doi:10.1046/j.1420-9101.2003.00641.x

Flowering time plasticity in *Arabidopsis thaliana*: a reanalysis of Westerman & Lawrence (1970)

J. R. STINCHCOMBE, L. A. DORN & J. SCHMITT

Ecology and Evolutionary Biology Department, Brown University, Box G-W, Providence, RI 02912, USA

Keywords:

adaptive plasticity;
Arabidopsis thaliana;
cost of plasticity;
flowering time;
genetic correlation;
genotypic selection;
phenotypic plasticity;
reaction norm;
selection gradients.

Abstract

Environmental variation in temperature can have dramatic effects on plant morphology, phenology, and fitness, and for this reason it is important to understand the evolutionary dynamics of phenotypic plasticity in response to temperature. We investigated constraints on the evolution of phenotypic plasticity in response to a temperature gradient in the model plant *Arabidopsis thaliana* by applying modern analytical tools to the classic data of Westerman & Lawrence (1970). We found significant evidence for two types of constraints. First, we detected numerous significant genetic correlations between plastic responses to temperature and the mean value of a trait across all environments, which differed qualitatively in pattern between the set of ecotypes and the set of mutant lines in the original sample. Secondly, we detected significant costs of flowering time plasticity in two of the three experimental environments, and a net pattern of selection against flowering time plasticity in the experiment overall. Thus, when explored with contemporary methods, the prescient work of Westerman & Lawrence (1970) provides new insights about evolutionary constraints on the evolution of plasticity.

Introduction

Phenotypic plasticity, or the ability of a single genotype to produce multiple phenotypes in response to environmental variation, has been the subject of intense investigation and debate for nearly 40 years (see e.g. Bradshaw, 1965; Schlichting, 1986; Scheiner, 1993; Sultan, 1995, 2000; Via *et al.*, 1995; Schmitt, 1997 for reviews). Phenotypic plasticity is considered adaptive if the pattern of natural selection differs between environments, and the plastic phenotypic response is in the direction favoured by selection (Schmitt *et al.*, 1995; Dudley & Schmitt, 1996; Dorn *et al.*, 2000). There have been several observations of adaptive plasticity as well as maladaptive and nonadaptive plasticity (e.g. Schmitt *et al.*, 1995; Warkentin, 1995; Dudley & Schmitt, 1996; Nunney & Cheung, 1997; Schmitt, 1997; Denver *et al.*, 1998; Donohue *et al.*, 2000a,b, 2001; Dorn *et al.*, 2000; Agrawal *et al.*, 2002), which raises an interesting paradox. That is, given the potential advantages of plasticity, and the observations of adaptive plasticity, why aren't organisms perfectly plastic?

Several potential constraints on the evolution of phenotypic plasticity have been identified. First, significant genetic correlations, either within or across environments (Via & Lande, 1985, 1987; Gomulkiewicz & Kirkpatrick, 1992; van Tienderen & Koelewijn, 1994) are capable of constraining or altering the evolutionary trajectory of the plastic trait. Secondly, it is possible that plasticity is costly (van Tienderen, 1991; DeWitt *et al.*, 1998; Scheiner & Berrigan, 1998). Interestingly, evidence for costs of plasticity in plant morphological/architectural traits is ambiguous or rare at best (e.g. Donohue *et al.*, 2000a; Dorn *et al.*, 2000) while costs of tolerance to herbivore damage (i.e. plasticity in fitness in response to herbivory) have been observed in a handful of cases (Simms & Triplett, 1994; Tiffin & Rausher, 1999; Stinchcombe, 2002; Weinig *et al.*, 2003). In addition, the evolution of phenotypic plasticity is critically dependent on the relative frequency of selective environments (e.g. Gomulkiewicz & Kirkpatrick, 1992). Unfortunately, the frequency of selective environments is rarely quantified, making it difficult to evaluate this potential

Correspondence: John R. Stinchcombe, Ecology and Evolutionary Biology Department, Brown University, Box G-W Providence, RI 02912, USA
e-mail: john_stinchcombe@brown.edu

constraint (but see Weis & Gorman, 1990; Kingsolver *et al.*, 2001; Arnold & Peterson, 2002; Huber *et al.*, in press). Although these potential constraints on the evolution of phenotypic plasticity have been identified, there are still comparatively few data on how frequently they are of sufficient magnitude or prevalence for selection to act against phenotypic plasticity (see Dorn *et al.*, 2000 for some exceptions). In other words, how frequently is a homeostatic response favoured and a plastic response selected against?

Westerman & Lawrence (1970) provided an early example of selection acting against plasticity in their study of plastic responses to temperature in the model plant, *Arabidopsis thaliana*. Westerman and Lawrence's paper is frequently cited as supporting evidence for the correlation between fruit and seed number in *Arabidopsis*, but in addition to that oft-cited correlation, Westerman and Lawrence (W&L in our shorthand) proposed and tested a comprehensive framework for understanding the fitness consequences of phenotypic plasticity. According to W&L, a 'developmentally flexible' genotype produced different phenotypes in different environments and had high average fitness over all environments, while 'developmentally inflexible' genotypes produced the same phenotype in different environments but had low average fitness. These classifications are essentially equivalent to the current concepts of adaptive plasticity and maladaptive homeostasis (Thompson, 1991; DeWitt, 1998; DeWitt *et al.*, 1998; Winn, 1999). W&L proposed that genotypes with low average fitness that produced different phenotypes in different environments were 'developmentally unstable,' while genotypes that produced the same phenotype in different environments but had high average fitness were 'developmentally stable.' These terms are essentially equivalent to the current concepts of maladaptive plasticity and adaptive homeostasis.

After developing this classification scheme, W&L proceeded to test the developmental flexibility and stability of height at flowering, rosette leaf number at flowering, and days until flowering in *Arabidopsis* in response to temperature, an important ecological variable for *Arabidopsis*. [For instance, temperature plays a key role in determining biogeographic range limits for *Arabidopsis* populations (e.g. Hoffman, 2002), in addition to mediating life history transitions, especially the transition from vegetative growth to flowering (see e.g. Johanson *et al.*, 2000; Blazquez *et al.*, 2003)]. W&L characterized the plasticity of flowering time, height, and leaf number in 33 inbred lines with joint regression analysis (see below). By plotting mean fruit number (over all environments) against a measure of plasticity from the joint regression analysis (their Fig. 3), W&L sought to determine fitness consequences of plasticity in flowering time, height, and leaf number in response to temperature. W&L did not present estimated regression coefficients or their statistical significance for the relationships between plasticity and fitness – although they note that one relationship is 'not quite significant', implying that statistical analyses were performed. As such, it is difficult to fully assess the magnitude and statistical significance of the relationships they presented. Moreover, they did not attempt to account for correlations among traits as potential constraints on the response to selection, or to distinguish direct selection from indirect selection on correlated traits (e.g. Lande & Arnold, 1983).

