Plant Species Biol. 11 : 69–85, 1996

PLANT SPECIES BIOLOGY

Developmental Reaction Norms: the Interactions among Allometry, Ontogeny and Plasticity

MASSOMO PIGLIUCCI[1], CARL D. SCHLICHTING, CYNTHIA S. JONES and KURT SCHWENK

Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs 06269 CT, U.S.A.
[1] *Current address: Department of Botany, University of Tennessee, Knoxville, TN, U.S.A.*

Abstract How micro- and macroevolutionary processes produce phenotypic change is without question one of the most intriguing and perplexing issues facing evolutionary biologists. We believe that roadblocks to progress lie A) in the underestimation of the role of the environment, and in particular, that of the interaction of genotypes with environmental factors, and B) in the continuing lack of incorporation of development into the evolutionary synthesis. We propose the integration of genetic, environmental and developmental perspectives on the evolution of the phenotype in the form of the concept of the *developmental reaction norm* (*DRN*) The *DRN* represents the set of multivariate ontogenies that can be produced by a single genotype when it is exposed to environmental variation. It encompasses: 1) the processes that alter the phenotype throughout the ontogenetic trajectory, 2) the recognition that different aspects of the phenotype are (and must be) correlated and 3) the ability of a genotype to produce phenotypes in different environments. This perspective necessitates the explicit study of character expression during development, the evaluation of associations between pairs or groups of characters (e.g., multivariate allometries), and the exploration of reaction norms and phenotypic plasticity. We explicitly extend the concept of the DRN to encompass adjustments made in response to changes in the internal environment as well. Thus, 'typical' developmental sequences (e.g., cell fate determination) and plastic responses are simply manifestations of different scales of 'environmental' effects along a continuum. We present: (1) a brief conceptual review of three fundamental aspects of the generation and evolution of phenotypes: the changes in the trajectories describing growth and differentiation (ontogeny), the multivariate relationships among characters (allometry), and the effect of the environment (plasticity); (2) a discussion of how these components are merged in the concept of the developmental reaction norm; and (3) a reaction norm perspective of major determinants of phenotypes: epigenesis, selection and constraint.

Key words: phenotypic evolution, reaction norm, allometry, development, ontogeny, heterochrony, plasticity, constraint, epigenetics, selection.

Evolution is ultimately defined at the genotypic level as the ensemble of processes that causes changes in allele frequencies. Yet, arguably the most fascinating aspect of evolutionary biology is the bewildering diversity and complexity observed at the phenotypic level. Given the only vaguely understood relationship between genotypes and phenotypes, the evolutionary modification of the phenotype remains one of the most intriguing problems in modern evolutionary biology. Our limited understanding stems from several different sources: the myriad genetic programs being carried out in the different tissues of a living organism and the necessity of integrating the products of such programs; the capacity of epigenetic systems to magnify the effects of simple genetic changes, or minimize effects of major ones; and the malleability of the developmental processes in the face of alterations in environmental conditions.

We believe that a comprehensive view of phenotypic evolution must explicitly incorporate three aspects of phenotypic expression: genetics, development and environment. A phenotype results from a complex and inextricable interaction between genes and environments throughout developmental time. Any given characteristic of an organism is the product of interactions among those genes and traits expressed during earlier stages, and the construction of a phenotype cannot be uncoupled from the particular environment in which it has developed. In this paper we combine the perspectives of genetics, development and environment in a representation of the phenotype we refer to as a *developmental norm of reaction*, i.e. the set of ontogenies that can be potentially produced by a single genotype when it is exposed to different environments. The developmental reaction norm encompasses: the processes that alter the phenotype throughout the ontogenetic trajectory; the recognition that different aspects of the phenotype are (and must be) correlated; and the ability of a genotype to produce phenotypes in different environments. We see three fundamental concepts arising from or congruent with this perspective:

of three fundamental aspects of the generation and evolution of phenotypes: the changes in the trajectories describing growth and differentiation (ontogeny), the multivariate relationships among characters (allometry), and the effect of the environment (plasticity); (2) a discussion of how, for a more comprehensive understanding of phenotypic evolution, these components are being and should be merged in the concept of the developmental reaction norm; and (3) a reaction norm perspective of two major determinants of phenotypes: epigenesis and constraints.

