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Progress in the detection of costs of phenotypic plasticity in plants

Among the most interesting biological phenomena is the fact that a genotype can develop different phenotypes depending on the environment in which this development takes place. Historically, however, the focus was on homeostasis by canalization of the phenotype to a presumed optimum (Waddington, 1960), and phenotypic plasticity was interpreted as deviation from such an optimum and therefore considered a nuisance. This has changed drastically with the insight that many plastic responses, such as stem elongation in response to shading, are actually adaptive strategies that increase fitness. Surprisingly, however, not all organisms are highly plastic, which suggests that the evolution of phenotypic plasticity is constrained either by a lack of heritable genetic variation or by limits and costs of plasticity, which outweigh its potential benefits (DeWitt *et al.*, 1998; van Kleunen & Fischer, 2005). In this context modelling studies (van Tienderen, 1991) emphasized the role of costs of plasticity. It is therefore very astonishing that empirical studies have found little evidence for the existence of such costs (van Kleunen & Fischer, 2005). Possibly, costs of plasticity are difficult to detect because genotypes burdened by high costs of plasticity have been purged from natural populations by natural selection (DeWitt *et al.*, 1998), and may only re-emerge after recombination (Fig. 1). This motivated Dechaine *et al.* (this issue, pp. 874–882) and two further recent studies (Callahan *et al.*, 2005; Weinig *et al.*, 2006) to test costs of plasticity with recombinant inbred lines rather than with natural plant genotypes.

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Costs of plasticity and their detection

A cost of plasticity is manifested as a reduction in fitness of a genotype as a consequence of the ability to express a certain phenotype through plastic rather than fixed development (van Tienderen, 1991; DeWitt *et al.*, 1998). In other words, if a genotype with fixed development always growing 10-cm-long leaves happens to grow in an environment where a plastic genotype also grows 10-cm-long leaves, costs of plasticity will become apparent as reduced fitness of the plastic genotype relative to the fixed genotype in this environment. This could be the result of maintenance costs of the sensory and regulatory machinery required for plasticity, less stable development of plastic genotypes and intrinsic genetic costs as a result of pleiotropy, linkage and epistasis involving genes relevant for variation in fitness and plasticity (van Kleunen & Fischer, 2005). However, to date there are no studies disentangling the relative importance of these mechanisms.

As illustrated by the example of leaf-length plasticity in the previous paragraph, a cost of plasticity is indicated by a

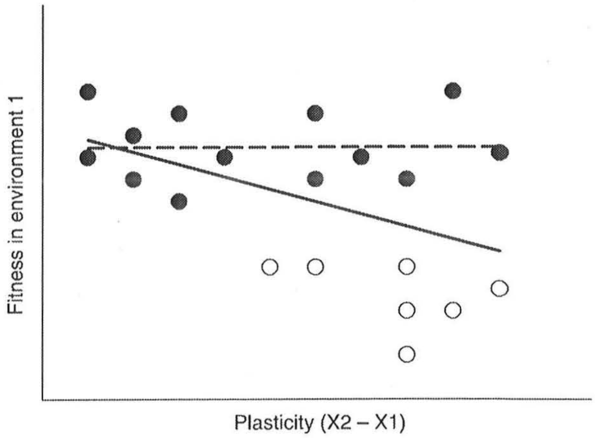

Fig. 1 Illustration of purged costs of plasticity and their re-emergence in segregating offspring. Costs of plasticity are indicated by a negative slope of the regression line of fitness in one of the test environments against plasticity in trait X across two environments. When genotypes with low fitness (open symbols) have been purged from the population, no cost of plasticity (dashed line) is found among the remaining genotypes (solid symbols). However, when the purged genotypes re-emerge in segregating offspring, a cost of plasticity (solid line) is found. Plasticity values are calculated by subtracting the mean trait value (X) of environment 1, in which low trait values are adaptive, from the mean trait value of environment 2, in which high trait values are adaptive.