Here we apply modern analytical tools to W&L's data, with the goal of filling in several gaps in their analyses. First, to evaluate genetic constraints on the evolution of plasticity, we estimated genetic correlations between the trait means, estimated over all environments, and plastic responses to temperature (see below), for all combinations of traits. Secondly, to test for costs of plasticity within individual environments in W&L's experiment, we utilized recently developed statistical approaches (e.g. van Tienderen, 1991; DeWitt, 1998; DeWitt *et al.*, 1998; Scheiner & Berrigan, 1998) that were unavailable at the time of W&L's study. In addition, to test for direct selection on plasticity, we estimated the pattern of natural selection on the trait means and plasticities in a multiple regression model that accounts for correlations between traits, taking advantage of the Lande & Arnold (1983) approach for estimating selection on correlated traits. By examining W&L'S remarkably prescient data in a contemporary framework, we were able to obtain new insights into the nature of genetic constraints on the evolution of flowering time plasticity in *Arabidopsis*.

Methods

Westerman and Lawrence's methods

Westerman and Lawrence used 33 inbred lines, 12 of which were mutant lines. Four of these lines were termed 'major mutant' lines by W&L, and included two that were glabrous (Coimbra-1 from Portugal and Wilna-2 from the U.S.S.R. in their terminology) and two others named *stellula*-1 and *apetala*. The remaining eight mutant lines were lines derived from radiation-induced mutants that had been exposed to cobalt-60, as described by Lawrence (1968a,b; note that the mutagenesis experiments were performed by C.W. Lawrence, not the co-author of W&L who was M.J. Lawrence). Most of the 21 nonmutant lines were drawn from the Laibach collection. A modified version of W&L's Table 2 describing the lines is presented in Table A1 in the appendix. Any correspondence between these mutants and inbred lines and current mutants and inbred lines of similar names that are available from *Arabidopsis* stock centers is uncertain.

Westerman and Lawrence planted seeds on agar medium in test tubes, and three treatments were applied: growth at 15, 20 and 25 °C (59, 68 and 77 °F,

respectively) in 'environmental cabinets' with 16 h daylengths and 80% relative humidity. Data available from the International Panel on Climate Change (http://ipcc-ddc.cru.uea.ac.uk/cru_data/visualisation/visual_index.html; for a description see New *et al.*, 1999) indicates that average daily maximum temperatures in the 15–20 °C range are found throughout the geographic range of Arabidopsis for several months of the growing season, although temperatures in the 20–25 °C range are only found in the SE United States and North Africa for a limited number of months. These climatological data suggest the environmental gradient used by W&L was ecologically relevant (cf. Arnold & Peterson, 2002). Westerman and Lawrence placed 10 seeds per line in two completely randomized blocks within each environmental cabinet. The traits W&L measured were flowering time (in days), height at flowering (mm), and rosette leaf number at flowering. To estimate fruit number, W&L counted the number of fruits for five plants in each line, block and cabinet. Westerman and Lawrence also counted seed number in a subset of these lines, and from these data W&L determined the frequently-cited correlation between fruit number and seed number.

Data

Westerman & Lawrence (1970) reported inbred line means for fruit number, flowering time, height, and leaf number in each environment; these data form the basis of our reanalysis. In addition, they reported the grand mean for each inbred line for all of these traits (i.e. each inbred line's mean over all environments) as well as the three environmental means (i.e. the mean value of these traits within each experimental environment). We refer to the grand mean of a given trait as a 'trait mean.' The grand mean of fruit number we considered an estimate of overall fitness; estimating fitness in this manner assumes that these environments are equally encountered by all genotypes.

Reanalysis

Measures of plasticity

Westerman & Lawrence (1970) present convincing evidence of significant inbred line × environment interactions for the traits of interest, indicating that plasticity was genetically variable in their sample ($F_{64,1324} \geq 7.1$, $P < 0.0001$ for the inbred line × environment interactions for flowering time, height, and leaf number and $F_{64,704} = 2.11$, $P < 0.0001$ for the inbred line × environment interaction for fruit number; F-statistics were calculated from the mean squares reported by W&L in their Table 5). These significant interactions cannot be explained by simple scaling effects or changes in trait variance across environments, because W&L performed their original ANOVA on square-root transformed data to stabilize the variances across all treatments. Moreover, graphical plots of the reaction norms show that inbred lines indeed changed rank order across environments (see Fig 1a–c). The observation of significant inbred line × environment interactions and crossing reaction norms is sufficient evidence to infer statistically significant heterogeneity among the measures of plasticity calculated for each inbred line (Falconer, 1990; Falconer & Mackay, 1996).

Following W&L, we estimated plasticity to temperature using joint regression analysis. Briefly, in this procedure an inbred line's mean value of a trait in each environment is regressed against the environmental means for that trait (i.e. the mean value of the trait within an environment over all inbred lines), and the resulting regression coefficient for each inbred line is taken as a measure of its plasticity or environmental sensitivity (Falconer, 1990; Falconer & Mackay, 1996). The joint-regression approach has a long history in traditional and agricultural quantitative genetics (see e.g. Yates & Cochran, 1938; Finlay & Wilkinson, 1963; Perkins & Jinks, 1968a,b; Zuberi & Gale, 1976; Mather & Jinks, 1982; Falconer, 1990; Falconer & Mackay, 1996, pp.133–134; Lynch & Walsh, 1998, pp.672–678; Gurganus *et al.*, 1998; Leips & Mackay, 2000) but has been largely lacking in recent studies seeking to measure selection on phenotypic plasticity.

Although it may appear unorthodox to estimate measures of plasticity from a regression containing three data points, we suggest that this approach is not altogether different from traditional measures of plasticity. Traditionally, plasticity is defined as the difference between an inbred line's mean value in environment 'A' and the same inbred line's mean value in environment 'B.' (e.g. Dorn *et al.*, 2000). Thus, if we only had data on the 15 and 25 °C environments, plasticity would be calculated as a simple difference in trait values between these environments. However, as the reaction norms to temperature in this study were largely linear, the presence of the intermediate 20 °C environment and estimates of the traits expressed in this environment allows a more accurate estimate of plasticity for each inbred line. These data allow us to fit an estimate of plasticity that reflects the expression of the traits in that environment, rather than simply assuming that it would be the midpoint between 15 and 25 °C environments, as would be the case if we estimated plasticity as a simple arithmetic difference. Using environmental means rather than temperature as the independent variable in these regressions also allows us to estimate an inbred line's plasticity relative to the average population responses to temperature, rather than as a mathematical function of temperature *per se* (Lynch & Walsh, 1998).