1. *Historical Overview*

The concepts of changes in the ontogenetic trajectory, correlations among characters, and response to different environmental conditions, which we combine into the definition of the developmental reaction norm, are not new. In fact, they all were proposed by the end of the last century or the beginning of this one. For historical and technical reasons, however, they have not been discussed within a common framework, and the few attempts to introduce them into the current paradigm of evolutionary biology have failed until recent times (Gottlieb, 1992; Hall, 1992; Rollo, 1994).

Three authors made significant contributions towards the synthesis that we are advocating, and they have been generally ignored, dismissed or considered as interesting peculiarities for half a century. Historically, ideas similar to some of those portrayed here were maturing by the end of the 1930's or the beginning of the 1940's in the work of three researchers: R.B. Goldschmidt in Germany, I.I. Schmalhausen in Russia, and C.H. Waddington in England.

In 1940 Goldschmidt published his very controversial book, *The material basis of evolution*, attacking the nascent 'modern' evolutionary synthesis on the basis that neodarwinism provided a parochial explanation of evolution, limited to microevolutionary phenomena (Goldschmidt, 1940). For him, the real challenge was explaining the evolution of new species and especially new body plans, a challenge he felt was clearly beyond the limits of the theoretical framework of Dobzhansky, Mayr and Simpson. His view of macroevolution, however, was much more comprehensive than the typical caricature of the "hopeful monster". He argued for a major role of chromosomal rearrangement, on the basis that, given the complexity of phenotypes, only such rearrangements of the already existing pieces were likely to bring workable alternative solutions to the morphology of an organism.

Goldschmidt reviewed examples of environmentally-induced phenotypes mimicking known mutations (he coined the term *phenocopy* for such cases), which in his view are a phenomenon of general biological importance, because they provide hints about gene action. If a mutation behaves exactly in the same way as the phenocopy, it is logical to conclude that this mutation induces a similar effect: the affected gene(s) work faster or slower. Goldschmidt also considered how the internal environment affects the creation of phenotypes, discussing in particular what was then known about hormonal action. He concluded that the regulatory action of both external and internal environments can produce astonishingly different phenotypes in genetically very similar backgrounds.

Goldschmidt finally discussed the effect of mutations on early development. He uses examples of the currently fashionable homeosis as a major explanation of how developmental mutants can trigger the production of radically altered phenotypes. The emphasis on action early in development was based on de Beer's (1940) idea that characters are much more interrelated at the beginning of the ontogenetic trajectory: mutation and selection acting at those stages would certainly be much more effective in reshaping the entire phenotype (Stebbins Jr., 1950; Arthur, 1988). This point has also been reemphasized recently by West-Eberhard (1989), and experimentally supported by Atchley (1984), (but see Raff et al., 1991; Wray, 1992, 1995).

Schmalhausen (1949) published his main contribution to evolutionary biology under the title *Factors of evolution: the theory of stabilizing selection*. His basic idea is simple and powerful: evolution is the process of how the developmental systems of living organisms are altered to change the norm of reaction to initially cope with, and later anticipate, the environmental stimuli. Schmalhausen begins by defining and discussing the concept of norm of reaction. He directs our attention to the manifold effects of mutation and to their environmentally-induced variability in expression (here coming close to the idea of phenocopy, so clearly expressed by Goldschmidt). He then introduces two important concepts: the distinction between a labile and a stable organism (i.e., a plastic and a non-plastic one); and the idea of a "normal phenotype", the result of past directional selection, and currently maintained by what he termed stabilizing selection, the selection against extreme phenotypes. A "new" norm of reaction arises initially because environmental changes expose a different portion of the existing reaction norm. There is then selection for mutations that improve the norm of reaction in the direction of the environmental change, followed by stabilizing selection on the new norm of reaction.

Schmalhausen emphasized that this sequence of events implies the shift from a reaction to the environment due to differential allelic sensitivity (a simple result of the biochemistry or physiology of the organism), to a more complex regulatory system. This system is capable not only of changing with the environmental conditions, but of *anticipating* the environmental demands (like the humidity-driven switch in many semi-aquatic plants between linear and dissected leaves, or the photoperiod-induced abscission of leaves

in many deciduous plants).

