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Phenotypic plasticity: linking molecular mechanisms with evolutionary outcomes

CARL D. SCHLICHTING[1]* and HARRY SMITH[2]
[1]*Ecology and Evolutionary Biology, U-43 University of Connecticut, Storrs, CT 06269-3043, USA;*
[2]*Division of Plant Sciences, University of Nottingham, Sutton Bonington Campus, Loughborough, LE1 7RH, UK*
*(*author for correspondence)*

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Abstract. We argue that phenotypic plasticity should be broadly construed to encompass a diversity of phenomena spanning several hierarchical levels of organization. Despite seemingly disparate outcomes among different groups of organisms (e.g., the opening/closing of stomata in leaves, adjustments of allocation to growth/reproduction, or the production of different castes in social insects), there are underlying shared processes that initiate these responses. At the most fundamental level, all plastic responses originate at the level of individual cells, which receive and process signals from their environment. The broad variations in physiology, morphology, behavior, etc., that can be produced by a single genotype, can be accounted for by processes regulating gene expression in response to environmental variation. Although evolution of adaptive plasticity may not be possible for some types of environmental signals, in many cases selection has molded responses to environmental variation that generate precise and repeatable patterns of gene expression. We highlight the example of responses of plants to variation in light quality and quantity, mediated via the phytochrome genes. Responses to changes in light at particular stages of plants' life cycles (e.g., seed germination, competition, reproduction) are controlled by different members of this gene family. The mechanistic details of the cell and molecular biology of phytochrome gene action (e.g., their effects on expression of other genes) is outlined. Plasticity of cells and organisms to internal and external environmental signals is pervasive, and represents not just an outcome of evolutionary processes, but also a potentially important molder of them. Phenotypes originally initiated via a plastic response, can be fixed through genetic assimilation as alternate regulatory pathways are shut off. Evolution of mechanisms of plasticity and canalization can both reduce genetic variation, as well as shield it. When the organism encounters novel environmental conditions, this shielded variation may be expressed, revealing hidden reaction norms that represent the raw material for subsequent evolution.

Key words: canalization, differential gene expression, environmental grain, gene regulation, genetic assimilation, hidden reaction norm, internal environment, macroevolution, molecular mechanisms of plasticity, phytochrome

The adaptive domain of developmental plasticity and contiguous phenomena

Phenotypic plasticity: definition and scope of the concept

Any discussion of the topic of phenotypic plasticity has to contend with the existence of numerous understandings of what the term 'means'. Here we argue that the broadest possible definition should be employed, which would encompass the 'contiguous phenomena' of developmental plasticity, metamorphosis, and differentiation. Thus, *phenotypic plasticity is any change in an organism's characteristics in response to an environmental signal.*

We make this proposal for several reasons. First, and most importantly, this broader definition encompasses phenomena, occurring at different levels of organization, that we believe are manifestations of a shared set of underlying processes. In this perspective, disparate definitions of plasticity arise largely because researchers have investigated different hierarchical levels. Second, it avoids artificial distinctions between types of characters or environments. There is no need to decide whether a character represents physiology, morphology or behavior; all receive equal treatment. Nor is there a need to categorize environmental factors as internal or external. Finally, this definition avoids the (very intractable) issue of whether responses are adaptive, by remaining neutral about their value or purpose (see below).

The continuity across levels of traits and across environmental boundaries arises because the mechanisms that enable the plastic responses at each of these levels are fundamentally the same. First, *all responses are stimulated by a signal from the environment*, whether the result is a change in protein production, physiological activity, growth or behavior. Second, *all 'environmental' signals, internal or external, must be received and processed at the level of individual cells*. Our perspective is summarized schematically in Figure 1.

We have chosen to emphasize the hierarchical levels *transcriptome* and *proteome*, which may be relatively unfamiliar to most ecologists, in order to focus attention on the fundamental processes that underlie plastic responses of all types. The transcriptome refers to those genetic sequences, in a cell at a specific time, that have been transcribed but not yet translated. The proteome then indicates the products of translation that are available for cellular metabolism or signaling. Why separate these processes that we have all learned as the single phrase 'transcription-and-translation'? Because (in a specific cell at a specific time) not all genes are necessarily transcribed, nor are all transcripts necessarily translated, nor indeed are all proteins/polypeptides necessarily functionally active. Cells and organisms can exert a remarkable degree of control over these processes. Differential control over the processes of transcription, translation and activation occurs dynamically in an integrated and coordinated manner as a response to internal and environmental influences.

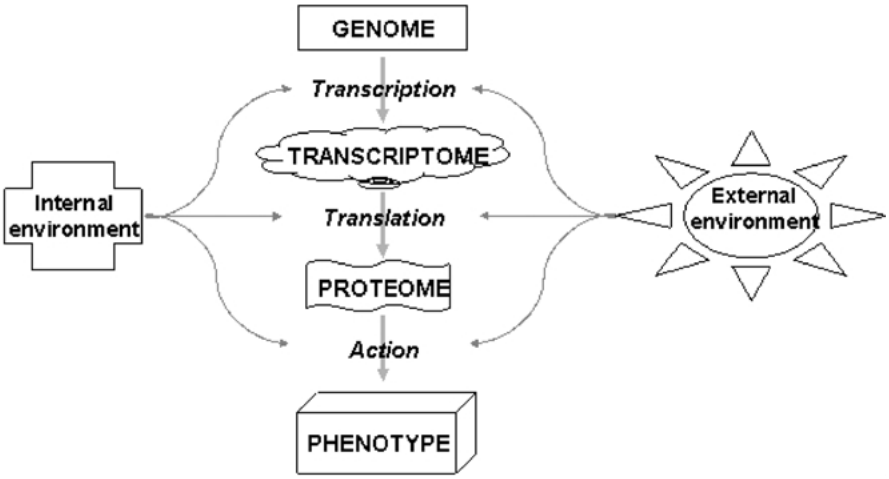

Figure 1. The hierarchy of gene expression underlying phenotypic plasticity.

We maintain that it is this control over differential gene expression that enables phenotypic plasticity at its various hierarchical levels (Schlichting, 1989a; Smith, 1990; Schlichting and Pigliucci, 1995b). The capacity for regulating gene expression in response to environmental change is ubiquitous across kingdoms, and among eukaryotes many of the mechanistic details are shared.

Given our definition, the concepts of differentiation, metamorphosis and plasticity are all interrelated (via the developmental reaction norm: Schlichting and Pigliucci, 1998). Development itself is viewed as a continuous reaction norm of the entire genotype. Even in the absence of external environmental changes, internal conditions change dynamically during the developmental process, exposing cells and tissues to very different 'environments' during ontogeny. The fate of each cell is a result of the selective expression of genetic information determined by internal controls and feedback circuits. Thus, *differentiation* can be achieved via two routes. There may be selective up- or down-regulation of particular genes, for example, certain genes may be turned off or on at particular points in development (e.g., *mab-5* is turned on and off several times in *Caenorhabditis elegans* – Salser and Kenyon, 1996). Alternatively, the patterns of expression or interaction of the same set of genes may be altered as internal conditions change. *Metamorphosis* represents a differentiation event where there is either a threshold, or a coordinated change in a suite of traits, again mediated through internal environmental change, but often initiated via an external environmental cue (e.g., Newman, 1992).

Overlaid upon this internal plasticity is plasticity due to external influences – the interplay of signals and constraints elicit modifications to the selective expression of genes from within a constant genotype. The resulting differentiation

events are thus cued by the external environment, but mediated by alterations in the internal environment via concomitant changes in gene expression and interaction. Such externally cued plasticity can be recognized at any level in the organismal hierarchy: biochemistry, physiology, morphology, or behavior.

