

Research review

Constraints on the evolution of adaptive phenotypic plasticity in plants

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Summary

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The high potential fitness benefit of phenotypic plasticity tempts us to expect phenotypic plasticity as a frequent adaptation to environmental heterogeneity. Examples of proven adaptive plasticity in plants, however, are scarce and most plastic responses actually may be 'passive' rather than adaptive. This suggests that frequently requirements for the evolution of adaptive plasticity are not met or that such evolution is impeded by constraints. Here we outline requirements and potential constraints for the evolution of adaptive phenotypic plasticity, identify open questions, and propose new research approaches. Important open questions concern the genetic background of plasticity, genetic variation in plasticity, selection for plasticity in natural habitats, and the nature and occurrence of costs and limits of plasticity. Especially promising tools to address these questions are selection gradient analysis, meta-analysis of studies on genotype-by-environment interactions, QTL analysis, cDNA-microarray scanning and quantitative PCR to quantify gene expression, and two-dimensional gel electrophoresis to quantify protein expression. Studying plasticity along the pathway from gene expression to the phenotype and its relationship with fitness will help us to better understand why adaptive plasticity is not more universal, and to more realistically predict the evolution of plastic responses to environmental change.

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'Alf was a jack-of-all trades, carpenter, tinsmith, black-smith, electrician, plasterer, scissors grinder, and cobbler. Alf could do anything, and as a result he was a financial failure although he worked all the time.'

John Steinbeck, 1952, East of Eden

Introduction

Sessile modular organisms, such as plants, which cannot migrate when environmental conditions change and whose modules may

experience different environments, benefit from mechanisms to cope with environmental heterogeneity. Because most organisms, and certainly plants, change their phenotype in response to environmental change, it is often assumed that phenotypic plasticity has frequently evolved as an adaptation to environmental heterogeneity. Many phenotypic responses to environmental stress, however, may be the consequence of passive reductions in growth due to resource limitation (Dorn *et al.*, 2000; van Kleunen *et al.*, 2000a). Moreover, phenotypic plasticity does not necessarily evolve as an adaptation but alternatively can evolve due to genetic correlations with other traits that are

under selection or due to genetic drift. Therefore, plasticity of a trait does not necessarily imply that it is adaptive (Sultan, 1987; Schmid, 1992).

A general consensus on the adaptive significance of plasticity exists only for a few plant traits. One example is the elongation of internodes and leaves in response to shading (Smith, 1982; Schmitt & Wulff, 1993; Dudley & Schmitt, 1995, 1996; Schmitt *et al.*, 1995). Another example is induced resistance against herbivores or pathogens (Agrawal, 1998; Agrawal *et al.*, 1999; Karban *et al.*, 1999; Tollrian & Harvell, 1999). For many other plant traits in which plasticity is assumed to be adaptive, such as changes in root morphology in response to soil nutrient levels (Hodge, 2004), it still has to be shown that they result in a fitness benefit for the plant.

In contrast to passive plastic responses, active plastic responses require a specific signal perception-transduction system allowing plants to respond by changing their development. Under some conditions, however, active and passive plastic responses may act at the same time (Fig. 1). For example, in response to shading imposed by competing plants a plant may actively elongate its stem internodes to position its leaves in the higher strata of the vegetation. However, at the same time the observed plastic response in internode length may be low due to passive reductions in growth because of resource limitation under competition. This implies that under some conditions the active plastic response may be large although the observed plastic response is relatively small or even of opposite sign, i.e. points in the other direction.

An ideal genotype would perform optimally under each environmental condition that it may potentially encounter in nature. However, the existence of specialist genotypes indicates that plastic generalist genotypes do not always evolve. Like the adaptive evolution of any trait, the prerequisites for adaptive evolution of phenotypic plasticity are genetic variation in and selection on it. Theoretical studies, however, show that even when these prerequisites are fulfilled the evolution of adaptive phenotypic plasticity may be constrained by costs and limits of phenotypic plasticity (van Tienderen, 1991, 1997; Moran, 1992; León, 1993; Padilla & Adolph, 1996; Tufto, 2000; Sultan & Spencer, 2002; Ernande & Dieckman, 2004). While several potential costs and limits of plasticity have been suggested (DeWitt *et al.*, 1998), empirical evidence for such costs and limits is very rare. Moreover, in the few cases where there is evidence for such costs it remained unclear what actually caused them.

Since the fundamental work on phenotypic plasticity by Schmalhausen (1949), many researchers have contributed to a better understanding of the evolution of phenotypic plasticity and laid the foundation for several important review articles (e.g. Bradshaw, 1965; Schlichting, 1986; Sultan, 1987; Stearns, 1989; Schmid, 1992; Scheiner, 1993; Schmitt & Wulff, 1993; Via *et al.*, 1995; Pigliucci, 1996). Here, we review studies testing for the prerequisites of, and constraints on, the evolution of phenotypic plasticity, consider the nature

