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Evolutionary Importance and Pattern of Phenotypic Plasticity

Insights Gained from Development

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Development and Phenotypic Plasticity

Phenotypic plasticity in morphological traits is fundamentally a developmental phenomenon; developmental pathways are expressed differently in response to specific environmental factors to produce continuously varying traits or discrete, alternative phenotypes. Although the study of phenotypic plasticity currently enjoys a renaissance in evolutionary biology, it is generally considered a substantially less interesting topic in developmental biology (Raff 1996) for two reasons. First, the goals of developmental biology are largely concerned with determining how proximate mechanisms regulate and integrate trait ontogeny and how alterations of these mechanisms produce changes at a macroevolutionary scale; developmental biology seldom considers how proximate mechanisms generate the phenotypic variation among individuals that is important in microevolution. Second, phenotypic variation among individuals, especially environmentally induced variation, is usually viewed as noise in developmental studies—something to be minimized when the research goal is to understand the proximate mechanisms regulating ontogeny. Consequently, the organisms chosen as models of development share several characteristics and are raised in ways that may generate bias, suggesting widespread conservation of developmental mechanisms across taxa and typically canalized development that generates invariant phenotypes within taxa (Bolker 1995; Grbi and Strand 1998). Such perceived absence of developmental (phenotypic) plasticity in the laboratories of developmental biologists, coupled with a lack of focus on variation among individuals, may have contributed substantially to the lack of information on, or even interest in, the proximate bases of plasticity.

The paucity of information regarding the proximate basis of observed phenotypic variation, however, is not necessarily viewed as important by evolutionary biologists. This is because the statistical techniques typically used to study adaptive evolution are thought to incorporate, albeit indirectly, the important effects of development on phenotype production and evolution. For example, the developmental mechanisms that influence the distribution of traits or trait suites are thought to be captured in the variance/covariance matrix (Cheverud 1984) used to predict the multivariate response of a population to selection. Strong correlations among traits reflect developmental integration, which ties the trait complexes together evolutionarily (Lande 1979; Lande and Arnold 1983).

Such indirect, statistical approaches to studying what is fundamentally a developmental phenomenon have generated problems for the study of phenotypic evolution, and the evolution of phenotypic plasticity in particular. One well-known problem is the controversy over the genetic basis for phenotypic plasticity (chapter 3; see also Scheiner 1993b; Schlichting and Pigliucci 1993; 1993a,b, 1994; Via et al. 1995; Pigliucci 1996a). Although other problems introduced by a purely statistical approach (discussed below) are more subtle, they may be important, if not perhaps more important. In the next section, we illustrate how such methods that consider developmental processes only indirectly have the potential to mislead investigators into underestimating the magnitude, and consequently the evolutionary importance, of phenotypic plasticity.

Development and Reaction Norms

To examine the effects of the developmental environment on the distribution of phenotypes in a population, reaction norms are typically generated for the focal trait (figure 5.1; see also figure 3.1). These measurements of the cross-environmental phenotypic variance are thought to reflect the developmental mechanisms producing trait variance and covariance. Strong correlations are interpreted as resulting from expression of the same developmental processes across environments; looser correlations result from different expression of developmental processes across environments.

Predictions of the evolutionary response of a population to selection that are based on estimations of the variance and cross-environmental covariance in phenotypes (as manifested in reaction norms) may be inaccurate for at least two reasons, both of which may be addressed by considering the developmental basis of the plastic response. First, interpreting the cross-environmental covariation in trait values is difficult without knowing the developmental basis of the correlation. Strong cross-environmental correlations in trait values are thought to constrain the evolution of a plastic responses, whereas weak correlations are less problematic for the independent evolution of trait values (Via and Lande 1985; Via 1987). However, the degree to which genetic correlations represent real constraints on the independent evolution of traits is determined by exactly how the traits are developmentally integrated (Wolf et al. 2001).

In many insects, for example, negative correlations in the size or shape of some traits can result from interactions among developing tissues at specific points in ontogeny (Klingenberg and Nijhout 1998; Nijhout and Emlen 1998; Stern and Emlen 1999). Even if the measured negative correlation is strong, the impact of such trade-offs on phenotypic evolution will depend on the proximate basis of preferential resource allocation,

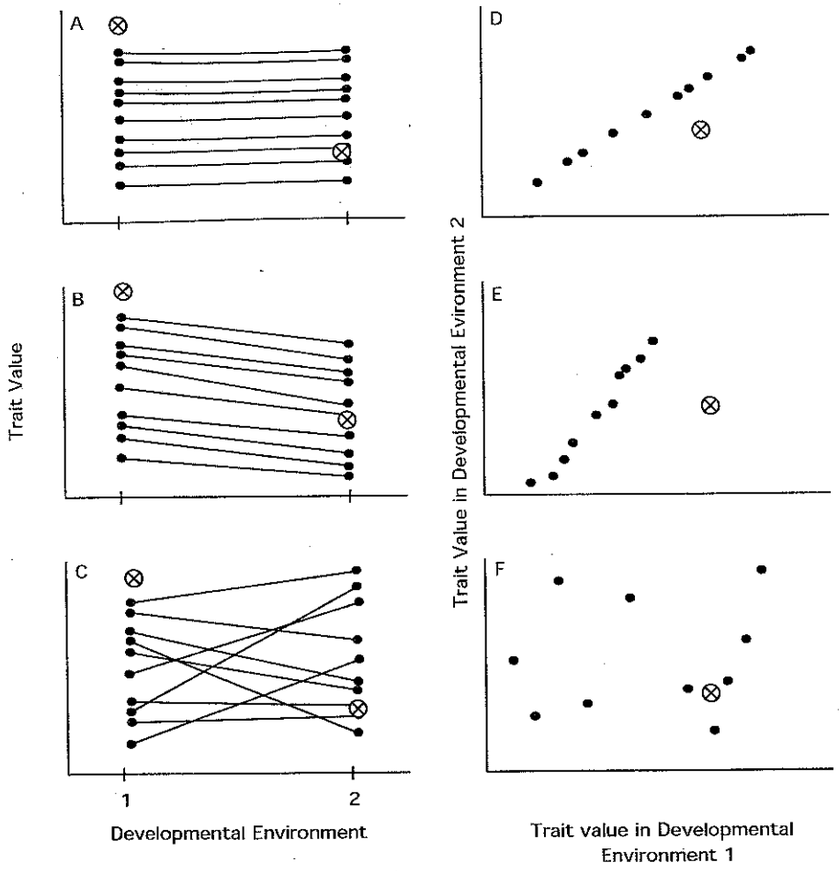

Figure 5.1. Representative linear reaction norms and cross-environmental trait correlations. Points indicate phenotypic values for hypothetical genotypes (or some surrogate such as sibship mean phenotype) in two developmental environments. Slopes in A–C represent levels of apparent phenotypic plasticity for a trait across environments. The phenotypic optimum in each environment is shown by the circle with the X. In D and E, this joint optimum is not achievable because the tightly correlated trait values prohibit that trait value combination, although it is easily achievable in E. [Reprinted with permission from Via (1987).]