Recent research using DNA microarray techniques (Ruan *et al*., 1998) that enable the expression of thousands of genes to be assayed simultaneously has emphasized the complexity and yet the organization underlying expression changes in plants in response to environmental signals. Some techniques allow whole-genome assays to be performed, whereas others are as yet restricted to smaller sets of genes. The number of genes whose expression is up- or down-regulated in response to simple environmental signals is remarkably large. For example, defined light signals that operate through single photoreceptors affect the expression of up to one-third of all genes in *Arabidopsis* (e.g., Ma *et al*., 2001; Tepperman *et al*., 2001). Defense responses investigated under defined conditions demonstrated changes in the expression of more than 2000 genes (Schenk *et al*., 2000) whereas drought and cold stress have been shown to affect the expression of at least 1300 genes (Seki *et al*., 2001). Within the range of genes so regulated are subsets that are affected with different dynamics; for example, far-red light treatment of *Arabidopsis* leads to changes in the expression of about 800 genes, 10% of which are affected within 1 h, whereas the expression of some genes is delayed for several hours or even days (Tepperman *et al*., 2001). Subsets of genes with related functions (e.g., transcription factors, signal transduction components, hormone-related genes, etc.) are also recognized within the transcriptome. Results indicate complex and sophisticated patterns of the coordinated and overlapping regulation of sets of genes. Furthermore, there is cross-talk between separate signaling pathways. A useful visualization of the organized complexity of gene expression responses can be seen in Figure 2 of Schenk *et al*. (2000), a Venn diagram depicting the overlapping subsets of gene regulation events in *Arabidopsis* following pathogen infection, or caused by treatment with individual elements considered to be potential signaling components of the defense response pathways. A similar visualization of overlapping gene expression responses to light treatment is given in Ma *et al*. (2001).

These studies in the laboratory are necessarily reductionist in approach, since only by defining conditions and eliminating variability as much as possible can fundamental processes at the molecular level be characterized. In the field, plants will be simultaneously challenged by many, different environmental signals, leading to almost unimaginable complexity in response at the gene expression level. Thus, although it is convenient to consider the plasticity of individual traits, it is important to remember that there must be feedback and coordination processes that link plasticity in related traits. Although this

coordination of phenotypic responses is clearly vital for determining the appropriateness of a set of plastic responses, there are only a few systems where explicit links have been documented between environmental cue, gene expression and the resulting phenotype. Examples include the phytochrome-mediated shade avoidance responses in plants (Morgan and Smith, 1978, 1979; Smith, 1982, 1995, 2000; Ballarè *et al*., 1987; Schmitt *et al*., 1995, 1999; Carabelli *et al*., 1996; Ballarè and Scopel, 1997; Casal, 2002); the formation of the dauer larva in *C. elegans* in response to food shortage (Larsen *et al*., 1995; Antebi *et al*., 1998; Gems *et al*., 1998; Inoue and Thomas, 2000; Walker *et al*., 2000) and the switch between wet and dry season morphs in the butterfly *Bicyclus anynana* (Brakefield *et al*., 1996, 1998; Roskam and Brakefield, 1999; Koch *et al*., 2000; Brakefield, 2001; Beldade *et al*., 2002).

When will plasticity be favored?

The direction and extent of plastic response are prime components of the 'adaptability' of the individual carrying the specific genotype, and as such are subject to strong selection. Certainly there are 'unavoidable responses' to limited substrates (i.e., reduced growth and reproduction) that may seem non-adaptive, but over evolutionary time even these seemingly non-functional responses may have been selected as preferred alternatives to more drastic outcomes – such as death. However, the *raison d'être* for plasticity is clearly adaptation to heterogeneous environmental conditions.

A perfectly plastic genotype (i.e., one that converts immediately to the optimal phenotype when conditions change) will always be favored to evolve whenever environments are heterogeneous. However, two limitations can probably be recognized in its description, namely the capacity for *immediate change* and the production of the *optimal phenotype*. Theoretical investigations have delineated sets of conditions favoring the evolution of generalist (i.e., plastic) vs. specialist genotypes (summarized in Lloyd, 1984; Schlichting and Pigliucci, 1998: Table 9.1). The outcome of selection in these studies has hinged upon several factors: the likelihood of environmental change, the predictiveness of the environmental cues (i.e., the correlation between the cue and future conditions), whether variation is spatial or temporal, and whether a change in conditions will be encountered within (fine-grained) or between (coarse-grained) generations.
Plasticity is selected against:

(1) if the environmental change is rare,
(2) if reliable environmental cues are lacking,
(3) if the environment fluctuates more rapidly than the typical response time,
(4) if a single phenotype is optimal in both environments,

(5) if the environment is spatially coarse-grained, and the organism can choose its habitat.

Plasticity is favored:

(1) if environmental change is frequent,
(2) if environmental cues are reliable,
(3) if environmental variation is temporally or spatially fine-grained,
(4) if environmental variation is temporally coarse-grained with predictive cues (polyphenism),
(5) if environmental variation is temporally fine-grained in a predictable sequence (heteroblasty).

Although historically there have been relatively few appropriate test studies of this body of theory, their numbers are increasing (Bell, 1997; Reboud and Bell, 1997; Kassen and Bell, 1998; Tucic *et al*., 1998; Donohue *et al*., 2000a, b; Weinig, 2000; Relyea, 2001; Avila-Sakar *et al*., 2001; Van Kleunen and Fischer, 2001). A key problem for evaluating adaptive plasticity is sorting functional from non-functional plasticity. Although this is exacerbated by the fact that alternatives to truly adaptive plasticity will be rather scarce in natural populations, recent advances have come from two approaches: employing mutant lines with altered plasticity and comparing their fitness with wild-type (e.g., Schmitt *et al*., 1995; Pigliucci and Schmitt, 1999), and manipulating phenotypes, e.g., by initiating growth in one environment and switching to a different one (Cipollini and Schultz, 1999; Schmitt *et al*., 1999; Van Buskirk and McCollum, 2000).

The production of genetic variation at the population level has often been considered as an alternative to plasticity for dealing with environmental heterogeneity. Phenotypic plasticity is a feature of an individual genotype, and the extent of plasticity of traits is determined by the particular genotype. There is ample evidence for genetic variation in plastic responses both within (van Kleunen *et al*., 2000; Smekens and van Tienderen, 2001) and between plant populations (Leiss and Müller-Schärer, 2001; Wilson, 2001; Botto and Smith, 2002). On the other hand, genotypic variability can only be detected *between* genotypes, and is a characteristic of a population. Thus the level at which selection will operate is also different. Variation in the plastic responses of particular genotypes will be subject to individual selection. On the other hand, the maintenance of genetic variability in a population as an adaptation for population success will be subject to group selection. Populations cannot 'decide' to be genetically variable to buffer themselves against environmental shifts or uncertainty, so there would need to be some form of 'species' selection that over evolutionary time favored lineages with this capacity (see, e.g., Eshel and Matessi, 1998). Note

that although genetic variability within a population can also be maintained via frequency dependent selection, this is the result of individual-based selection: the variability is not maintained to benefit the population.

Types of developmental plasticity

Plasticity of traits to produce homeostasis of fitness

It is vital to distinguish between the plasticity of fitness, and plasticity of its components (Lortie and Aarssen, 1996; Taylor and Aarssen, 1988). Selection will always act to increase fitness, and to maintain it at high levels. Thus, in theory, selection should operate to reduce the plasticity of fitness (Lewontin, 1957), in the sense of maximizing the mean and minimizing the variation in production of offspring across environments. However, because fitness is a conglomerate trait, summing positive and negative contributions of numerous other characteristics of the organism, such selection may favor considerable plasticity of the components of fitness.

Traits highly correlated with fitness should have low plasticity. Characters lower in the hierarchy (i.e., whose correlations with fitness are much less than 1) can be selected to be more or less plastic depending on the relationships of their plasticity with overall fitness – i.e., how appropriate are the phenotypes to the environmental variation. Examples of maintenance of fitness through plasticity of other traits are the papers by Lechowicz and Blais (1988) and Johnson and Koehl (1994). For the giant kelp, *Nereocystis*, an important fitness component is its ability to avoid being dislodged. Johnson and Koehl found that several traits affected this (e.g., blade shape, 'ruffliness', and stipe diameter). They found that these traits were differentially modified depending on the local conditions of waves, tides, and currents. Thus *Nereocystis* modified different traits in different locations, in order to maintain a similar ratio of force to cross-sectional area – thus mechanical stresses were quite comparable for the kelp in different environments.

Adding to the complexity of this relationship is the fact that the correlations between traits and the correlations between fitness and these traits can themselves be altered by the environment. The magnitudes of the contributions of traits to fitness are not necessarily fixed, being to a greater or lesser degree dependent upon the environment in which they were expressed (Schlichting, 1989a, b; Schlichting and Pigliucci, 1998). In some cases even the signs of the relationship of a trait with fitness can change (Pigliucci and Schlichting, 1998; van Hinsberg, 1998; Donohue *et al*., 2000b).

Nereocystis is an example of an organism that appears to have developed responses suitable to a range of commonly occurring conditions. However,

there are numerous factors that will determine the ability of an organism to respond appropriately – genetic machinery for sensing and responding to cues, availability of the cues themselves, lag times between reception of cues and the production of the new phenotype, and costs of the plastic responses (DeWitt *et al*., 1998; Schlichting and Pigliucci, 1998; see **Mechanisms** below for further discussion). A deficiency in any of those categories may favor canalization of the trait instead, that is, selection for no response to environmental change.