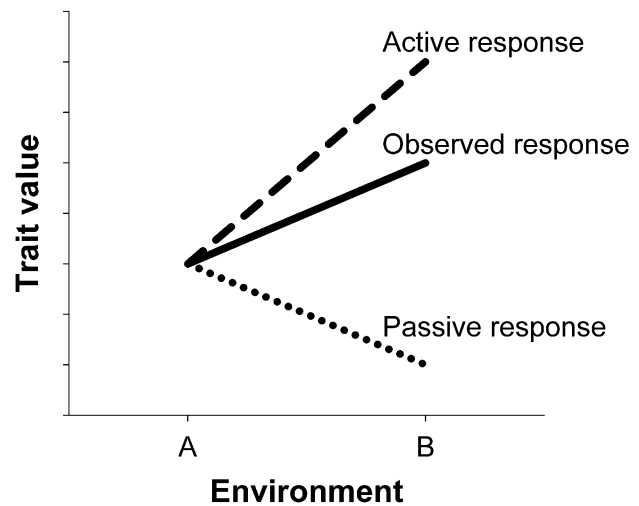


Fig. 1 Illustration of how an observed plastic response may be the result of both passive plasticity as a consequence of resource limitation, and active plasticity as a consequence of changes in allocation.

of several constraints, and indicate open research questions. We suggest how conventional methods could be used and optimized to better understand the evolution of phenotypic plasticity. Moreover, we will discuss how new tools of functional genomics, including cDNA microarrays and two-dimensional gel electrophoresis, for quantifying gene and protein expression, respectively (Colebatch *et al.*, 2002), may help to improve the understanding of the evolution of phenotypic plasticity and constraints thereon.

Genetic variation in phenotypic plasticity

Phenotypic plasticity in a trait can only evolve when there is sufficient genetic variation (Via, 1987; Via *et al.*, 1995). By now, hundreds of studies on genetic variation in plasticity, often measured as genotype-by-environment interactions in analysis of variance, have been published and allow general conclusions to be drawn. For example, although heritabilities of plasticity are generally lower than those of mean trait values (Scheiner, 1993), most studies show that there is genetic variation in plasticity. Such variation can be found even over spatial scales of only a few metres (Stratton, 1994, 1995; van Kleunen & Fischer, 2001).

Despite the progress in methods to analyse multiple independent studies together (i.e. meta-analysis; Hedges & Olkin, 1985) the large number of studies on genotype-by-environment interactions has not yet been fully explored for general patterns. Relevant questions that could be answered by meta-analysis are whether genetic variation in phenotypic plasticity has been more strongly reduced by selection in sessile organisms than in free-moving organisms and in clonal than in nonclonal organisms, and whether it has been more strongly reduced for presumably adaptive plasticity than for nonadaptive plasticity.

Most studies on genotype-by-environment interactions used replicated genotypes or full-sib families and thus quantified broad-sense heritabilities of phenotypic plasticity. Only a few used half-sib families to quantify narrow-sense heritabilities. Still fewer studies tested for narrow-sense heritabilities of phenotypic plasticity by studying realized evolutionary responses to selection on plasticity. Such studies are especially scarce for plants, as a consequence of their relatively long generation times.

Some studies selected indirectly for reduced plasticity (often called canalization) by crossing individuals that have high trait values in an environment that induces low trait values with individuals that have low trait values in an environment that induces high trait values (Waddington & Robertson, 1966; Thompson & Rook, 1988). Other studies selected indirectly for low or high plasticity as a correlated trait to selection on trait values in single environments. A line of *Plantago lanceolata* selected for long leaves under a low red-far red ratio of light mimicking shading by plants, and another line selected for short leaves under a high red-far red ratio were more plastic in leaf length than lines that had been selected in opposite directions in the respective light environments (van Hinsberg, 1996). This shows that phenotypic plasticity can evolve indirectly as a correlated response to selection on trait mean values. However, it does not necessarily imply that this mode of the evolution of plasticity is the most common one.

For *Drosophila melanogaster* there is evidence for evolutionary responses to direct selection on phenotypic plasticity in scutellar bristle number (Waddington, 1960; Kindred, 1965; Druger, 1967) and thorax size in response to temperature (Scheiner & Lyman, 1991), and body weight in response to larval food conditions (Hillesheim & Stearns, 1991). For plants, however, there is less evidence for responses to selection on phenotypic plasticity. Brumpton *et al.* (1977) found that plasticity in flowering time and height of *Nicotiana rustica* in response to sowing date had significantly changed after two generations of selection. However, after two more generations of selection only plasticity in height responded whereas plasticity in flowering time had decreased instead of increased (Jinks *et al.*, 1977). Further, plasticity in reproductive allocation and growth form of the clonal herb *Ranunculus reptans* in response to competition with a naturally co-occurring grass did not respond to artificial selection for three generations (van Kleunen *et al.*, 2002; Fischer *et al.*, 2004). It might be, however, that in these studies the heritability of plasticity or the number of generations was too low to result in a significant evolutionary change.

In addition to the above-mentioned conventional methods for estimating genetic variation, the progress in molecular genetic and statistical techniques has made it possible to estimate the number and position of loci affecting trait variation (i.e. quantitative trait loci; QTL) and the importance of each of these loci. Several studies have done QTL mapping for plants grown in different environments to study whether effects of QTL differ between environments in sign and magnitude.