resource competition, or other form of communication among developing traits (Nijhout and Emlen 1998; Stern and Emlen 1999; Wolf et al. 2001). The same arguments apply to the evolution of phenotypic plasticity in some trait; tight cross-environmental correlations may or may not represent constraints on the evolution of a plastic response, depending on the proximate basis of trait development in each environment.

Second, statistical estimation of the relationship between allelic and phenotypic variation may be inaccurate when based on the variance reflected in the slopes of reaction norms. The degree of phenotypic plasticity in some trait is often measured as the magnitude of the slope of a reaction norm (see chapter 3). However, some traits are environmentally canalized, where developmental regulatory action counters or resists the influences of the developmental environment through ontogeny.

For example, reaction norms with a zero slope are interpreted as the developmental environment not affecting the final trait value or no developmental response to the environment. However, the production of an invariant trait across developmental environments (figure 5.1A) may represent environmental canalization (Wagner et al. 1997), meaning that the mechanisms regulating development may counter environmental effects, reducing the impact of variation in the developmental environment on the final value of the trait. In such a case, significant effects of the environment on development are present; however, adaptive compensatory mechanisms act to resist the environmental perturbation. Here, a flat reaction norm represents adaptive (developmental) phenotypic plasticity; in the absence of developmental compensation, a different and less fit phenotype would be produced.

Countergradient variation, where genetically based developmental compensation resists environmentally induced phenotypic variation (reviewed in Conover and Schultz 1995), is one example of such compensation. Completely different, alternative ontogenies can also be employed to resist or compensate for effects of the developmental environment on terminal phenotype. For example, under stressful conditions some individuals may adopt alternative juvenile phenotypes that may differ dramatically in form and behavior and yet still converge on the same adult phenotype (e.g., Collins 1979; Pfennig 1992). In cases of countergradient variation and these environmentally induced polymorphisms (polyphenisms), environmental effects on trait development would manifest in the absence of such adaptive developmental compensation, transforming the reaction norm in figure 5.1A, for example, into that of figure 5.1, B and C. Hence, care must be taken when making inferences about the evolution of phenotypic plasticity based solely on reaction norms; flat reaction norms may sometimes conceal extremely interesting adaptive developmental plasticity underlying trait production. It is therefore critical to identify when developmental compensation alters the slope of a reaction norm, because failure to do so risks misinterpreting the effects of the environment on trait expression or, worst still, misunderstanding instances of cryptic adaptive plasticity in a focal trait.

Together, these two points illustrate that measuring the terminal phenotypes produced across a range of environments may be initially informative, but more information is needed to understand the evolutionary potential of an observed plastic response. Below, we describe a research program designed to determine the role of proximate developmental mechanisms in shaping the evolution of plastic responses.

The Research Program

The research program has three basic steps. First, putative developmental regulators believed to underlie a plastic response must be identified. Second, a series of experiments are performed to establish the hypothesized role of the focal developmental mechanism in producing the plastic response. Third, similar manipulations of development are applied to other lineages to determine if the same developmental mechanisms regulate similar plastic responses in those lineages and to detect a developmental bias or predisposition in lineages to produce given forms of phenotypic plasticity.

Step 1. Identification of the Hypothesized Proximate Basis of a Plastic Response

The first step in dissecting the proximate basis of a plastic response requires at least some knowledge of developmental regulation of the traits in question. Such understanding can narrow the field of inquiry and facilitate the identification of critical windows or sensitive periods during ontogeny, where developmental decisions or evolutionarily important trade-offs are likely to occur. Also, considerations of development can aid in the identification of a plastic response not reflected in a flat reaction norm, that is, the occurrence of countergradient variation or other form of resistance to environmental effects on development that may not be apparent from the examination of reaction norms. Once hypotheses about specific proximate mechanisms are made, one can establish the role of the putative developmental basis of a plastic response. Ideally, experiments can be designed such that some estimate of the evolutionarily important variation in the relevant developmental mechanisms can be made as well. There is a growing body of knowledge that can provide the required kinds of detailed information (Gilbert 2000; Stern 2000). In particular, investigators may have to consider the developmental modules, or constituent parts, that comprise the complex mosaic trait that is of evolutionary interest. Most morphological traits that are of evolutionary interest are composed of developmental modules; considering these modules separately may make the proximate regulation of trait ontogeny more developmentally tractable (Stern 2000) and more informative in a quantitative genetic setting (Wolf et al. 2001).

Step 2. Investigating the Developmental Basis of the Plastic Response

Just as there are well-established criteria for identifying a trait as an adaptation (Gould and Lewontin 1979), there are strict criteria for establishing the causal role of some process in development (Gilbert 2000). To meaningfully connect development to evolution requires a melding of both fields of inquiry. Failure to do so risks misdirecting entire research programs because it can lead to the description of false evolutionary patterns and a flawed understanding of how development shapes evolutionary change (Bolker 1995; Zera 1999; Zera and Huang 1999).

We recommend that investigators collaborate across evolutionary and developmental biology, bringing together their respective views and expertise on developmental regulation, experimental tools, phenotypic variation, environmental variation, and the complexities of selection and population structure. Through collaboration, developmentally tractable traits that exhibit evolutionarily interesting phenotypic plasticity can be identified and the proximate mechanisms regulating plasticity can be dissected. In the remainder of this section, we briefly review some of the most promising techniques for investigating the proximate basis of plastic responses in animals at a few different levels of biological organization. We focus on techniques not usually employed by classically trained evolutionary biologists; more standard approaches (e.g., the use of bioassays or more precise techniques to quantify levels of hormone among groups of interest) are probably familiar and are easily gleaned from reviews or textbooks (e.g., Nijhout 1994; Rose 1999; Gilbert 2000).