Measuring plasticity in this manner has several advantages. First, it allows a straightforward estimate of overall plasticity that uses all of the data, and produces a single plasticity estimate for cases in which more than two experimental environments are used. Second, the mean

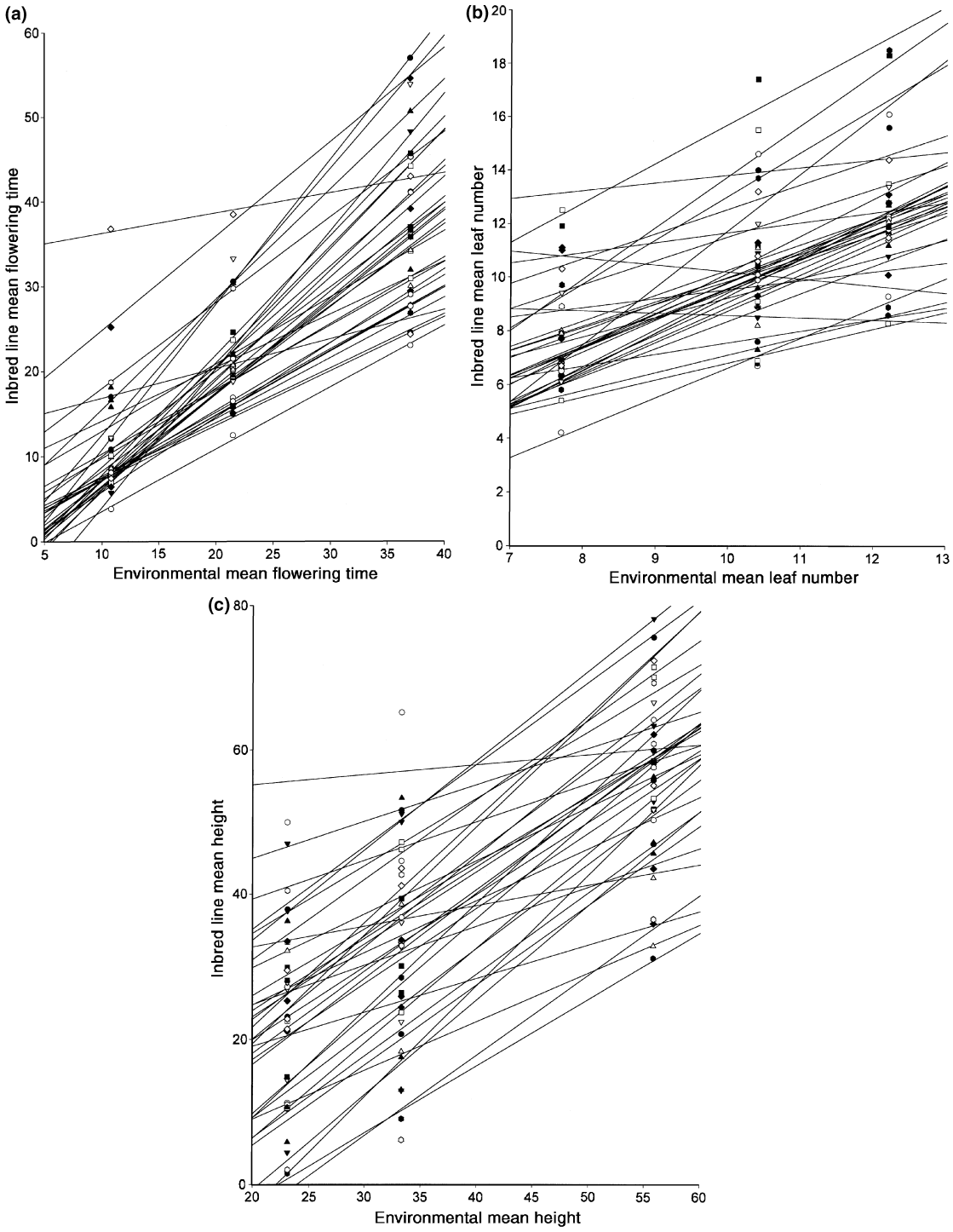

Fig. 1 Reaction norms for flowering time, leaf number, and plant height as functions of the environmental means for these traits. Inbred line means for the traits are portrayed on the vertical axis, while the environmental means for the traits in the three experimental treatments are portrayed on the horizontal axis. For panels (a and b), environmental means increased with decreasing temperature; for panel (c), environmental means increased with increasing temperature. Regression lines show the plastic response of inbred lines across environments. (a) Flowering time, (b) leaf number, (c) plant height.

plasticity of the population is by definition 1 and plasticity is dimensionless, easing comparisons across traits and species (Falconer, 1990). Finally, as shown by Falconer (1990), the variances and covariances of means and plasticities are easily calculated from the manipulation of variances and covariances, and apply equally to phenotypic and genotypic data. These mathematical expressions allow one to determine if any genetic correlations between means and plasticities are of biological significance rather than numerical/statistical artifact (see appendix B of Tiffin & Rausher (1999) for a discussion of artifactual correlations between means and plasticities when plasticity is defined as the difference between two means).

Estimates of genetic correlations

To estimate genetic correlations between trait means and plasticities, we evaluated inbred-line mean correlations between trait means and plasticities for all combinations of traits. We used simple Pearson's correlations of inbred line means as an approximation of genetic correlations, as the inbred line means were the only data available. Given the limited sample sizes and already low power of our analyses, we do not apply a Bonferroni correction, which can be prohibitively conservative (e.g. Moran, 2003).

Estimates of costs of plasticity

To estimate costs of plasticity within individual environments, we modified the techniques originally proposed by van Tienderen (1991), and subsequently developed by DeWitt *et al.* (1998) and Scheiner & Berrigan (1998). To test for costs of plasticity in this framework, an inbred line's mean fitness *within* an individual environment is regressed on the inbred line means of the trait within that environment and a measure of plasticity, that is:

$$w_{i,e} = \beta_1 X_{i,e} + \beta_2 \mathrm{pl}X_i + \mathrm{Error} \qquad (1)$$

where $w_{i,e}$ is the fitness of genotype i in environment e, $X_{i,e}$ is the mean value of the trait for genotype i in environment e, $\mathrm{pl}X_i$ is a measure of the plasticity of genotype i, and β_1 and β_2 are estimated regression coefficients. A significant, negative regression coefficient for plasticity (that is, $\beta_2 << 0$) indicates that more plastic inbred lines have lower fitness in the environment under consideration, even when controlling for the mean value of the trait in this environment – in other words, plasticity is costly (van Tienderen, 1991). To extend this approach to consider multiple traits simultaneously, one simply includes additional terms for the means and plasticities of those traits in the statistical model. In the case of two experimental environments, a genotype's plasticity is usually measured as the difference between its mean in both environments. However, measuring plasticity as a difference between means quickly becomes cumbersome with a large number of environments, as the number of measures of plasticity for each genotype increases quite quickly with the number of experimental environments. Accordingly, to test for costs of plasticity within individual environments, we used the regression coefficients from the joint regression analysis as our estimates of $\mathrm{pl}X_i$ for equation (1). This approach also allowed us to use the same measure of plasticity in our analysis of costs of plasticity in all three environments.

