



A developmental morphologist's perspective on plasticity

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Abstract. This series of essays addresses plasticity from the perspective of developmental morphology. The first essay deals with the problem of distinguishing between plasticity and other types of ontogenetic variation. In a temporally varying environment, morphological plasticity may be expressed as the production of a succession of different metamers. However, even in a constant environment, plant metamers can vary dramatically, a phenomenon known as heteroblasty. Because heteroblasty and plasticity can yield similar patterns of ontogenetic variation, the two are often confounded in analyses of developmental plasticity. The second essay discusses the integration of plant phenotypic responses and finds that the evidence for integration is equivocal. The third section shows that developmental properties can constrain the expression of morphological plasticity. Developmental lags and the 'epiphenotype problem' are particularly important features for analyses of the evolution and expression of plasticity. Finally, in answer to the question of strategies for studying plasticity, I emphasize the need for research at multiple levels and for the inclusion of a historical or phylogenetic perspective.

Key words: architectural effect, developmental integration, epiphenotype, heteroblasty, lag-time, limits to plasticity, morphology, plasticity, preformation, reaction norm

The adaptive domain of developmental plasticity and contiguous phenomena

Developmental plasticity, metamorphosis, and intra-organismic variation

Prior to a discussion of the 'adaptive domain' of developmental plasticity, plasticity must be distinguished from the other forms or sources of organismic variation listed in the subheading of this question: metamorphosis, differentiation, and genetic variation. I wish to expand upon the category 'metamorphosis' and the relation between metamorphosis and developmental plasticity.

If metamorphosis can be interpreted as genetically determined ontogenetic changes in whole-organism phenotypes (other definitions are provided by Schlichting and Smith, 2002), then plasticity and metamorphosis in plants can

yield similar patterns of intra-individual phenotypic variation. Metamorphosis in plants includes the phenomenon of heteroblasty, i.e., genetically programmed changes among successive metamers that occur as a normal expression of whole-plant ontogeny (Ashby, 1948; Jones, 1999). Heteroblastic changes can include such traits as shoot orientation, phyllotaxy, leaf size, shape, anatomy and biochemistry, internode length and width, the fates of lateral meristems, and the capacity to flower (Goebel, 1900; Arber, 1919; Allsopp 1965, 1967). The term heteroblasty has also been extended to include more subtle shifts in qualitative and quantitative features of vegetative shoots (Poethig, 1988; 1990; Kerstetter and Poethig, 1998; reviewed in Diggle, 1999) and even to morphological changes in floral form within inflorescence axes (Lord, 1979). It has long been appreciated that heteroblasty and plasticity can yield very similar phenotypes (for reviews of the history of confusion between these phenomena, see Arber, 1919; Cook, 1969; Jones, 1999) and that both plastic and heteroblastic variation along the length of a shoot may represent adaptation to environmental heterogeneity (Goebel, 1900; Winn, 1996, 1999b).

Because genetically programmed ontogenetic changes in form and function (e.g., metamorphosis) can be similar in pattern to environmentally induced changes (plasticity), the two phenomena are often confounded in analyses of phenotypic variation in plants. Although the phenomenon of heteroblasty is appreciated by plant biologists, there is a persistent underlying assumption that because plants are composed of repeating metameric units, all metamers should be inherently phenotypically similar. As a consequence, much intra-individual phenotypic variation among metamers is interpreted as phenotypically plastic responses to changes in the internal or external environment without careful consideration and testing of alternative explanations.

For example, a review of the literature on variation in floral form and function within inflorescences (Diggle, 1995), found that most authors attributed such variation exclusively to plasticity of resource allocation. With a few notable exceptions (Macnair and Cumbes, 1990; Wolfe, 1992; Mossop *et al.*, 1994; Diggle, 1991a; see also Mazer and Delesalle, 1996; Nishikawa, 1998; Ladio and Aizen, 1999; Ashman and Hitchens, 2000), the possibility that there are ontogenetic shifts (heteroblasty/metamorphosis) of form and reproductive potential among flowers within inflorescences was not considered. Those experiments designed specifically to discriminate between plasticity and 'metamorphosis' demonstrated that some part of the variation routinely attributed to plasticity of resource allocation was, in fact, due to inherent differences among flowers and fruits within inflorescence axes. While such variation falls within the definition of metamorphosis, I have termed the inherent variation within axes 'architectural effects' in recognition of the association of this variation with the position of the flower, fruit, or other structures within the overall architecture of a plant (Diggle, 1995, 1997b, 2002).