Such differences, i.e. QTL-by-environment interactions, were indeed found for several traits such as inflorescence traits in *Arabidopsis thaliana* (Ungerer *et al.*, 2003), and relative growth rate and its components in *Hordeum spontaneum* (Elberse *et al.*, 2004). However, differential importance of certain QTL between different environments does not necessarily need to indicate genetic variation in phenotypic plasticity. For example, if one locus is responsible for variation in one environment and another for variation in another environment, the resulting phenotypes may still be the same for both environments. Therefore, Elberse *et al.* (2004) tested whether there are QTL for plasticity itself and found them for plasticity in relative growth rate and some of its components in *H. spontaneum*. This indicates that there are genes that directly affect plasticity and that there is variation at these loci. This would provide a good starting point for artificial selection for plasticity, analogous to the use of QTL mapping in agricultural plant breeding. To date, however, nobody has tested experimentally whether the mapping of QTL for plasticity is a more useful way of detecting genetic variation therein than the quantitative genetic methods estimating heritability and additive genetic variation mentioned in the previous three paragraphs.

In conclusion, most studies on genetic variation in phenotypic plasticity in plants indicate at least broad-sense genetic variation. Moreover, there is evidence that plasticity can evolve indirectly as a correlated response. However, what is missing are long-term selection experiments to test whether and how far phenotypic plasticity of plants does actually respond to direct selection.

Natural selection on phenotypic plasticity

Phenotypic plasticity of a trait is generally assumed to be under selection when one environment selects for a different trait value than does another environment. As a consequence, selection on phenotypic plasticity has generally been inferred from comparisons of selection on trait values in different environments (Scheiner, 1989; Dudley & Schmitt, 1996; Dudley, 1996; Donohue *et al.*, 2000; Dorn *et al.*, 2000; Steinger *et al.*, 2002; Huber *et al.*, 2004). Most such studies were concerned with natural phenotypic variation and used selection gradient analysis, in which the fitness of individuals is regressed on the trait of interest separately for different environments (Lande & Arnold, 1983). Other studies were concerned with studying fitness effects of phenotypic variation induced by experimental manipulation (Schmitt *et al.*, 1999) or of mutant and transgenic plants (Schmitt *et al.*, 1995; Galen *et al.*, 2004) in different environments. All these methods are powerful in determining whether a plastic response *per se* would be beneficial. However, they do not unequivocally prove that the plastic genotypes are selected for, rather than two groups of specialist genotypes.

Evolution of adaptive plasticity requires that plastic genotypes have the highest global fitness averaged over the

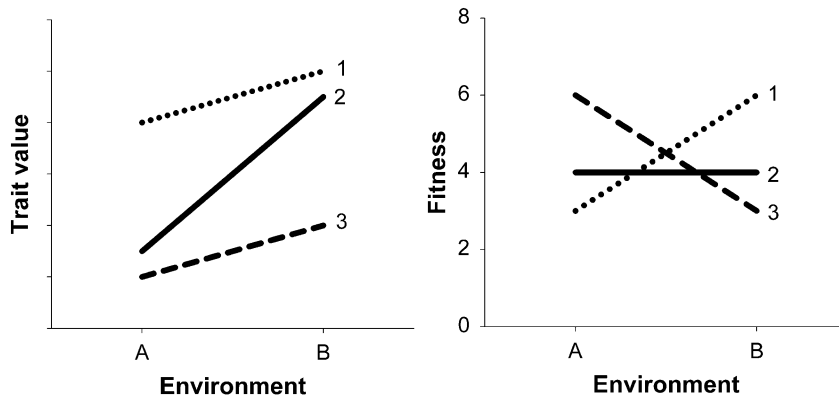


Fig. 2 Illustration of a situation where selection pressures on a trait in two different environments are opposing suggesting that plasticity would be beneficial but where overall there is no selection for phenotypic plasticity in this trait. Summed over both environments the fitness of the plastic genotype 2 (fitness = 8) is lower than the one of the less plastic genotypes 1 and 3 (fitness = 9). This might be a consequence of nonlinear relations between the trait and fitness or costs of plasticity.

environments (Releya, 2002) rather than the highest fitness in each environment separately. It is quite well possible that under some circumstances the most plastic genotype has a lower global fitness than the specialist genotypes. Such an example is illustrated in Fig. 2 where in environments A and B genotypes with small and high trait values, respectively, have the highest fitness, i.e. are selected for. This indicates that phenotypic plasticity would be beneficial and suggests selection for phenotypic plasticity. However, in this example, the most plastic genotype has a lower global fitness averaged over both environments than both of the less plastic genotypes, and as a consequence this plastic genotype will not be selected for. Such a situation might arise when the plastic genotype does not produce as extreme trait values as the less plastic specialist genotypes in each environment (Fig. 2) or when the trait of interest is not linearly related to fitness, which is mostly the case (Stearns & Hoekstra, 2000). Finally, even if the plastic genotype would reach exactly the same trait values as the most specialized genotype in each environment, its fitness may still be lower as a consequence of costs of having the capacity for plasticity (see section on constraints on the evolution of phenotypic plasticity).

The example above serves to show that selection on phenotypic plasticity cannot unequivocally be tested by using selection gradient analyses on mean trait values in each environment separately. Given that genotypes differ in their plasticity, it might be better to use selection gradient analysis in which the global fitness of genotypes or seed families averaged over the environments is regressed on their plasticity values (van Kleunen & Fischer, 2001; Stinchcombe *et al.*, 2004). For two reasons, however, averaging of fitness over environments is a potential problem with this method. First, in nature the chance that a plant encounters one environment or another may differ. Therefore, fitness should be weighted by the frequency in which the species occurs in each environment (see Sultan & Spencer, 2002). Second, the accuracy with which a genotype will produce a certain trait value and have corresponding fitness may differ between environments. Therefore, when the variance in fitness differs between

environments, it might be better to use the geometric rather than the arithmetic mean of fitness over environments.

