is adaptive. Finally, understanding the evolution of plastic response to the environment requires a broad analysis of trait expression, in addition to the functional response of specific traits.

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