Physiology

Hormones coordinate the development of traits within an organism, regulate the production of continuous and discrete phenotypic variation, and create ontogenetic windows or sensitive periods during which plastic responses occur (see recent review in Nijhout 1999b). Establishing the role of a given endocrine mechanism in development is challenging, because it requires a variety of *in vitro* and *in vivo* tests. These include (but are not restricted to) documenting the endocrine profiles of individuals expressing typical and plastic ontogenies, and manipulating the circulating hormone titer through a variety of techniques, including elimination of endogenous sources of the hormone and replacement with endogenous hormone (removal and phenotype rescue). The specifics of such tests will differ with the biological system under study, and any single test may not provide sufficient information to unequivocally establish the role of a hormones in development (for an overview, see Gilbert 2000). For example, although differences in hormone titer may not be apparent between individuals expressing typical and alternative trait ontogenies, differences in the amount of physiologically active hormone may be achieved through alteration of hormone-binding proteins, receptor densities, and so forth. Each of these possibilities must be examined in detail to clearly establish the role (or nonrole) of a given hormone in trait development.

One approach typically not used in studies of phenotypic plasticity is cell or tissue culture. In culture studies, targets (cells or organs) are raised in cocktails that differ in chemical composition. By comparing the ontogeny of the targets in media, the physiological signals that affect trait ontogeny can be isolated. For example, cultures of amphibian tails and limbs have identified the role of different hormones and genes in organ regulation at amphibian metamorphosis (reviewed in Tata 1993; Rose 1999) and in the physiological basis of differences in development rate among amphibians (Buchholtz and Hayes 2002).

Embryology

Embryonic manipulations involving the destruction of incipient traits or the transfer of structures (e.g., limb buds, neural crest cells, imaginal disks, etc.) among individuals to create chimeras offer a powerful set of techniques for the dissection of signal–target interactions. These manipulations can be used to identify key factors that initiate, terminate, or regulate a plastic response to the environment.

Laser ablation and microsurgical approaches can be used to identify the role of a structure in the expression of other traits. Such experiments can determine, for example, if a group of cells act as a morphogen source or sink, and the ontogenetic timing of the release or uptake of the morphogenic factor. Somewhat surprisingly, major organs can be removed, such as the brain of an insect (e.g., Endo and Kamata 1985) or thyroid gland of a larval amphibians (e.g., Kanki and Wakahara 1999), or more subtle manipulations can be applied to smaller putative signaling centers (e.g., Nijhout 1980; Nijhout and Emlen 1998; Dichtel et al. 2001; Patel et al. 2002). Such experiments are particularly powerful when coupled with exogenous replacement of the putative signal to "rescue" the normal phenotype.

Transplant experiments involve removing structures from individuals possessing one phenotype and placing the structure into, or into contact with, a recipient differing in

phenotype. Donors and recipients can be from different species, selected lines, seasonal morphs, instars, and so on. Transplant experiments can be of two general types. In the first kind of transplant experiment, the focus is on the effect of transplanted tissue on the development of the host phenotype. In the second, the focus is on what the host phenotype does to the transplanted tissue. Again, these types of manipulations can be dramatic, for example, involving the transplantation of brain tissue into the abdomen of a host or the fusing of two host types to identify a morphogen source that induces an alternative seasonal morph (e.g., Endo and Funatsu 1985; Endo and Kamata 1985). Alternatively, such manipulations can be more subtle. For example, Monterio et al. (1997) transferred signaling centers among individual *Bicyclus anynana* butterflies to determine the degree to which the response to artificial selection on wing pattern was due to changes in the signal or in the response cells. These techniques were also used to investigate how the artificial selection affected the expression of alternative seasonal wing pattern morphs in this butterfly (Brakefield et al. 1996). Gilbert (2000) has a thorough review of transplant experiments, and how they can be applied to address evolutionary questions.

Molecular Genetics

There are a number of molecular genetic techniques that can be used to investigate the proximate basis of phenotypic plasticity. Some model genetic systems exhibit evolutionarily interesting forms of plasticity and consequently provide an enormously powerful resource for understanding the relationship between proximate mechanism and the expression of alternative phenotypes. One of the genetic model systems, the nematode *Caenorhabditis elegans*, develops continuously from larva to adult or can employ an alternative life history and diapause as a dauer (Cassida and Russel 1975; Golden and Riddle 1984). The developmental switch is determined by physiological signals, especially the threat of starvation, during a particular point in larval ontogeny. The current model for this regulatory system involves signaling from the transforming growth factor (TGF)^2 pathway and from an insulin receptor signaling pathway, impinging *daf-12*, a gene that acts as a life history switch in conjunction with heterochronic genes that regulate life staging (Antebi et al. 1998; Gerisch et al. 2001). This research is being extended to homologous processes in other nematodes. Because the genetics are available, a mechanistic view of phenotype switching works is thus possible in *C. elegans* beyond that currently possible even for temperature-dependent sex determination in reptiles (Crews 1994, 1996; Godwin and Crews 1997). A number of technological advances developed for use in such model systems also hold promise for nonmodel systems.

Injection of novel oligonucleotide reagents (Summerton 1999) or RNA inhibition (Brown et al. 1999; Fire et al. 1998; Kennerdell and Carthew 1998; Paddison et al. 2002) may allow investigators to perform genetic manipulations in at least some nongenetic model systems. These reagents inhibit the action of a target gene, mimicking the effect of a knockout mutation. If injected early in ontogeny, this manipulation can prevent expression of the target gene in the entire organism; if it is applied later, mosaics can be created where some cells or tissues express the gene whereas others do not. These manipulations can be used to investigate the genetic basis of plastic responses and could be particularly powerful if coupled with other manipulations of development, such as the removal and phenotype rescue approach discussed above.

Genomic approaches offer new ways of investigating evolutionary changes in genetic architecture among closely related species or populations of nonmodel organisms (Peichel et al. 2001). The growing number of gene chips available for model systems also can be used to study the proximate mechanisms underlying plastic responses in nonmodel systems (Gibson 2002). Extractions from a given tissue or a whole organism are passed over a chip that contains an array of genes from the model organism. Levels of gene expression in the sample are indicated by the degree of illumination of each receptor in the array. Comparing the illumination pattern generated by individuals expressing typical and alternative phenotypes reveals correlations between specific gene products and the production of the plastic response. Although the treatment of the data generated by such chip tests is an active area of research in itself, the technology offers promise because many genes of interest (e.g., hormone receptors) are conserved phylogenetically (Hacia et al. 1999; Rast et al. 2000). Hence, gene chips developed for a model system may be used to investigate nonmodel systems.