An example of the potential for architectural effects to confound the study of phenotypic plasticity is provided by analyses of sex expression in andromonoecious *Solanum hirtum* (Solanaceae). Experimental manipulation of fruit set demonstrated that the production of staminate flowers in *S. hirtum* is phenotypically plastic (Diggle, 1993, 1994). Under conditions of no fruit-set plants produce only, or predominantly, hermaphrodite flowers. Under conditions of high fruit-set plants produce staminate flowers (with reduced, non-functional gynoecia) in distal positions within each inflorescence (Diggle, 1991b). Thus, gynoecial development of distal flowers is plastic in response to the fruiting status of the plant (Diggle, 1991a, 1994). In addition to the non-functional gynoecium, however, staminate flowers also are smaller than the proximally located hermaphrodite flowers within the same inflorescence (Diggle, 1991b). Is this reduction in size also a plastic response to the presence of developing fruit (as is the loss of female function)? Or, is the variation in flower size due to a difference in location (hermaphrodite flowers are proximal whereas staminate flowers are distal within each inflorescence), that is, is this an architectural effect? Quantitative analyses of floral organ lengths that included appropriate architectural controls (Diggle, 1991b, 1995) showed that declining petal length is a plastic response, whereas declining anther length is an architectural effect; anthers of distal flowers are smaller than those of proximal flowers, regardless of treatment. The decline in ovary size of staminate flowers is due to both plasticity and position (Diggle, 1991b, 1995). Similarly, Ashman and Hitchens (2000) show substantial declines in floral organ size within inflorescences of *Fragaria virginiana* and this pattern is identical in fruiting and non-fruiting plants. Thus, in these taxa (and others reviewed by Diggle, 2002 (in press)) variation is architectural, rather than a plastic response to resource preemption.

For these studies and numerous others reviewed by Diggle (2002 in press) the absence of architectural analysis would have led to the incorrect conclusion that all of the proximal to distal variation of floral organs is attributable to phenotypically plastic responses to the presence of developing fruit. The pattern of intra-inflorescence variation produced by plasticity and by 'metamorphosis' may be identical, and only careful experimentation can reveal the actual source of morphological or functional variation. Perhaps even more significant to the study of plasticity, similar experiments with *Arabidopsis thaliana* (Brassicaceae) have demonstrated that architectural effects may not only mimic plasticity, as for example in *S. hirtum* and *F. virginiana* but may mask the true magnitude of plasticity, and/or cause misinterpretation of the direction of plasticity (Diggle, 1997b).

Experimental analyses of vegetative organs have also demonstrated the overlap between metamorphosis and plasticity. Winn (1996) has examined sources of intra-individual variation in leaf morphology, anatomy and

physiology in *Dicerandra linearifolia* (Lamiaceae). Consistent differences in these traits are observed between leaves produced in the winter and summer, and several of these differences are consistent with adaptive responses to seasonal changes in temperature. The observed pattern of change could be a manifestation of heteroblasty (metamorphosis), or of plasticity, or both.

Experiments that controlled for both leaf position (architecture, sensu Diggle, 1995) and environment, demonstrated that although environment (temperature), leaf position, and their interaction all contribute to variation in leaf traits, position has by far the greatest effect. Although the observed pattern of intra-individual variation in leaf traits is consistent with plastic responses to a changing thermal environment, this variation is primarily the result of a fixed ontogenetic progression of leaf types. Winn (1996) suggests that when the sequence of environments encountered by an individual is predictable, heteroblasty may be favored over plasticity (see Alpert and Simms 2002 for further discussion of environmental predictability and the evolution of plasticity). This hypothesis cannot yet be evaluated. Winn's (1996) study is one of the few to differentiate experimentally between the effects of plasticity and 'metamorphosis' on vegetative traits. Interestingly, Winn (1999a, b) shows that there is no evidence that the production of alternative leaf types is adaptive for this species. Thus, metamorphosis in *D. linearifolia* may persist because there is not selection against it.