Estimates of selection on plasticity

To estimate the net pattern of selection on trait means and plasticities, we used a multiple regression analysis to control for correlations between independent variables (e.g. Lande & Arnold, 1983; Rausher, 1992). In these analyses we used the grand mean of fruit set for each inbred line (calculated over all environments) as our estimate of fitness, and our estimates of trait means and plasticities as the independent variables. In essence, this approach measures the net pattern of selection on plasticity, and is the commonly used method to measure selection on plastic responses to herbivore damage (e.g. Mauricio *et al.*, 1997; Tiffin & Rausher, 1999; Stinchcombe & Rausher, 2002; Weinig *et al.*, 2003). We detected no evidence of quadratic selection and for this reason present only linear selection gradient analyses. Preliminary analyses indicated a significant main effect of whether an inbred line was a mutant or not, and differences in the pattern of selection between mutant and nonmutant lines. Accordingly, we performed separate analyses for mutant and nonmutant lines; in the mutant category we pooled radiation-induced mutants, the named mutants (*stellula* and *apetala*), and the glabrous mutants.

Results

Genetic correlations between Trait Means and Plasticity

Mutant lines

Genetic correlations between trait means and plasticities in mutant lines were either small and nonsignificant or large and significant (Table 1 above the diagonal). For instance, plasticity in flowering time showed a significant, positive genetic correlation with mean flowering time across all temperatures ($r = 0.63$, $P < 0.05$) and plasticity in leaf number also showed a significant, positive genetic correlation with mean leaf number across all temperatures ($r = 0.61$, $P < 0.05$).

Correlations between trait means did not necessarily reflect the correlations among their plasticities. Leaf number and flowering time are usually positively correlated in this species, such that leaf number is often used as a surrogate measure of flowering time (e.g. El-Assal *et al.*, 2001). However, for the mutant lines in this experiment the trait means for flowering time and leaf number over all environments were only marginally correlated, but plasticity in leaf number and

	Flowering time plasticity	Height plasticity	Leaf number plasticity	Mean flowering time	Mean height	Mean leaf number
Flowering time plasticity	–	0.26	0.80***	0.63**	0.003	0.48
Height plasticity	−0.60***	–	0.60**	−0.51†	0.18	−0.04
Leaf number plasticity	0.72***	−0.11	–	0.19	−0.25	0.61**
Mean flowering time	0.18	−0.31	−0.01	–	−0.16	0.50†
Mean height	0.47**	−0.45†	0.21	0.42†	–	−0.80***
Mean leaf number	0.20	−0.30	0.10	0.88***	0.28	–

†$P \leq 0.1$; **$P < 0.05$; ***$P < 0.005$.

Table 1 Genetic correlations between plasticities and trait means in Westerman and Lawrence's experiment. Data from mutant lines ($n = 12$) are shown above the diagonal while data from nonmutant lines ($n = 21$) are shown below the diagonal.

plasticity in flowering time were positively correlated ($r = 0.80$, $P < 0.005$) – in other words, inbred lines with higher plasticity in leaf number also had higher plasticity in flowering time. Inbred lines with high mean leaf number over all environments tended to have low mean height over all environments, as indicated by the highly significant, negative correlation between these two trait means ($r = -0.80$, $P < 0.01$); however, the plasticities of these two traits were positively correlated ($r = 0.60$, $P < 0.05$). Plasticity in flowering time and plasticity in height were not significantly correlated with each other.

Nonmutant lines

Nonmutant lines demonstrated a qualitatively different pattern of genetic correlations (Table 1, below the diagonal). In contrast to the mutant lines, in the nonmutant lines there was only one significant correlation between a trait mean and a trait's plasticity, in this case a negative correlation between average height over all environments and plasticity in height ($r = -0.45$, $P < 0.05$) – i.e. taller inbred lines exhibited less height plasticity. In addition, there was a significant positive genetic correlation between average height and plasticity in flowering time ($r = 0.47$, $P < 0.05$), the only significant genetic correlation between one trait's mean and another trait's plasticity in the nonmutant lines. In contrast to the mutant lines, the correlation between mean flowering time and rosette leaf number was significant and positive ($r = 0.88$, $P < 0.005$), as was the correlation between plasticity in flowering time and plasticity in rosette leaf number ($r = 0.72$, $P < 0.005$).

Similar to what was observed in the mutant lines, the correlations between trait means across environments was not necessarily indicative of the correlation between those traits' plasticities. For instance, average flowering time and average height across all temperatures showed a marginally significant positive correlation ($r = 0.42$, $P < 0.10$) while the plasticities of those traits were negatively correlated ($r = -0.60$, $P < 0.005$).

Costs of plasticity

Mutant lines

Our analysis of costs of plasticity within individual experimental environments revealed no evidence for costs of plasticity in the mutant lines (cf. nonsignificant plasticity terms in the left column of Table 2). However, we did detect significant selection acting to decrease height in the 20 °C environment and to increase leaf number at bolting in the 25 °C environment.

Nonmutant lines

In the nonmutant lines, we observed similar patterns of selection within the three experimental environments. For instance, there was significant selection against taller inbred lines in both the 15 and 25 °C environments; selection was also acting against height in the 20 °C environment, although this selection gradient was not significant. Inbred lines that exhibited greater plastic

Table 2 Analysis of costs of plasticity within the three temperature environments used by Westerman and Lawrence. For each temperature environment, fitness *within* that environment was regressed on the terms shown.

