Evol Ecol (2010) 24:1321–1337
DOI 10.1007/s10682-010-9373-6

ORIGINAL PAPER

The plasticity of phenotypic integration in response to light and water availability in the pepper grass, *Lepidium bonariense*

Kylie L. Mallitt · Stephen P. Bonser · John Hunt

Received: 21 May 2009 / Accepted: 20 February 2010 / Published online: 17 March 2010

Abstract Organisms that live in a heterogeneous environment face a number of important challenges. On one hand, they require the flexibility to respond to environmental conditions and change their phenotype accordingly. On the other, they are required to be robust in their overall body plan to ensure an integrated, functional organism. Here, we examine the relationship between phenotypic plasticity and integration in the common peppergrass, *Lepidium bonariense*, by examining the multivariate response of a series of functional traits to a combination of light and water treatments. *Lepidium bonariense* displayed considerable variation in phenotype in response to water and light availability with the extraction of the first two principal components retaining 85% of the total variation in our data set. Principal component 1 (PC1) largely reflects the negative genetic correlation between specific leaf area and overall plant size, whereas PC2 was typical of a shade-avoidance syndrome displayed by many species of vascular plants. Both PC1 and PC2 exhibited considerable variation among genotypes in phenotypic plasticity in response to the combined effect of light and water availability. Despite complex plasticity in this species, we demonstrate that variation in light and water availability did not significantly influence patterns of functional trait integration, with the genetic variance–covariance matrix remaining stable across environments.

Keywords Phenotypic plasticity · Phenotypic integration · Plant functional traits · Light · Water · Evolution · *Lepidium bonariense*

K. L. Mallitt · S. P. Bonser
Evolution and Ecology Research Centre, School of Biological, Earth and Environmental Sciences, The University of New South Wales, Sydney, NSW 2052, Australia

J. Hunt (✉)
Centre for Ecology and Conservation, The University of Exeter in Cornwall, Tremough Campus, Penryn TR10 9EZ, UK
e-mail: J.Hunt@exeter.ac.uk

Introduction

Understanding how complex phenotypes evolve in heterogeneous environments is a central question in evolutionary ecology (Raff 1996; Wagner 2001; Pigliucci and Preston 2004; Schlosser and Wagner 2004). Complex phenotypes consist of numerous traits that can be viewed as either integrated or non-integrated (Wagner 2001; Pigliucci and Preston 2004). Integrated traits are highly dependent on each other because of shared genetic or developmental pathways, competition for limited resources during development and/or due to a common functional role (Cheverud 1982; Pigliucci 2003). In contrast, non-integrated traits are functionally and developmentally independent of each other (Cheverud 1996; Wagner 2001).

The degree to which traits form an integrated unit has two major implications for our understanding of the adaptation and evolution of complex phenotypes. Firstly, quantitative genetic theory predicts that a history of persistent stabilising selection will facilitate integrated traits to evolve genetic correlations that are consistent with their functional and/or developmental relationships (Lande 1979, 1980; Cheverud 1982, 1984, 1996). Consequently, patterns of phenotypic integration may provide information on the nature of selection that has shaped the current phenotype of the organism (Armbruser and Schwaegerle 1996). Secondly, an understanding of integration enables predictions of the capacity for complex phenotypes to adapt to new environments (Lande and Arnold 1983; Wagner 2001; Pigliucci and Preston 2004). On one hand, phenotypic integration can drive adaptive evolution by reducing the environmental variance in each of the integrated traits. Variance that is orthogonal to the adaptive direction of evolutionary change for the entire structure is therefore reduced (Lande 1980, 1984; Wagner 1988). On the other hand, if integration reflects a history of invariant developmental processes, the high degree of genetic canalization can reduce the available levels of genetic variation. This constrains the response of a complex structure to present selection (Lande 1979; Arnold 1992; Wagner et al. 1997).

Despite much recent progress on this topic (Pigliucci and Preston 2004; Schlosser and Wagner 2004), most empirical studies have only studied patterns of phenotypic integration in a single environment (Pigliucci and Preston 2004; but see Pigliucci et al. 1995; Liu et al. 2007). However, phenotypic plasticity (the ability of a genotype to modify phenotypic expression depending on the environment (Bradshaw 1965), can be an adaptive strategy to cope with a heterogeneous environment (Bradshaw 1965; Moran 1992; Scheiner 1993; Pigliucci 2001; DeWitt and Scheiner 2004). Whenever an organism is exposed to a variety of environmental conditions, the optimal reaction is to express the phenotype best suited to each particular set of conditions (Schlichting 1989). Individual traits that comprise an integrated unit in one environment may, due to differential expression, be loosely integrated in alternate environments. It may then be necessary to alter the correlations amongst traits to approach the optimum phenotype (Schlichting 1989). Alternatively, traits may respond in a coordinated fashion to environmental conditions (i.e. high levels of integration). The correlation structure amongst traits may then be conserved even though the expression of the traits may change per se. Despite the importance that changes in the correlation structure can have for evolutionary change (Lande and Arnold 1983), we still know surprisingly little about how the environment influences levels of phenotypic integration.

Due to their sessile existence, plants unavoidably experience fluctuations in their external environment and this may facilitate the evolution of phenotypic plasticity (Pigliucci 2001). Variation in the expression of plant functional traits (such as stems, leaves and roots) significantly influence plant fitness by altering their ability to acquire and allocate resources (Reich et al. 2003). Water and light are two environmental resources that

are known to be particularly important to the functional responses of plants (Pigliucci 2001; DeWitt and Scheiner 2004). Reduced light quality is correlated with the presence and proximity of neighbours and has been shown to initiate stem elongation to improve light inception (Schmitt and Wulff 1993). Likewise, reduced water availability has been shown to induce changes in plant phenotype, generally limiting total biomass (Rajakaruna et al. 2003). Therefore, it is not surprising that numerous studies have quantified the independent effects of light (e.g. Callahan and Waller 2000; Pigliucci and Kolodynska 2002a) and water availability (e.g. Pigliucci and Kolodynska 2002b; Kolodynska and Pigliucci 2003; Mommer and Visser 2005) on levels of phenotypic plasticity and integration in plants. Fewer studies have, however, examined the combined effects of light and water availability on levels of phenotypic plasticity (e.g. Noda et al. 2004; Monaco et al. 2005; Valladares et al. 2005) and none have, to our knowledge, examined the combined effects of these environments on levels of phenotypic integration. This is important because natural organisms often experience a myriad of environmental factors and recent studies suggest that multiple environmental factors can often have a very different effect on the phenotype compared to either environment in isolation (Sih et al. 2004).

Here, we examine the relationship between phenotypic plasticity and integration in the common peppergrass, *Lepidium bonariense*, by examining the multivariate response of a series of functional traits to a combination of light and water treatments. We discuss our findings in relation to the importance of phenotypic plasticity and integration to the evolution of complex phenotypes in heterogeneous environments.

Materials and methods

Study species and seed collection

The common peppergrass, *Lepidium bonariense* L. (Brassicaceae), is an annual or perennial herbaceous weed found throughout New South Wales, Australia (Harden 2000). The rapid growth, local abundance and a fully inbred self-fertilising life-history (providing discrete genotypes) make it an ideal species for laboratory studies. *Lepidium bonariense* develops as a basal rosette of leaves, and flowers develop from terminal stalks up to 50 cm in height (Harden 2000).

We collected a single individual of *L. bonariense* from each of thirteen sites around the eastern suburbs of Sydney, Australia. There was a minimum distance of 1 km between collections sites. Thus, each plant is likely to represent a discrete genotype. We obtained genotypes from roadside footpaths and all locations were characterised by disturbed and exposed environmental conditions. Maternal effects may induce plastic responses in offspring that are adaptive in the maternal environment. This may confound the expression of plasticity by offspring when exposed to a novel environment. To standardize maternal effects in our study, we derived the largest seed at each site by sampling the largest plant (see Roach and Wulff 1987). Even so, we must acknowledge the potential for maternal effects to confound our genetic estimates.