As limited as our understanding of plasticity is, the evolution and mechanics of canalization are even less well understood. Two forms of canalization have been recognized (Stearns and Kawecki, 1994; Stearns *et al*., 1995; Wagner *et al*., 1997): (1) systems to buffer the effects of mutations and (2) systems to buffer the effects of environmental variability. Wagner *et al*. (1997) have suggested that canalization is more likely to evolve in response to environmental fluctuation than to offset mutational variability, because of the vast difference in the frequencies of these sources of variation. Thus, both plasticity and canalization can be the result of selective pressures exerted by heterogeneous environments, and there will be a continual tension between the need for plasticity vs. canalization.

Mechanisms of developmental plasticity

Basic mechanisms in plants and animals

The challenges for *any* organism are to process environmental information (internal or external), to correctly 'assess' its significance, and to respond (or not) appropriately. Needless to say, this is no small task. Certainly the rapidity of long-distance information transfer is a major advantage of a nervous system. However, this does not necessarily mean that integrating diverse internal and external environmental signals will be any less problematic. And recently, rapid long-distance signaling has been detected in plants (Wu *et al*., 2002). When multiple signals are received, both nervous and 'non-nervous' organisms must prioritize and integrate information. In both, the nature of prioritization and integration of information, as well as the 'data processing' equipment will have been molded by past selection.

In order for plasticity to be adaptive, there are several key components of an *infrastructure* for plastic response: signal reception, signal transduction, signal translation, and the resulting product(s). Such systems of response evolve to produce repeatable responses to reliable cues. At its foundation, phenotypic plasticity employs the same mechanisms in all organisms: reception of environmental signals is a stimulus for altering patterns of gene expression. Methylation of DNA is a mechanism used widely across eukaryotes (Mattick,

2001). Both plants and animals employ Polycomb-group genes to enforce expression patterns of homeotic genes, and share many homologous genes for proteins such as histones that modify chromatin structure (Meyerowitz, 2002). There are however, many differences in components of the infrastructure between plants and animals, such as mechanisms of signal reception or cell-to-cell signaling (Meyerowitz, 2002). Many of these dissimilarities have no doubt arisen due to the differences in the types of signals to be received (e.g., soil nutrient status).

Although not nearly as rapid as nerve signal transmission, we know that modular organisms such as plants can send signals to distant regions, e.g., via hormones (Carland and McHale, 1996). The unraveling of the mechanisms of signal reception and transduction is an area of research that is of paramount interest (Gilroy and Trewavas, 2001), and the details are being elucidated at all levels: molecular, structural, and whole organism (Anderson and Beddington, 1997; Ballarè and Scopel, 1997; Batschauer, 1998; Zhang and Forde, 1998).

Perhaps the simplest answer to the question of how organisms integrate signals throughout the whole organism is that generally they do not! This is clearly the case for modular organisms – decisions are typically made only for the affected module, or for those subsequently produced (Watson and Casper, 1984). It is probably also true in general for non-modular organisms. This notion of locally controlled decision making is a foundation of several models of animal development as well (Kauffman, 1993; Raff, 1996; Britten, 1998). This of course does not solve the conundrum of integration, it just reduces the scale at which it must be achieved. There has been substantial recent work at the molecular genetic end of the scale showing that regulatory loci are of fundamental importance in controlling cascades of events (Arnone and Davidson, 1997; Graba *et al*., 1997; Bent, 2001; Morris, 2001). Environmental control of these regulators has also been demonstrated for various processes (Hsu *et al*., 2001; Malamy and Ryan, 2001). Once a cascade is triggered, only one of the possible developmental pathways is followed. Other mechanisms of integration include the various feedback loops that balance the production or activities of gene products (Bhalla and Iyengar, 1999; Becksei and Serrano, 2000).

A focus on local integration within modules does not exclude the importance of integration between modules. In clonal plants, Stuefer *et al*. (1996) showed for *Trifolium*, that plants whose ramets were only in low water conditions had different responses than low water ramets that were attached to ramets in low light treatments. Van Kleunen *et al*. (2000) found that maintenance of connections between paired ramets significantly increased survival abilities, demonstrating the ecological relevance of selection on integration.

Studies on patterns of integration at the whole organism level have been largely correlative in nature. One general finding that has emerged is a recognition of stronger patterns of integration within functional groups of traits

(e.g., within flowers) rather than between (e.g., between flowers and leaves) (Olson and Miller, 1959; Berg, 1960; Waitt and Levin, 1993; Cheverud, 1995; Conner and Sterling, 1995; Nicotra *et al.*, 1997; Leamy *et al.*, 1999; but see Armbruster *et al.*, 1999; Herrera *et al.*, 2002). These results support the proposals that the majority of trait interactions will be confined to local networks. Note however, that most of these studies still found some levels of connection between functional groups of traits.

We have predicted (Schlichting, 1989a; Smith, 1990) that when environments change, the patterns of integration must be flexible as well. This can occur if patterns of gene expression can be altered based on environmental signals. There have now been numerous demonstrations of the environment dependence of correlations among traits (e.g., Callahan and Waller, 2000; Merilä *et al.*, 2000; Albert *et al.*, 2001) (for a general review of work on phenotypic integration see Schlichting and Pigliucci, 1998: Chapter 7). Although significant work remains, results suggest that broad patterns of integration can be modified to produce phenotypes appropriate for the current environmental conditions.

The view from the cell

The mechanisms of plasticity must reside within those processes that define and regulate cellular processes. The consequences of plasticity at the population level may seem so divorced from cellular processes that the connection is nebulous, but to understand the way individual organisms express plastic phenotypes from an unchanging genotype we must look to the mechanisms of gene expression and its regulation. Furthermore, speculation on the evolution of phenotypic plasticity should be based upon a comprehensive knowledge of the molecular and cellular mechanisms by which individual organisms express flexibility in the face of a changing environment.

Fundamental to phenotypic plasticity is plurality. Selection of specific developmental pathways is in both cases the definitive step in the assumption of plasticity. So, selection of particular developmental pathways from multiple available pathways defines a plastic response, and if we understand the process of pathway selection we understand much of the mechanistic detail. In other words, we need to know not only the road map of development, but also the directional signs on that map.

What underlies the plurality implicit in this view of plasticity? Most plant genes occur as members of multigene families (i.e., evolved from a common precursor), and it has been suggested that this lies provides a basis for adaptive plasticity (Smith, 1990). Gene families commonly code for gene products whose functions are related, but considerable functional divergence can be found. Genes encoding structural proteins, enzymes, transcription factors,

photoreceptors and signal transduction components are typically found as gene families. Evolved through gene duplication and modification, or through polyploidization, gene families provide a huge capacity for the multiple regulation of the expression of genes whose gene products may have overlapping – or redundant – functions, or whose functions have diverged substantially (Pickett and Meeks-Wagner, 1995; Wagner, 1999).

Redundant genes are usually detected by deleterious mutations, particularly in regulatory genes, that produce no deleterious phenotype. This does not necessarily mean that a redundant gene has *no* function: even though its function may overlap that of others within the same family under normal conditions, it is possible that its existence becomes important under other environmental conditions. Because completely redundant genes should be lost during evolution through selection against accumulating mutations in one copy of a duplicated gene (e.g., Tautz, 1992; Lynch and Conery, 2000), those that are retained over long periods of evolutionary time would appear to have gained an adaptive function (Oberholzer *et al*., 2000; Dermitzakis and Clark, 2001; Van De Peer Y *et al*., 2001). In this way, regulation of the expression of genes that are regarded as genetically redundant may contribute importantly to the mechanisms of phenotypic plasticity.

An instructive example here is the regulation of plant development and behavior by light signals, mediated by the phytochrome family of photoreceptors. The phytochrome genes encode a family of chromo protein photoreceptors with crucial functions in the acclimation of plants to their natural environment (Smith 1995, 2000). They detect unique and unambiguous signals from the light environment, and induce cellular and developmental responses that provide the individual plant with a comprehensive strategy for survival in a complex and changeable photic environment. The multiple responses mediated by the phytochromes represent one of the most intensively studied examples of phenotypic plasticity in plants, and indeed the significance of this research area to modern considerations of the evolution of plasticity has been explicitly recognized (Callahan *et al*., 1997).