Waddington's ideas on development and evolution appeared in definitive form much later than the other two authors (*The strategy of the genes*, Waddington, 1957), but his basic intuitions and the experiments supporting them were published starting from the early 1940s. Central to the way in which Waddington perceives development is the idea of canalization: the tendency of a genotype to follow the same developmental path even in the face of internal or external perturbations. Canalization, in his conception, is clearly the result of natural selection, and from both its definition and the examples portrayed to support it (e.g., environmentally cued metamorphosis in amphibians, heterophylly in plants), the concept of canalizing selection is very similar to the idea of stabilizing selection on the new norm of reaction proposed by Schmalhausen (who acknowledged profound similarities between his thinking and Waddington's). The process described by Schmalhausen of change from the old norm to the new one through an initially environmentally-induced response is basically Waddington's notion of genetic assimilation (Waddington's later attempt [1961] to distinguish the two notwithstanding). In fact, genetic assimilation was originally defined as the process by which a phenotypic character initially produced as a response to some environmental influence, is stabilized due to natural selection and finally occurs even in the absence of the previously necessary external influence (Waddington, 1942). This is an explicit view of reaction norms as the objects of selection.

Waddington demonstrated the occurrence of genetic assimilation with his famous series of experiments on *Drosophila* subjected to heat shock during larval development. He began with a stock of flies that produced a novel phenotype in low frequency when subjected to heat shock at an early larval stage. After several generations, he observed the appearance of the novel phenotype in the absence of the heat shock (Waddington, 1952, 1953, 1959). He later generalized such findings to different traits and environmental stimuli (Waddington, 1961).

2. *Current Approaches to Phenotypic Evolution*

The conceptual framework proposed by Goldschmidt, Schmalhausen and Waddington has not been incorporated into current models of phenotypic evolution. Most of these have been derived from the statistical-genetic underpinnings of the modern synthesis established by Fisher, Wright and Haldane and promulgated by Dobzhansky, Mayr and Simpson. The emphasis in early models and their current elaborations has been on simple genetic systems—mostly many alleles with small additive effects. One main reason for this emphasis is that the mathematical treatment of these systems can be based on sets of simple linear equations, which allow analytical solutions of general validity. Few explicit attempts have been made to include development and epigenetics into these models because they introduce non-linear effects leading to mathematical intractability and solutions that are not generalizable.

There are two main current approaches, optimization modelling and quantitative genetics. Although optimization models allow the fitness function (i.e., the mapping of phenotypes onto fitness, or the adaptive landscape) to change depending on the conditions of the population (e.g., frequency-dependent selection), their major limitation is that they do not address at all the genetic basis of phenotypic evolution. These models assume that the genetic variation necessary for selection to operate will be present in the population. This implies that there are no genetic constraints (and thus no development or epigenetics) and that phenotypic evolution is governed only by conflicting selective pressures.

Quantitative genetics, on the other hand, specifies a particular genetic basis for phenotypes, but generally assumes both temporal and spatial constancy of the genetic variance-covariance matrix, and of the fitness surface (i.e., the relationship between character states and fitness is fixed). Also, the genetic system governing phenotypic traits is assumed to be very simple (but see Barton and Turelli, 1989). These limitations have been recognized (Turelli, 1988; Schlichting and Pigliucci, 1995a), and attempts have been made to merge the two approaches (Roff, 1994). However, as Charlesworth (1990) argued, a simple mapping of genetic constraints (quantitative genetics) onto functional limitations (optimization models) is possible only in very simple situations (e.g., the case of two traits governed by a trade-off, based on a negative genetic correlation). Additionally, when we consider a complex multivariate phenotype, simple genetic correlations are not necessarily reliable indicators of functional constraints or tradeoffs anymore, as pointed out by Houle (1991).

Other attempts have been made to include a more complex view of the phenotype within the quantitative genetics framework. Although explicitly recognizing the role of developmental processes in evolution (e.g., Atchley and Hall, 1991; Cowley and Atchley, 1992), the inclusion of more statistical terms (e.g., "epigenetic" components) into the model does not address the fundamental issues either. What is needed is a mechanistic analysis of how variation arises and is filtered through development: selection operates throughout the life cycle, and a view limited to the adult phenotype is therefore insufficient to account for its effects in shaping phenotypic evolution. From this point of view, one problem with the current synthesis is an imbalance between an emphasis on population-level phenomena (dealing with how novelties spread) vs. individual-level processes (dealing with the origin of phenotypic novelties).