Experimental design

We placed seeds from each of the thirteen genotypes on wet filter paper in sealed petri dishes. We refrigerated these at 10°C for 36 h, and then placed them into a greenhouse to germinate. We transplanted several seedlings to pots (10 cm high, 7.5 cm diameter) at the

emergence of the cotyledons. Pots contained soil consisting of approximately 30% coconut peat, 50% river sand, 15% composted organics and 5% dynamic lifter (Yates®, Australia) and a slow release fertiliser. We arranged the pots on open benches in a glasshouse, and assigned pots randomly to one of four environmental treatments in a complete factorial design: (a) High Water and High Light (HW:HL), (b) High Water and Low Light (HW:LL), (c) Low Water and High Light (LW:HL) and (d) Low Water and Low Light (LW:LL). To maximise the establishment success, we grew all plants in ambient greenhouse light and watered them daily for 2 weeks after germination. Once the seedlings were established, we removed extra plants so that there was only a single plant in each pot. We assigned a single replicate of each of the 13 genotypes to each treatment to create a block (13 genotypes $\times$ 4 treatments = 52 pots per block). We replicated blocks 10 times (52 pots $\times$ 10 blocks = 520 plants).

We watered the plants in the high water treatments with 50 ml every day. We watered the plants in the low water treatment with 50 ml every second day until 12 weeks of age and then every third day until harvest. This watering regime provided a relatively low amount of water to the treated plants, while minimising water stress induced mortality. We provided each plant in the high light treatment with ambient greenhouse light. We surrounded each plant in the low light treatment with a cylinder of translucent green plastic film, 30 cm high and 8.5 cm in diameter (Lee Filters, Andover UK, no. 121 Lee Green). This filter simulated the effect of light filtered through a canopy of competing plants, reducing photosynthetic photon flux density by 36% and the red to far-red ratio (R: FR) from 1.0 to 0.2. We harvested plants at 21 weeks of age. Unfortunately, no plants reached reproductive maturity by this stage.

Trait measurements

At harvest, we measured a total of 12 morphological traits on each plant. For every leaf per plant, we measured petiole length (PL), petiole diameter (PD), leaf length (excluding the petiole) (LL) and leaf width (LW) to the nearest 0.01 mm using digital callipers, and leaf area (including the petiole) (LA) using a Li-Cor leaf area meter (Li-3000A, LiCor Biosciences, Lincoln, Nebraska). We calculated plant means for each leaf measurement and used these values in all subsequent analyses. For each plant, we also measured shoot diameter (SD) at the surface of the soil, plant height measured from the surface of the soil to the highest point on the plant (Height), root mass (RM), leaf mass (including petiole) (TM) and leaf number (LN). Individual plants were dried to a constant weight in a drying oven at 60°C for 36 h prior to mass measurements.

Using the above measurements, we also calculated specific leaf area (SLA) (calculated as leaf area divided by leaf mass for each individual leaf per plant) and total plant mass (the total above and below ground biomass). We did not observe stem elongation during the time frame of our study, with the petioles of each leaf arising in a rosette directly from the soil horizon. Consequently, stem length or mass measurements were not possible.

Statistical analysis

Traditionally, G $\times$ E studies including multiple phenotypic traits has been analysed using multivariate analysis of variance (MANOVA). Univariate ANOVA's are then used to determine how each variable contributes to the overall MANOVA model (e.g. Pigliucci and Hayden 2001). One of the major assumptions of MANOVA is that the phenotypic traits being examined are statistically independent (Field 2000). Our data, however,

exhibited strong multi-collinearity, as illustrated by the average variance inflation factor (VIF) statistic being substantially greater than 1 (mean VIF across all traits = 98.32; Bowerman and O'Connell, 1990) and more than 75% of the tolerance values falling below 0.2 (Menard 1995). This fact alone suggests a high degree of integration in the plant traits we examined. There are two approaches that can be taken to account for multi-collinearity in a multivariate data set (Field 2000). Firstly, one possible solution is to omit the offending variable(s) from the data set. This approach is only useful, however, when a small number of variables are responsible for the observed multi-collinearity (Field 2000). This was not the case in our data set as the sequential deletion of variables did not significantly reduce the VIF scores.

A second approach, and the one adopted here, is to use principal component analysis (PCA) to compress the original data into a set of linear variables and then use the resulting principal component scores in subsequent statistical analyses (Dunteman 1989; Field 2000). As our experiment was run in a series of blocks, we standardized all phenotypic trait measures by block mean prior to principal component analysis to remove any variation due to block effects and also to conserve degrees of freedom in our final model (see below). Only principal components yielding an eigenvalue greater than 1 were retained for analysis (in our case the first two principal components) and we interpreted factor loadings with an absolute value greater than 0.4 as biologically relevant (Stevens 1992). We then used a generalized linear mixed-model (GLMM) to examine the effects of genotype (random effect), water environment (fixed effect), light environment (fixed effect) and all interaction terms (i.e. Genotype × Light (random effect), Genotype × Water (random effect), Light × Water (fixed effect), Genotype × Light × Water (random effect)) on the factor loadings derived from the principal component analysis. All statistical analyses were performed in JMP (version 7).

We used Pearson product-moment correlation coefficients to calculate the genetic correlations between phenotypic traits in each of our four light and water treatment combinations. We based correlations on the average value of each phenotypic trait for each genotype in each of the environments and calculated Jacknifed standard errors using Poptools (version 2.6.9; freely available at http://www.cse.csiro.au/poptools). We calculated variance-covariance matrices (**G**) for each treatment using the VC(matrix) function in Poptools. Various methods are currently available to assess differences in **G** in multidimensional space (Krzanowski 1979; Cohn 1999; Phillips and Arnold 1999). Most recently, the common principal component (CPC) model has become a popular and effective statistical tool in evolutionary studies (Phillips and Arnold 1999). CPC is a hierarchical series of tests that evaluates whether two or more matrices share one, two or more principal components in common or whether they are directly proportional or equal (Phillips and Arnold 1999). Each hypothesis is evaluated using a standard χ^2 analysis using a critical α value of 0.05 (Phillips and Arnold 1999). To examine differences in trait integration across environments we compared **G** matrices in a pair-wise manner using the jump-up approach outlined in Phillips and Arnold (1999) (freely available at http://darkwing.uoregon.edu/~pphil/software.html). A potential criticism of this approach is that it includes all eigenvectors of the two matrices being compared, so that any similarity between matrices may be driven by a similarity between principal components that are characterised by small eigenvalues in one matrix and large eigenvalues in the other (Blows et al. 2004). Consequently, it has been argued that CPC may provide an overly conservative approach when comparing **G** matrices (e.g. Pigliucci and Kolodynska 2002b; Kolodynska and Pigliucci 2003). Therefore, in addition to CPC we also compared **G** in each of the environments using the geometric approach of Krzanowski (1979).

Krzanowski's (1979) method compares two *k*-dimensional subspaces by calculating the angles between the best-matched pairs of orthogonal axes. Let the subsets of matrices 1 and 2 be represented by **A** and **B**, respectively. To compare the similarity of matrices, we first normalised the eigenvalues of **A** and **B** by dividing the coefficients of each eigenvector by the square root of the sums of squares of the coefficients of the respective eigenvector. The two sets of principal components were then be compared by constructing a matrix **S** according to the following:

$$S = A^T B B^T A$$

The matrix **S** finds the minimum angles between an arbitrary set of orthogonal vectors in the subspace of **A** and a set of orthogonal vectors closest to the same directions in the subspace of **B**. These arbitrary vectors are termed the principal vectors in the subspace of **A** and **B**. The eigenvalues of **S** can then be used to determine the similarity between the two subspaces. The smallest angle between any pair of orthogonal axes of **A** and **B** is then defined as $\cos^{-1}\sqrt{\lambda_1}$, where λ_1 is the largest eigenvalue of **S**.