negative selection gradient for plasticity (Fig. 1) when, for a large number of genotypes, fitness in one environment is related to phenotypic plasticity in an ecologically relevant trait measured by comparing the trait in two or more environments (for more details, see Dechaine *et al.*). The low percentage of cases where costs of plasticity were detected might indicate that costs are rare or difficult to detect (van Kleunen & Fischer, 2005). Alternatively, as mentioned earlier, genotypes burdened by high costs of plasticity might often have been purged from natural populations by natural selection (DeWitt *et al.*, 1998). However, even if currently purged, such costs might still constrain evolution of plasticity if they regularly re-emerge after recombination. This is especially likely for the genetic costs of plasticity that result from linkage or epistasis. Therefore, Callahan *et al.* (2005) proposed to use recombinant inbred lines (RILs) instead of natural genotypes for tests of costs of plasticity. RILs are created by crossing two parental genotypes to create an F1 offspring that is then selfed to create an F2-offspring generation. Each of the F2 offspring is then selfed for multiple generations to create (nearly) identical homozygous offspring for each RIL. The latter procedure offers the advantage that phenotypic plasticity can then be assessed at the genotype level.

Twelve previous studies using natural genotypes of plants detected costs of plasticity in 43 of 333 analyses (eight studies reviewed in van Kleunen & Fischer, 2005; Caruso *et al.*, 2006; Griffith & Sultan, 2006; Weijschede *et al.*, 2006; Avramov *et al.*, 2007). Compared with these studies, three recent studies using RILs found more evidence for costs of plasticity. Callahan *et al.* (2005) grew RILs of *Arabidopsis thaliana* that had undergone different vernalization treatments, and found significant costs of plasticity in one of four analyses (for one of two traits). Weinig *et al.* (2006) grew RILs of *A. thaliana* at low and high densities, and found significant costs of plasticity in four out of 12 analyses (for three out of six traits) and Dechaine *et al.* grew RILs of *Brassica rapa* at low and high densities, and found significant costs of plasticity in four out of 12 analyses (for three out of six traits). This suggests that intrinsic genetic costs of plasticity were purged from natural populations and re-emerged in segregating progeny. However, in comparison to most other studies on costs of plasticity, the studies by Weinig *et al.* (2006) and Dechaine *et al.* also used more stressful environments, which might have increased the chances of detecting costs of plasticity (van Kleunen & Fischer, 2005). Therefore, although the use of RILs appears very promising, it is still too early to conclude that it really increases the likelihood of detecting costs of plasticity.

Dechaine *et al.* argued that purging of intrinsic genetic costs of plasticity is most likely in highly selfing species that only rarely produce segregating offspring in which costs would reappear. Similarly, one would expect such purging also to be likely in species that predominantly reproduce clonally. However, no such pattern emerges in the 12 studies on costs of plasticity of natural genotypes of plants. Of the 114 analyses on the highly selfing *A. thaliana*, 16.7% revealed costs of plasticity. Of the 44 analyses on the highly clonal *Ranunculus reptans* and *Trifolium repens*, 9.1% revealed costs of plasticity. Of the 175 analyses on species with other reproductive strategies, including *Impatiens capensis, Iris pumila, Lobelia cardinalis, Lobelia siphilitica, Plantago coronopus, Picea omorika, Polygonum persicaria, Polgygonum hydropiper Raphanus raphanistrum* and *Sinapis arvensis*, 11.4% revealed costs of plasticity. Clearly, however, there are too few studies to draw any strong conclusions on differences in costs of plasticity between species of different life histories.

Benefits of plasticity or benefits and costs of homeostasis?

The studies by Dechaine *et al.* and Weinig *et al.* (2006) also serve to illustrate nicely another important issue in studies of costs of plasticity. While both mainly found negative selection gradients for plasticity, indicating costs of plasticity, they also found positive ones. Interestingly, summed over all studies of costs of plasticity in plants, significantly positive selection gradients for plasticity (45 out of 361 analyses) are almost as common as significantly negative ones (52 out of 361 analyses). As it is hard to imagine why potential plastic responses should benefit fitness as long as they are not expressed, it may be asked whether positive selection gradients reported in some studies are artifacts having come about by chance. Because positive and negative selection gradients are found with similar frequency, this could imply that negative selection gradients would also be artifacts. However, because costs of plasticity are very plausible, negative selection gradients cannot all be the result of chance effects alone. This situation draws our attention to the exact biological interpretation of positive selection gradients.