Developmental Reaction Norms: Allometry, Ontogeny and Plasticity

Coined by Woltereck in 1909 (Woltereck, 1909), the term reaction norm defines a suite of phenotypes (potentially or actually) produced by a single genotype when the development occurs in a given range of environments. (It is interesting to note that Woltereck used the existence of reaction norms as an argument against the concept of the genotype [Johannsen, 1911]). From a practical standpoint, reaction norms can be measured as average responses of genotypes, populations, or species, depending on the level of analysis one is interested in (much in the same way that simple character means measured within an environment can be averages at any of the same levels).

The determinants of the reaction norm that we will consider are: (a) ontogenetic trajectories, describing the unfolding of the developmental program; (b) allometry, measuring the relationships among individual traits of the whole organism; and (c) plasticity, as a measure of the magnitude and pattern of the response to the external environment.

1. *Ontogeny*

The ontogeny of an organism includes all events, both quantitative and qualitative, occurring from the single cell through the adult stages. As a field of a study, developmental biology is much older than evolutionary biology itself, and its practitioners have been interested in not only documenting patterns, but in identifying general rules for the production of organismal form, as well as evolutionary relationships. It is, however, a field in rapid expansion because of the very recent advances of molecular developmental genetics in both animal and plant model systems (e.g., Akam et al., 1994b).

The quantitative events that occur during the ontogeny of an individual (e.g., increase in size) can be visualized as an ontogenetic trajectory (Alberch et al., 1979), with the qualitative events (e.g., differentiation, metamorphosis, etc.) corresponding to particular points on the trajectory (e.g. (Creighton and Strauss, 1986; Strauss, 1990). The trajectory can be described mathematically, usually by means of a sigmoidal function (although other functions may be used as well) (Fig. 2). As the link between the genes and the adult phenotype, ontogeny represents the ensemble of processes by which local genetic rules are translated into functional phenotypes, that is the epigenetic system.

2. *Allometry*

Allometry is a concept originally developed by Huxley (1932) in reference to the proportional growth of one character relative to a second character (e.g., changes in aspects of the horse skull (Radinsky, 1984)), or to some measure of overall organ/body size (e.g., brain:body size; Lande, 1979). In Huxley's view, allometry referred specifically to phenomena in which the rate of growth of one feature does not equal the rate of growth of the second feature (i.e., the allometric coefficient is greater than or less than one), in contrast to isometry, which represents the special case where two features increase in size at exactly the same rate (i.e., the allometric coefficient equals one). Huxley's description has since been generalized (Cock, 1966; Gould, 1966; Cheverud, 1982) to include regression analyses based on measures on multiple individuals taken at a specific developmental stage, often maturity (static allometry: Fig. 3), as well as on species means along a phylogeny (evolutionary allometry). Allometric associations have been used to infer shared develop-

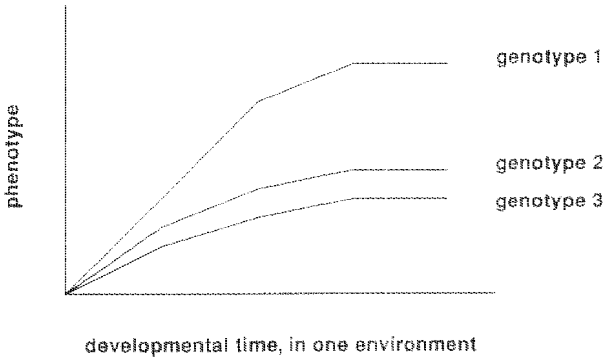

Fig. 2. A depiction of the ontogenetic trajectories of three genotypes grown in a single enviroment. The phenotypic trait increases through time, eventually reaching a constant value at maturity. Genotype 1 has a steeper growth trajectory, and achieves a larger final size.

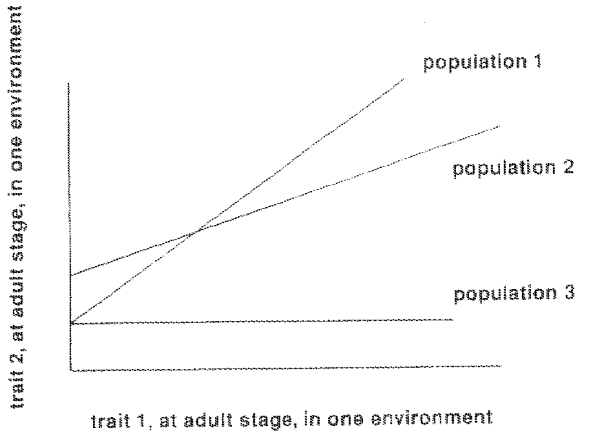

Fig. 3. A representation of static allometry at the adult stage in a single environment. The three populations differ in the relationships between traits 1 and 2. Populations 1 and 2 show a positive relationship, whereas in population 3, there is no correlation between the two traits.

mental mechanisms and to examine evolutionary phenomena underlying the observed patterns.