A key property of the matrix **S** is that the sum of the eigenvalues of **S** equals the sum of squares of the cosines of the angles between the two sets of orthogonal axes. This sum must lie in the range of 0 to *k*, as all eigenvalues will have values between 0 and 1. This equates to an angle between 0° and 90°, respectively. If the sum is close to 0, the two subspaces are dissimilar and are orthogonal, while a sum equal to *k* would suggest that the two original matrices share a common orientation. It is important to note, however, that *k* must not equal *n* in this analysis, because including more than half of the *n* principal components will constrain the analysis to recover common dimensions (i.e. angles of 0°). In our matrix comparisons, we include only the first half of the principal components ($n = 6$), which explain over 99% of the variation contained in each of our four **G** matrices (range from 99.979 to 99.989%).

Results

Of the original 520 plants included in our design, 373 (72%) survived to 21 weeks of age. Although there was strong environment-specific mortality, with significantly more plants dying in the LW:LL treatment compared to the other three treatments (% mortality: HW:HL = 15.38%, HW:LL = 21.54%, LW:HL = 18.46%, LW:LL = 57.69%; $\chi^2 = 276.81$, $df = 3$, $P = 0.0001$), mortality in each of the environmental treatments did not significantly differ across genotypes ($\chi^2 = 7.92$, $df = 36$, $P = 0.99$).

Plasticity in response to light and water availability

Lepidium bonariense displayed considerable variation in phenotype in response to light and water availability. The phenotype of plants grown in the different light and water environments differed in two-dimensional space (Fig. 1). Extraction of the first two principal components retained 85.33% of the total variation in our data set (Table 1). The first principal component (PC1) was positively correlated with all phenotypic traits, with the exception of SLA, that had a significant negative factor loading (Table 1). Positive PC1 scores represents larger plants with reduced SLA while negative PC1 scores represent smaller plants with increased SLA. Generalised linear mixed-model (GLMM) indicated a large effect of both environments on the magnitude of this negative genetic correlation

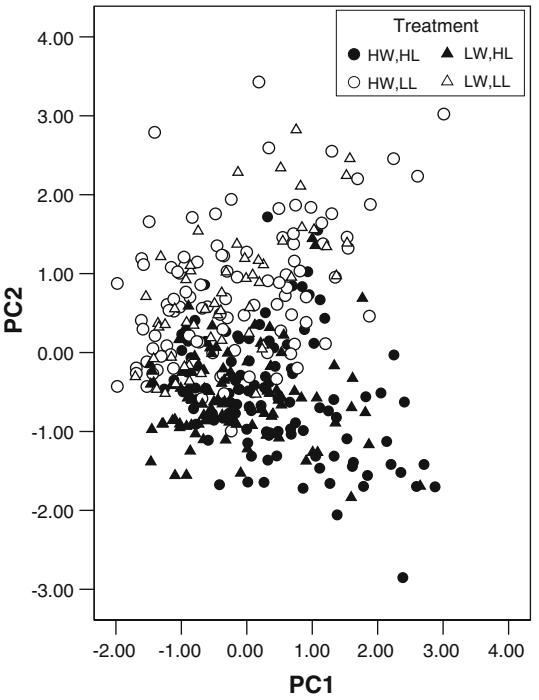

Fig. 1 Principal component analysis of *Lepidium bonariense* grown under our four different light and water treatment combinations: high water, high light (HW, HL = *closed circles*), high water, low light (HW, LL = *open circles*), low water, high light (LW, HL = *closed triangle*) and low water, low light (LW, LL = *open triangle*). The first and second principal component scores are derived from the measurement of twelve functional traits on each plant (see "Methods") at 21 weeks of age

Table 1 Rotated factor loadings of the first two principal components of phenotypic traits in *Lepidium bonariense* reared under different light and water availability

	PC 1	PC 2
Eigen-values	9.06	1.18
% of variance explained	75.50	9.83
Factor loadings		
Petiole length (mm)	**0.81**	**0.53**
Petiole diameter (mm)	**0.81**	0.06
Leaf length (mm)	**0.88**	**0.45**
Leaf width (mm)	**0.92**	0.27
Stem diameter (mm)	**0.92**	−0.23
Leaf area (cm^2)	**0.96**	0.04
Height (mm)	**0.85**	0.23
Leaf number	**0.74**	**−0.46**
Leaf mass (g)	**0.98**	−0.08
Root mass (g)	**0.91**	−0.27
Total plant mass (g)	**0.95**	−0.21
SLA (cm^2/g)	**−0.63**	**0.43**

Factor loadings greater than 0.4 are interpreted as biologically significant (Stevens 1992) and are presented in bold

(Table 2). On average, plants grown in the high light environments were relatively larger in size and had a reduced SLA relative to those grown in the low light environments (mean ± SE PC1 score: HL = 0.15 ± 0.07; LL = −0.20 ± 0.08) (Fig. 2; Table 2). Similarly, plants grown in the high water environments were relatively larger in size and had a reduced SLA relative to those grown in the low water environments (mean ± SE PC1

Table 2 Generalised linear mixed-model (GLMM) of phenotypic variation (summarised as principal component 1) in *Lepidium bonariense* genotypes in response to variation in water and light availability

Source	Principal component 1			Principal component 2		
	df	*F* ratio	*P* value	*df*	*F* ratio	*P* value
Genotype (G)	12,0.27	8.27	0.61	12,0.79	8.78	0.32
Water (W)	1,12.76	5.19	0.04	1,13.89	7.68	0.02
Light (L)	1,12.54	45.51	0.0001	1,12.45	125.23	0.0001
W × L	1,12.56	0.77	0.40	1,12.84	3.78	0.07
G × W	12,12.00	0.67	0.75	12,12.01	0.41	0.93
G × L	12,12.01	0.53	0.86	12,12.01	0.96	0.52
G × W × L	12,322	2.40	0.006	12,322	1.87	0.04

score: HW = 0.15 ± 0.07; LW = −0.19 ± 0.07) (Fig. 2; Table 2). Interestingly, the interaction between water and light availability on the negative genetic correlation between SLA and plant size was not significant (Table 2). Although there was no effect of genotype per se on plant phenotype or the way that specific genotypes responded to either light or water availability in isolation, there was a significant genotype by environment response when both environmental factors were examined together (Fig. 2; Table 2).

The second principal component (PC2) was positively correlated with petiole length, leaf length and SLA and negatively correlated with leaf number (Table 1). GLMM

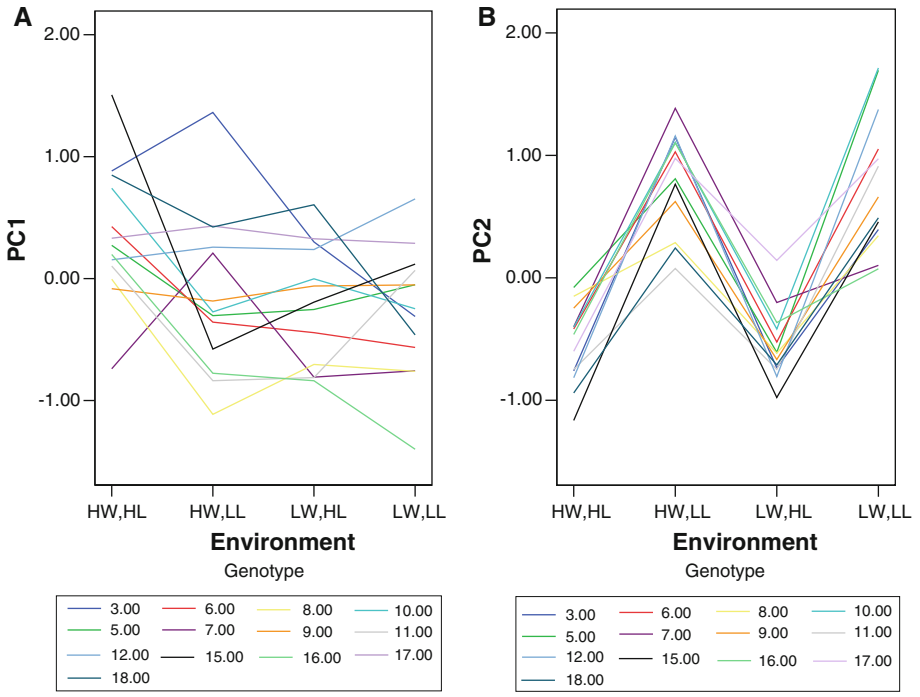

Fig. 2 Multivariate reaction norms plotting **a** principal component 1 scores and **b** principal component 2 scores for each of thirteen *Lepidium bonariense* genotypes in each environment. The multivariate reaction norm for each genotype is represented by a unique colour