The 'spheres' of fixed ontogenetic changes (metamorphosis) and plasticity not only overlap because they potentially can yield the same progressive change in the phenotype of a metamerism organism, they overlap because metamorphosis is subject to plasticity. The timing of ontogenetic changes is subject to environmental influence (Allsopp, 1967; Lee and Richards, 1991; Jones 1995; and examples given by Alpert and Simms, 2002). The best known examples concern the transition from 'juvenile' to 'adult' growth forms. In many taxa there are changes in leaf size, shape, phyllotaxy, etc. associated with this phase change (Goebel 1900; Jones 1999). These changes are genetically fixed, however, the absolute time at which they occur may be modified by the environment.

Confusion between metamorphosis and plasticity also arises because the same morphological changes can be heteroblastic (fixed) at one stage of ontogeny and plastic at another. For example, species of the genus *Monstera* are markedly heteroblastic vines (Madison, 1977; Lee and Richards, 1991). In most species, seeds germinate to form a plagiotropic stolon that produces minute scale leaves and long internodes. When the shoot encounters and begins to ascend a tree trunk (or other support), it produces overlapping asymmetric foliage leaves that are flattened against the tree and completely cover the stem. The heteroblastic sequence ends with a transition to the production of short internodes and adult foliage: large erect lobed or perforated leaves. Production of the adult foliage type continues unless the vine outgrows its support. Un-

supported adult axes have long internodes and bear reduced leaves. Thus, the production of small leaves may be fixed early in ontogeny (although the timing of the transition is plastic) but plastic in the adult phases of ontogeny and responsive to the presence or absence of a host or growth support.

Differentiation also may have some overlap with plasticity. In plants, the term differentiation, by definition the process of becoming different, might be most appropriately used to describe processes occurring in determinate structures such as individual organs or tissues, rather than to whole, indeterminate organisms. Thus, differentiation may overlap with patterns of plasticity expressed at sub-organismal levels. In fact, Schlichting and Pigliucci (1998) have suggested that the process of differentiation can be viewed as a developmental reaction norm, with developmental processes being a set of plastic responses to changes in the internal organismal environment; changes brought about by the process of development itself.

If, as argued by Schlichting and Pigliucci (1998), Cohen (1999, Chapter 17) and Schlichting and Smith (2002), development is a form of plastic response to a changing internal environment, then are the phenomena that I have referred to as metamorphosis/heteroblasty/architectural effects merely different forms or levels of plasticity? And, if so, is it useful to distinguish between these forms of fixed ontogenetic variation and plasticity? I would argue that this distinction is critical because it focuses attention on the particular trait and cue under study. Empirical analyses of plasticity should be done in reference to particular traits and particular environmental cues (external or internal). For example, in the discussion of intra-inflorescence variation above, the traits of interest were flower form and potential female function. The hypothesis was that observed intra-inflorescence variation is a phenotypically plastic response to the presence of developing fruit. That hypothesis has been falsified for numerous taxa (Diggle, 2002 in press), variation occurs even in the absence of developing fruit (the specific cue under study), that is, such a pattern is not plasticity as defined in the hypothesis. Nevertheless, there is clearly some underlying cause for the observed variation. That cause may well be a gradient in the 'internal environment' and intra-inflorescence variation could be a plastic response to this, as yet unidentified, internal cue. Thus, my call for distinguishing between fixed ontogenetic variation and plasticity is, in essence, complementary to the views of Schlichting and Pigliucci (1998). That is, variation among metamers during ontogeny might be explained as a plastic response to a gradient in the internal environment. However, if we continue to view intra-organismal variation as plastic responses to the external environment, we will never ask, let alone answer, questions about other sources (internal) of developmental variation (see also discussion of 'ontogenetic contingency' below).

If plant biologists wish to understand 'the adaptive domain' and 'functional limits' of differentiation, metamorphosis, and plasticity, we must be cognizant

of the potential to confound these phenomena and, when studying plasticity, use carefully designed experiments that control for developmental, and ontogenetic/architectural effects (e.g., Macnair and Cumbes, 1990; Wolfe, 1992; Diggle 1991a, 1995, 1997b; Mossop *et al.*, 1994; Winn 1996; Mazer and Del-salle, 1996; Ashman and Hitchens, 2000). Plasticity and metamorphosis likely interact or overlap to produce the range of intra-individual phenotypic variation observed among plants. Yet, until phenotypically plastic responses and proximate cues are isolated both conceptually and experimentally from other levels of response, a complete understanding of the evolution and function of plasticity, and its interaction with other processes that yield a variable phenotype in metameric organisms, is not possible.

Mechanisms of developmental plasticity

Are plant phenotypes integrated?