The empirical study of allometry allows us to determine how characters are correlated and therefore potentially integrated into the whole phenotype. Recently, the idea of phenotypic integration has been at the center of increasingly sophisticated multivariate approaches to the study of allometry (e.g., Jolicoeur, 1989; Klingenberg and Zimmermann, 1992; Klingenberg and Spence, 1993). It is now possible to describe the complex interrelationships among many traits simultaneously, as well as the variation in natural populations for the way integration is achieved.

3. *Plasticity*

The concept of phenotypic plasticity is so tightly connected with the idea of reaction norm that the two are often considered to be synonymous, although they are technically distinct. The idea of reaction norms is as old as the distinction between genotype and phenotype introduced by Johanssen (1911) at the beginning of the century. It was greatly elaborated by Schmalhausen (1949), and brought again to the evolutionary debate by Bradshaw (1965), followed more recently by a number of other researchers (e.g., Schlichting, 1986; Sultan, 1987; Stearns, 1989; West-Eberhard, 1989; Schlichting and Pigliucci, 1995b).

Conceptually, the reaction norm can be imagined as a genotype-specific function defined on a cartesian plane with some measure of the environment on the abscissa, and a measure of the phenotypic trait on the ordinate (Fig. 4). Plasticity is a particular attribute of the reaction norm describing any case in which the reaction norm of a genotype is not flat (i.e., it is not a line parallel to the environmental axis). There are currently two classes of phenotypic plasticity recognized (Smith-Gill, 1983): *phenotypic modulation*, where the plastic responses are a continuous and proportional function of the environmental stimulus (e.g., plant height influenced by nutrient availability, water or light; thorax length in *Drosophila* influenced by temperature); and *developmental conversion*, where responses are of a threshold type and are not proportional to the stimulus (e.g., an initial environmental cue triggers a series of developmental events in the organism such as germination, flowering, shade avoidance response, and heterophylly in plants).

Empirically, reaction norm plots allow the researcher to trace the way different genotypes respond to a given set of environmental changes (e.g., Gupta and Lewontin, 1982). The spread and amount of crossing of the reaction norms in such diagrams (Fig. 4) is a representation of the genetic variation for character means and plastic responses in the given population (de Jong, 1995).

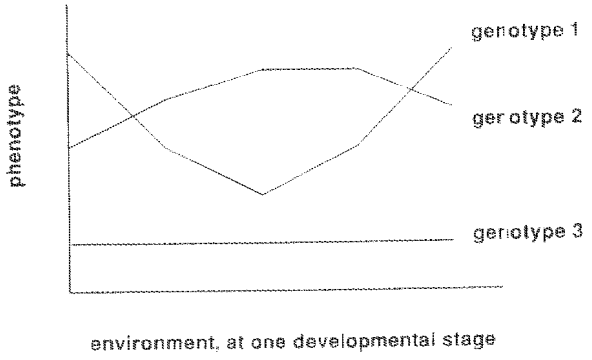

Fig. 4. A typical reaction norm diagram representing the expression of a single phenotypic trait (e.g., plant height) measured at one point in time in several environments (e.g., across a nutrient gradient). In this example, there is variation among the three genotypes: 1 and 2 display different plastic responses, while 3 is non-plastic.