revealed a large and significant effect of both environments on PC2 (Table 2). On average, plants grown in the low light environments had longer leaves and petioles, an increased SLA and a reduced number of leaves relative to those grown in the high light environment (LL = 0.77 ± 0.07; HL = −0.56 ± 0.05) (Fig. 2; Table 2). Similarly, on average, plants grown in the high water environments had longer leaves and petioles, an increased SLA and a reduced number of leaves compared to those grown in the low water environments (LW = −0.11 ± 0.07; HW = 0.08 ± 0.07) (Fig. 2; Table 2). Again, there was no overall effect of genotype per se on plant phenotype nor did specific genotypes differ in their response to either light or water availability in isolation. However, there were significant genotype-specific responses when both environmental factors were examined in combination (Fig. 2; Table 2).

The plasticity of phenotypic integration in response to light and water availability

Genetic correlations for the two high water (HW,HL and HW,LL) and low water (LW,HL and LW,LL) treatments, along with jacknifed standard errors, are provided in Tables 3 and 4, respectively. With the exception of SLA which is negatively genetically correlated with all other traits, all four correlation matrices are characterised by strong positive genetic correlations between phenotypic traits (Tables 3 and 4). Although the mean size of phenotypic traits varied across our four environmental treatments, common principal component (CPC) analysis demonstrated that the structure of the genetic variance-covariance matrix (**G**) did not change across our environmental treatments (i.e. the eigenvectors of **G** are equal and proportional across environmental treatments) (Table 5). Qualitatively similar findings were attained using the less conservative methodology of Krzanowski (1979), with consistently high sums of eigenvalues and small angles between the dominant eigenvectors in each pair-wise matrix comparison (Table 5).

Discussion

Organisms living in a heterogeneous environment encounter a number of challenges. On one hand, they require the flexibility to respond to environmental conditions and change their phenotype accordingly (Pigliucci 2001; DeWitt and Scheiner 2004). On the other, they are required to be robust in their overall body plan to ensure an integrated, functional organism (Pigliucci and Preston 2004). Here, we show in the peppergrass, *Lepidium bonariense*, that these two processes (i.e. phenotypic plasticity and integration) do not have to be mutually exclusive. Although *L. bonariense* genotypes exhibit considerable plasticity in response to water and light availability, we show that the pattern of covariation amongst functional traits did not differ across environments. Thus, the complex phenotype of *L. bonariense* responds to environmental change in a tightly integrated manner.

The plasticity of complex phenotypes in a heterogeneous environment

Plant functional traits in *L. bonariense* responded to water and light availability in a manner that is largely predictable based on physiological processes. We showed that PC1 largely reflects the negative genetic correlation between overall plant size and specific-leaf area (SLA). PC1 scores were more negative (i.e. relatively smaller plants but larger SLA) in the low water and light environments. Generally, in low water environments plants show responses that include a reduction in all above ground and below ground biomass

Table 3 Genetic correlation matrices for the two high water treatments

	PL	PD	LL	LW	SD	LA	Height	LN	LM	RM	TM	SLA
PL		0.84	**0.99**	**0.96**	**0.89**	**0.95**	**0.92**	0.60	**0.93**	**0.87**	**0.89**	**−0.62**
		(0.50)	**(0.41)**	**(0.39)**	**(0.42)**	**(0.30)**	**(0.31)**	(0.42)	**(0.26)**	**(0.23)**	**(0.24)**	**(0.29)**
PD	**0.62**		0.85	**0.87**	**0.84**	**0.80**	0.74	0.51	0.71	0.67	0.68	−0.55
	(0.22)		(0.59)	**(0.43)**	**(0.38)**	**(0.40)**	(0.42)	(0.40)	(0.75)	(0.54)	(0.65)	(0.38)
LL	**0.99**	**0.65**		**0.98**	**0.93**	**0.97**	**0.93**	0.66	**0.95**	**0.90**	**0.92**	**−0.67**
	(0.43)	**(0.22)**		**(0.34)**	**(0.40)**	**(0.27)**	**(0.40)**	(0.43)	**(0.36)**	**(0.27)**	**(0.30)**	**(0.30)**
LW	**0.86**	**0.66**	**0.89**		**0.95**	**0.97**	**0.92**	**0.69**	**0.94**	**0.89**	0.92	**−0.69**
	(0.37)	**(0.30)**	**(0.32)**		**(0.37)**	**(0.35)**	**(0.48)**	**(0.41)**	**(0.61)**	**(0.34)**	(0.45)	**(0.31)**
SD	**0.80**	**0.65**	**0.80**	0.64		**0.95**	**0.93**	**0.81**	**0.94**	**0.93**	**0.95**	**−0.84**
	(0.37)	**(0.26)**	**(0.37)**	(0.37)		**(0.41)**	**(0.34)**	**(0.43)**	**(0.54)**	**(0.41)**	**(0.58)**	**(0.26)**
LA	**0.88**	**0.74**	**0.90**	**0.88**	**0.87**		**0.93**	**0.80**	**0.98**	**0.91**	**0.95**	**−0.69**
	(0.30)	**(0.24)**	**(0.26)**	**(0.42)**	**(0.38)**		**(0.30)**	**(0.45)**	**(0.42)**	**(0.32)**	**(0.37)**	**(0.31)**
Height	**0.73**	**0.73**	**0.76**	**0.63**	**0.90**	**0.86**		**0.74**	**0.95**	**0.96**	**0.96**	**−0.75**
	(0.24)	**(0.22)**	**(0.21)**	**(0.27)**	**(0.28)**	**(0.39)**		**(0.42)**	**(0.24)**	**(0.27)**	**(0.23)**	**(0.28)**
LN	**0.75**	0.58	**0.78**	0.65	**0.85**	**0.88**	**0.84**		**0.80**	**0.78**	0.81	**−0.73**
	(0.25)	(0.31)	**(0.23)**	(0.36)	**(0.45)**	**(0.52)**	**(0.46)**		**(0.53)**	**(0.46)**	(0.51)	**(0.37)**
LM	**0.89**	**0.73**	**0.91**	**0.85**	**0.89**	**0.99**	**0.90**	**0.89**		**0.96**	**0.99**	**−0.77**
	(0.37)	**(0.22)**	**(0.36)**	**(0.38)**	**(0.38)**	**(0.43)**	**(0.43)**	**(0.46)**		**(0.22)**	**(0.29)**	**(0.29)**
RM	**0.76**	**0.71**	**0.79**	**0.69**	**0.95**	**0.90**	**0.97**	**0.87**	**0.92**		**0.99**	**−0.83**
	(0.25)	**(0.24)**	**(0.22)**	**(0.25)**	**(0.24)**	**(0.41)**	**(0.39)**	**(0.69)**	**(0.47)**		**(0.16)**	**(0.30)**
TM	**0.81**	**0.72**	**0.84**	**0.75**	**0.94**	**0.94**	**0.96**	**0.90**	**0.96**	**0.99**		**−0.81**
	(0.27)	**(0.22)**	**(0.24)**	**(0.27)**	**(0.27)**	**(0.37)**	**(0.46)**	**(0.51)**	**(0.44)**	**(0.56)**		**(0.29)**
SLA	**−0.87**	−0.39	**−0.86**	**−0.71**	**−0.77**	**−0.78**	**−0.75**	**−0.78**	**−0.82**	**−0.75**	**−0.79**	
	(0.37)	(0.26)	**(0.38)**	**(0.31)**	**(0.44)**	**(0.35)**	**(0.30)**	**(0.40)**	**(0.33)**	**(0.31)**	**(0.29)**	