In *Arabidopsis thaliana* there are five members of the phytochrome family, phytochromes A (*phyA*) through E (*phyE*) (Mathews and Sharrock, 1997). The evolution of the gene family has been traced to presumed progenitors in the photosynthetic eubacteria, and the modern diversity has appeared as a result of a number of gene duplication events coinciding with the evolution of seed plants and later of the flowering plants. Gene duplication is continuing, and the divergence of *phyB* and *phyD* in *A. thaliana* was a comparatively recent event. Indeed, evolution within the phytochrome family appears to be occurring at rates somewhat higher than those of other plant nuclear genes (Alba *et al*., 2000). By analysis of mutant and transgenic plants, functions have been assigned to four of the five *A. thaliana* phytochromes, and we are beginning to

achieve a consensus of the overall significance of this gene family to phenotypic plasticity (Whitelam and Devlin, 1997).

We will present a brief but detailed view of the range of physiological functions of the phytochrome family, with the intention of deriving general concepts for the mechanisms of phenotypic plasticity. First, a synopsis of the molecular structure of the phytochromes is required (for review see Quail, 1997). The gene products are large polypeptides (apoproteins) of ca. 125 kD, which are assembled in the cytosol with chromophores (light-absorbing prosthetic groups). This is the integral photoreceptor (holoprotein), which is capable of absorbing light and triggering the processes of signal transduction. The region of the protein to which the chromophore binds has been conserved throughout evolution. The other parts of the molecule have evolved much more rapidly and acquired distinct physiological functions over time. So, the picture is of a molecule that is roughly divided into two halves – a conserved 'sensory' domain that perceives light signals, and a variable 'regulatory' domain that interacts with transduction components to initiate different functions depending on family member.

There are four principal processes during the plant life cycle during where the perception of environmental light signals can be crucial – germination, seedling establishment, shade avoidance and photoperiodism.

Germination – particularly of small seeds with minimal stored resources – is often dependent upon exposure to light. The sensitivity to light can be extreme – with some seeds remaining buried, sometimes for years, in the soil until disturbance exposes them to light (Scopel *et al*., 1991). In these cases, often as little as a millisecond of light is sufficient to initiate the complex developmental pathways of germination, arrested in the dark-imbibed seed in the soil bank. This opportunistic response is mediated principally by phytochrome A.

Seedling establishment is a program of development that enables the seedling to become fully photoautotrophic. The seedling that germinates under the soil grows in an etiolated manner, with rapidly elongating stem and suppressed leaf development. Light signals perceived by the phytochromes (mainly phytochrome B) and other photoreceptors initiate and control the coordinated inhibition of stem elongation, the expansion of leaves and the development of the photosynthetic apparatus.

Shade avoidance is particularly important during the main period of vegetative growth, when plants detect the presence and proximity of neighbors through the perception of light signals reflected from leaves (Smith 1982, 1995). This evokes the 'shade avoidance syndrome', which comprises rapid control of stem elongation growth, the re-allocation of resources and the timing of flowering. Shade avoidance is one of the major strategic components of competition in dense populations, and signal perception is mediated by phytochrome B, with redundant action of phyD and phyE (Smith and Whitelam, 1997).

Photoperiodism, through which the induction of flowering and bud dormancy is mediated by the perception of the relative lengths of the day and night, involves signal perception by the phytochromes and integration with the biological clock. Recognition of photoperiodism, mediated by phyA or phyB (for long- or short-day responses, respectively) is crucial for the timing of flowering and dormancy as a function of latitude.

There may be arguments as to whether these phytochrome-mediated processes are truly plastic phenomena, or are simply 'responses' to the environmental signals – such arguments are, at this level, largely semantic. All four developmental processes outlined here are ecologically critical, and variation in the 'response' can be observed for individual genotypes in different environments.

Through evolution, the phytochrome family has acquired new members with increasingly discrete physiological functions. To home in on shade avoidance, we have here a classic single-input multiple-output syndrome. The unambiguous spectral signal of radiation scattered from nearby vegetation evokes a range of plastic outputs – enhanced elongation, strengthened apical dominance, elevated leaf angle, altered resource allocation, and accelerated flowering. The range of plasticity is huge, and it is reasonable to assume that such plasticity is adaptive. However, the adaptive nature of shade avoidance is difficult to test because the plasticity itself prevents the expression of inappropriate phenotypes in any given environment. Mutant (Ballarè & Scopel, 1997; Pigliucci and Schmitt, 1999) and transgenic (Schmitt *et al*., 1995) plants disabled in signal perception (i.e., lacking functional phyB) have been used in experiments to test the adaptive plasticity hypothesis, and in these cases it was shown that phenotypes that were inappropriate were maladaptive. Mutant plants that were elongated in open situations were unfit in that environment, and transgenic plants that were always dwarfed were unfit in crowded environments.

Recent observations seem to suggest that microevolution may operate differentially on the pathways emanating from the same signal. In a study of more than 100 ecotypes (accessions) of *A. thaliana*, although very wide variation in response was observed, there was no correlation between the responses of elongation and responses of flowering to far red proximity signals (Botto and Smith, 2002). In other words, ecotypes that responded strongly in elongation did not necessarily respond strongly in floral acceleration, and *vice versa*. What is the molecular basis of such adaptive diversity within a single species? Several targets of interest emerge. First, molecular variation in the photoreceptor genes themselves, leading to differential transduction of reflection signals, may potentially underlie ecotype differences in plasticity. Such variation in photoreceptor gene sequences does exist, in some cases generating completely non-functional alleles. For example, the Wassilewskija ecotype of *A. thaliana* has a non-functional phytochrome D gene (due to a large deletion within the

structural gene), yet is perfectly viable, although the absence of functional phyD results in observable differences in phenotype (Aukerman *et al*., 1997). Yet another ecotype has been found that appears to have a non-functional gene for phytochrome A (Maloof *et al*., 2001); again this accession from the natural environment survives well in competition with others but the absence of a functional phyA modifies the responses of this accession to light signals. Outside the phytochromes, but still a photoreceptor gene, El-Assal *et al*. (2001) have found using QTL studies a variant allele of the CRY2 gene, which encodes one of the cryptochrome, blue-light detecting photoreceptors. This allele is responsible for the difference in flowering behavior between a European ecotype (Landsberg *erecta*, L*er*) and a mid-Atlantic ecotype (Cape Verde Isles, Cvi) of *Arabidopsis*. In this case the molecular difference between the CRY2 alleles of L*er* and Cvi was the substitution of a single amino acid.

Second, downstream target genes specific to photomorphogenesis may vary between ecotypes to provide the observed variation. In a study of recombinant inbred lines of *A. thaliana* generated from crosses of L*er* × Cvi we have located a number of QTL that contribute significantly to shade avoidance responses (J. Botto and H. Smith, unpublished data). These loci indicate that variation in downstream components must exist, and it will be an important task to track down the actual genes for further investigation.

Third, regulatory genes located in the basic ground plan of development, but whose activity comes under the influence of the phytochromes, may have been under selection pressure that resulted in differential output from the common signal. In this general regard, regulatory genes (i.e., loci that control the expression of other genes) have become of increasing interest to evolutionary biologists in recent years. Such genes may be expected to play central roles in the genetic architecture of development, and functional polymorphism among such genes within species is considered likely to contribute significantly to microevolution (McSteen and Hake, 1998; Purugganan, 2000; Lukens and Doebley, 2001). Regulatory genes encode sequence-specific DNA-binding transcriptional activators or cell–cell signalling molecules (Meyerowitz 2002) and some have been demonstrated to evolve more rapidly than structural genes (Purugganan *et al*., 1995; Ting *et al*., 1998; Barrier *et al*., 2001).

What are the general lessons from this consideration of the specifics of phytochrome-mediated phenotype plasticity? First, it is in principle possible to explain the processes of phenotypic plasticity on the basis of known elements of cell and molecular biology – namely that the processes regulating gene expression in response to environmental signals are sufficiently sophisticated to account for the observed selection of widely differing phenotypes from a constant genotype. Second, the fundamental significance of plurality in plasticity can be explained, at least to a large extent, through the evolution of gene families encoding products of related, overlapping and sometimes redundant

function. The phytochrome family comprises only five members and yet encompasses a wide range of function – what of the complexity that a family of hundreds of transcription factors could generate? Plurality and variation are the essential elements of plasticity.