These approaches, in combination with the use of more traditional methods drawing on the resources developed for model systems such as *in situ* hybridization or antibody staining, can be combined with traditional gene mapping (candidate gene approaches or genomewide searches using techniques such as subtractive hybridization or linkage map building) to relate quantitative variation in plastic responses to some genes associated with their production. A theoretical link between candidate gene approaches and microevolution is now being forged (Haag and True 2001).

Investigators may wish to focus attention on the *cis*-regulatory regions that control gene expression. Evolution of *cis*-regulatory regions are responsible for quantitative differences between species (Stern 1998; Sucena and Stern 2000) and between sexes within species (Kopp et al. 2000). The evolution of the *cis*-regulatory region allows for quantitative, independent changes in gene expression across tissues or times in ontogeny (Stern 2000). Consequently, *cis*-regulatory regions probably regulate expression of plastic responses at some level in both continuously varying traits and alternative, discrete traits.

Step 3. Investigation of Other Lineages

Once the developmental basis of the plastic response in a focal lineage has been identified, at least in part, other lineages can be investigated to address how proximate mechanisms may have shaped the evolution of the plastic response. Comparisons can be made among lineages (e.g., taxa, selected lines, mutants) that differ in the threshold for induction of a plastic response or the degree to which trait plasticity is exhibited. Such investigations can indicate what parts of a developmental cascade are involved in the microevolution of the plastic response. However, using the comparative method in combination with experimental manipulations also has the potential to reveal the role of development in the generation of phylogenetic pattern of adaptive phenotypic plasticity.

The degree of phenotypic plasticity exhibited by any trait will be related to how trait ontogeny is regulated. It follows that some aspects of developmental regulation may facilitate the evolution of phenotypic plasticity in some traits or periods of ontogeny and restrict it in other traits or times in the life cycle. We can identify latent potential in the existing developmental system that may facilitate the evolution of plasticity by comparing the response of individuals from several lineages to environmental and develop-

mental manipulations. If done in a broad phylogenetic context, such comparisons may reveal a correspondence between mode of developmental regulation, action of some gene, and so forth, and the specific form of plasticity under investigation. Eventually, these comparisons may explain phylogenetic pattern in phenotypic plasticity and hint at the general evolutionary importance of a given plastic response.

One obvious way these topics can be addressed is to apply steps 1 and 2 above to lineages that exhibit independently derived, convergent plasticities. If the developmental basis of the plastic response is the same across lineages, then there may be a narrow range of developmental routes that can be taken to produce the plastic response, or it may be that repeated co-option of the same proximate mechanisms is responsible for the convergent plasticities. This approach has revealed convergence in the proximate basis of plasticity in pupal color across families of butterflies (Starnecker and Hazel 1999). Conversely, a variety of developmental processes underlying convergent plasticities would indicate that development may play little role in shaping or constraining the evolution of the plastic response.

A second way to investigate phylogenetic pattern in plastic responses is to perform manipulations similar to those described in step 2 to individuals from lineages that do not naturally exhibit the plastic response. Potential biases in the evolution of a plastic response introduced by the proximate mechanisms regulating development would be indicated when individuals from a lineage normally canalized for the focal trait exhibits plasticity in response to the manipulation. If the manipulation induces some degree of plasticity in the focal trait, this suggests that there is at least the potential to produce the plastic response in the outgroup via the same developmental means as in the focal lineage.

The degree of similarity between the naturally occurring plasticity in the focal lineage, the experimentally induced plasticity of manipulated individuals in the focal lineage, and the experimentally induced plasticity in the outgroup suggests how easily that form of trait plasticity might evolve, and perhaps how easily it occurred in the ancestor of the focal lineage. A high degree of similarity in response to the manipulation between individuals from the outgroup and the focal lineage indicates that the evolution of the plastic response was probably relatively easy and could be repeated among other members of the clade. Disparity in the response indicates that the evolution of the plastic response may represent more of a developmental, and consequently and evolutionary, challenge.

Using environmental manipulations, this approach has been used investigate the evolution of seasonal polyphenic wing color patterns in *Pieris* and the closely related *Tatochila* butterflies. Some species in these groups are polyphenic, producing melanized (cool season) adults during short day conditions and adults lacking melanization (immaculates) under long day conditions. Other species in these genera are monomorphic, expressing either the melanized or immaculate morph. Selection imposed by the thermal environment often conveys higher fitness to the melanized form in the cool season and the immaculate form during the dry season in *Pieris* species (reviewed in Kingsolver and Huey 1998), and this is likely to be the case with other, less studied *Pieris* and *Tatochila* species. In some lineages that do not exhibit the melanized phenotype, cold shock induces a phenotype superficially similar to the cold-season morph (reviewed in Shapiro 1976, 1980; Nijhout 1991). Differences between the typical and induced patterns indicate that the alternative morphs are not homologous (Shapiro 1980). It would be very interesting to know the degree to which the proximate basis of morph determination is

shared between the typically expressed seasonal morphs and the induced, novel alternative forms, however, because this would suggest how easily the polyphenism can evolve in these speciose and widely distributed butterflies.

In the remaining portion of the chapter, we explore how determining the phylogenetic distribution and proximate basis of trait plasticity in single and composite traits can be particularly informative for investigations into how development shapes the evolution of adaptive plasticity. In so doing, we describe what is known about the proximate basis of adaptive plasticity in some amphibian and insect systems and illustrate the kind of integrative research approach we advocate.