The high light treatment (HW, HL) is presented below the diagonal and the low light treatment (HW, LL) is presented above the diagonal. Jacknifed standard errors are provided in parenthesis. Values provided in bold are significant at $\alpha = 0.05$

PL petiole length, *PD* petiole diameter, *LL* leaf length, *LW* leaf width, *SD* shoot diameter, *LA* leaf area, *Height* plant height, *LN* leaf number, *LM* leaf mass, *RM* root mass, *TM* total mass, *SLA* specific leaf area

components, leaf dimensions, the number of leaves produced, stem diameter and absolute height (Preston and Ackerly 2003). Moreover, arid conditions typically increase the risk of cavitation (i.e. breakage in the hydraulic pathway) and plants frequently adapt by reducing SLA and stem diameter to minimise water loss (Reich et al. 1997). Contrary to this, however, we found that plants in a low water environment showed an increase in SLA. The benefits of relatively high SLA (and high leaf productivity) declines with increasing plant size (Bonser 2006). The observed SLA differences in high and low water treatments could simply be due to differences in plant size across treatments (larger plants in high water treatments). Considerably more research, however, is required to understand exactly how water availability influences the physiological basis of this negative genetic correlation in plants.

In contrast to PC1, PC2 reveals finer detail about how *L. bonariense* adjusts its phenotype in response to varying light availability. The ability to detect reduced light

Table 4 Genetic correlation matrices for the two low water treatments

	PL	PD	LL	LW	SD	LA	Height	LN	LM	RM	TM	SLA
PL		0.61	**0.99**	**0.96**	**0.87**	**0.95**	**0.86**	0.39	**0.93**	**0.76**	**0.87**	−0.55
		(0.39)	**(0.30)**	**(0.28)**	**(0.38)**	**(0.35)**	**(0.44)**	(0.33)	**(0.60)**	**(0.39)**	**(0.44)**	(0.38)
PD	0.55		0.60	0.59	**0.79**	0.55	0.68	**0.72**	0.61	0.70	0.66	−0.68
	(0.39)		(0.38)	(0.34)	**(0.31)**	(0.38)	(0.38)	**(0.28)**	(0.37)	(0.39)	(0.41)	(0.39)
LL	**0.99**	0.59		**0.97**	**0.86**	**0.96**	**0.85**	0.39	**0.93**	**0.75**	**0.86**	−0.53
	(0.11)	(0.41)		**(0.26)**	**(0.43)**	**(0.39)**	**(0.42)**	(0.33)	**(0.43)**	**(0.40)**	**(0.45)**	(0.39)
LW	**0.87**	**0.68**	**0.92**		**0.76**	**0.94**	**0.78**	0.28	**0.87**	0.65	**0.79**	−0.42
	(0.33)	**(0.32)**	**(0.37)**		**(0.39)**	**(0.40)**	**(0.40)**	(0.33)	**(0.50)**	(0.41)	**(0.42)**	(0.41)
SD	0.81	0.50	0.86	**0.87**		**0.84**	**0.91**	0.47	**0.91**	**0.92**	**0.94**	**−0.78**
	(0.56)	(0.43)	(0.54)	**(0.30)**		**(0.38)**	**(0.38)**	(0.30)	**(0.31)**	**(0.26)**	**(0.34)**	**(0.27)**
LA	0.80	0.53	0.86	**0.94**	**0.87**		**0.86**	0.56	**0.97**	**0.79**	**0.91**	−0.49
	(0.55)	(0.42)	(0.61)	**(0.36)**	**(0.41)**		**(0.30)**	(0.32)	**(0.35)**	**(0.39)**	**(0.40)**	(0.40)
Height	**0.83**	0.59	**0.86**	0.83	**0.87**	0.78		0.48	**0.90**	**0.93**	**0.94**	**−0.70**
	(0.25)	(0.33)	**(0.29)**	(0.53)	**(0.20)**	(0.47)		(0.33)	**(0.46)**	**(0.33)**	**(0.47)**	**(0.36)**
LN	0.55	**0.73**	0.59	**0.58**	**0.75**	**0.74**	**0.60**		**0.63**	**0.61**	**0.65**	−0.31
	(0.53)	**(0.33)**	(0.46)	**(0.21)**	**(0.27)**	**(0.20)**	**(0.24)**		**(0.31)**	**(0.30)**	**(0.33)**	(0.31)
LM	**0.85**	0.52	**0.90**	**0.93**	**0.92**	**0.99**	**0.85**	**0.75**		**0.89**	**0.97**	−0.65
	(0.41)	(0.47)	**(0.47)**	**(0.44)**	**(0.47)**	**(0.29)**	**(0.43)**	**(0.23)**		**(0.34)**	**(0.35)**	(0.39)
RM	**0.84**	0.49	**0.89**	**0.91**	**0.96**	**0.92**	**0.93**	**0.78**	**0.95**		**0.97**	**−0.80**
	(0.37)	(0.39)	**(0.38)**	**(0.33)**	**(0.45)**	**(0.41)**	**(0.22)**	**(0.23)**	**(0.36)**		**(0.32)**	**(0.41)**
TM	**0.85**	0.50	**0.90**	**0.92**	**0.95**	**0.95**	**0.91**	**0.78**	**0.97**	**0.99**		**−0.74**
	(0.42)	(0.42)	**(0.47)**	**(0.31)**	**(0.45)**	**(0.43)**	**(0.29)**	**(0.24)**	**(0.34)**	**(0.40)**		**(0.45)**
SLA	**−0.78**	−0.38	**−0.79**	−0.65	**−0.80**	**−0.61**	**−0.84**	−0.63	**−0.72**	**−0.83**	**−0.80**	
	(0.32)	(0.42)	**(0.35)**	(0.36)	**(0.35)**	**(0.31)**	**(0.25)**	(0.37)	**(0.37)**	**(0.24)**	**(0.27)**	

The high light treatment (LW, HL) is presented below the diagonal and the low light treatment (LW,LL) is presented above the diagonal. Jacknifed standard errors are provided in parenthesis. Values provided in bold are significant at $\alpha = 0.05$. Trait abbreviations as outlined in Table 3

availability, particularly in response to shading by neighbours, appears common in many species of terrestrial plants (Casal et al. 1994; Ballaré et al. 1995, 1997; Smith 1982; Smith and Whitelam 1997). Shaded plants experience a reduced red to far-red light ratio compared with individuals exposed to full sun (Smith 1982; van Hinsberg and van Tienderen 1997). This cue for light competition triggers a cascade of phenotypic responses including an increase in functional traits such as petiole length, leaf length and width, SLA and height and a decrease in traits such as stem diameter, root mass and the number of leaves produced (Casal et al. 1994; Ballaré et al. 1995, 1997; Smith 1982; Smith and Whitelam 1997). The contribution of each plant trait to PC2 (see Table 1) suggests that the response shown by *L. bonariense* to a low light environment is largely consistent with this typical shade avoidance syndrome. This syndrome is defined by an elongate phenotype that enables individuals to counter the effects of shading before it seriously reduces photosynthesis (Ballaré et al. 1997; Smith and Whitelam 1997; Dorn et al. 2000). The shade avoidance phenotype exhibited by *L. bonariense* is also consistent with the much broader guerrilla strategy adopted by plants foraging under resource limitation, facilitating the

Table 5 Statistical comparison of the genetic variance–covariance matrices (**G**) in each of the four environmental treatments