Developmental plasticity and evolution

Phenotypic plasticity: micro- and macro-evolutionary aspects

Microevolutionary effects: The key microevolutionary role that the evolution of appropriate plastic responses to environmental variation is to increase the range of conditions in which an organism can flourish. Examples include adding a new host plant, expanding the range of tolerance to formerly stressful conditions, inducing resistance to predators, increasing competitiveness or facilitating range expansion. Although these abilities may not lead to diversification, they may represent key adaptations to exploit new niches. One case in point is the evolution of castes in the social insects. The different morphs are produced by means of plastic responses to different cues perceived early in development (Evans and Wheeler, 2001). There is little doubt about the eventual evolutionary significance of such plastic capabilities.

Plasticity may shield genetic variability from selection, available for release in the future. If a particular form of plasticity is favored, selection can result in the convergence of different genotypes on the same phenotype, thus leading to the maintenance of genetic variability. The unexposed genetic variation is thus neutral in this environment. However, if these genetic variants differ in their *hidden reaction norms* in response to other parts of the environmental spectrum, future evolution may be facilitated (Schlichting, in press).

The presence of hidden reaction norms is in essence the basis for the *genetic assimilation* scenarios of Schmalhausen (1949) and Waddington (1953): hidden phenotypic variation is exposed by new environmental conditions, and if the new phenotype is closer to the optimum for those conditions, adaptation, or at least tolerance, may be provided. Selection can act on mutational variation that arises subsequently; in the extreme, the ability to produce the original phenotype can be lost, even if the original environmental conditions recur. The concept of genetic assimilation is experiencing a revival, with workers from various disciplines suggesting that it plays an important role in adaptive evolution (Gottlieb, 1992; Schlichting and Pigliucci, 1998; Pál and Miklos, 1999; Robinson and Dukas, 1999; Tardieu, 1999; Chapman *et al*., 2000).

Macroevolutionary effects: Correlations among traits are themselves plastic, and the plastic responses of traits are not necessarily directly coupled. This could lead to novel combinations of traits that are not predictable from

phenotypic expression in 'typical' environments. Truly novel environmental conditions may exceed the limits of systems for canalization, thus exposing new parts of the reaction norm and resulting in novel phenotypes (Schlichting and Pigliucci, 1998). Waddington's genetic assimilation experiments provide examples – e.g., his production of bithorax adult flies following exposure of the eggs to ether. In plants, Serna and Fenoll (1997) found that, by limiting gas exchange of *Arabidopsis* plants, unusual clusters of stomata are produced that resemble the effects of known mutants. More generally, alterations in developmental programs that result in heterochrony (changes in timing of gene expression; McNamara, 1995; Itoha *et al.*, 1998), or heterotopy (changes in location of gene expression: Sachs, 1988; Li and Johnston, 2000) will also be likely candidates for producing novel phenotypes (Hall, 1998).

West-Eberhard (1986, 1989) and Wimberger (1994) have proposed that both behavioral plasticity and polyphenism are important facilitators of speciation. In their scenarios, analogous to genetic assimilation, the plasticity that has evolved to produce distinct morphological polyphenic forms in different seasons, or in response to different types of food, are obvious starting points for rapid evolution. A species that produces distinct, environmentally induced phenotypes could easily become genetically polymorphic via *loss* of plasticity in either of the two environments. A non-plastic form could be produced, for example, if one of the environmental states began to occur only rarely in some parts of the range – this population would tend to become a specialist. Such specialization could subsequently lead to reproductive isolation on the basis of niche preferences and morphological distinctiveness. One can imagine such a scenario in plant populations adapted to frequent inundation – individuals respond plastically to the wet/dry cycles (e.g., via heterophylly). Long-term wet or dry periods could then lead to forms (i.e., ecotypes) that do not maintain the plastic responses. For further discussion of plasticity as a stimulator of diversification see Schlichting in press.

Strategies in studying developmental plasticity

Plasticity begins at the molecular and cell level

The raison d'être of this collection of articles is that plasticity is a definable phenomenon, specifically selected through evolution and conferring advantages in competition and survival. Searching for unified mechanisms or models of plasticity implies that there must be specific processes involved, identifiable as components of 'plasticity', and that the rest of what goes on in the organism is somehow separate and different. This is unlikely to be the case: if internal and external environments do not in fact differ fundamentally, then, as we have

argued above, developmental events in response to internal cues differ only in the source of the cue, not the processes generating the responses. This continuity serves to emphasize a crucial point: *the relationship between genotype and phenotype is meaningless in the absence of an environmental context* (Schlichting, 2002). It is those different environmental contexts that can generate the various signals for differential gene expression.

In the phytochrome model, we know that there are at least three signal transduction pathways emanating from phytochrome A, with strong interactions and both positive and negative cross-talk between them (Bowler *et al.*, 1994). We would be unwise to assume that the mechanisms of action of the remaining members of the phytochrome family are any less-closely controlled. Interactions between the phytochromes, both at the physiological and the molecular level, are well established (e.g., Cerdan *et al.*, 1999). Spreading the net a little wider, there are interactions between phytochrome-mediated light-sensing mechanisms and other 'internal' sensory systems, such as sucrose-sensing systems (Dijkwel *et al.*, 1997), the brassinosteroids (Li and Chory, 1999), and other phytohormones (Møller and Chua, 1999). Overlaying all of these interactions is the control that is exerted by the endogenous clock, intimately involving photoreceptors (Millar 1999).

If phenotypic plasticity is a consequence of plurality at the molecular-genetic level, then mechanisms for plasticity would seem to be super-abundant. Thousands of genes are organized into hundreds of gene families; multiple mechanisms exist for the regulation of gene expression; modular promoters combine response-specific elements conferring sensitivity to multiple environmental and internal signals; arrays of environmental sensors sit at the tips of multi-branched signal transduction pathways channeling information to the genome. All of these phenomena simply represent the molecular facts of life – complexity works because it is flexible. Controlling and selecting the network of regulatory possibilities is the heart of functional plasticity and its exploitation for survival.

The emphasis in this consideration of phenotypic plasticity has been on the ways in which plurality is generated, maintained, selected and controlled. With plurality the opportunity for variation, and therefore flexibility, is provided. Without plurality the organism is vulnerable to unpredictable exigencies – with unlimited plurality, chaos ensues! Natural selection has prevented the chaos, but has preserved the flexibility. The basis of a molecular-genetic theory of plasticity should therefore be those regulatory processes that underlie plurality and, most importantly, keep it under control (via canalization). Such a theory can be explicitly connected to models of the evolution of organisms in heterogeneous environments: molecular mechanisms of developmental plasticity and canalization will respond to selection pressures depending on the grain, predictiveness and frequency of environmental change (see The adaptive domain of developmental plasticity and contiguous phenomena section).

References

Alba, R., Kelmenson, P.M., Cordonnier-Pratt, M.M. and Pratt, L.H. (2000) The phytochrome gene family in tomato and the rapid differential evolution of this family in angiosperms. *Mol. Biol. Evol.* **17**, 362–373.

Albert, M.J., Escudero, A. and Iriondo, J.M. (2001) Female reproductive success of narrow endemic *Erodium paularense* in contrasting microhabitats. *Ecology* **82**, 1734–1747.

Anderson, K. and Beddington, R. (1997) Pattern formation and developmental mechanisms. *Cur. Opin. Gen. Dev.* **7**, 455–458.

Antebi, A., Culotti, J.G. and Hedgecock, E.M. (1998) *daf-12* regulates developmental age and the dauer alternative in *Caenorhabditis elegans*. *Development* **125**, 1191–1216.

Armbruster, W.S., Di Stilio, V.S., Tuxill, J.D., Flores, T.C. and Velásquez Runk, J.L. (1999) Covariance and decoupling of floral and vegetative traits in nine neotropical plants: a re-evaluation of Berg's correlation-pleiades concept. *Am. J. Bot.* **86**, 39–55.

Arnone, M.L. and Davidson, E.H. (1997) The hard-wiring of development: organization and function of genomic regulatory systems. *Development* **124**, 1851–1864.

Aukerman, M.J., Hirschfeld, M., Wester, L., Weaver, M., Clack, T., Amasino, R.M. and Sharrock, R.A. (1997) A deletion in the PHYD gene of the *Arabidopsis* Wassilewskija ecotype defines a role for phytochrome D in red/far-red light sensing. *Plant Cell* **9**, 1317–1326.

Avila-Sakar, G., Krupnick, G.A. and Stephenson, A.G. (2001) Growth and resource allocation in *Cucurbita pepo* ssp *texana*: effects of fruit removal. *Internat. J. Plant. Sci.* **162**, 1089–1095.