4. *Allometry and Ontogeny*

The first two-way interaction among the three basic concepts just reviewed is that between allometry and ontogeny. Allometry is often measured as a developmentally static entity, usually using data from adult individuals; at the same time, most published papers on ontogenetic trajectories consider one character at a time. If we combine the two perspectives (Fig. 5), we appreciate that allometric coefficients can show complex dynamics throughout ontogeny, as recently pointed out by a number of authors (Dragavtsev and Utemisheva, 1975; Zelditch, 1988; Kellogg, 1990; Klingenberg and Zimmermann, 1992; Jones, 1993; Ki-

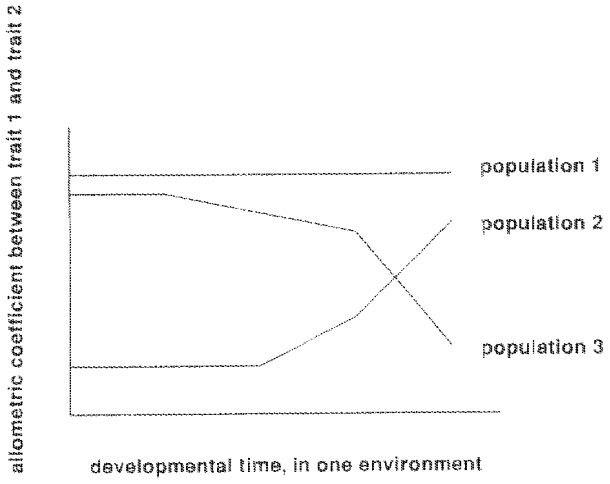

Fig. 5. Combining allometry and ontogeny: a representation of the relationship between traits (e.g., plant height and new leaf production) as a function of developmental time, measured in a single environment. The two traits have a constant relationship for population 1 throughout development, but the relationship changes in each of the other populations.

ingenberg and Spence, 1993; Bonser and Aarssen, 1994). For example, Pigliucci et al. (1996) found for *Lobelia siphilitica* grown under high nutrients that the correlation between leaf production and plant height declined from 0.63 to 0.02 between the fourth and tenth week of development.

The study of such diagrams yields a better understanding of how phenotypic integration in the adult is forged throughout development. Furthermore, in this way it is possible to pinpoint periods of the ontogeny when the correlation between two traits is stronger or weaker. If this correlation has a genetic basis, this means that there will be more or less favorable opportunities for selection to act, depending on which ontogenetic stage is going to be affected by selective pressures (as has been pointed out by Atchley, 1984). The detailed knowledge of the occurrence of such "windows" can add an entirely new dimension to the evolutionary consequences of character correlations, as well as point to alternative strategies of plant or animal breeding (e.g., in cases in which one wants to apply selection on two traits in a direction that would be opposed by the existing correlation at the adult stage).

5. *Allometry and Plasticity*

The connection between allometry and plasticity opens the way to the understanding of the other side of variation in allometric coefficients: their environmental dependence. Allometry is usually measured in a single environment ("common garden" conditions), the intent being to characterize the genetic variation in phenotypic correlations, leaving out "environmental noise". At the same time, plasticity is usually studied on single characters, with reaction norms for separate traits plotted against the environmental variable(s) and then compared in a qualitative way. However, we now know that both phenotypic and genetic correlations (and therefore allometric coefficients) may change dramatically when the same set of genotypes is raised in different environments (Lechowicz and Blais, 1988; Schlichting, 1989a,b; Stearns et al., 1991; Platenkamp and Shaw, 1992; Thompson, 1992; Andersson and Shaw, 1994; Bonser and Aarssen, 1994; Miller et al., 1994; Schmid and Dolt, 1994; Windig, 1994; Cheplick, 1995; Hakkarainen and Korpimaki, 1995; Pigliucci et al., 1995; Schlichting and Pigliucci, 1995b). For example, in the previously mentioned study on *Lobelia* (Pigliucci et al., 1996), the correlation of leaf number and height at week ten was 0.02 in the high nutrient treatment, but 0.66 under low nutrients.

These kinds of data tell an analogous story to the one related by the ontogenetic changes of allometric coefficients: allometry is not an invariant property of certain characters. It can be altered when the environmental conditions are changed (Fig. 6). This has again both evolutionary and practical consequences on the correlated response of several traits to selection, which