Comparison	CPC				Krzanowski (1979)	
	Accepted hypothesis	χ^2	*df*	*P*-value	Sum of eigenvalues	Angle
HW,HL vs. HW,LL	Equality	48.09	78	1.0	5.30	0.51°
HW,HL vs. LW, HL	Equality	25.53	78	1.0	5.17	0.32°
HW,HL vs. LW,LL	Equality	59.87	78	1.0	5.19	1.05°
HW,LL vs. LW,HL	Equality	33.98	78	1.0	5.12	0.02°
HW,LL vs. LW,LL	Equality	30.35	78	1.0	4.95	0.11°
LW,HL vs. LW,LL	Equality	42.97	78	1.0	5.70	0.04°

Common principle component (CPC) analysis is based on Flury's hierarchy (jump-up approach) (Phillips and Arnold 1999). The highest statistically acceptable hypothesis in the hierarchy is specified, along with the chi-square value, degrees of freedom and *P*-values associated with the rejection of the next hypothesis in the hierarchy. For each comparison, the matrices were tested against an unrelated structure (Phillips and Arnold 1999). In all cases, the highest level in the hierarchy (i.e. a test for equality) was rejected meaning that all matrices were equal and proportional. Krzanowski's (1979) methodology is geometric approach that compares two *k*-dimensional subspaces by calculating the angles between the best-matched pairs of orthogonal axes. The closer the sum of the eigenvalues is to *k* (i.e. in our case 6), the more similar are the matrices (Krzanowski 1979). The angle between the dominant orthogonal axes in the two matrices being compared was calculated as $\cos^{-1}\sqrt{\lambda_1}$, where λ_1 is the largest eigenvalue of the matrix **S**

rapid and directional expansion of individuals from a poor to a better quality habitat (de Kroon and Hutchings 1995; Fischer and van Kleunen 2002).

Our finding that the multivariate phenotypic response (PC1 and PC2) of specific genotypes was plastic across water and light environments is not, in itself, a particularly surprising outcome. This is because most traits in the majority of species are likely to show some degree of plasticity (Pigliucci 2004). Indeed, phenotypic plasticity in response to a wide variety of abiotic (e.g. *light*: Pigliucci and Kolodynska 2002a; *water*: Pigliucci and Kolodynska 2002b; *nutrients*: Pigliucci and Schlichting 1998; *temperature*: Collins et al. 2002) and biotic environmental factors (e.g. *herbivory*: Agrawal 2004; *competition*: Cahill et al. 2005; *touch*: Pigliucci 2002) appears particularly common in plants. What is of particular interest, however, is the fact that *L. bonariense* genotypes differed in their plasticity to the combined effect of light and water availability (i.e. Genotype × Light × Water). Several recent studies on plants (e.g. Agrawal and Van Zandt 2003; Agrawal 2004; Huber et al. 2004; Cahill et al. 2005; McGuire and Agrawal 2005) and animals (e.g. Kristan and Hammond 2000; Alvarex and Nicieza 2002; Relyea and Mills 2001; Boone and Semlitsch 2001, 2002) suggest that this phenotypic response to multiple environmental factors may, in fact, be more commonplace than is currently appreciated. For example, in a recent manipulative experiment examining the interplay between competition with grass and beetle herbivory in the milkweed (*Asclepias syriaca*), Agrawal (2004) demonstrated that while neither factor had an individual impact, they jointly reduced the growth of milkweed plants. While our laboratory greenhouse study only addresses a small part of the environmental complexity that exists in nature (Sih et al. 2004), it illustrates the need to consider any interactions that may exist between multiple environmental factors and the way that different genotypes respond to them. Such interactions should be incorporated when trying to understand the evolution of phenotypic plasticity in organisms that occupy a heterogeneous environment.

The plasticity of phenotypic integration

It is generally accepted that environmental conditions can influence the patterns of genetic covariance between traits, as well as levels of genetic variance in the individual traits themselves (Pigliucci 2004; Sgrò and Hoffmann 2004). Here, we show that despite exhibiting considerable phenotypic plasticity, the patterns of covariation between functional traits in *L. bonariense* did not differ across environments. The published literature on plants is very much equivocal with regard to how trait means and covariances should respond to different environmental conditions. Some species show a change in mean trait size and covariances across environments (Lechowicz and Blais 1988; Schlichting 1989; Pigliucci et al. 1997; Pigliucci and Schlichting 1998; Callahan and Waller 2000; Pigliucci and Hayden 2001; Pigliucci 2002; Tonsor and Scheiner 2007; Nicotra et al. 2007; Brock and Weinig 2007), while others only exhibit a change in mean trait size with the covariance structure remaining constant (Waitt and Levin 1993; Herrera et al. 2002; Pigliucci and Kolodynska 2002a, b; Kolodynska and Pigliucci 2003; Bidart-Bouzat et al. 2004; Bossdorf and Pigliucci 2009). It is likely that the relationship between plasticity and trait integration will be species-specific making generalizations on this relationship difficult, if not impossible.

The predictive value of the genetic variance-covariance matrix (**G**), however, largely depends on whether it remains constant over evolutionary time and space and/or if it evolves, does it do so in a predictable manner (Steppan et al. 2002). Traditionally, high levels of trait integration within and between environments, such as demonstrated here for *L. bonariense*, have been viewed as major constraints to the rate of adaptive evolution (Arnold 1992; Wagner et al. 1997; Merilä and Björklund 2004). However, the structure of **G** alone does not provide definitive evidence that integration will constrain or facilitate evolution in alternate environments (Sgrò and Hoffmann 2004). The response of a suite of genetically correlated traits to selection is the product of **G** combined with the vector of linear selection gradients (β) and the matrix of nonlinear selection gradients (γ) (Lande and Arnold 1983). Schluter (1996) illustrated the effect of **G** on the multivariate response to selection using the concept of a genetic line of least resistance ($\mathbf{g_{max}}$). This vector, quantified as the first principal component through **G**, describes the direction in multivariate space in which the most additive genetic (co)variance exists (Schluter 1996). A given amount of selection will result in the greatest evolutionary response when $\mathbf{g_{max}}$ is aligned with the dominant vector of selection (Schluter 1996). The closer this angle is to 90°, however, the greater the response to selection will be constrained in magnitude and potentially biased in its direction (Schluter 1996). Thus, knowledge of both multivariate selection and **G** is central to our understanding of the adaptive significance of phenotypic integration (Lande and Arnold 1983).

Although a number of studies have addressed the fitness consequences of phenotypic plasticity in alternate environments (Kingsolver 1995; Pigliucci and Schichting 1996; Dorn et al. 2000; Agrawal et al. 2002; Relyea and Auld 2005; Wolf and Mazer 2005; Kraft et al. 2005), quantifying the selection that operates on phenotypic integration is an inherently more difficult task. This is because natural selection is expected to remove unfavourable trait combinations over evolutionary time (Lande and Arnold 1983). This inevitably reduces trait variances and lowers the power of formal selection analyses (Lande and Arnold 1983). A powerful, yet under-utilised, approach to addressing this question is the phenotypic manipulation of trait covariances (Dudley and Schmitt 1996; Sinervo and Basolo 1996; Schmitt et al. 1999; Brooks et al. 2005). While such an approach may not be conducive to all species being studied, its application to an appropriate range of

ecologically relevant environments may provide insight into the selective pressure shaping patterns of integration in complex phenotypes (Sinervo and Basolo 1996).

Conclusion

In conclusion, our work shows that the common peppergrass, *Lepidium bonariense*, exhibits considerable plasticity in its multivariate phenotypic response to light and water availability. Importantly though, genotypes differed in plasticity to the combined effect of light and water availability. This finding suggests that laboratory studies that only manipulate a single environmental factor are likely to underestimate the degree of plasticity. Moreover, it suggests that plasticity is likely to be far greater in field where environmental factors are more complex and typically vary in multiple dimensions. Despite finding considerable plasticity in this species, light and water availability did not alter the degree of phenotypic integration. Thus, although these environmental factors influenced the mean size of phenotypic traits, the covariance structure remained the same. This suggests that the relationship between traits remains stable across environments to maintain their functional integrity but further knowledge on how selection operates on these traits in different environments is needed before we can ascertain whether this will drive or constrain phenotypic evolution.