I would turn the first part of this question around and ask, to what extent do plants 'integrate multiple environmental and internal stimuli' and whether they have 'coordinated adaptive plastic responses'. At the morphological level, integration can refer to at least two different phenomena. An integrated plastic response can refer to coordinated quantitative changes of suites of morphological characters in response to environmental cues, or integration can refer to communication among parts of a metameric organism that is experiencing spatial or temporal heterogeneity. Can plants respond to 'multiple environmental stimuli' with either form of integration, and is it advantageous to do so?

If spatial variation in the environment is fine grained with respect to plant size, then metamers (or other units of morphological organization) may each develop under very different conditions and the organism as a whole will experience multiple environments. Clonal plants often have been used to examine the potential for integrated plastic responses to spatial environmental variability (see extensive discussion of clonal integration in the contributions by Alpert and Simms (2002), and Sachs (2002)). Physiological studies tend to show a high degree of integration among ramets (e.g., Lötscher and Hay, 1997; Humphrey and Pyke, 1997; de Kroon *et al.*, 1998; Stoll and Schmid, 1998). For example, nutrients tend to move from areas of higher to lower supply. In contrast, morphological and developmental analyses of the frequency and angle of branching and internode elongation show little integration among ramets (e.g., de Kroon and Schieving, 1990; Evans and Cain, 1995; Kembell and Marshall, 1995; Birch and Hutchings, 1999). Morphological units are generally relatively autonomous (Watson and Casper, 1984; Schlichting and Smith, 2002). In fact, the concept of clonal foraging in plants is dependent on a

high degree of ramet developmental independence (de Kroon and Schieving, 1990). Thus, the answer to the question of integration differs for physiological vs. morphological plasticity.

Empirical research on coordinated changes in suites of morphological characters has also been inconclusive. Many of these studies have been conducted with short-lived annuals where entire plants encounter a single environment. Various traits are measured and the relationship among traits across environments is subject to multivariate analysis. The hypothesis under examination is that functionally, developmentally, or genetically related traits should show correlated plastic responses. The results of these studies have been mixed. Many have shown that character correlations are not stable across environments (Adams, 1967; Primack and Antonovics, 1981; Antonovics and Primack, 1982; Singh and Chowdhury, 1983; Schlichting 1986, 1989a, b; Ariyo *et al.*, 1987; Lechowicz and Blais, 1988; Andersson, 1989; Pigliucci *et al.*, 1991, 1995; Pigliucci and Schlichting, 1998; Callahan and Waller, 2000; Pigliucci and Marlow, 2001). Other studies support the hypothesis of character integration: they have shown stability of correlations (Winn and Evans, 1991; Meerts, 1992; Kudoh *et al.*, 1996), that putatively functionally or developmentally related traits are more highly correlated across environments than other traits (Waitt and Levin, 1993); and that within populations, those individuals with the greatest integration of characters across environments have the highest fitness (Schlichting, 1989b). There is no information on the heritability of such correlations, and no analyses of large or long-lived plants.

To the extent that plant phenotypes, and phenotypic responses maybe integrated, what are the potential mechanisms? Metamers, and the meristems that produce them, do not develop in isolation. As stated in the question, the internal environment of the plant is a critical determinant of developmental response. The internal environment in which a meristem develops is influenced, in turn, by the past history of the individual on which it is borne, including ontogenetic stage and previous plastic responses, by the position of the meristem within the architectural ground plan (see section 1), and by other competing sinks (ontogenetic contingency; Diggle, 1994; Watson *et al.*, 1995; Pigliucci, 1998). The internal environment, therefore, provides the context in which a metamer/meristem develops and will play a large role in determining the morphological and/or anatomical response of that metamer to external environmental variables. Thus, 'ontogenetic contingency' may provide a mechanism of apparent morphological integration of plastic responses among plant parts. Sachs (2002) and Schlichting and Smith (2002) discuss the importance of long distance transport of information bearing molecules (hormones, mRNAs, etc.) as a potential mechanism for integration. These molecules form part of the 'internal environment' in which development occurs. Sachs (2002) also points out that the appearance of integration maybe an

inherent consequence of the correlative nature of plant development. If this is true, then 'integration', to the extent that it occurs in plants, is a ubiquitous character rather than the result of natural selection in particular taxa and should not be termed a 'coordinated adaptive plastic response'.

How do mechanisms of plasticity vary with ontogeny?