Ballarè, C.L. and Scopel, A.L. (1997) Phytochrome signalling in plant canopies: testing its population-level implications with photoreceptor mutants of *Arabidopsis*. *Funct. Ecol.* **11**, 441–450.

Ballarè, C.L., Sánchez, R.A., Scopel, A.L., Casal, J.J. and Ghersa, C.M. (1987) Early detection of neighbour plants by phytochrome perception of spectral changes in reflected sunlight. *Plant Cell Env.* **10**, 551–557.

Barrier, M., Robichaux, R.H. and Purugganan, M.D. (2001) Accelerated regulatory gene evolution in an adaptive radiation. *Proc. Nat. Acad. Sci. USA* **98**, 10208–10213.

Batschauer, A. (1998) Photoreceptors of higher plants. *Planta* **206**, 479–492.

Becksei, A. and Serrano, L. (2000) Engineering stability in gene networks by autoregulation. *Nature* **405**, 590–593.

Beldade, P., Brakefield, P.M. and Long, A.D. (2002) Contribution of *Distal-less* to quantitative variation in butterfly eyespots. *Nature* **415**, 315–318.

Bell, G. (1997) Experimental evolution in *Chlamydomonas*. I. Short-term selection in uniform and diverse environments. *Heredity* **78**, 490–497.

Bent, A.F. (2001) Plant mitogen-activated protein kinase cascades: negative regulatory roles turn out positive. *Proc. Nat. Acad. Sci. USA* **98**, 784–786.

Berg, R.L. (1960) The ecological significance of correlation pleiades. *Evolution* **14**, 171–180.

Bhalla, U.S. and Iyengar, R. (1999) Emergent properties of networks of biological signaling pathways. *Science* **283**, 381–387.

Botto, J.F. and Smith, H. (2002) Differential genetic variation in adaptive strategies to a common environmental signal in *Arabidopsis* accessions: phytochrome-mediated shade avoidance. *Plant Cell Env.* **25**, 53–63.

Bowler, C., Yamagata, H., Neuhaus, G. and Chua, N.H. (1994) Phytochrome signal-transduction pathways are regulated by reciprocal control mechanisms. *Gen. Dev.* **8**, 2188–2202.

Brakefield, P.M. (2001) Structure of a character and the evolution of butterfly eyespot patterns. *J. Exp. Zool.* **291**, 93–104.

Brakefield, P.M., Gates, J., Keys, Z., Kesbeke, F., Wijngaarden, P.J., Monteiro, A., French, V. and Carroll, S.B. (1996) Development, plasticity and evolution of butterfly eyespot patterns. *Nature* **384**, 236–242.

Brakefield, P.M., Kesbeke, F. and Koch, P.B. (1998) The regulation of phenotypic plasticity of eyespots in the butterfly *Bicyclus anynana*. *Am. Nat.* **152**, 853–860.

Britten, R.J. (1998) Underlying assumptions of developmental models. *Proc. Nat. Acad. Sci. USA* **95**, 9372–9377.

Callahan, H.S. and Waller, D.M. (2000) Phenotypic integration and the plasticity of integration in an amphicarpic annual. *Internat. J. Plant Sci.* **161**, 89–98.

Callahan, H.S., Pigliucci, M. and Schlichting, C.D. (1997) Developmental phenotypic plasticity: where ecology and evolution meet molecular biology. *BioEssays* **19**, 519–525.

Carabelli, M., Morelli, G., Whitelam, G.C. and Ruberti, I. (1996) Twilight-zone and canopy shade induction of the *Athb-2* homeobox gene in green plants. *Proc. Nat. Acad. Sci. USA* **93**, 3530–3535.

Carland, F.M. and McHale, N.A. (1996) LOP1: a gene involved in auxin transport and vascular patterning in *Arabidopsis*. *Development* **122**, 1811–1819.

Casal, J. (2002) Environmental cues affecting development. *Cur. Opi. Plant Biol.* **5**, 37–42.

Cerdan P.D., Yanovsky, M.J., Reymundo, F.C., Nagatani, A., Staneloni, R.J., Whitelam, G.C. and Casal, J.J. (1999) Regulation of phytochrome B signaling by phytochrome A and FHY1 in *Arabidopsis thaliana*. *Plant J.* **18**, 499–507.

Chapman, L.J., Galis, F. and Shinn, J. (2000) Phenotypic plasticity and the possible role of genetic assimilation: Hypoxia-induced trade-offs in the morphological traits of an African cichlid. *Ecol. Lett.* **3**, 387–393.

Cheverud, J.M. (1995) Morphological integration in the saddle-back tamarin (*Saguinus fuscicollis*) cranium. *Am. Nat.* **145**, 63–89.

Cipollini, D.F. and Schultz, J.C. (1999) Exploring cost constraints on stem elongation in plants using phenotypic manipulation. *Am. Nat.* **153**, 236–242.

Conner, J.K. and Sterling, A. (1995) Testing hypotheses of functional relationships: a comparative survey of correlation patterns among floral traits in five insect-pollinated plants. *Am. J. Bot.* **82**, 1399–1406.

Dermitzakis, E.T. and Clark, A.G. (2001) Differential selection after duplication in mammalian developmental genes. *Mol. Biol. Evol.* **18**, 557–562.

DeWitt, T.J., Sih, A. and Wilson, D.S. (1998) Costs and limits to benefits as constraints on the evolution of phenotypic plasticity. *Trends Ecol. Evol.* **13**, 77–81.

Dijkwel, P.P., Huijser, C., Weisbeek, P.J., Chua, N.H. and Smeekens, S.C.M. (1997) Sucrose control of phytochrome A signaling in *Arabidopsis*. *Plant Cell* **9**, 583–595.

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

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

Eshel, I. and Matessi, C. (1998) Canalization, genetic assimilation and preadaptation. A quantitative genetic model. *Genetics* **149**, 2119–2133.

Evans, J.D. and Wheeler, D.E. (2001) Gene expression and the evolution of insect polyphenisms. *BioEssays* **23**, 62–68.

Gems, D., Sutton, A.J., Sundermeyer, M.L., Albert, P.S., King, K.V., Edgley, M.L., Larsen, P.L. and Riddle, D.L. (1998) Two pleiotropic classes of *daf-2* mutation affect larval arrest, adult behavior, reproduction and longevity in *Caenorhabditis elegans*. *Genetics* **150**, 129–155.

Gilroy, S. and Trewavas, A. (2001) Signal processing and transduction in plant cells: the end of the beginning? *Nature Rev. Mol. Cell Biol.* **2**, 307–314.

Gottlieb, G. (1992) *Individual Development and Evolution*: *The Genesis of Novel Behavior*. Oxford University Press, New York, NY.

Graba, Y., Aragnol, D. and Pradel, J. (1997) *Drosophila* Hox complex downstream targets and the function of homeotic genes. *BioEssays* **19**, 379–388.

Hall, B.K. (1998) *Evolutionary Developmental Biology*. 2nd ed. Chapman and Hall, London.

Herrera, C.M., Cerdá, X., García, M.B., Guitián, J., Medrano, M., Rey, P.J. and Sánchez-Lafuente, A.M. (2002) Floral integration, phenotypic covariance structure and pollinator variation in bumblebee-pollinated *Helleborus foetidus*. *J. Evol. Biol.* **15**, 108–121.

Hsu, T.C., Liu, H.C., Wang, J.S., Chen, R.W., Wang, Y.C. and Lin, B.L. (2001) Early genes responsive to abscisic acid during heterophyllous induction in *Marsilea quadrifolia*. *Plant Mol. Biol.* **47**, 703–715.

Inoue, T. and Thomas, J.H. (2000) Suppressors of transforming growth factor-ß pathway mutants in the *Caenorhabditis elegans* dauer formation pathway. *Genetics* **156**, 1035–1046.

Itoha, J.-I., Hasegawab, A., Kitanob, H. and Nagatoa, Y. (1998) A recessive heterochronic mutation, *plastochron1*, shortens the plastochron and elongates the vegetative phase in rice. *Plant Cell* **10**, 1511–1522.

Johnson, A.S. and Koehl, M.A.R. (1994) Maintenance of dynamic strain similarity and environmental stress factor in different flow habitats: thallus allometry and material properties of a giant kelp. *J. Exp. Biol.* **195**, 381–410.

Kassen, R. and Bell, G. (1998) Experimental evolution in *Chlamydomonas*. IV. Selection in environments that vary through time at different scales. *Heredity* **80**, 732–740.