Evolutionary Pattern in Phenotypic Plasticity

We have argued that examination of the phylogenetic distribution of adaptive trait plasticity can help to illuminate the role of development in shaping plastic responses and in creating phylogenetic pattern in plasticity. However, it can be a daunting undertaking to compare the proximate basis of trait plasticity across lineages, in large part because of the difficulty in identifying the "trait" of interest for a given plastic response. One way to approach this problem is to consider traits differently to address different research questions. For example, comparisons can be made for both morphologically and ecologically defined traits. Comparisons of plasticity among homologous morphological traits will indicate the role of development in producing a range of phenotypes from a single structure. Comparing ecologically defined traits (i.e., mosaic traits that may be composed of different, nonhomologous elements across lineages) can elucidate how development affects the evolution of plasticity in similarly adaptive phenotypes assembled from different constituent parts. Such comparisons can also suggest certain types of ontogenies or particular aspects of developmental regulation that may facilitate the evolution of adaptive developmental plasticity in particular traits. In the subsections that follow, we examine each of these topics in detail and provide empirical examples to illustrate our points.

Evolutionary Pattern in the Proximate Basis of Adaptive Plasticity

A first step toward understanding the evolutionary importance of phenotypic plasticity is to document the phylogenetic distribution of plastic responses. Knowing the ancestor–descendent relationship for plastic responses is central to the formulation and testing of hypotheses addressing the adaptive evolution of plasticity (Doughty 1995; Gotthard and Nylin 1995). However, identifying the point of origin of a plastic response may also hint at the general evolutionary impact of plasticity in the focal trait. Clusters of homologous plastic responses may suggest that trait plasticity could have facilitated adaptive radiation (i.e., plasticity in the focal trait represents a key innovation *sensu* Liem 1990). Widespread homoplasies (convergences or independent evolutionary origins), in particular, plastic responses, might indicate that the response is relatively easy to evolve when favored ecologically.

Once the phylogenetic pattern of plastic responses is known, comparing the proximate basis underlying trait plasticity could prove enlightening. For example, paedomor-

phosis, a life history strategy in which salamanders retain the aquatic, larval morphology into reproductive maturity has evolved independently several times among, and even within, the main urodele clades (reviewed in Shaffer and Voss 1996). Although there are numerous hypotheses addressing the conditions favoring the evolution of obligate and facultative paedomorphosis (Sprules 1974; Whiteman 1994), all hypotheses share the basic tenet that reproduction as a paedomorph confers higher fitness than reproduction as a terrestrial adult. When larval habitats can change in quality over the course of an individual's lifetime or when the relative success of the reproductive strategy is context dependent, then phenotypically plastic development, facultative paedomorphosis, should be favored (Whiteman 1994).

The suite of morphological traits that compose the paedomorph is the same across lineages. Yet the evolutionary loss of metamorphosis (i.e., the trait of larval reproductive mode) has occurred through modification of different proximate mechanisms among lineages. Investigations of paedomorphosis focusing on several species of *Ambystoma* suggest details about the genetic basis of alternative phenotype production. Amplified fragment length polymorphisms studies suggest that the switch to paedomorphosis in *A. mexicanum* involves one gene of major effect, and possibly numerous genes of smaller effect (Voss and Shaffer 1997). Differences among populations in the polygenic basis of facultative paedomorphosis in *A. talpoideum* are suggested by common garden experiments (Harris et al. 1990; Semlitsch and Gibbons 1986), as are genetically based differences in sensitivity to environmental conditions that induce metamorphosis in facultatively paedomorphic lineages (Semlitsch et al. 1990).

Mechanistically, paedomorphosis could result from any one of numerous changes that prevent metamorphosis (Rose 1996; Shaffer and Voss 1996). Timing and coordination of developmental events in amphibians are regulated by interactions of thyroid hormones (TH) with other hormones and response tissues (reviewed in White and Nicoll 1981; Galton 1992a; Shi 1994; Kaltenbach 1996; Rose 1999). As reviewed by Shaffer and Voss (1996), the independent evolution of paedomorphic life histories resulted from a variety of changes in proximate developmental mechanisms, ranging from a reduction of thyroid activity in some lineages to a decrease of tissue sensitivity to TH in other lineages. There are several candidate mechanisms that could lead to reduced tissue sensitivity to TH, including changes in the binding affinity or number of TH receptors, and alterations of enzyme systems that regulate or otherwise effect exposure of specific tissues to circulating TH levels (Galton 1985, 1992b; Rose 1996). Hence, the paedomorph phenotype is composed of changes in the same suite of morphological traits, but the ontogeny of these traits has been altered through modification of different mechanisms across lineages. This suggests that it is relatively easy for salamanders to truncate or delay somatic development when such ontogenies are favored.

Such variation in the proximate basis of the paedomorphic ontogeny stands in stark contrast to the apparent constancy in the basis of adaptive acceleration of development rate exhibited by many larval amphibians. Amphibians from lineages (populations, species) that typically experience changing environments during the larval stage can alter ontogeny in response to stresses in the developmental environment, whereas lineages that usually develop in more constant environments often lack such plasticity (Denver 1997b; Duellman and Trueb 1986). Declines in larval habitat quality can be signaled by decreases in per capita food availability or water volume; increases in larval density, water temperature, or concentration of chemicals; and the presence of chemical cues associated with predators or injured larvae (reviewed in Newman 1992; Denver 1997b). The proximate basis of the adaptive acceleration of larval ontogeny has been examined extensively for the developmental response to habitat desiccation, particularly in spadefoot toad larvae that exhibit both exceptionally high growth rates and exceedingly short larval periods (Buchholz and Hayes 2000, 2002; Morey and Reznick 2000). In response to declining water levels, production of corticotropin-releasing hormone (CRH) in the brain precociously activates the thyroid and interrenal axes, which in turn coordinate accelerated larval ontogeny by raising TH and corticosterone (B) levels (Denver 1997a, 1998a). TH regulates larval differentiation, pacing the approach toward metamorphosis by controlling patterns of gene expression in virtually all tissues (Kikuyama et al. 1993; Rose 1999). Corticosterone (B) seems to synergize the effects of TH, although the mechanism by which this occurs is not clear (reviewed in Denver 1997b; Kikuyama et al. 1993; Rose 1999). The conservation of the neuroendocrine response to habitat desiccation in amphibians is interesting because, from a developmental perspective, there are several other possible mechanisms that could be used to achieve the same effect (reviewed in Denver 1997b).