	Mutant lines			Nonmutant lines		
Term	β	SE	P	β	SE	P
15 Degrees						
Flowering time (FT)	0.04	0.66	0.949	0.08	0.23	0.733
Height (HT)	−0.04	0.11	0.726	**−0.20**	**0.09**	**0.046**
Leaf number (LN)	0.49	1.17	0.691	−0.05	0.77	0.947
FT plasticity	3.64	18.67	0.853	−9.87	6.10	0.128
HT plasticity	−2.13	6.23	0.746	−3.75	3.11	0.248
LN plasticity	−1.46	6.31	0.827	2.49	3.92	0.536
20 Degrees						
Flowering time (FT)	−0.10	0.24	0.704	−0.21	0.24	0.409
Height (HT)	**−0.15**	**0.06**	**0.041**	−0.10	0.07	0.157
Leaf number (LN)	−0.37	0.59	0.559	0.25	0.59	0.673
FT plasticity	3.50	3.67	0.383	*−9.33*	*4.49*	*0.0567*
HT plasticity	−3.98	3.93	0.357	−4.65	2.72	0.109
LN plasticity	−1.09	2.75	0.707	0.45	3.08	0.886
25 Degrees						
Flowering time (FT)	−0.22	0.23	0.378	−0.09	0.17	0.626
Height (HT)	−0.01	0.04	0.855	**−0.14**	**0.07**	**0.0486**
Leaf number (LN)	**1.28**	**0.50**	**0.049**	0.20	0.55	0.718
FT plasticity	1.37	1.76	0.472	**−8.39**	**3.79**	**0.044**
HT plasticity	−2.37	2.65	0.412	−0.30	2.26	0.897
LN plasticity	0.94	1.42	0.537	2.22	2.29	0.348

Significant terms ($P < 0.05$) are shown in bold and marginally significant terms ($0.05 < P < 0.10$) in italics.

flowering time plasticity had significantly lower fitness in the 20 °C environment and a marginally significant trend for lower fitness in the 25 °C environment ($P = 0.0567$), supporting the hypothesis that flowering time plasticity is costly in those environments. In addition, in the 15 °C environment, there was a nonsignificant trend for inbred lines with greater flowering time plasticity to have lower fitness. Although there was a cost to flowering time plasticity in two environments, we detected no evidence that mean flowering time was under selection in any of the experimental treatments.

Net patterns of selection on plasticity and trait means

Mutant lines

Natural selection in the mutant lines favoured increased average leaf number across all environments, while marginally significant selection was acting to decrease plasticity in leaf number (Table 3a). These results suggest that selection to increase leaf number in the 25 °C environment was sufficiently strong to lead to selection for increased leaf number over all environments. In addition, selection to increase leaf number in the 25 °C treatment but not the other treatments would have the effect of decreasing plasticity in leaf number, as plants in that treatment produced fewer leaves than did plants in the 15 and 20 °C treatments (that is, the plastic response and the direction of selection are of opposite sign). The evolutionary response to this pattern of natural selection, however, would likely have been constrained because average leaf number across all environments and plasticity of leaf number exhibited a positive genetic correlation (Table 1), yet selection was acting in opposite directions for each of them. In addition, marginally significant selection was acting in opposite directions for plasticity in flowering time and plasticity in leaf number, two traits that were positively correlated with each other.

Table 3 Selection analysis for trait means and plasticity traits in Westerman and Lawrence's experiments. (a) Mutant Lines, (b) Nonmutant lines.

Trait	β	SE	*P*-value
(a) Mutant lines			
Flowering time plasticity	*5.17*	*2.50*	*0.094*
Height plasticity	−2.30	1.81	0.259
Leaf number plasticity	*−3.09*	*1.33*	*0.067*
Mean flowering time	−0.29	0.17	0.136
Mean height	0.015	0.04	0.721
Mean leaf number	**1.61**	**0.45**	**0.016**
(b) Nonmutant lines			
Flowering time plasticity	**−8.03**	**3.55**	**0.039**
Height plasticity	−3.13	2.05	0.149
Leaf number plasticity	2.17	2.40	0.381
Mean flowering time	−0.0098	0.16	0.951
Mean height	**−0.14**	**0.06**	**0.032**
Mean leaf number	0.007	0.50	0.989

Significant terms ($P < 0.05$) are shown in bold and marginally significant terms ($0.05 < P < 0.10$) in italics. Error degrees of freedom were five for (a) and 14 for (b).

Nonmutant lines

In contrast to the pattern seen in the mutant lines, natural selection in the nonmutant lines was acting in same direction on two positively correlated traits – selection acted against height at flowering, and against plasticity in flowering time (Table 3b). These results are entirely consistent with the pattern of selection observed within the individual experimental treatments: within the 15 and 25 °C treatments significant directional selection was acting against height, while within the 20 and 25 °C treatments selection was acting against inbred lines with greater flowering time plasticity. The pattern of selection on plasticity in flowering time in the nonmutant lines is in the opposite direction of the pattern of selection in mutant lines (cf. signs of flowering plasticity terms in Table 3a vs. b). As we observed in the within-environment analyses, selection acted on flowering time plasticity but not mean flowering time.

Discussion

Constraints on the evolution of plasticity

Our reanalysis of W&L's data with current statistical methods for measuring natural selection and estimating costs of phenotypic plasticity allows a rigorous assessment of their work in light of the current context on the evolution of phenotypic plasticity. Several important results emerge from our reanalysis. First, in our reanalysis of W&L's data in a statistical model that accounts for multiple correlated traits, we still detected significant evidence for selection acting against plasticity of flowering time. Secondly, by analysing the mutant and nonmutant lines separately, our reanalysis showed that the negative relationship between flowering time plasticity and fitness observed by W&L is not an artifact of mutant lines having greater plasticity and lower overall fitness: the negative relationship between flowering time plasticity and fitness holds for the nonmutant lines. Thirdly, our analysis of the costs of flowering time plasticity suggested that there is a significant costs of plasticity in the 20 and 25 °C environments. These results illustrate the complementary nature of analyses of the net pattern of selection on phenotypic plasticity (over all environments) and the costs of phenotypic plasticity as expressed in individual environments. Finally, our analysis of the genetic correlations between trait means and plasticities identified several possible genetic constraints on the evolution of these traits.

These data clearly illustrate two potential constraints on the evolution of phenotypic plasticity: first, selection can act against plasticity directly (due to costs of plasticity) and secondly, genetic correlations between

plasticity and other traits may constrain the response to selection. The simplest potential constraint on the evolution of plasticity is that selection can act against plasticity directly – as described by W&L originally and confirmed by our reanalysis and our analyses of costs of plasticity within the individual experimental treatments. Interestingly, these costs were most apparent in the high temperature treatments representing the upper end of *A. thaliana's* climatic range, suggesting a possible role of temperature stress in their expression. It is doubtful that the pattern of selection acting against flowering time plasticity is due to genetic correlations between plasticity in flowering time and other traits which W&L measured and reported. For instance, we detected no significant correlational selection gradients that would indicate that selection was acting against flowering time plasticity and in favour positively correlated traits (or acting against both flowering time plasticity and other negatively correlated traits). The possibility that selection is acting against plasticity in flowering time through a correlation with mean flowering time can be discounted because we included mean flowering time in our selection analyses and failed to detect any selection on it in both the within-environment analyses and in the analysis of the net pattern of selection. As such there is no evidence that strong selection on mean flowering time is creating negative selection on plasticity in flowering time. In addition, we detected no evidence for the negative genetic correlations which would be necessary to support this potential explanation: plasticity in flowering time is positively correlated with mean flowering time in the mutant lines, and not at all in the nonmutant lines.