In conclusion, evidence for opposing selection forces in different environments suggests that there may be selection for phenotypic plasticity. Alternatively, however, it could indicate that different groups of specialist genotypes should evolve in different environments. To distinguish between these alternatives, it needs to be tested whether plastic genotypes have the highest global fitness across all environments. Of course, even if this is the case plasticity may not respond to selection, depending on the rate of migration between populations (Sultan & Spencer, 2002).

Natural genetic differentiation in phenotypic plasticity

In nature, adaptive phenotypic plasticity is likely to evolve in environments that are heterogeneous in space or time. Several studies compared the outcome of evolution in heterogeneous and homogeneous environments (reviewed in Kassen, 2002) of organisms with short generation times such as *Escherichia coli* (Bennet *et al.*, 1992), *Chlamydomonas reinhardtii* (Reboud & Bell, 1997; Kassen & Bell, 1998), and *Drosophila melanogaster* and *D. simulans* (Joshi & Thompson, 1997). Most of these experimental studies showed that in heterogeneous environments generalists with high global fitness evolved (Kassen, 2002). However, they did not reveal which underlying physiological or morphological traits had actually evolved a higher adaptive plasticity. Moreover, to our knowledge no such study has addressed plants.

A few studies tested whether plants from more heterogeneous natural environments exhibit higher adaptive plasticity than ones from more homogeneous environments. Dudley & Schmitt (1995) and Donohue *et al.* (2003) showed that plants of *Impatiens capensis* from an open habitat with a vertical shading gradient, where plastic internode elongation in response to density may position leaves in better light conditions, showed higher adaptive plasticity than plants from a woodland habitat, where internode elongation in response to density would hardly be effective because of the closed

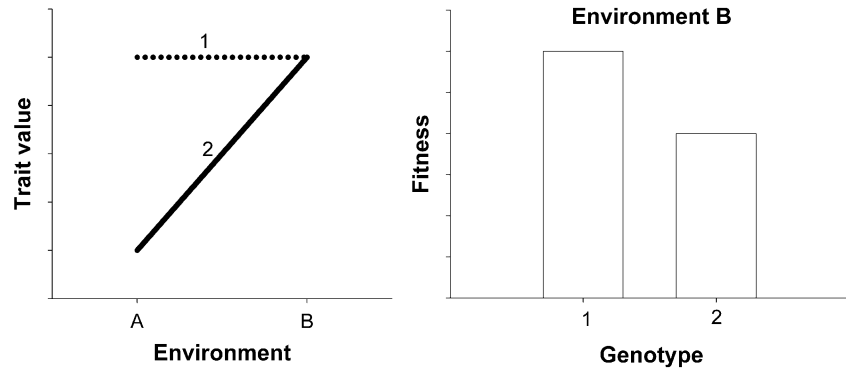


Fig. 3 Illustration of a cost of plasticity. The fixed genotype 1 and the plastic genotype 2 have the same phenotype in environment B. The cost of plasticity is indicated by a lower fitness of the plastic genotype 2 than the fixed genotype 1 in this environment.

canopy. Weinig (2000) compared plants of *Abutilon theophrastii* from cornfields, where plastic internode elongation should only be effective early in the season, with plants from weedy sites where plastic responses should be more effective later in the season. Indeed, early in plant life plasticity in internode length in response to shading was higher for plants from cornfields than for plants from weedy sites, whereas it was lower at later life stages. We compared the response to competition of genotypes of *Ranunculus reptans* microhabitats, which were competitively heterogeneous due to patches of competing grasses, with the response of genotypes from homogeneous microhabitats without competing grasses (van Kleunen & Fischer, 2001). Plasticity in traits that enable escape from competitors, such as specific internode length and vertical angle of stolons, turned out to be higher for genotypes from the heterogeneous microhabitat. Because these studies show that more plastic genotypes had evolved in more heterogeneous environments, they indicate that adaptive evolution of phenotypic plasticity is possible in nature, even at small spatial scales.

Constraints on the evolution of phenotypic plasticity

Theoretical studies indicate that the evolution of plasticity may be constrained by costs and limits of plasticity, and that as a consequence specialist genotypes or intermediately plastic ones may evolve instead of highly plastic generalist genotypes (van Tienderen, 1991, 1997; Moran, 1992; León, 1993; Padilla & Adolph, 1996; Tufto, 2000; Sultan & Spencer, 2002; Ernande & Dieckman, 2004). So far, there have only been a few studies that empirically tested for costs and limits of plasticity. Even fewer of these studies addressed the nature of these costs and limits of plasticity, although this is absolutely essential for a better understanding of the evolution of phenotypic plasticity.

Nature of the costs of plasticity

In a stimulating review article, DeWitt *et al.* (1998) distinguished five potential costs and four potential limits of plasticity. Newer insights question whether all of these costs and limits should be classified separately, suggesting that not

all of them are relevant for phenotypic plasticity in plants, and suggesting that the list was not complete.