Elevation of CRH levels is part of a generalized, phylogenetically ancient and conserved response to stress in vertebrates (Denver 1997a). It would be interesting to know if the same basic endocrine mechanisms underlie the production of the environmentally induced, adaptive phenotypes exhibited by many larval amphibians. Trophic polymorphisms in tadpoles (e.g., Pfennig 1992) and salamander larvae (e.g., Collins and Holomuzki 1984; Loeb et al. 1994; Walls and Blaustein 1995) are produced under stressful larval habitats. Predators also induce developmental acceleration in many amphibian larvae (e.g., Skelly 1992; Warkentin 1995) as well as polyphenisms in larval fin morphology (McCollum and Van Buskirk 1996). In each of these cases, as well as in the environmentally induced metamorphosis in paedomorphic salamanders described above, alteration of typical ontogeny occurs when larvae experience stressful conditions. It seems likely that, at least on some level, the generalized stress response produced by increases in CRH levels will be involved in these cases of adaptive plasticity.

In summary, adaptive plasticity can have the same or different developmental basis across lineages. Facultative paedomorphs are morphologically similar among lineages, yet somatic development is interrupted differently to achieve reproduction in the aquatic environment. The independently evolved, morphologically divergent, stress-induced responses to declines in the larval habit quality may share involvement of the thyroid and interrenal axes, as mediated by CRH. Contrasting the developmental basis of these cases of adaptive plasticity raises the issue of how proximate developmental mechanisms can generate phylogenetic pattern in phenotypic plasticity. The variety of proximate mechanisms involved across lineages of facultatively paedomorphic salamanders is not surprising because this life history strategy represents an inhibition of metamorphosis or truncation of typical somatic ontogeny; there are many ways to interrupt typical development. The repeated co-option of the generalized CRH-mediated stress response may suggest that evolutionarily plastic novelties are constrained in how they can develop. It also suggests that there is something about the proximate basis of amphibian development that facilitates the evolution of adaptive plasticity in this group, and that this has allowed amphibians to exploit a variety of habitats. Interestingly, two genera of spadefoot toads (*Scaphiopus* and *Spea*) develop rapidly because of high levels of circulating TH and greater tissue sensitivity to this hormone (D. R. Buchholz, in

preparation), suggesting that there is variation in the developmental cascade downstream and perhaps even semi-independent of CRH on which selection has acted. Moreover, the rapid development conferred by these changes may have facilitated diversification of the clade in xeric environments (Buchholz and Hayes 2002).

Developmental Windows and Morphological Plasticity

Adaptive flexibility in ontogeny or in the development of a trait may be characteristic of some lineages and wholly absent in others. The phylogenetic breadth and variety of traits exhibiting adaptive plasticity suggest that the problematic absence of detectable, reliable predictors of the future selective environment, not patterns of selection, limit the evolution of adaptive plasticity (Moran 1992). For such cues to be effective, they must be detected before or at a point in ontogeny when development can be altered to produce the most appropriate phenotype for the future selective environment. This necessary temporal match between the detection of cues in the developmental environment and ontogenetic points where adaptive adjustments in development can be made has the potential to produce developmental constraints on, and phylogenetic pattern in, the evolution of adaptive plasticity.

For example, the stress-induced modulation of the thyroid and interrenal axes of amphibian larvae discussed above can take place exceptionally quickly, allowing adaptive plasticity in development rate through much of larval ontogeny. Changes in the circulating levels of TH are detectable in tadpoles of *Scaphiopus hammondii* within hours of a decrease in the water level (Denver 1997a). These changes in TH titer rapidly alter patterns of organ-specific gene expression (Denver 1998b), which manifest as changes in larval ontogeny as quickly as 3 days (Denver 1998a). Furthermore, these ontogenetic responses are proportional to the relative change in water level and are reversible—the developmental response closely tracks environmental changes in real time (Denver et al. 1998).

However, there are limits to plasticity in developmental rate in amphibians. Adaptive acceleration of ontogeny in amphibians involving precocious activation of the thyroid and interrenal axes requires that these axes are developed and that tissues are competent to respond to changes in signal. Absence of a fully developed thyroid–interrenal axis may explain why amphibians do not alter development rate in response to changes in the environment early in larval ontogeny. Later in ontogeny, a developmental commitment is made to metamorphose, after which larvae are again unable to respond adaptively to changes in the larval environment. These two developmental events, the maturation of the thyroid and interrenal axes and the commitment to begin the endocrine–genetic cascade toward metamorphosis, bracket a window of ontogeny during which adaptive plastic responses are possible. Such ontogenetic windows of environmental sensitivity during which adaptive changes in development can occur are commonly observed in amphibian ontogenies (e.g., Hensley 1993; Denver et al. 1998; Morey and Reznick 2000).

The continuous development of amphibians contrasts with the ontogenies of insects where saltatory morphological change is a necessary consequence of moving stepwise through discrete instars. Because insects can only realize morphological changes at a molt, alteration in the development of a larval or adult structure necessarily precedes the appearance of the structure, sometimes by great periods of time. For example, male *Othophagus taurus* beetles facultatively produce horns, depending on their projected adult size. Size at pupation in *O. taurus* is determined by the amount and quality of dung with which each larvae has been provisioned (Moczek 1998), similar to other *Othophagus* beetles (Emlen 1994, 1997b), and only large males develop horns, which they use to secure matings (Moczek 1998; Emlen and Nijhout 1999; Moczek and Emlen 2000; see chapter 9 for a review of the ecology this system). As larvae deplete the finite amount of dung with which they have been provisioned, male phenotype (long or short horned) is determined and the developmental commitment to pupate is made (Emlen and Nijhout 1999, 2001). Male morph determination is probably affected by the general mechanisms that regulate development in insects. Below, we discuss some of these mechanisms generally and then explore specifically how they influence the evolution of morphological plasticity in horn beetle morphology.

In insects, differentiation and the molting cycle are controlled primarily by the interaction of target tissues with two hormones, juvenile hormone (JH) and ecdysone (see reviews in Nijhout 1994, 1999b). JH is produced by the corpora allata and is generally thought to exist for only a short time in circulation. Because it has a short half-life in the hemolymph, reductions in JH production can lead to swift declines in circulating JH levels. Low titers of circulating JH induce the production of prothoracicotropic hormone (PTTH), which in turn initiates the synthesis and release of ecdysone, a steroid hormone that begins the cascade of developmental events leading to a molt. If the circulating levels of JH are above some threshold when ecdysone interacts with target cells, then the tissues retain their larval commitment and a larval molt ensues. If JH is below this threshold, then cell fates change and differentiation occurs; this is when changes in phenotype or shifts to different forms of instars (e.g., pupa) can occur. In hemimetabolous insects (e.g., true bugs, crickets) such a molt leads to an adult, whereas in holometabolous insects (e.g., butterflies, beetles) a pupa or adult is produced. This interaction of JH and ecdysone with target cells during specific points in ontogeny generates critical periods or developmental windows during which cell fates are determined (Nijhout 1994, 1999a,b).