Plant ontogeny and construction must always be considered in any search for mechanisms of developmental plasticity. Morphological plasticity is inherently the result of developmental processes, and meristems and primordia are the sites of many aspects of development. Thus, it appears logical to target developmental processes occurring at the level of meristems for an examination of mechanisms of plasticity. However, studies that have addressed phenotypic plasticity from an explicitly developmental perspective demonstrate that analyses should occur at multiple levels of morphological and anatomical organization and must consider whole-organism ontogeny.

For example, analyses of sex expression in andromonoecious *S. hirtum* (introduced under question 1) show that a complete understanding of the mechanism of phenotypic plasticity cannot be obtained from analysis at the meristem level alone. Experimental analyses of *S. hirtum* at the level of whole-plants show that the numbers and proportions of hermaphrodite and staminate flowers produced by individuals vary with environment (Diggle, 1993). Developmental studies demonstrate that this plasticity of whole-plant sex expression is due to developmental plasticity at the level of individual floral meristems; gynoecial development is responsive to environmental conditions (Diggle, 1991a, 1994).

A meristem level approach, however, cannot completely explain the expression of plasticity observed at the whole-plant level. Architectural analyses that focus on the distribution of flower types within and among inflorescences of *S. hirtum*, demonstrate that not all floral primordia are plastic (Diggle, 1994). Only those flowers developing in distal positions within each inflorescence are capable of responding to environmental conditions by altering gynoecial development. Floral primordia initiated at basal positions within each inflorescence are invariably hermaphrodite. The development of these primordia is fixed and cannot be modified by the environment. Thus, flower-level plasticity varies ontogenetically as each new inflorescence develops.

In order to understand plasticity of sex expression in *S. hirtum* the analysis of 'mechanism' must take place on at least two levels, the level of the floral meristem and the level of whole plant (or whole branch) ontogeny. At the meristem level, the mechanism of whole-plant plasticity involves regulation of gynoecial development. Simultaneously, at the level of the inflorescence the mechanism of plasticity involves regulation of which meristems are capable of

response. This distinction has repercussions for the search for mechanisms at other levels. For example, a physiological or molecular genetic analysis should focus on what makes only some flowers plastic as well as how gynoecium development is regulated in those flowers that are plastic.

Interpretation of 'mechanisms' of vegetative plasticity also depends on ontogenetic analysis. For example, leaf shape in *Cucurbita argyrosperma* subspecies *sororia* (Cucurbitaceae) is phenotypically plastic (Jones, 1995). This taxon is heteroblastic and leaf shape normally changes from slightly lobed early in ontogeny to highly lobed later in ontogeny (Jones, 1992). Under shaded conditions, however, all leaves are slightly lobed. Historically, this type of response to shade in a heteroblastic species has been thought to result from a prolongation of the juvenile phase of whole-plant development (e.g., Goebel, 1908; see Jones, 1995, for a review of these ideas). Developmental analysis of *C. argyrosperma* (Jones, 1995) shows this hypothesis to be incorrect. Comparison of plants grown under shaded conditions with plants grown in full sun shows that both sun and shade plants go through the same heteroblastic changes of early leaf development. The less lobed leaves of the shaded plants were the result of responses to shade occurring in late stages of the development of individual leaves. Thus, the differences in whole plant morphology between sun and shade grown plants are the result of plastic responses occurring at the level of individual developing organs rather than at the level of whole-plant transition from juvenile to adult stages. Identification of 'mechanism' in this case depends explicitly on understanding the development of individual leaves within the heteroblastic context of whole-plant ontogeny.

Developmental plasticity and evolution

Developmental limits to morphological plasticity

Plasticity of morphological and/or anatomical traits is inherently the result of environmental modification of development, yet we know little of the role of development in the evolution of phenotypic plasticity. Are there particular patterns or modes of plant development that may either enable or constrain the evolution of plasticity? A number of constraints, including both costs and limits, on the evolution of phenotypic plasticity have been suggested (reviewed by DeWitt *et al.*, 1998) and several hypothesized limits to the benefits of plasticity may be related to developmental features of plants. These include: (a) lag-time limit; (b) developmental range limit; and (c) epiphenotype problem.