A cost of plasticity is the reduction in fitness of a genotype as a consequence of expressing a certain phenotype through plastic rather than fixed development (Fig. 3). First, there may be costs of acquiring information about the environment by actively sampling it. Such costs, however, are likely to be more widely spread for free-moving organisms such as most animals, but not for sessile organisms such as plants. Second, there may be costs of maintenance of the sensory and regulatory machinery required for plastic responses. Third, production costs of structures through plastic development may be higher than the ones through fixed development. This, however, is an unlikely cost in modular organisms such as plants, in which there is no obvious reason why the production of, say, a new 10-cm long leaf through plastic development should be more expensive than the production of such a new leaf through fixed development. Such production costs may only exist when the timing of the production of new structures differs between plastic and nonplastic genotypes and takes place at a different developmental stage of the plant where costs may differ (S. M. Scheiner as cited in DeWitt, 1998). Fourth, the capacity for plastic development may result in less stable development (i.e. developmental instability), which in turn may result in reduced fitness (Tarasjev, 1995; Møller, 1997, 1999). Fifth, there may be so-called genetic costs of plasticity caused by negative genetic correlations between phenotypic plasticity in a trait and fitness as a consequence of pleiotropy, or of linkage or epistasis involving genes relevant for variation in fitness and phenotypic plasticity. Because plasticity costs due to information acquisition, maintenance, production, and developmental instability will all become apparent as negative genetic correlations between plasticity and fitness, the fifth category of so-called genetic costs should more narrowly be termed intrinsic genetic costs, to clearly distinguish them from negative genetic correlations brought about by the other mechanisms.

Nature of the limits of plasticity

In addition to these potential costs of plasticity, DeWitt *et al.* (1998) distinguished four potential limits of plasticity. These

limits differ from costs of plasticity in that there is a cost of the trait value expressed in a single environment as a consequence of plasticity rather than a cost of having the potential for plastic development *per se*.

First, when the information from an environmental cue is unreliable, a plastic response may result in a mal-adaptive phenotype (Lively, 1986; Tufto, 2000; Weinig, 2000). Although this is a limit and not a cost, it is often referred to as an ecological cost of plasticity (e.g. Cipollini *et al.*, 2003). A mal-adaptive phenotype may also be produced in response to an environmental cue if this cue is not specific to a particular environmental change and this may be 'misunderstood'. For example, plants of *Ranunculus reptans* that occur in temporarily flooded habitats with different levels of interspecific competition elongate their internodes in response to shading. This elongation response is adaptive when shade is imposed by a naturally co-occurring grass (van Kleunen & Fischer, 2001). However, when it is imposed by the water column during inundation, it is maladaptive because plants under water cannot afford the higher respiration needed for longer internodes (Lenssen *et al.*, 2004). Second, plastic change requires time and during this time lag the phenotype of the organism is mal-adaptive. Third, it has been argued that because plastic genotypes have more developmental baggage to carry, they may not be able to produce trait values as extreme as non-plastic genotypes (Wilson & Yoshimura, 1994). However, this so-called developmental-range limit has to be the consequence of maintenance or production costs of plasticity, and therefore is no separate limit of plasticity. Fourth, there may be the so-called epiphenotype problem in which a structure added to an organism as consequence of plastic development is weaker than one that is integrated during early development. This, however, is a rather specific limit that is only likely to apply to a few traits such as secondary spine development in zooplankton in the presence of predators (DeWitt *et al.*, 1998).

A study of Weinig & Delph (2001) implicitly provided evidence for another limit of plasticity that was not listed in the review by DeWitt *et al.* (1998). They showed for plants of *Abutilon theophrastii* that induction of plastic internode elongation by a low red-far red ratio of light early in life limited further plastic internode elongation to the same light cue later in life. Because this shows that the potential for plastic responses depends on the history of plastic reactions of the plant, we call this new limit the plasticity-history limit.

In conclusion, the costs and limits of plasticity that are most relevant for plants are genetic costs including maintenance and developmental instability costs, and plasticity-history, environmental-reliability, and lag-time limits. The latter two have been studied most frequently, often by using phenotypic manipulation (Schmitt *et al.*, 1999), and generally show that there are costs of expressing the wrong phenotype (Cipollini *et al.*, 2003). Therefore, we now focus on empirical tests of the poorly studied costs of plasticity.

Phenomenological studies of costs of plasticity

In a focal environment, a cost of plasticity becomes apparent when a more plastic genotype exhibits lower fitness in this focal environment than a less plastic genotype with the same trait value (Fig. 3; DeWitt *et al.*, 1998). This can be tested in each focal environment (k) separately by regression of the mean fitness of a genotype (j) in the focal environment ($\bar{W}_{j,k}$) on the genotypic mean trait value in the focal environment ($\bar{Z}_{j,k}$) and the genotypic mean plasticity over both environments ($\bar{P}_j = \bar{Z}_{j,k=2} - \bar{Z}_{j,k=1}$; equation 1; van Tienderen, 1991; Scheiner & Berrigan, 1998).

$$\bar{W}_{j,k} = \text{Constant}_k + \alpha_k \bar{Z}_{j,k} + \beta_k \bar{P}_j \quad \text{Eqn 1}$$

Because the mean trait value is included in this regression model, a significantly negative regression coefficient (i.e. a negative selection gradient) for the plasticity term indicates a genetic cost of plasticity (including maintenance, production and developmental instability costs) *per se* rather than a cost of expressing a certain phenotype.