As is the case with most adult structures in holometabolous insects, the horns of horned beetles develop from imaginal disks (Emlen and Nijhout 1999). Early in larval ontogeny, epidermal fields are sequestered into pockets in the body. These pockets become the imaginal disks, which grow slowly and semi-independently from the rest of the insect until late in the final larval instar, when their cell populations grow exponentially and differentiate. Eventually, the structures in disks develop cuticle and expand to form nearly all the external adult morphological structures (e.g., eyes, wings, antennae) (Nijhout 1994).

The current developmental model that explains phenotype development in *O. taurus* postulates two critical windows late in juvenile ontogeny (Emlen and Nijhout 2001). The first involves the setting of horn disk sensitivity to JH and the second involves JH-mediated horn disk growth. Late in larval ontogeny, male morph is determined by projected adult size. As the limited food resource approaches depletion, male larvae that fall beneath a critical threshold mass and all female larvae (i.e., larvae that are destined to become hornless adults) experience a transient a spike in ecdysone production. Male larvae that maintain a mass above the threshold (i.e., larvae destined to produce horned adults) experience no such spike. Just before the prepupal stage, JH levels climb, producing horns on large males and hornless small males. The role of JH in morph determination during this second critical window has been confirmed by producing small

horned males via topical application of a JH mimic (methoprene) at this time (Nijhout and Emlen 1998; Emlen and Nijhout 1999, 2001).

Although JH has been implicated as being involved in the facultative development of horns and the critical window of JH action has been defined, the mechanism(s) through which JH affects male phenotype has not been established. As was the case with altered TH action in amphibians, there are many (nonexclusive) JH-related mechanisms that underlie the differences between horn morphs (Emlen and Nijhout 1999, 2001). First, JH levels may be the same across all male sizes, but imaginal disk sensitivity to JH may differ between morphs. The transient spike in ecdysone that occurs in presumptive hornless males could reprogram cells in the horn disks, decreasing disk sensitivity to JH. This hypothesis is supported by changes in JH sensitivity that result from topical treatment with methoprene during the first critical period; treated males experience an increase in the size threshold for horn induction (Emlen and Nijhout 2001). If this hypothesis is correct, it means that the methoprene treatment during the second critical period induces horn production by greatly exceeding the higher threshold required for JH response in small males. Second, rates of JH production or elimination may differ between morphs such that large males have higher JH levels than small males during the critical period. Indeed, levels of JH synthesis scale linearly with the size of the corpora allata, which scales with body size in other insects (Emlen and Nijhout 2001). This suggests that larger (horned) males may have higher JH levels during the second critical period. Third, JH may be produced for a longer period of time in horned males, increasing the period of population growth for the cells that comprise the horn disks in these individuals. Finally, juvenile hormone esterase (JHE), an enzyme involved in JH degradation, could have greater titers or activity in small males. Differences in JHE are involved in polyphenism expression in other systems (discussed below).

Determining which of these, or other, mechanisms regulate horn ontogeny in this and other species of beetles exhibiting facultative horn production could greatly inform our understanding of the evolution of switch points. It would be also be interesting to know where in the developmental cascade response to artificial selection and natural selection on plasticity in horn morphology (e.g., Emlen 1996) have occurred. Furthermore, how genetic correlations shape or limit the evolution of phenotypic plasticity (and morphological traits in general) can be explored with this and other insect systems where individuals facultatively express alternative phenotypes in which the relative sizes of morphological traits differ among morphs.

There is a trade-off, represented by a negative genetic correlation, between horn size and adult eye size (Nijhout and Emlen 1998). These structures develop from imaginal disks sharing close proximity to one another, and manipulation experiments indicate that altering the size of one disk causes an inverse change in the size of the structures produced by adjacent, but not distant disks (Nijhout and Emlen 1998). Although the proximate basis of these interactions is not known, the evolutionary potential of the response will be determined largely by the mechanistic basis of the trade-off (Wolf et al. 2001). For example, resource competition or chemically mediated epigenetic effects among disks could have different effects on evolutionary outcomes (Nijhout and Emlen 1998). Also, slight changes in the sensitivity of one set of disks to regulatory hormones (i.e., shifts in thresholds or critical periods) may alter the relative timing among disks of the initiation or termination of the exponential growth phase. Small temporal changes of this type are likely to enhance the growth of one set of disks at the expense of adjacent disks, whereas larger temporal changes may effectively dissociate the ontogeny of the disks, possibly eliminating the trade-off altogether. Because disk growth is restricted to a small window of time late in juvenile ontogeny (Emlen and Nijhout 1999, 2001), such large temporal dissociation among structures may not be possible in this system. The restrictions imposed by development on the possibilities for altering this trade-off raise the issue of how the proximate mechanisms that regulate and integrate trait ontogeny can produce phylogenetic pattern in morphology or plasticity in morphological traits.

Comparative work indicates that the negative relationship between the size of horns and traits that develop in proximity holds across the *Onthophagus* phylogeny (Emlen 2001), suggesting that the interactions among developing disks produce phylogenic patterns in phenotypes. It appears likely that *Onthophagus* beetles have broken the developmental constraint imposed by disk–disk interactions by moving the horns to regions where they interfere the least with the development of other ecologically relevant traits (Emlen 2001). Nocturnal species of beetle are more likely than diurnal species to have horns near their eyes. Presumably this is because the reduction in eye size caused by large horns has little impact on relative fitness in nocturnal species. In these cases of facultative horn production, integrating an understanding of the proximate basis of trait development with the ecology of the morphologies helps to explain the phylogenetic pattern in phenotypes and plasticity of the traits (Emlen 2001; Emlen and Nijhout 2000).

A well-characterized facultative polymorphism in insects for understanding proximate endocrine mechanisms, life history trade-offs, and quantitative genetic parameters involves a set of dispersal polymorphisms in crickets (reviewed in Zera and Denno 1997; Zera 1999). Dispersal polymorphisms, where some individuals facultatively develop wings and flight musculature, occur in a variety of insects, differ in the environments that induce them, and differ in the postdispersal maintenance of flight muscles. Furthermore, because the sensitive period for wing induction differs widely across taxa, ranging from embryonic development through each juvenile instar (Zera and Denno 1997), wing polymorphisms represent another promising experimental model for investigating how the relationships among suites of traits are affected by the proximate mechanisms that create ontogenetic windows during which plastic responses can occur.