The potential influence of genetic correlations acting as constraints on the evolution of plasticity can also be seen in the mutant lines – in multiple cases, the evolutionary response to selection would have been constrained because of genetic correlations between plasticity in one trait and plasticity in another trait, or because of genetic correlations between the mean value of a trait and its plasticity. The potential influence of this latter type of constraint has been emphasized recently by Pollard, Pigliucci, and Cruzan (e.g. Pollard *et al.*, 2001; Pigliucci *et al.*, 2003). The consequences of these correlations have been clearly described by Lynch & Walsh (1998), pp. 675–676): if selection favours an increase in the trait mean (e.g. mean leaf number across all environments), a correlated response to that selection will be increased phenotypic plasticity, in this example, of leaf number. Any subsequent deterioration in environmental conditions, because of the increased plasticity to environmental conditions, will often lead to populations with lower mean values of the focal trait (e.g. Simmonds, 1981; Lynch & Walsh, 1998).

Insight from mutant lines

The analysis of the mutant lines presents some evidence about the potential for random mutation to potentially change the genetic variances and co-variances governing the evolution of plasticity and quantitative traits (also see Camara & Pigliucci, 1999; Pigliucci & Schmitt, 1999). Although the use of mutagens is not without drawbacks (see Pigliucci, 2003 for a review), C.W. Lawrence's original papers (Lawrence, 1968a,b) provide some detail that suggests the mutant lines analysed by W&L were not unusually aberrant. First, C.W. Lawrence discarded lines in which crosses suggested a single gene of major effect on flowering time. Secondly, as originally noted, the flowering times of the mutagenized lines were well within the typical flowering times of ecotypes in the Laibach collection at the time. Given these caveats and mitigating factors, it is interesting that the mutant lines used by W&L showed dramatically different patterns of genetic correlations between means and plasticities than the nonmutant lines. Despite the low sample size in these analyses, we were able to detect several significant genetic correlations, perhaps due to the increased variance and because of the mutagenized lines. In like fashion, we also detected significant and marginally significant selection on trait means and plasticities in the mutant lines despite these small sample sizes. Interestingly, the signs of the significant and marginally significant selection gradients differed between the mutant and nonmutant lines in two of three cases. Although the observation that mutation creates new genetic variation in populations is a truism, we find it striking that mutation in these lines created novel patterns of genetic covariances between quantitative traits and plasticities.

Prescient features of Westerman and Lawrence

As has been noted before, (Pigliucci *et al.*, 1995), the growing conditions used by W&L – especially the agar test tubes – were less than ideal approximations of natural growing conditions. Nevertheless, their study was conceptually sophisticated and remarkably prescient in several ways. First, W&L's analysis of the relationship between mean fruit number and plasticity and their classification scheme for developmental flexibility/stability essentially represents a very early approach to testing the adaptive plasticity hypothesis, 15 years before Via & Lande's (1985, 1987) pioneering papers, which in turn inspired the experimental tests of the adaptive plasticity hypothesis usually cited in the current literature (e.g. Dudley & Schmitt, 1996). Secondly, the analysis on line means performed by W&L to test the adaptive plasticity hypothesis can be thought of as an early use of genotypic selection analysis (Rausher, 1992; Stinchcombe *et al.*, 2002), approximately 19 years before this technique was first performed in its current context (Rausher & Simms, 1989). Thirdly, W&L helped to pioneer the use of *Arabidopsis* mutants and ecotypes for addressing questions in ecological and evolutionary genetics approximately 25 years before the current renaissance (e.g.

Pigliucci *et al*., 1995; Mauricio & Rausher, 1997). Their prescient approach produced a classic data set that yields novel insights of contemporary interest when explored with modern analytical methods, and more contributions than a simple correlation between fruit number and seed number.

Acknowledgments

We thank Eric von Wettberg and Robert J. Baker (University of Saskatchewan) for informative discussions of joint regression analysis, although any errors are entirely our own. Two anonymous reviewers and D. Fairbairn made numerous helpful comments on an earlier version of the manuscript. This work has been supported by NSF grants DEB 0079932, DEB 9976997, and DEB 0129018.

References

Agrawal, A.A., Janssen, A., Bruin, J., Posthumus, M.A. & Sabelis, M.W. 2002. An ecological cost of plant defence: attractiveness of bitter cucumber plants to natural enemies of herbivores. *Ecol. Lett.* **5**: 377–385.

Arnold, S.J. & Peterson, C.R. 2002. A model for optimal reaction norms: the case of the pregnant garter snake and her temperature-sensitive embryos. *Am. Nat.* **160**: 306–316.

Blazquez, M.A., Ahn, J.H. & Weigel, D. 2003. A thermosensory pathway controlling flowering time in *Arabidopsis thaliana*. *Nat. Genet.* **33**: 168–171.

Bradshaw, A.D. 1965. Evolutionary significance of phenotypic plasticity in plants. *Adv. Genet.* **13**: 115–155.

Camara, M.D. & Pigliucci, M. 1999. Mutational contributions to genetic variance-covariance matrices: an experimental approach using induced mutations in *Arabidopsis thaliana*. *Evolution* **53**: 1692–1703.

Denver, R.J., Mirhadi, N. & Phillips, M. 1998. Adaptive plasticity in amphibian metamorphosis: response of *Scaphiopus hammondii* tadpoles to habitat desiccation. *Ecology* **79**: 1859–1872.

DeWitt, T.J. 1998. Costs and limits of phenotypic plasticity: tests with predator- induced morphology and life history in a freshwater snail. *J. Evol. Biol.* **11**: 465–480.

DeWitt, T.J., Sih, A. & Wilson, D.S. 1998. Costs and limits of phenotypic plasticity. *Trends Ecol. Evol.* **13**: 77–81.

Donohue, K., Hammond Pyle, E., Messiqua, D., Heschel, M.S. & Schmitt, J. 2000a. Evidence of adaptive divergence in plasticity: density- and site-dependent selection on shade avoidance responses in *Impatiens capensis*. *Evolution* **54**: 1956–1968.