Although this method for estimating costs of plasticity was proposed by van Tienderen already in 1991 first applications have only been published quite recently. For the snail *Physa heterostropha* (DeWitt, 1998), the crustacean *Daphnia pulex* (Scheiner & Berrigan, 1998), and tadpoles of *Rana sylvatica* (Releya, 2002) there is empirical evidence for costs of plasticity in response to predator cues in some of the studied morphological and behavioral characteristics.

For plants, most studies of costs of plasticity considered morphological traits in response to shading (Table 1). Genotypes of *Ranunculus reptans* that in response to competition with a naturally co-occurring grass were more plastic in internode length produced on average fewer rosettes and flowers when grown with competition (van Kleunen *et al.*, 2000b). Genotypes of *Impatiens capensis* that were more plastic in leaf length and internode length in response to plant density produced fewer seeds when grown at high natural density (Donohue *et al.*, 2000). Genotypes of *Simapsis arvensis* that were more plastic in specific leaf area in response to light intensity produced fewer seeds when grown at low light intensity (Steinger *et al.*, 2002). These three studies found costs of plasticity only in the more stressful test environments. This suggests that costs of plasticity are most likely to be detected under resource limitation, where plants cannot compensate for them. Somewhat analogously, studies on inbreeding depression have shown that genetic differences in fitness are more pronounced under stressful than under benign environmental conditions (Dudash, 1990; Hauser & Loeschcke, 1996). On the other hand, Dorn *et al.* (2000) found evidence for costs of plasticity in response to plant density, light intensity and light spectral composition for morphological and phenological traits of *Arabidopsis thaliana* in both stressful and less stressful environments (Table 1). In response to

Table 1 Overview of studies on plants finding experimental evidence for costs of plasticity by using Eqn 1

Species	Environmental variable	Prop. of analyses indicating costs	Fitness measure	Traits with significant costs of plasticity thereof
<i>Impatiens capensis</i> (Donohue <i>et al.</i> , 2000)	Plant density	3/32	Number of seeds	Internode length, leaf length
<i>Arabidopsis thaliana</i> (Dorn <i>et al.</i> , 2000)	Plant density	4/12	Number of fruits	Number of basal branches, leaf length, number of inflorescence branches
	Foliage shade	6/28	Number of fruits	Number of rosette leaves, leaf length, number of basal branches, flower interval, days to bolting
	Red-far red-ratio	3/28	Number of fruits	Number of basal branches, flower interval
<i>Ranunculus reptans</i> (van Kleunen <i>et al.</i> 2000b)	Light intensity	4/28	Number of fruits	Number of inflorescence branches, number of basal branches, leaf length
	Competition	2/12	Number of rosettes, number of flowers	Stolon internode length
<i>Plantago coronopus</i> (Smekens & van Tienderen, 2001)	Salt stress	1/28	Spike length	Leaf thickness
<i>Picea omorika</i> (Tucić & Stojković, 2001)	Plant density	1/20	Total plant dry weight	Epicotyl length
<i>Raphanus raphanistrum</i> (Agrawal <i>et al.</i> , 2002)	Herbivory	1/1* ¹	Fruit mass	Glucosinolate concentration
<i>Sinapsis arvensis</i> (Steinger <i>et al.</i> 2003)	Light intensity	1/6	Number of seeds	Specific leaf area
<i>Arabidopsis thaliana</i> (Stinchcombe <i>et al.</i> , 2004)	Temperature	2/18* ²	Number of fruits	Flowering time

For each species (reference between parentheses) the table reports the environmental cue, the traits with significant costs of plasticity thereof, and the fitness measure used to quantify costs of plasticity. For each environmental cue, the table reports the proportion of analyses indicating significant or marginally significant costs of plasticity. The total number of analyses includes different traits, different test environments, and different regression models.

*¹The cost of plasticity of glucosinolate concentration in *Raphanus raphanistrum* was only marginally significant ($P = 0.051$).

*²One of the costs of plasticity of flowering time in *Arabidopsis thaliana* was only marginally significant ($P = 0.057$).

density Tucić & Stojković (2001) found evidence for costs of plasticity in epicotyl elongation of *Picea omorika* seedlings for plants in the less stressful low-density environment. This shows that costs of plasticity can be so severe that they are even expressed in benign environments.

Tucić & Stojković (2001) also tested whether costs of plasticity change with the mean trait value in a focal environment by including the interaction term between plasticity and mean trait value in the regression model. They found a significantly positive selection gradient for this interaction term of epicotyl length of the *P. omorika* seedlings. This indicates that for a fixed plasticity in this trait, plants with large mean trait values in an environment have a higher fitness than plants with low mean trait values. In other words, and in contrast to the interpretation of the authors and of Scheiner & Berrigan (1998), this means that the costs of plasticity are smaller for genotypes with large mean trait values in the focal environment.

Only a few studies have tested for costs of plasticity in response to factors other than shading (Table 1). Agrawal *et al.* (2002) found marginally significant costs of plasticity in the amount of chemical defenses of *Raphanus raphanistrum* in response to herbivory by caterpillars. Smekens & van

Tienderen (2001) detected costs of plasticity in leaf thickness of *Plantago coronopus* in response to salt stress. Further, in a reanalysis of previously published data of Westerham & Lawrence (1970) on the response of inbred lines of *Arabidopsis thaliana* to temperature, Stinchcombe *et al.* (2004) found evidence for costs of plasticity in flowering time. More studies on costs of plasticity in a larger variety of plant traits and in a larger variety of environments are required before general conclusions can be drawn on the kind of traits and environments for which such costs do exist.