Lag-time limits occur when there is a substantial time interval between the perception of an environmental cue and the production of an altered phenotype (see also the extensive discussion of this problem from an alternative point

of view in Alpert and Simms (2002)). Such delayed responses may result in a mismatch between the current environment and the phenotype. Theoretical models (Padilla and Adolph, 1996) show that lag-times can constrain the evolution of plasticity even when there is no cost to plasticity. The duration of a lag-time will be determined, in part, by the rate and duration of development of the structures that are responding. The interval between perception of an environmental change and a resulting morphological change is likely to be shortest in plants that initiate and mature new structures rapidly (see also Ackerly, 1997 for a discussion of physiological plasticity and lag-time). For example, changes in the light environment (e.g., by formation of forest gaps during tree falls) may result in the development of 'sun' leaves on shoots that had been producing 'shade' leaves (see Lichtenthaler, 1985 for a discussion of differences between sun and shade leaves). The rapidity of this response will be determined by the normal plastochron interval of the plant. Plastochron intervals may range from less than 2 days in rapidly growing annuals, to weeks in slowly growing, long-lived perennials (Dale and Milthorpe, 1983). The capacity to alter the plastochron interval has been demonstrated for some annuals (Ackerly *et al.*, 1992; Jones, 1995; Ma *et al.*, 1997; Roderiguez *et al.*, 1998; Pengelly *et al.*, 1999), however the change is a small fraction of the total time between leaf initiation events. The capacity for change in plastochron intervals of perennials is unknown.

In addition to the effect of growth rate, lag-times will also be influenced by the duration of growth, that is the total time period required to construct a new organ or other structure. An extreme example is provided by the common developmental pattern known as preformation, the initiation of determinate structures (e.g., leaves, inflorescences, flowers) one or more growing seasons prior to their complete maturation and function (Hallé *et al.*, 1978; Diggle, 1997a). As a result of this prolonged period of development (potentially spanning portions of at least 2 years), responses to environmental variation may be delayed significantly (Diggle, 1997a; Aydellote and Diggle, 1997; Geber *et al.*, 1997). Preformation is common among temperate trees, shrubs and herbaceous perennials and is nearly ubiquitous among taxa of the arctic and alpine tundra (reviewed by Sørensen, 1941; Diggle, 1997a). In *Polygonum viviparum* (Polygonaceae), a common component of many arctic and alpine plant communities, development of leaves and inflorescences requires 4 years from initiation to maturation (Diggle, 1997a).

The potential for such extreme preformation to limit plasticity in *P. viviparum* was tested by experimental defoliation. Plants are unable to respond vegetatively to this treatment within a growing season. No additional leaves are matured to replace those removed despite the presence of preformed primordia within the apical bud (Diggle, unpublished data). Developmental analysis, however, shows that plants do respond to defoliation both within and among

years. The rate of leaf initiation by the shoot apical meristem, and the growth rates of individual primordia are reduced immediately following defoliation. Nevertheless, this reduction in growth is not evident on an ecologically relevant scale until those leaf primordia develop to functional maturity, 1 and 2 years following the treatment. Although plastic developmental responses may be initiated rapidly in response to an environmental cue (e.g., leaf loss), the manifestation of these responses as an altered mature phenotype is delayed by preformation and the length of time required to produce a new organ. Experiments with other preforming alpine perennials have shown similar delays in morphological response (Bowman and Connant, 1994; Aydelotte, 1996).

Because preformation is also extremely common among temperate perennials, it is likely to impose significant time-lags on the expression of plasticity in these environments as well. For example, in temperate *Populus* species, preformed (proleptic) and neoformed (sylleptic) shoots can occur on the same individual. Preformed shoots are far less plastic than neoformed shoots (Wu and Stettler, 1998). Additional analyses are required to determine if the time-lags associated with preformation and/or slow growth result in a mismatch between the phenotype and the environment in *Populus*.

'Developmental range' has also been suggested to limit the evolution of phenotypic plasticity when fixed (non-plastic) development is better able than plastic development to produce an extreme phenotype (DeWitt *et al.*, 1998). While this hypothesis obviously concerns development, I know of no features of development expressed at the morphological or anatomical level that would lead, a priori, to this conclusion, nor do I know of any empirical tests of this phenomenon using plants. Consideration of developmental genetic mechanisms, however, may provide a different perspective. Maintenance of a system capable of producing multiple developmental outcomes may come at the expense of maximizing the efficiency of producing any one outcome.