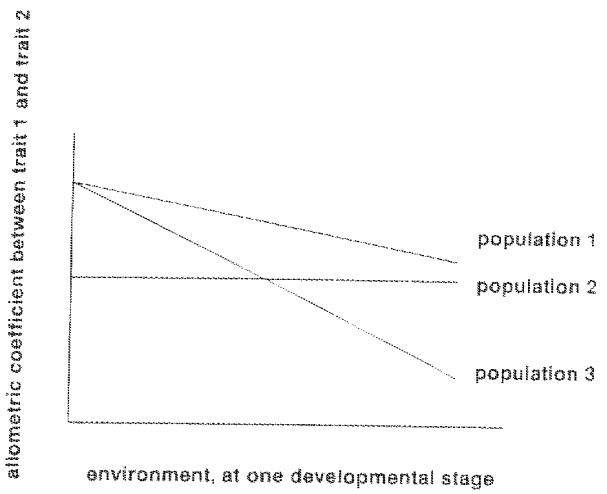

Fig. 6. Combining allometry and plasticity: a representation of the effect of changing the environment (e.g., decreasing food supply) on the relationship between traits (e.g., body size and reproductive output), measured at a single developmental stage. The allometric coefficient is unaffected by the environment for Population 2, but changes (is plastic) in the other two populations.

is going to be different in different environments, as elegantly demonstrated by Neyfakh and Hartl (1993).

6. *Ontogeny and Plasticity*

Reaction norms are usually studied at the adult stage, mostly for simplicity (following several genotypes in different environments throughout their growth can be cumbersome). Analogously, ontogenies are often characterized for organisms raised in one—usually standardized—environment. But, like any other aspect of the phenotype, reaction norms originate through a developmental process, and should therefore be studied from a developmental perspective in order to be properly understood (Stearns, 1982, 1983). Some researchers have recently addressed the plasticity of ontogenetic trajectories in a few model systems (Matthies, 1990; Diggle, 1991a,b, 1993, 1994; Jones, 1992, 1993, 1995; Pigliucci and Schlichting, 1995; Pigliucci et al., 1996). Jones (1995), utilizing a detailed ontogenetic analysis, demonstrated that less-lobed, more juvenile looking shade leaves of *Cucurbita* arose through plastic responses rather than as a prolongation of the juvenile phase.

Such analyses directly provide insights into how the parameters affecting growth (onset, offset, and rate of developmental events) are altered by changes in environment (Fig. 7). Some characters may have a very stable ontogeny across a wide array of conditions, while others can be extremely sensitive to changes in the environment. A knowledge of these differences would help our understanding of the developmental

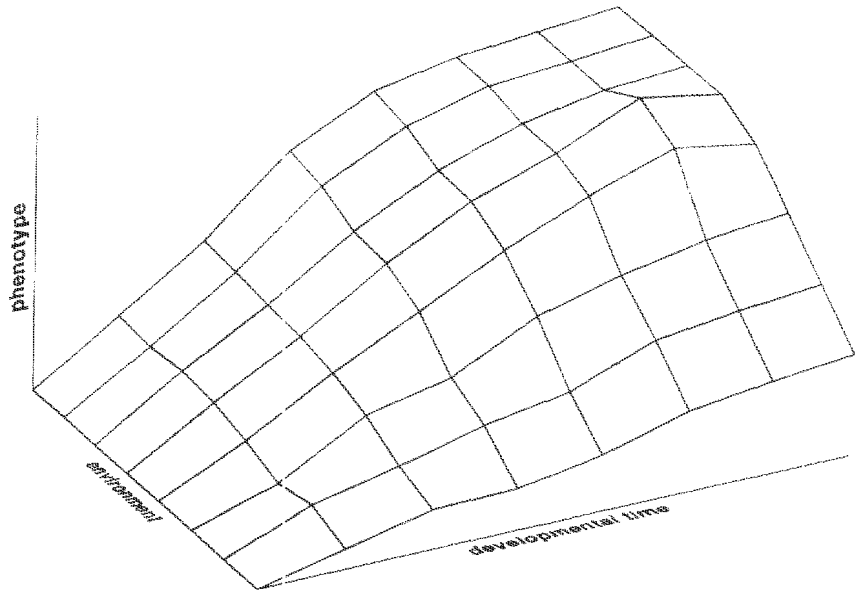

Fig. 7. Combining ontogeny and plasticity: the lines along the development time axis represent the ontogenetic trajectories for a single trait (e.g., body mass) measured across several different environments (e.g., different temperatures). The trait attains low values in some environments and higher values in others.

mechanism itself, and of how some parts of it evolved to either be resistant or to respond flexibly to heterogenous conditions.