Kauffman, S.A. (1993) *The Origins of Order: Self-organization and Selection in Evolution*. Oxford University Press, New York.

Koch, P.B., Lorenz, U., Brakefield, P.B. and ffrench-Constant, R.H. (2000) Butterfly wing pattern mutants: developmental heterochrony and co-ordinately regulated phenotypes. *Dev. Gen. Evol.* **210**, 536–544.

Larsen, P.L., Albert, P.S. and Riddle, D.L. (1995) Genes that regulate both development and longevity in *Caenorhabditis elegans*. *Genetics* **139**, 1567–1583.

Leamy, L.J., Routman, E.J. and Cheverud, J.M. (1999) Quantitative trait loci for early- and late-developing skull characters in mice: a test of the genetic independence model of morphological integration. *Am. Nat.* **153**, 201–214.

Lechowicz, M.J. and Blais, P.A. (1988) Assessing the contributions of multiple interacting traits to plant reproductive success: environmental dependence. *J. Evol. Biol.* **1**, 255–273.

Leiss, K.A. and Müller-Schärer, H. (2001) Performance of reciprocally sown populations of *Senecio vulgaris* from ruderal and agricultural habitats. *Oecologia* **128**, 210–216.

Lewontin, R.C. (1957) The adaptations of populations to varying environments. *Cold Spring Harbor Symp. Quant. Biol.* **22**, 395–408.

Li, J.M. and Chory, J. (1999) Brassinosteroid actions in plants. *J. Exp. Bot.* **50**, 275–282.

Li, P. and Johnston, M.O. (2000) Heterochrony in plant evolutionary studies through the twentieth century. *Bot. Rev.* **66**, 57–88.

Lloyd, D.G. (1984) Variation strategies of plants in heterogeneous environments. *Biol. J. Linn. Soc.* **21**, 357–385.

Lortie, C.J. and Aarssen, L.W. (1996) The specialization hypothesis for phenotypic plasticity in plants. *Internat. J. Plant Sci.* **157**, 484–487.

Lukens, L. and Doebley, J. (2001) Molecular evolution of the teosinte branched gene among maize and related grasses. *Mol. Biol. Evol.* **18**, 627–638.

Lynch, M. and Conery, J.S. (2000) The evolutionary fates and consequences of duplicate genes. *Science* **290**, 1151–1155.

Ma, L.G., Li, J.M., Qu, L.J., Hager, J., Chen, Z.L., Zhao, H.Y. and Deng, X.W. (2001) Light control of *Arabidopsis* development entails coordinated regulation of genome expression and cellular pathways. *Plant Cell* **13**, 2589–2607.

Malamy, J.E. and Ryan, K.S. (2001) Environmental regulation of lateral root initiation in *Arabidopsis*. *Plant Physiol.* **127**, 899–909.

Maloof, J.N., Borevitz, J.O. and Dabi, T. (2001) Natural variation in light sensitivity of *Arabidopsis*. *Nature Genet.* **29**, 441–446.

Mathews, S. and Sharrock, R.A. (1997) Phytochrome gene diversity. *Plant Cell Env.* **20**, 666–671.

Mattick, J.S. (2001) Non-coding RNAs: the architects of eukaryotic complexity. *EMBO Reports* **2**, 986–991.

McNamara, K.J. (1995) *Evolutionary Change and Heterochrony*. John Wiley and Sons, Chichester.

McSteen, P. and Hake, S. (1998) Genetic control of plant development. *Cur. Opi. Biotech.* **9**, 189–195.

Merilä, J., Laurila, A., Pahkala, M., Rasanen, K. and Laugen, A.T. (2000) Adaptive phenotypic plasticity in timing of metamorphosis in the common frog *Rana temporaria*. *Ecoscience* **7**, 18–24.

Meyerowitz, E.M. (2002) Plants compared to animals: the broadest comparative study of development. *Science* **295**, 1482–1485.

Millar, A.J. (1999) Biological clocks in *Arabidopsis thaliana*. *New Phytol*. **141**, 175–197.

Møller, S.G. and Chua, N.H. (1999) Interactions and intersections of plant signaling pathways. *J. Mol. Biol*. **293**, 219–234.

Morgan, D.C. and Smith, H. (1978) The function of phytochrome in the natural environment. VII. The relationship between phytochrome photo-equilibrium and development in light-grown *Chenopodium album* L. *Planta* **142**, 187–193.

Morgan, D.C. and Smith, H. (1979) A systematic relationship between phytochrome-controlled development and species habitat for plants grown in simulated natural radiation. *Planta* **145**, 253–259.

Morris, P.C. (2001) MAP kinase signal transduction pathways in plants. *New Phytol*. **151**, 67–89.

Newman, R.A. (1992) Adaptive plasticity in amphibian metamorphosis. *BioScience* **42**, 671–678.

Nicotra, A.B., Chazdon, R.L. and Schlichting, C.D. (1997) Patterns of genotypic variation and phenotypic plasticity of light response in two tropical *Piper* species. *Am. J. Bot*. **84**, 1542–1552.

Oberholzer, V., Durbin, M.L. and Clegg, M.T. (2000) Comparative genomics of chalcone synthase and Myb genes in the grass family. *Genes Genet. Syst*. **75**, 1–16.

Olson, E.C. and Miller, R.L. (1958) *Morphological Integration*. University of Chicago Press, Chicago, IL.

Pál, C. and Miklos, I. (1999) Epigenetic inheritance, genetic assimilation and speciation. *J. Theor. Biol*. **200**, 19–37.

Pickett, F.B. and Meeks-Wagner, D.R. (1995) Seeing double – appreciating genetic redundancy. *Plant Cell* **7**, 1347–1356.

Pigliucci, M. and Schlichting, C.D. (1998) Reaction norms of *Arabidopsis*. V. Flowering time controls phenotypic architecture in response to nutrient stress. *J. Evol. Biol*. **11**, 285–301.

Pigliucci, M. and Schmitt, J. (1999) Genes affecting phenotypic plasticity in *Arabidopsis*: pleiotropic effects and reproductive fitness of photomorphogenic mutants. *J. Evol. Biol*. **13**, 551–562.

Purugganan, M.D. (2000) The molecular population genetics of regulatory genes. *Mol. Ecol*. **9**, 1451–1461.

Purugannan, M.D., Rounsley, S.D., Schmidt, R.J. and Yanofsky, M.F. (1995) Molecular evolution of flower development: diversification of the plant MADS-box regulatory gene family. *Genetics* **140**, 354–356.

Quail, P.H. (1997) The phytochromes: a biochemical mechanism of signaling in sight? *BioEssays* **19**, 571–579.

Quail, P.H. (2002) Phytochrome photosensory signalling networks. *Nature Rev. Mol. Cell Biol*. **3**, 85–93.

Raff, R.A. (1996) *The Shape of Life*: *Genes*, *Development*, *and the Evolution of Animal Form*. University of Chicago Press, Chicago, IL.

Reboud, X. and Bell, G. (1997) Experimental evolution in *Chlamydomonas*. III. Evolution of specialist and generalist types in environments that vary in space and time. *Heredity* **78**, 507–514.

Relyea, R.A. (2001) The lasting effects of adaptive plasticity: predator-induced tadpoles become long-legged frogs. *Ecology* **82**, 1947–1955.

Robinson, B.W. and Dukas, R. (1999) The influence of phenotypic modifications on evolution: the Baldwin effect and modern perspectives. *Oikos* **85**, 582–589.

Roskam, J.C. and Brakefield, P.M. (1999) Seasonal polyphenism in *Bicyclus* (Lepidoptera: Satyridae) butterflies: different climates need different cues. *Biol. J. Linn. Soc*. **66**, 345–356.

Ruan, Y., Gilmore, J. and Conner, T. (1998) Towards *Arabidopsis* genome analysis: monitoring expression profiles of 1400 genes using cDNA microarrays. *Plant J*. **15**, 821–833.

Sachs, T. (1988) Ontogeny and phylogeny: phytohormones as indicators of labile changes. In L.D. Gottlieb and S.K. Jain (eds) *Plant Evolutionary Biology*. Chapman and Hall, London.

Salser, S.J. and Kenyon, C. (1996) A *C. elegans Hox* gene switches on, off, on and off again to regulate proliferation, differentiation and morphogenesis. *Development* **122**, 1651–1661.

Schenk, P.M., Kazan, K., Wilson, I., Anderson, J.P., Richmond, T., Somerville, S.C. and Manners, J.M. (2000) Coordinated plant defense responses in *Arabidopsis* revealed by microarray analysis. *Proc. Nat. Acad. Sci. USA* **97**, 11655–11660.