Crickets are hemimetabolous insects, and although they lack the imaginal disks discussed above, the wings of crickets develop largely during the last instar. As reviewed by Zera and Denno (1997), the juvenile hormone–wing morph hypothesis (Southwood 1961; Wigglesworth 1961) posits that JH levels above some threshold late in juvenile development act to inhibit growth and development of the wings and associated flight muscles, producing a short-winged (nondispersing) adult. In the cricket *Gryllus rubens*, wing development can be inhibited by experimental increases in JH titer late in the final larval instar (reviewed in Zera and Denno 1997; Zera 1999). Furthermore, JHE activity is greater and associated with increased levels of JH degradation in juveniles that develop into long-winged crickets compared with those that become short-winged morphs. These data are consistent with the juvenile hormone–wing morph hypothesis, but Zera (Zera and Denno 1997; Zera 1999) notes that these data are not definitive because the difference in JH levels between developing dispersing and nondispersing morphs is small enough to call into question its functional significance. Interestingly, ecdysone titers rise earlier and to a greater degree in the final juvenile instar of immatures destined to develop long wings—suggesting a role for ecdysone in morph determination similar to that suggested for the transient pulse of ecdysone in horned morphs of *O. taurus*.

As a description of the endocrine basis of development in this dramatic morphological, behavioral, and life-historical polymorphism, these investigations are valuable. However, these studies are made more informative because they, and those of other investigators (e.g., Fairbairn and Yadlowski 1997; Roff et al. 1997), have estimated several quantitative genetic parameters of the endocrine system and even performed elaborate selection experiments on components of the endocrine system (reviewed in Zera 1999; Zera and Huang 1999). Wings, flight muscles, and ovarian tissues trade off, as do other insect traits that develop in close proximity to one another (e.g., Klingenberg and Nijhout 1998; Nijhout and Emlen 1998). However, because development of these traits is dissociated temporally, the observed negative relationships among them probably results from division of a fixed energy budget among competing sinks (Zera and Denno 1997), not from interactions among traits during ontogeny.

Thus far we have discussed only discrete facultative polymorphisms in insects. Insects also illustrate continuous plastic responses to the environment. Diapause, where insects undergo a virtual developmental arrest, is a particularly dramatic developmental response to the environment present in nearly all insect orders, and is often facultative (Denlinger 1985; Nijhout 1994). Among species, diapause occurs in every stage of development, and it is usually associated with a response to poor or harsh environmental conditions, such as periods of low food availability or water scarcity (Nijhout 1999b). The genetic architecture underlying facultative diapause has been thoroughly described for populations of the pitcher-plant mosquito, *Wyeomyia smithii* (reviewed in Bradshaw and Holzapfel 2000). Across its geographic range, *W. smithii* differs in the photoperiod required to initiate and maintain larval diapause, and the threshold for diapause induction is polygenic (Bradshaw and Lounibos 1977; Hard et al. 1992, 1993). A cline in photoperiodic response exists such that mosquitoes from the more northern, derived populations differ from the southern, ancestral populations in the minimum critical photoperiod for diapause induction by 10 standard deviations (Bradshaw and Holzapfel 2000).

Extensive studies of the proximate basis of larval diapause have been on the final instar larvae of just a few lepidopterans (reviewed in Nijhout 1999b). Diapause is achieved developmentally by continued JH production, which inhibits PTTH production, resulting in developmental arrest. Developmentally, diapause is broken when environmental cues trigger the cessation of JH production, which in turn initiates PTTH production and the resumption of ontogeny. This pattern suggests that the mechanisms that regulate the timing of the typical molting cycle have been co-opted to achieve larval diapause (Nijhout 1999b), as seems to be the case for wing polyphenisms discussed above. It would be interesting to know the relationships between the well-characterized genetic differentiation among populations in critical photoperiod discussed above for mosquitoes and the mechanisms believed to underlie diapause regulation. Of particular interest would be to know the degree to which different aspects of the endocrine system are involved in the evolution of the switch point for diapause induction or resumption of development.

Summary and Conclusions

Phenotypic plasticity is a developmental phenomenon, and as such, the likelihood of the evolution and maintenance of adaptive plastic responses will depend not only on the ecological patterns of selection favoring plasticity on some trait but also on broad aspects of the regulation of ontogeny and the proximate mechanisms underlying trait development and phenotypic integration. We advocate the inclusion of developmental mechanism to deepen our understanding of the forces that shape the evolution of plastic responses.

Knowing how proximate mechanisms regulate trait ontogeny and integrate it with that of other traits will help elucidate how developmental processes might bias evolutionary outcomes. Such bias can be in the form of constraints; development defines the ontogenetic windows during which environmental signals must be perceived and processed, thereby limiting the possibilities for timely plastic responses. Development also determines the limitations on the degree of trait plasticity that can be exhibited. Bias can also be positive, facilitating the expression of plasticity in some traits, perhaps promoting diversification of lineages that regulate ontogeny of traits or life histories in particular ways. Understanding the proximate basis of trait ontogeny and integration also allows a more accurate interpretation of the evolutionary impact of the genetic correlations among traits believed to affect the evolution of plastic responses.

To more fully understand the evolutionary importance of phenotypic plasticity, we advocate an examination of the proximate basis of adaptive plasticity in traits and trait suites across taxa. Documenting phylogenetic pattern in the proximate basis of adaptive plasticity can suggest hypotheses regarding the relative impact of development on adaptive evolution, which can then be tested through direct manipulations of development. We propose a research program that crosses the boundaries of many subdisciplines in biology. It requires that investigators classically trained in evolutionary and developmental biology consider phenotypic variation and phenotype development in new ways. By fostering collaboration across disciplines, the proposed research program has the potential to increase our understanding not only of phenotypic plasticity but also of phenotypic evolution in general. In particular, species that exhibit discrete polyphenisms—where the differences between phenotypes are so great that alternative morphs have occasionally been misdescribed as separate species—offer powerful tools for investigating how proximate mechanisms affect the generation and evolution of morphological diversity.

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