Donohue, K., Hammond Pyle, E., Messiqua, D., Heschel, M.S. & Schmitt, J. 2000b. Density dependence and population differentiation of genetic architecture in *Impatiens capensis* in natural environments. *Evolution* **54**: 1969–1981.

Donohue, K., Hammond Pyle, E., Messiqua, D., Heschel, M.S. & Schmitt, J. 2001. Adaptive divergence in plasticity in natural populations of *Impatiens capensis* and its consequences for performance in novel habitats. *Evolution* **55**: 692–702.

Dorn, L.A., Hammond Pyle, E. & Schmitt, J. 2000. Plasticity to cues and resources in *Arabidopsis thaliana*: testing for adaptive value and costs. *Evolution* **54**: 1982–1994.

Dudley, S.A. & Schmitt, J. 1996. Testing the adaptive plasticity hypothesis: Density-dependent selection on manipulated stem length in *Impatiens capensis*. *Am. Nat.* **147**: 445–465.

El-Assal, S.E.D., Alonso-Blanco, C., Peeters, A.J.M., Raz, V. & Koornneef, M. 2001. A QTL for flowering time in *Arabidopsis* reveals a novel allele of *CRY2*. *Nat. Genet.* **29**: 435–440.

Falconer, D.S. 1990. Selection in different environments: effects on environmental sensitivity (reaction norm) and on mean performance. *Genet. Res.* **56**: 57–70.

Falconer, D.S. & Mackay, T.F.C. 1996. *Introduction to Quantitative Genetics*. Longman Group, Essex, U.K.

Finlay, K.W. & Wilkinson, G.N. 1963. The analysis of adaptation in a plant breeding program. *Aust. J. Agric. Res.* **14**: 742–754.

Gomulkiewicz, R. & Kirkpatrick, M. 1992. Quantitative genetics and the evolution of reaction norms. *Evolution* **46**: 390–411.

Gurganus, M.C., Fry, J.D., Nuzhdin, S.V., Pasyukova, E.G., Lyman, R.F. & Mackay, T.F.C. 1998. Genotype-environment interaction at quantitative trait loci affecting sensory bristle number in *Drosophila melanogaster*. *Genetics* **149**: 1883–1898.

Hoffman, M.H. 2002. Biogeography of *Arabidopsis thaliana* (L.) Heynh. (Brassicaceae). *J. Biogeogr.* **29**: 125–134.

Huber, H., Kane, N., Heschel, M.S., von Wettberg, E.J., Banta, J., Leuck, A. & Schmitt, J. Frequency and microenvironmental pattern of selection on shade avoidance traits in a natural population of *Impatiens capensis*. *Am. Nat.* in press.

Johanson, U., West, J., Lister, C., Michaels, S., Amasino, R. & Dean, C. 2000. Molecular analysis of *FRIGIDA*, a major determinant of natural variation in *Arabidopsis* flowering time. *Science* **290**: 344–347.

Kingsolver, J.G., Gomulkiewicz, R. & Carter, P.A. 2001. Variation, selection and evolution of function-valued traits. *Genetica* **112**: 87–104.

Lande, R. & Arnold, S.J. 1983. The measurement of selection on correlated characters. *Evolution* **37**: 1210–1226.

Lawrence, C.W. 1968a. Radiation-induced polygenic mutation in *Arabidopsis thaliana*. I. Selection for flowering time. *Heredity* **23**: 321–337.

Lawrence, C.W. 1968b. Radiation-induced polygenic mutation in *Arabidopsis thaliana*. II. Analysis of lines selected for flowering time. *Heredity* **23**: 573–589.

Leips, J. & Mackay, T.F.C. 2000. Quantitative trait loci for life span in *Drosophila melanogaster*: interactions with genetic background and larval density. *Genetics* **155**: 1773–1788.

Lynch, M. & Walsh, B. 1998. *Genetics and the Analysis of Quantitative Traits*. Sinauer Associates, Sunderland, MA, USA.

Mather, K. & Jinks, J.L. 1982. *Biometrical Genetics*. Chapman and Hall, London.

Mauricio, R. & Rausher, M.D. 1997. Experimental manipulation of putative selective agents provides evidence for the role of natural enemies in the evolution of plant defense. *Evolution* **51**: 1435–1444.

Mauricio, R., Rausher, M.D. & Burdick, D.S. 1997. Variation in the defense strategies of plants: are resistance and tolerance mutually exclusive? *Ecology* **78**: 1301–1311.

Moran, M.D. 2003. Arguments for rejecting the sequential Bonferroni in ecological studies. *Oikos* **100**: 403–405.

New, M., Hulme, M. & Jones, P. 1999. Representing twentieth-century space-time climate variability. Part I: Development of a 1961–90 mean monthly terrestrial climatology. *J. Climate* **12**: 829–856.

Nunney, L. & Cheung, W. 1997. The effect of temperature on body size and fecundity in female *Drosophila melanogaster*: evidence for adaptive plasticity. *Evolution* **51**: 1529–1535.

Perkins, J.M. & Jinks, J.L. 1968a. Environmental and genotype-environmental components of variability. III. Multiple lines and crosses. *Heredity* **23**: 339–356.

Perkins, J.M. & Jinks, J.L. 1968b. Environmental and genotype-environmental components of variability. IV. Non-linear interactions for multiple inbred lines. *Heredity* **23**: 525–535.

Pigliucci, M. 2003. Studying selection in a model system: evolutionary and ecological genetics of flowering time in *Arabidopsis thaliana*. *Ecology* **84**: 1700–1712.

Pigliucci, M. & Schmitt, J. 1999. Genes affecting phenotypic plasticity in *Arabidopsis*: pleiotropic effects and reproductive fitness of photomorphogenic mutants. *J. Evol. Biol.* **12**: 551–562.

Pigliucci, M., Whitton, J. & Schlichting, C.D. 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., Pollard, H. & Cruzan, M.B. 2003. Comparative studies of evolutionary responses to light environments in *Arabidopsis*. *Am. Nat.* **161**: 68–82.

Pollard, H., Cruzan, M. & Pigliucci, M. 2001. Comparative studies of reaction norms in Arabidopsis. I. Evolution of response to daylength. *Evol. Ecol. Res.* **3**: 129–155.

Rausher, M.D. 1992. The measurement of selection on quantitative traits: biases due to environmental covariances between traits and fitness. *Evolution* **46**: 616–620.

Rausher, M.D. & Simms, E.L. 1989. The evolution of resistance to herbivory in *Ipomoea purpurea*. I. Attempts to detect selection. *Evolution* **43**: 563–572.

Scheiner, S.M. 1993. Genetics and evolution of phenotypic plasticity. *Annu. Rev. Ecol. Syst.* **24**: 35–68.