Schlichting, C.D. (1989a) Phenotypic integration and environmental change. *BioScience* **39**, 460–464.

Schlichting, C.D. (1989b) Phenotypic plasticity in *Phlox*. II. Plasticity of character correlations. *Oecologia* **78**, 496–501.

Schlichting, C.D. (in press) The role of phenotypic plasticity in diversification. In T.J. DeWitt and S.M. Scheiner (eds) *Phenotypic Plasticity*: *Functional and Conceptual Approaches*. Oxford University Press, Oxford.

Schlichting, C.D. and Pigliucci, M. (1995a) Lost in phenotypic space: environment-dependent morphology in *Phlox drummondii* (Polemoniaceae). *Internat. J. Plant Sci.* **156**, 542–546.

Schlichting, C.D. and Pigliucci, M. (1995b) Gene regulation, quantitative genetics and the evolution of reaction norms. *Evol. Ecol.* **9**, 154–168.

Schlichting, C.D. and Pigliucci, M. (1998) *Phenotypic Evolution*: *A Reaction Norm Perspective*. Sinauer Associates, Sunderland, MA.

Schmalhausen, I.I. (1949) *Factors of Evolution*. Blakiston, Philadelphia, PA.

Schmitt, J., McCormac, A.C. and 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.

Schmitt, J., Dudley, S.A. and Pigliucci, M. (1999) Manipulative approaches to testing adaptive plasticity: Phytochrome-mediated shade-avoidance responses in plants. *Am. Nat.* **154**(Supplement), S43–S54.

Scopel, A.L., Ballare, C.L. and Sanchez, R.A. (1991) Induction of extreme light sensitivity in buried weed seeds and its role in the perception of soil cultivations. *Plant Cell Env.* **14**, 501–508.

Seki, M., Narusaka, M., Abe, H., Kasuga, M., Yamaguchi-Shinozaki, K., Carninci, P., Hayashizaki, Y. and Shinozaki, K. (2001) Monitoring the expression pattern of 1300 *Arabidopsis* genes under drought and cold stresses by using a full-length cDNA microarray. *Plant Cell* **13**, 61–72.

Serna, L. and Fenoll, C. (1997) Tracing the ontogeny of stomatal clusters in *Arabidopsis* with molecular markers. *Plant J.* **12**, 747–755.

Smekens, M.J. and van Tienderen, P.H. (2001) Genetic variation and plasticity of *Plantago coronopus* under saline conditions. *Acta Oecol.* **22**, 187–200.

Smith, H. (1982) Light quality, photoreception, and plant strategy. *Ann. Rev. Plant Physiol.* **33**, 481–518.

Smith, H. (1990) Signal perception, differential expression within multigene families and the molecular basis of phenotypic plasticity. *Plant Cell Env.* **13**, 585–594.

Smith, H. (1995) Physiological and ecological function within the phytochrome family. *Ann. Rev. Pl. Physiol. Plant Mol. Biol.* **46**, 289–315.

Smith, H. (2000) Phytochromes and light signal perception by plants – an emerging synthesis. *Nature* **407**, 585–591.

Smith, H. and Whitelam, G.C. (1997) The shade avoidance syndrome: multiple responses mediated by multiple phytochromes. *Plant Cell Env.* **20**, 840–844.

Stearns, S.C. and Kawecki, T.J. (1994) Fitness sensitivity and the canalization of life history traits. *Evolution* **48**, 1438–1450.

Stearns, S.C., Kaiser, M. and Kawecki, T.J. (1995) The differential genetic and environmental canalization of fitness components in *Drosophila melanogaster*. *J. Evol. Biol.* **8**, 539–557.

Stuefer, J.F., de Kroon, H. and During, H.J. (1996) Exploitation of environmental heterogeneity by spatial division of labour in a clonal plant. *Funct. Ecol.* **10**, 328–344.

Tardieu, C. (1999) Ontogeny and phylogeny of femoro-tibial characters in humans and hominid fossils: Functional influence and genetic determinism. *Am. J. Phys. Anthropol.* **110**, 365–377.

Tautz, D. (1992) Redundancies, development and the flow of information. *Bioessays* **14**, 263–266.

Taylor, D.R. and Aarssen, L.W. (1988) An interpretation of phenotypic plasticity in *Agropyron repens* (Gramineae). *Am. J. Bot.* **75**, 401–413.

Tepperman, J.M., Zhu, T., Chang, H.-S., Wang, X. and Quail, P.H. (2001) Multiple transcription-factor genes are early targets of phytochrome A signaling. *Proc. Nat. Acad. Sci. USA* **98**, 9437–9442.

Ting, C.T., Tsaur, S.C., Wu, M.L. and Wu, C.I. (1998) A rapidly evolving homeobox at the site of a hybrid sterility gene. *Science* **282**, 1501–1504.

Tucic, B., Tomic, V., Avramov, S. and Pemac, D. (1998) Testing the adaptive plasticity of *Iris pumila* leaf traits to natural light conditions using phenotypic selection analysis. *Acta Oecol.* **19**, 473–481.

Van Buskirk, J. and McCollum, S.A. (2000) Functional mechanisms of an inducible defence in tadpoles: morphology and behaviour influence mortality risk from predation. *J. Evol. Biol.* **13**, 336–347.

Van De Peer, Y, T.J., Braasch, I. and Meyer, A. (2001) The ghost of selection past: rates of evolution and functional divergence of anciently duplicated genes. *J. Mol. Evol.* **53**, 436–446.

van Hinsberg, A. (1998) Maternal and ambient environmental effects of light on germination in *Plantago lanceolata*: correlated responses to selection on leaf length. *Funct. Ecol.* **12**, 825–833.

van Kleunen, M. and Fischer, M. (2001) Adaptive evolution of plastic foraging responses in a clonal plant. *Ecology* **82**, 3309–3319.

van Kleunen, M., Fischer, M. and Schmid, B. (2000) Costs of plasticity in foraging characteristics of the clonal plant *Ranunculus reptans*. *Evolution* **54**, 1947–1955.

Waddington, C.H. (1953) Genetic assimilation of an acquired character. *Evolution* **7**, 118–126.

Wagner, A. (1999) Redundant gene functions and natural selection. *J. Evol. Biol.* **12**, 1–16.

Wagner, G.P., Booth, G. and Bagheri-Chaichian, H. (1997) A population genetic theory of canalization. *Evolution* **51**, 329–347.

Waitt, D.E. and Levin, D.A. (1993) Phenotypic integration and plastic correlations in *Phlox drummondii* (Polemoniaceae). *Am. J. Bot.* **80**, 1224–1233.

Walker, D.W., McColl, G., Jenkins, N.L., Harris, J. and Lithgow, G.R. (2000) Evolution of lifespan in *C. elegans*. *Nature* **405**, 296–297.

Watson, M.A. and Casper, B.B. (1984) Morphogenetic constraints on patterns of carbon distribution in plants. *Ann. Rev. Ecol. Syst.* **15**, 233–258.

Weinig, C. (2000) Limits to adaptive plasticity: temperature and photoperiod influence shade-avoidance responses. *Am. J. Bot.* **87**, 1660–1668.

West-Eberhard, M.J. (1986) Alternative adaptations, speciation and phylogeny. *Proc. Nat. Acad. Sci. USA* **83**, 1388–1392.

West-Eberhard, M.J. (1989) Phenotypic plasticity and the origins of diversity. *Ann. Rev. Ecol. Syst.* **20**, 249–278.

Whitelam, G.C. and Devlin, P.F. (1997) Roles of different phytochromes in *Arabidopsis* photomorphogenesis. *Plant Cell Env.* **20**, 752–759.

Wilson, R.S. (2001) Geographic variation in thermal sensitivity of jumping performance in the frog *Limnodynastes peronii*. *J. Exp. Biol.* **204**, 4227–4236.

Wimberger, P.H. (1994) Trophic polymorphisms, plasticity, and speciation in vertebrates. In D.J. Stouder, K. Fresh and R.J. Feller (eds). *Theory and Application in Fish Feeding Ecology*. University of South Carolina Press, Columbia, SC.

Wu, X., Weigel, D. and Wigge, P.A. (2002) Signaling in plants by intercellular RNA and protein movement. *Genes Dev.* **16**, 151–158.

Zhang, H. and Forde, B.G. (1998) An *Arabidopsis* MADS box gene that controls nutrient-induced changes in root architecture. *Science* **279**, 407–409.