The eight empirical studies on costs of plasticity in plants mentioned in the previous three paragraphs found evidence for costs of plasticity in 27 of the total of 207 analyses (Table 1), i.e. the fraction of analyses revealing costs of plasticity (13.0%) is higher than would be expected by chance based on an error rate of 5%. While most authors of these studies concluded that costs of plasticity seem to be rather irrelevant, we advocate taking costs of plasticity more seriously for two reasons. First, in our opinion even a proportion of 13.0% of cases in which costs of plasticity matter indicates that such costs are an important factor in the evolution of plasticity. Second, this low proportion of significant costs of plasticity might be an underestimate as a consequence of three potential

weaknesses in the analyses. First, the estimates of fitness may not have been accurate because some of the studies did not use life-time seed production and because all but the studies on obligate selfers neglected the paternal fitness contribution of a plant. Second, plasticity may have been quantified inaccurately as a consequence of a low number of replicates per genotype. Moreover, many studies on costs of plasticity (e.g. Dorn *et al.*, 2000; Agrawal *et al.*, 2002; Steinger *et al.*, 2002) used absolute values of plasticity instead of signed ones. This does not matter when all genotypes respond in the same direction, as was the case in most of these studies. However, using absolute plasticity values will be incorrect when this is not the case. For example, a genotype that in response to shading by competing plants passively decreases its leaf length by 1 cm as a consequence of resource limitation should not be assigned the same plasticity value as one that actively increases its leaf length by 1 cm. Third, just as for every selection gradient analysis (Lande & Arnold, 1983), selection gradients of plasticity depend on the covariance structure between different traits, their plasticity and fitness. Therefore, selection gradient analysis may erroneously either obscure existing costs of plasticity or suggest nonexistent ones. In conclusion, the analyses for costs of plasticity can still be optimized by using more precise estimates of fitness and plasticity, and by including more relevant traits and their plasticity.

Towards mechanistic studies of constraints on the evolution of phenotypic plasticity

Even though there is some evidence that costs of plasticity exist (Table 1), we still lack a mechanistic understanding of their nature. It is unknown, whether the observed costs of plasticity are costs of having the genetic or physiological machinery for plastic responses or rather a consequence of genetic correlations with other traits affecting fitness. To find this out, a better understanding of the genetics of phenotypic plasticity is required.

Three possible genetic mechanisms were proposed underlying phenotypic plasticity (see Scheiner, 1993). The first idea was that plasticity might increase with homozygosity because homozygous individuals may be less well buffered against environmental change than heterozygous individuals (Lerner, 1954). However, empirical evidence supporting this mechanism is very scarce. Fischer *et al.* (2000) found that plants of *Ranunculus reptans* from small populations with reduced molecular genetic variation and most likely a higher degree of homozygosity had reduced, but not increased, adaptive phenotypic plasticity in leaf length. Wu (1998) found increased within-environmental variation for homozygotes when compared to heterozygotes, which the author interpreted as higher phenotypic plasticity of homozygotes without, however, providing information about between-environment variation. We do not consider homozygosity as a relevant genetic mechanism underlying phenotypic plasticity, and especially not adaptive plasticity.

Instead of the degree of homozygosity, more likely genetic mechanisms for phenotypic plasticity are: (i) allelic sensitivity in which the transcription and translation of structural genes affecting the phenotype depend on the environment; and/or (ii) the action of regulatory genes which are sensitive to the environment and regulate the transcription and translation of structural genes epistatically (Schmalhausen, 1949; Schlichting & Pigliucci, 1993; Schlichting & Smith, 2002). Although both of these potential mechanisms differ in whether the environment affects the transcription of structural genes directly or indirectly through regulatory genes, ultimately they rely on plasticity in gene expression.

Comparative studies of plasticity of wild-type organisms with mutant or transgenic organisms that differ for known reasons in their plasticity are powerful tools for testing both the adaptive value of phenotypic plasticity (Schmitt *et al.*, 1995; Galen *et al.*, 2004) and the nature of costs of phenotypic plasticity. A good example for the latter is a study on costs of having the genetic machinery for induced resistance against tetracycline, which was done with strains of the bacterium *Escherichia coli*. Some strains carried additional plasmids with an operon coding for induced resistance, others carried additional plasmids with a nonfunctional copy of this operon, others carried additional plasmids without this operon or did not carry additional plasmids at all (Nguyen *et al.*, 1989). Although this study found evidence for costs of expressing tetracycline resistance (not a cost of plasticity *per se*), it did not find evidence for costs of carrying the operon for induced tetracycline resistance (i.e. no evidence for costs of carrying the genetic machinery for plasticity). Nevertheless, it found evidence for costs of carrying extra genetic material in the form of extra plasmids, indicating that there may be costs of carrying genetic machinery in general. Possibly, such costs might be relatively smaller for organisms with larger genomes, e.g. plants, than they are for bacteria.