Acknowledgments We thank Mark Blows for bringing the methodology of Krzanowski's (1979) matrix comparison to our attention and Rob Brooks and David Hosken for helpful comments and discussions that improved this manuscript. KLM was supported by an Australian Postgraduate Award, SPB was supported by a UNSW faculty research grant and JH was supported by NERC and a University Royal Society Fellowship.

References

Agrawal AA (2004) Resistance and susceptibility of milkweed: competition, root herbivory, and plant genetic variation. Ecology 85:2118–2133

Agrawal AA, Van Zandt PA (2003) Ecological play in the coevolutionary theatre: genetic and environmental determinants of attack by a specialist weevil on milkweed. J Ecol 91:1049–1059

Agrawal AA, Conner JK, Johnson MTT, Wallsgrove R (2002) Ecological genetics of an induced plant defense against herbivores: additive genetic variance and costs of phenotypic plasticity. Evolution 56:2206–2213

Alvarex D, Nicieza AG (2002) Effects of induced variation in anuran larval development on postmetamorphic energy reserves and locomotion. Oecologia 131:186–195

Armbruser WS, Schwaegerle KE (1996) Causes of covariation of phenotypic traits among populations. J Evol Biol 9:261–276

Arnold SJ (1992) Constraints on phenotypic evolution. Am Nat 140:S85–S107

Ballaré CL, Scopel AL, Roush ML, Radosevic SR (1995) How plants find light in patchy canopies. A comparison between wild-type and phytochrome-b-deficient mutant plants of cucumber. Funct Ecol 9:859–868

Ballaré CL, Scopel AL, Sanchez RA (1997) Foraging for light: photosensory ecology and agricultural implications. Plant Cell Environ 20:820–825

Bidart-Bouzat MG, Portnoy S, DeLucia EH, Paige KN (2004) Elevated CO_2 and herbivory influence trait integration in *Arabidopsis thaliana*. Ecol Lett 7:837–847

Blows MW, Chenoweth SF, Hine E (2004) Orientation of the genetic variance-covariance matrix and the fitness surface for multiple male sexually selected traits. Am Nat 163:329–340

Bonser SP (2006) Form defining function: interpreting leaf functional variability in integrated plant phenotypes. Oikos 114:187–190

Boone MD, Semlitsch RD (2001) Interactions of an insecticide with larval density and predation in experimental amphibian communities. Conserv Biol 15:228–238
Boone MD, Semlitsch RD (2002) Interactions of an insecticide with competition and pond drying in amphibian communities. Ecol Appl 12:307–316
Bossdorf O, Pigliucci M (2009) Plasticity to wind is modular and genetically variable in *Arabidopsis thaliana*. Evol Ecol 23:669–685
Bowerman BL, O'Connell RT (1990) Linear statistical models: an applied approach. Belmont, California
Bradshaw AD (1965) Evolutionary significance of phenotypic plasticity in plants. Adv Genet 13:115–155
Brock MT, Weinig C (2007) Plasticity and environment-specific covariances: an investigation of floral-vegetative and within flower correlations. Evolution 61:2913–2924
Brooks R, Hunt J, Blows MW, Smith MJ, Bussiere LF, Jennions MD (2005) Experimental evidence for multivariate stabilizing sexual selection. Evolution 59:871–880
Cahill JF, Kembel SW, Gustafson DJ (2005) Differential genetic influences on competitive effect and response in *Arabidopsis thaliana*. J Ecol 93:958–967
Callahan HS, Waller DM (2000) Phenotypic integration and the plasticity of integration in an amphicarpic annual. Int J Plant Sci 161:89–98
Casal JJ, Ballaré CL, Tourn M, Sanchez RZ (1994) Anatomy, growth and survival of a long hypocotyl mutant of *Cucumis sativus* deficient in phytochrome B. Ann Bot 73:569–575
Cheverud JM (1982) Phenotypic, genetic and environmental morphological integration in the cranium. Evolution 36:499–516
Cheverud JM (1984) Quantitative genetics and developmental constraints on evolution by selection. J Theor Biol 110:155–171
Cheverud JM (1996) Developmental integration and the evolution of pleiotropy. Am Zoologist 36:44–50
Cohn RD (1999) Comparisons of multivariate relational structures in serially correlated data. J Agri Biol Environ Stat 4:238–257
Collins RP, Helgadottir A, Fothergill M, Rhodes I (2002) Variation amongst survivor populations of white clover collected from sites across Europe: growth attributes and physiological responses to low temperature. Ann Bot 89:283–292
De Kroon H, Hutchings MJ (1995) Morphological plasticity in clonal plants—the foraging concept reconsidered. J Ecol 83:143–152
DeWitt TJ, Scheiner SM (2004) Phenotypic plasticity: functional and conceptual approaches. Oxford University Press, Oxford
Dorn LA, Pyle EH, Schmitt J (2000) Plasticity to light cues and resources in *Arabidopsis thaliana*: testing for adaptive value and costs. Evolution 54:1982–1994
Dudley SA, Schmitt J (1996) Testing the adaptive plasticity hypothesis: density-dependent selection on manipulated stem length in *Impatiens capensis*. Am Nat 147:445–465
Dunteman GE (1989) Principal components analysis. SAGE Publishing, California
Field A (2000) Discovering statistics using SPSS for windows. SAGE publications, London
Fischer M, van Kleunen M (2002) On the evolution of clonal plant life histories. Evol Ecol 15:565–582
Harden GJ (2000) Flora of New South Wales, vol 1. The University of New South Wales Press, Sydney
Herrera CM, Cerda X, Garcia MB, Guitian J, Medrano M, Rey PJ, Sanchez-Lafuente AM (2002) Floral integration, phenotypic covariance structure and pollinator variation in bumblebee-pollinated *Helleborus foetidus*. J Evol Biol 15:108–121
Huber H, Kane NC, Heschel MS, von Wettberg EJ, Banta J, Leuck AM, Schmitt J (2004) Frequency and microenvironmental pattern of selection on plastic shade-avoidance traits in a natural population of *Impatiens capensis*. Am Nat 163:548–563
Kingsolver JG (1995) Fitness consequences of seasonal polyphenism in western white butterflies. Evolution 49:942–954
Kolodynska A, Pigliucci M (2003) Multivariate response to flooding in *Arabidopsis*: an experimental evolutionary investigation. Funct Ecol 17:131–140
Kraft PG, Franklin CE, Blows MW (2005) Predator-induced phenotypic plasticity I tadpoles: extension or innovation? J Evol Biol 19:450–458
Kristan DM, Hammond KA (2000) Combined effects of cold exposure and sub-lethal intestinal parasites on host morphology and physiology. J Exp Biol 203:3495–3504
Krzanowski WJ (1979) Between-groups comparison of principal components. J Am Stat Assoc 75:703–707
Lande R (1979) Quantitative genetic analysis of multivariate evolution, applied to brain:body size allometry. Evolution 33:402–416
Lande R (1980) The genetic covariance between characters maintained by pleiotropic mutations. Genetics 94:203–215