Scheiner, S.M. & Berrigan, D. 1998. The genetics of phenotypic plasticity. VIII. The cost of plasticity in *Daphnia pulex*. *Evolution* **52**: 368–378.

Schlichting, C.D. 1986. The evolution of phenotypic plasticity in plants. *Annu. Rev. Ecol. Syst.* **17**: 667–693.

Schmitt, J. 1997. Is photomorphogenic shade avoidance adaptive? Perspectives from population biology. *Plant Cell Environ.* **20**: 826–830.

Schmitt, J., McCormac, A.C. & Smith, H. 1995. A test of the adaptive plasticity hypothesis using transgenic and mutant plants disabled in phytochrome-mediated elongation responses to neighbors. *Am. Nat.* **146**: 937–953.

Simmonds, N.W. 1981. Genotype (*G*), environment (*E*), and *GE* components of crop yields. *Exp. Agric.* **17**: 355–362.

Simms, E.L. & Triplett, J. 1994. Costs and benefits of plant responses to disease: resistance and tolerance. *Evolution* **48**: 1973–1985.

Stinchcombe, J.R. 2002. Environmental dependency in the expression of costs of tolerance to deer herbivory. *Evolution* **56**: 1063–1067.

Stinchcombe, J.R. & Rausher, M.D. 2002. The evolution of tolerance to deer herbivory: modifications caused by the abundance of insect herbivores. *Proc. R. Soc. Lon. B Biol. Sci.* **269**: 1241–1246.

Stinchcombe, J.R., Rutter, M.T., Burdick, D.S., Tiffin, P., Rausher, M.D. & Mauricio, R. 2002. Testing for environmentally induced bias in phenotypic estimates of natural selection: Theory and practice. *American Naturalist* **160**: 511–523.

Sultan, S.E. 1995. Phenotypic plasticity and plant adaptation. *Acta Botanica Neerlandica* **44**: 363–383.

Sultan, S.E. 2000. Phenotypic plasticity for plant development, function and life history. *Trends Plant Sci.* **5**: 537–542.

Thompson, J.D. 1991. Phenotypic plasticity as a component of evolutionary change. *Trends Ecol. Evol.* **6**: 246–249.

van Tienderen, P.H. 1991. Evolution of generalists and specialists in spatially heterogenous environments. *Evolution* **45**: 1317–1331.

van Tienderen, P.H. & Koelewijn, H.P. 1994. Selection on reaction norms, genetic correlations and constraints. *Genet. Res.* **64**: 115–125.

Tiffin, P. & Rausher, M.D. 1999. Genetic constraints and selection acting on tolerance to herbivory in the common morning glory *Ipomoea purpurea*. *Am. Nat.* **154**: 700–716.

Via, S. & Lande, R. 1985. Genotype-environment interaction and the evolution of phenotypic plasticity. *Evolution* **39**: 505–522.

Via, S. & Lande, R. 1987. Evolution of genetic variability in a spatially heterogenous environment: effects of genotype-environment interaction. *Genet. Res.* **49**: 147–156.

Via, S., Gomulkiewicz, R., Dejong, G., Scheiner, S.M., Schlichting, C.D. & Vantienderen, P.H. 1995. Adaptive phenotypic plasticity: consensus and controversy. *Trends Ecol. Evol.* **10**: 212–217.

Warkentin, K.M. 1995. Adaptive plasticity in hatching age: A response to predation risk trade-offs. *Proc. Natl. Acad. Sci. U. S. A.* **92**: 3507–3510.

Weinig, C., Stinchcombe, J.R. & Schmitt, J. 2003. Evolutionary genetics of resistance and tolerance to natural herbivory in Arabidopsis thaliana. *Evolution* **57**: 1270–1280.

Weis, A.E. & Gorman, W.L. 1990. Measuring selection on reaction norms: an exploration of the *Eurosta-Solidago* system. *Evolution* **44**: 820–831.

Westerman, J.M. & Lawrence, M.J. 1970. Genotype-environment interaction and developmental regulation in *Arabidopsis thaliana*. I. Inbred lines; description. *Heredity* **25**: 609–627.

Winn, A. 1999. Is seasonal variation in leaf traits adaptive for the annual plant *Dicerandra linearifolia*? *J. Evol. Biol.* **12**: 306–313.

Yates, F. & Cochran, W.G. 1938. The analysis of groups of experiments. *J. Agric. Sci.* **28**: 556–580.

Zuberi, M.I. & Gale, J.S. 1976. Variation in wild populations of *Papaver dubium*. X. Genotype-environment interaction associated with differences in soil. *Heredity* **36**: 359–368.

Received 22 May 2003; revised 17 July 2003; accepted 31 July 2003

Appendix Table A1 Modified version of Westerman and Lawrence's Table 2, containing information about the inbred lines used in their experiment.

Line number	Name	Country of origin	Source	Mutant
1	Enkheim	Germany	Laibach collection	0
2	Eifel	Germany	Laibach collection	0
3	S2E	–	Lawrence, 1968a,b	1
4	A3L2	–	Lawrence, 1968a,b	1
5	Estland	USSR	Laibach collection	0
6	C3L2	–	Lawrence, 1968a,b	1
7	Henley-in-Arden	England	Natural populations	0
8	Maine	France	Natural populations	0
9	S1L2	–	Lawrence, 1968a,b	1
10	C2L1	–	Lawrence, 1968a,b	1
11	Langridge	–	–	0
12	C3E	–	Lawrence, 1968a,b	1
13	Limburg	Germany	Laibach collection	0
14	A1E	–	Lawrence, 1968a,b	1
15	S3L1	–	Lawrence, 1968a,b	1
16	Landsberg-1	Germany	Laibach collection	0
17	Bologna-1	Italy	Laibach collection	0
18	Coimbra-1	Portugal	Laibach collection	1 (glabrous)
20	Le Mans-2	France	Laibach collection	0
22	Palermo-1	Italy	Laibach collection	0
23	Burghhaun	Germany	Laibach collection	0
24	Eifel-6	Germany	Laibach collection	0
25	Gückingen	Germany	Laibach collection	0
26	Wilna-2	USSR	Laibach collection	1 (glabrous)
27	Oystese	Norway	Laibach collection	0
28	Estland-1	USSR	Laibach collection	0
29	Enkheim-2	Germany	Laibach collection	0
33	Pitztal-2	Germany	Laibach collection	0
34	Antwerp-1	Belgium	Laibach collection	0
35	Göttingen	Germany	Laibach collection	0
37	Dijon	France	Laibach collection	0
39	*stellula*-1	–		1
40	*apetala*	–		1