Scheiner & Berrigan (1998) suggested that a plastic increase of a trait should be as costly as a plastic decrease. Consequently, many studies of costs of plasticity used absolute values of plasticity. In such analyses, a positive selection gradient for plasticity can unambiguously be interpreted as a cost of homeostasis (Dorn *et al.*, 2000). However, it should also be considered whether the plastic response is active, in the sense that it has evolved because of its higher fitness compared with any fixed phenotype, such as increased leaf length in the shade, or whether it is passive, such as a reduction in leaf length resulting from resource deficiency (Fischer *et al.*, 2000; van Kleunen & Fischer, 2005). Because it is unlikely that the costs of a plastic trait increase and a plastic decrease are the same when the increase is achieved by an active adaptive plastic response while a decrease comes about by a passive plastic response, tests for the costs of plasticity should use signed rather than absolute values (van Kleunen *et al.*, 2000; van Kleunen & Fischer, 2005; Weinig *et al.*, 2006; Dechaine *et al.*). In such

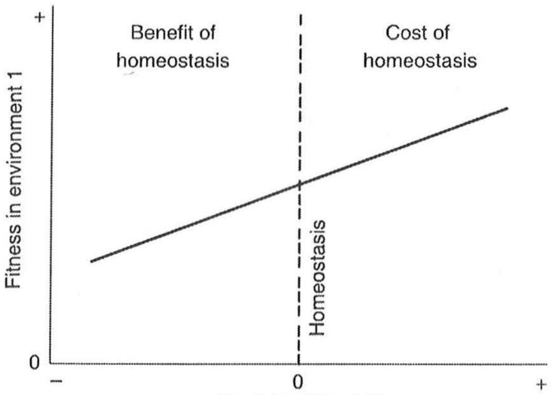

Fig. 2 Illustration of the interpretation of a positive selection gradient of plasticity. A positive selection gradient indicates a benefit of plasticity. Considered in more detail within the framework of homeostasis and active and passive plasticity (see text for further details), the interpretation of a positive selection gradient depends on whether signed plasticity values are positive or negative. A positive selection gradient indicates a benefit of homeostasis when all data points are in the left half of the graph, and it indicates a cost of homeostasis when all data points are in the right half of the graph. Note that analogous considerations apply for the interpretation of negative selection gradients. Plasticity values are calculated by subtracting the mean trait value (X) of environment 1, in which low trait values are adaptive, from the mean trait value of environment 2, in which high trait values are adaptive.

analyses, however, the interpretation of a positive selection gradient of plasticity is less straightforward. When signed plasticity values are all positive, a positive selection gradient indicates costs of homeostasis (right half in Fig. 2), as is the case for absolute plasticity values. This is exemplified by the positive selection gradient of plasticity in number of branches in response to density reported by Dechaine *et al.* However, when plasticity values are all negative, a positive selection gradient indicates that there are benefits of homeostasis (left half of Fig. 2). This appears to be the case for the positive selection gradient of plasticity in apical inflorescence height of *A. thaliana* (Weinig *et al.*, 2006). These considerations indicate that selection gradients for plasticity are of high biological relevance, but that they need to be interpreted very carefully. Clearly, to understand whether costs and benefits of plasticity really do exist, the conceptual framework of homeostasis and plasticity and the issue of active vs passive plasticity need to be considered when interpreting future selection gradient analyses. Similarly, previous studies need to be carefully reinterpreted if they did not comment on these issues.

Perspectives

Callahan *et al.* (2005), Weinig *et al.* (2006) and Dechaine *et al.* used RILs derived from only two parental genotypes, and as a consequence of this limited genetic variation, the observed costs constitute a minimum estimate of the costs that could occur in natural populations (Dechaine *et al.*). Future studies could therefore further increase the chance of detecting costs of plasticity by using segregating offspring from multiple parent pairs. Moreover, also including the parent genotypes in the experiment will allow for testing explicitly whether costs of plasticity have indeed increased in the segregating offspring relative to the parent generation.

Unfortunately, separate analyses for different traits and studies on single pairs of environments, as discussed here, may reveal only part of the picture. Therefore, among the many interesting topics that still need to be addressed in the context of costs of plasticity, potential trade-offs between plastic responses to different environmental stimuli and between plastic responses of different traits sharing the same sensory and response pathways deserve particular attention. Ultimately, this may involve insight into the exact molecular basis of costs of plasticity (van Kleunen & Fischer, 2005). Clearly, many questions remain on the evolution of phenotypic plasticity and its constraints. Studies using new approaches, such as the one by Dechaine *et al.*, are especially important, as they stimulate further progress in this exciting research field.

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