Lande R (1984) The genetic correlation between characters maintained by selection, linkage, and inbreeding. Genet Res 44:309–320
Lande R, Arnold SJ (1983) The measurement of selection on correlated characters. Evolution 37:1210–1226
Lechowicz MJ, Blais PA (1988) Assessing the contributions of multiple interacting traits to plant reproductive success: environment dependence. J Evol Biol 1:255–273
Liu Y, Schieving F, Stuefer JF, Anten NPR (2007) The effects of mechanical stress and spectral shading on the growth and allocation of ten genotypes of a stoloniferous plant. Ann Bot 99:121–130
McGuire R, Agrawal AA (2005) Trade-offs between the shade-avoidance response and plant resistance to herbivores? Tests with mutant *Cucumis sativus*. Funct Ecol 19:1025–1031
Menard S (1995) Applied logistic regression analysis. SAGE Publishing, New York
Merilä J, Björklund M (2004) Phenotypic integration as a constraint and adaptation. In: Pigliucci M, Preston K (eds) Phenotypic integration: studying the ecology, evolution of complex phenotypes. Oxford University Press, New York, pp 107–129
Mommer L, Visser EJW (2005) Underwater photosynthesis in flooded terrestrial plants: a matter of leaf plasticity. Ann Bot 96:581–589
Monaco TA, Johnson DA, Creech JE (2005) Morphological and physiological responses of the invasive weed *Isatis tinctoria* to contrasting light, soil-nitrogen and water. Weed Res 45:460–466
Moran NA (1992) The evolutionary maintenance of alternative phenotypes. Am Nat 139:971–989
Nicotra AB, Hermes JP, Jones CS, Schlichting CD (2007) Geographic variation and plasticity to water and nutrients in *Pelargonium australe*. New Phytol 176:136–150
Noda H, Muraoka H, Washitani I (2004) Morphological and physiological acclimation response to contrasting light and water regimes in *Primula sieboldii*. Ecol Res 19:331–340
Phillips PC, Arnold SJ (1999) Hierarchical comparison of genetic variance–covariance matrices. I. Using the Flury hierarchy. Evolution 53:1506–1515
Pigliucci M (2001) Phenotypic plasticity: beyond nature and nurture. The Johns Hopkins University Press, London
Pigliucci M (2002) Touchy and bushy: phenotypic plasticity and integration in response to wind stimulation in *Arabidopsis thaliana*. Int J Plant Sci 163:399–408
Pigliucci M (2003) Phenotypic integration: studying the ecology and evolution of complex phenotypes. Ecol Lett 6:265–272
Pigliucci M (2004) Studying the plasticity of phenotypic integration in a model organism. In: Pigliucci M, Preston K (eds) Phenotypic integration: studying the ecology, evolution of complex phenotypes. Oxford University Press, New York, pp 107–129
Pigliucci M, Hayden K (2001) Phenotypic plasticity is the major determinant of changes in phenotypic integration in Arabidopsis. New Phytol 152:419–430
Pigliucci M, Kolodynska A (2002a) Phenotypic plasticity to light intensity in *Arabidopsis thaliania*: invariance of reaction norms and phenotypic integration. Evol Ecol 16:27–47
Pigliucci M, Kolodynska A (2002b) Phenotypic plasticity and integration in response to flooded conditions in natural accessions of *Arabidopsis thaliana* (L.) Heynh (Brassicaceae). Ann Bot 90:199–207
Pigliucci M, Preston K (2004) Phenotypic integration: studying the ecology and evolution of complex phenotypes. Oxford University Press, Oxford
Pigliucci M, Schichting CD (1996) Reaction norms of *Arabidopsis*. 4. Relationships between plasticity and fitness. Heredity 76:427–436
Pigliucci M, Schlichting CD (1998) Reaction norms of *Arabidopsis.* V. Flowering time controls phenotypic architecture in response to nutrient stress. J Evol Biol 11:285–301
Pigliucci M, Whitton J, Schlichting CD (1995) Reaction norms of *Arabidopsis*. I. Plasticity of characters and correlations across water, nutrient and light gradients. J Evol Biol 8:421–438
Pigliucci M, Diiorio P, Schlichting CD (1997) Phenotypic plasticity of growth trajectories in two species of *Lobelia* in response to nutrient availability. J Ecol 85:265–276
Preston KA, Ackerly DD (2003) Hydraulic architecture and the evolution of shoot allometry in contrasting climates. Am J Bot 90:1502–1512
Raff RA (1996) The shape of life: genes, development and the evolution of animal form. University of Chicago Press, Chicago
Rajakaruna N, Baldwin BG, Chan R, Desrochers AM, Bohm BA, Whilton J (2003) Edaphic races and polygenic taxa in the *Lastheria californica* complex (Asteracea Heliantheae): a hypothesis of parallel evolution. Mol Ecol 157:1675–1679
Reich PB, Walters MB, Ellsworth DS (1997) From tropics to tundra: global convergence in plant functioning. Proc Nat Acad Sci USA 94:13730–13734
Reich PB, Wright IJ, Cavender-Bares J, Craine JM, Oleksyn J, Westoby M, Walters MB (2003) The evolution of plant functional variation: traits, spectra and strategies. Int J Plant Sci 164:S143–S164

Relyea RA, Auld JR (2005) Predator and competitor-induced plasticity: how changes in foraging morphology affect phenotypic trade-offs. Ecology 86:1723–1729
Relyea RA, Mills N (2001) Predator-induced stress makes the pesticide carbaryl more deadly to gray treefrog tadpoles. (Hyla versicolor). Proc Nat Acad Sci USA 98:2491–2496
Roach DA, Wulff RD (1987) Maternal effects in plants. Ann Rev Ecol Syst 18:209–235
Scheiner SM (1993) Genetics and evolution of phenotypic plasticity. Ann Rev Ecol Syst 24:35–68
Schlichting CD (1989) Phenotypic integration and environmental change. Bioscience 39:460–464
Schlosser G, Wagner GP (2004) Modularity in development and evolution. The University of Chicago Press, Chicago
Schluter D (1996) Adaptive radiation along genetic lines of least resistance. Evolution 50:1766–1774
Schmitt J, Wulff RD (1993) Light spectral quality, phytochrome and plant competition. TREE 8:47–51
Schmitt J, Dudley SA, Pigliucci M (1999) Manipulative approaches to testing adaptive plasticity: phytochrome-mediated shade-avoidance responses in plants. Am Nat 154:S43–S54
Sgrò CM, Hoffmann AA (2004) Genetic correlations, tradeoffs and environmental variation. Heredity 93:241–248
Sih A, Bell AM, Kerby JL (2004) Two stressors are far deadlier than one. TREE 19:274–276
Sinervo B, Basolo A (1996) Testing adaptation using phenotypic manipulation. In: Rose MR, Lauter GV (eds) Adaptation. Academic Press, London, pp 149–185
Smith H (1982) Light quality, photoreception and plant strategy. Ann Rev Plant Physiol 33:481–518
Smith H, Whitelam GC (1997) The shade avoidance syndrome: multiple responses mediated by multiple phytochromes. Plant Cell Environ 20:840–844
Steppan SJ, Phillips PC, Houle D (2002) Comparative quantitative genetics: evolution of the G matrix. TREE 17:320–327
Stevens JP (1992) Applied multivariate statistics for the social sciences. Hillsdale, New Jersey
Tonsor SJ, Scheiner SM (2007) Plastic trait integration across a CO_2 gradient in *Arabidopsis thaliana*. Am Nat 169:E119–E140
Valladares F, Dobarro I, Sanchez-Gomez D, Pearcy RW (2005) Photoinhibition and drought in Mediterranean woody saplings: scaling effects and interaction in sun and shade phenotypes. J Exp Biol 56:411–485
Van Hinsberg A, van Tienderen PH (1997) Variation in growth form in relation to spectral light quality (red/far-red ratio) in *Plantago lancelata* L. in sun and shade populations. Oecologia 111:452–459
Wagner GP (1988) The influence of variation and of developmental constraints on the rate of multivariate phenotypic evolution. J Evol Biol 1:45–66
Wagner GP (2001) The character concept in evolutionary biology. Academic Press, San Diego
Wagner GP, Booth G, Bagheri-Chaichian H (1997) A population genetic theory of canalization. Evolution 51:329–347
Waitt DE, Levin DA (1993) Phenotypic integration and plastic correlations in *Phlox drummondii*. Am J Botany 80:1224–1233
Wolf LM, Mazer SJ (2005) Patterns of phenotypic plasticity and their fitness consequences in wild radish (*Raphanus sativor*: Brassicaceae). Int J Plant Sci 166:631–640