In the example above, plasticity in tetracycline resistance of *E. coli* is regulated by the expression of a single gene. However, it is likely that plasticity in quantitative traits is usually the result of the up- or downregulation of a large number of regulatory and structural genes. New molecular tools of functional genomics such as cDNA microarrays have opened the possibility to study genome-wide expression of thousands of genes simultaneously (Schena *et al.*, 1995; Colebatch *et al.*, 2002). For the plant *Arabidopsis thaliana*, it has been shown that hundreds of genes are differently expressed in environments differing in light quantity (Tepperman *et al.*, 2001), light quality (Ma *et al.*, 2001), and the absence or presence of pathogens (Schenk *et al.*, 2000) and herbivores (Reymond *et al.*, 2000). These studies show that gene expression is phenotypically plastic, although none of the involved molecular biologists formulated it in this way.

Some of the studies on cDNA microarrays compared gene expression of 'wild-type' genotypes with mutants or genetically engineered plants (Reymond *et al.*, 2000; Ma *et al.*,

2001; Tepperman *et al.*, 2001) but only a few assessed natural genetic variation in gene expression (Bochdanovits *et al.*, 2003; Oleksiak *et al.*, 2002; Fay *et al.*, 2004). More surprisingly, only a few studies have quantitatively assessed the relationship between variation in gene expression and variation in phenotypic traits. A notable exception is a recent study of Bochdanovits *et al.* (2003) that showed that adult body size of *Drosophila melanogaster* is positively correlated with levels of gene expression, as assessed with cDNA microarrays. This illustrates that an integration of genomics and quantitative genetics is possible. However, it has never been tested whether more plastic genotypes express more genes than less plastic genotypes, or express genes at higher levels, or both.

A logical next step would be to assess the relations between gene expression, plasticity in gene expression, phenotypic plasticity, and fitness. By quantification of gene expression and plasticity therein for a large number of genotypes differing in their phenotypic plastic responses, it can be tested whether phenotypic plasticity is correlated with a stronger expression of and a larger number of constitutively expressed genes. When this is true and when plants with a constitutively high gene expression have reduced fitness when the plastic response is not required, this will be evidence for genetic costs of maintenance of the sensory and regulatory machinery required for a phenotypic plastic response. Moreover, costs of plasticity may not only come about because of constitutively higher expression of genes, but also because of higher plasticity in gene expression.

A limitation of the use of cDNA microarrays for quantifying gene expression in a large number of samples is their high cost. Once candidate genes are known, it is possible to reduce the costs by using smaller arrays probing only these genes, or by using cheaper alternatives such as quantitative PCR (Freeman *et al.*, 1999; Raymaekers, 2000).

Gene expression is assessed by quantifying the amount of transcriptome, i.e. mRNA. However, an increase in the amount of mRNA does not necessarily imply that more of the final gene product (proteins) will be produced because mRNA may be immobilized (i.e. post-transcriptional gene silencing) or the number and activity of ribosomes may differ between plants. Methods used in proteomics such as two-dimensional gel electrophoresis (Colebatch *et al.*, 2002; Kersten *et al.*, 2002) can be used to determine the expression of hundreds of proteins in plant tissues. Protein expression profiles may differ between plants grown in different environments (Santoni *et al.*, 1994; Majoul *et al.*, 2003). Therefore another logical step, similar to the one proposed for gene expression, would be to assess the relationships between protein expression, plasticity thereof, phenotypic plasticity and fitness to test for costs of having the physiological machinery required for phenotypic plasticity. Moreover, if phenotypic plasticity is a consequence of higher plasticity in gene and protein expression, it would be interesting to test whether there is more machinery and substrate for transcription processes,

such as ribosomes and amino acids, present in more plastic plants than in less plastic plants.

Conclusions

We hope to have made it clear that phenotypic plasticity does not necessarily have to be adaptive. We also hope to have increased the awareness of the importance of potential constraints on the evolution of phenotypic plasticity. Moreover, our review indicates, despite the large number of theoretical and empirical high quality studies on phenotypic plasticity, that our understanding of its evolution and constraints on such evolution is still limited.

We identified several open questions in this context. Essential questions are whether genetic variation for phenotypic plasticity differs between sessile and free-moving and clonal and nonclonal organisms, and between traits whose plasticity is adaptive or nonadaptive. Moreover, we need many more empirical studies testing potential examples of the evolution of adaptive plasticity in nature. Interestingly, although it is highly likely, to date we do not even have much proof that there is direct natural selection on phenotypic plasticity itself. Furthermore, our knowledge of the nature and magnitude of costs and limits of phenotypic plasticity is very limited.

To answer these questions, research on phenotypic plasticity in plants needs to increasingly turn away from the phenomenological approaches that largely ignore molecular mechanisms responsible for changes in development. Rather, research on phenotypic plasticity should start to benefit from genomic and proteomic methods such as cDNA microarray scanning, quantitative PCR and two-dimensional gel electrophoresis of proteins. *Vice versa*, genomic and proteomic research could benefit from such interdisciplinary work, especially as this research is still mainly method-oriented rather than hypothesis-oriented (Colebatch *et al.*, 2002), and mostly neglects genetic variation in gene and protein expression.

Ultimately, to understand the mechanisms of phenotypic plasticity and its costs, we need to integrate data on plasticity at all levels along the pathway from genotype to phenotype, and relate these plasticities to each other and to fitness. Such research will help us to better understand why adaptive plasticity is not more universal, and to more realistically predict the evolution of plastic responses to environmental change.

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