'The epiphenotype problem' occurs if a phenotype built by an add-on to a mature organism (in response to an altered environment) is not as good as one that is integrated during ontogeny (as in a fixed phenotype; DeWitt *et al.*, 1998). Whereas this may be a significant problem in organisms with unitary development, the metameric nature of plant morphology may alleviate this limit. Additional units of plant phenotypes are normally 'added on' by the process of apical indeterminate growth. Thus, plastic and non-plastic phenotypes may both be achieved by the same mechanism and would not be expected to differ in epiphenotype. Conversely, however, plants may suffer from the 'epiphenotype problem' if the turnover of metamers is slow relative to the duration of the new environmental conditions. Because of indeterminate accretion of parts, plants may express both appropriate and inappropriate phenotypes when new organs are added but older organs persist longer than the environment that induced them. A shoot may produce shade leaves when

overtopped by a neighbor, but may retain the older (proximal) sun leaves as well. The duration of this overlap will depend on both the rate of leaf initiation and the rate of leaf senescence. For example, *Cecropia obtusifolia* and *Heliocarpa appendiculatus* are tropical pioneer species that differ in rates of leaf turnover. Experimental manipulation of light levels showed that it took saplings of *Cecropia* 35–40 days to replace 50% of its leaf area, whereas it took *Heliocarpa* only 20 days (Ackerly, 1997). In these cases, the limitations of the epiphenotype have not limited plasticity. Lineages, in which the rates of leaf replacement are variable among taxa, should be examined for a relationship between rates of leaf turnover and plasticity of individual leaves.

The ‘epiphenotype problem’ may also include instances when plastic responses at one stage of ontogeny limit plasticity at subsequent stages. For example, Wenig and Delph (2001) have shown that plastic stem elongation of *Abutilon theophrastis* that occurs in response to shade early in ontogeny limits plastic responses to the same cue later in ontogeny. Early elongation may reduce subsequent responses if mechanical stability is affected. Wenig and Delph (2001) refer to this as an ‘opportunity cost’ of plasticity. Similarly, Geber (1989) has shown that early allocation of meristems to flower production rather than production of vegetative branches limits future flower production. These experiments did not test for plasticity, but clearly in taxa for which flowering is plastic, early plasticity can limit opportunities for plasticity later.

Consideration of these three potential developmental limits shows that lag-times clearly exist and can constrain phenotypic responses to environmental change. Developmental range and epiphenotype may also affect the evolution and expression of plasticity, and this appears to be a fertile area for future research.

Strategies in studying developmental plasticity

Progress in understanding developmental plasticity will undoubtedly be the result of research at multiple levels and from diverse perspectives (Pigliucci, 1996; Schlichting and Pigliucci, 1998). The formulation of molecular-genetic models is but one aspect of such a multifaceted approach and certainly will be useful in focusing attention on particular aspects of the developmental plasticity (Via *et al.*, 1995, but see de Jong, 1995), but the genetic basis of plasticity is ultimately an empirical question, and is most likely to yield to a molecular genetic approach. This approach must be pluralistic, however, and consider quantitative control as well as major genes (Stratton, 1998).

Integration of research at multiple organizational levels will depend critically on the recognition that ‘development’ can be studied meaningfully at each of

multiple levels. There is no one 'true' method or level of developmental analysis. For example, to an evolutionary ecologist, a developmental analysis might encompass measurements of plant height, branch number, and leaf area over time (e.g., Pigliucci and Schlichting, 1995; Kudoh *et al.*, 1996; Pigliucci *et al.*, 1997; Wayne *et al.*, 1998; Thomas *et al.*, 1999), to a morphologist, a developmental approach may involve the examination of the unfolding of plant architecture or organ and tissue level processes (e.g., Cook, 1969), and a developmental geneticist may study the progression of gene expression (reviewed by Pigliucci, 1996, 1998). Each researcher is studying development, each may observe plastic responses, and each may formulate valid conclusions about mechanism. Discussion among those involved in research at different levels, and respect for the contributions of each level of inquiry should breach most 'interdisciplinary barriers'.

Although research at many levels, from the molecular through the population, is currently quite active, an approach that is missing from evolutionary analyses of developmental plasticity is a historical or phylogenetic one. There are few analyses of the distribution of plasticity or of the range of expression of plasticity within clades of organisms (Jockusch, 1998; Pigliucci *et al.*, 1999). We do not know when plasticity has arisen within particular groups, what the antecedent developmental processes might be, and how developmental plasticity has diversified within lineages of plants. Phylogenetic biologists should also be included in the attempt to fully understand the evolution of developmental plasticity.

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