7. *The Three-way Integration: Complexity in the Study of Phenotypes*

We have presented the progression from isolated views of phenotypes based on allometry, ontogeny and plasticity, to the possible two-way interactions between them. The logical extension of this progression leads to the complete integration of the three aspects of phenotypes so far discussed (Pigliucci and Schlichting, 1995; Pigliucci et al., 1996). Ontogeny can be measured for multivariate traits and across environments; allometric coefficients can be calculated under different conditions and throughout the ontogeny; and reaction norms of character correlations can be plotted at different times during the development (Fig. 8).

Clearly, both the environmental and the phenotypic axes are themselves multidimensional in the real world, and we recognize that it is impossible to capture the whole of organismal complexity in a single graph. The point, however, is not just to present a different or more complete way to graphically visualize the phenotype. The important message is that the concept of the developmental reaction norm as characterized by the three-way interaction can provide us with new insights into how phenotypes are generated and evolve.

This integrated view explicitly addresses the potential for: (1) the environment to modify developmental trajectories; (2) different developmental stages to be more plastic than others; and (3) the changes of correlated character complexes to be substantially more intricate than single traits considered in isolation. Thus, we suggest an integrated view of the phenotype as the genotype-specific response of ontogenetic trajectories to environments. We consider the developmental reaction norm as the object of selection, leading to the conclusion that selection can differentially affect the phenotype depending on its time and place of action, a result that, while obvious in this context, is virtually ignored in current models of phenotypic evolution.

Admittedly, few researchers will find it necessary or even possible to address all these facets within a single study. However, what we are advocating is a ***change in perspective***, leading to the continuous awareness that all those pieces are there even if we ignore some of them for the sake of convenience. Ultimately, our ability to understand the intricacies of phenotypic evolution will depend on our capacity to integrate its separate components. After all, even though we are forced to focus on particular aspects of the organism at any given time, natural selection continues to operate on the complex whole.

Evolution of Developmental Reaction Norms

In our view, the evolution of developmental reaction

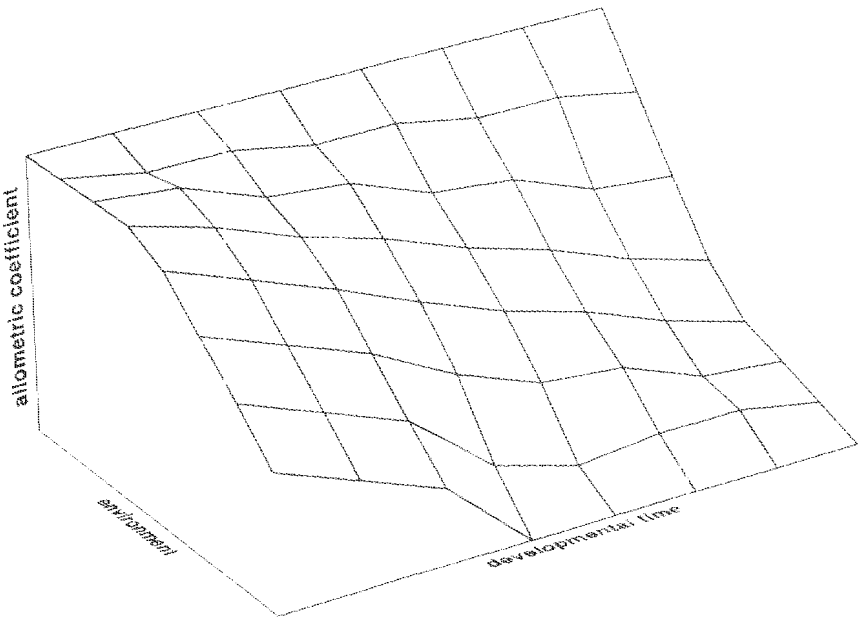

Fig. 8. The developmental reaction norm: Combining allometry, ontogeny and plasticity. This is a diagrammatic representation of the change in a multivariate phenotype (e.g., the correlation between flower production and plant height) through development, and in different environments (e.g., a nutrient gradient). The allometric coefficient is unaltered through time in one environment, but the two traits become more and more uncoupled through development as the nutrient level changes.

norms proceeds by means of alterations of the epigenetic system. In this section we discuss the mechanistic bases of epigenetic systems, and ultimately of phenotypes. We then consider the means by which developmental reaction norms can be altered, and finally we discuss the evolutionary outcomes of the selective forces and the constraints acting on phenotypes.

Evolution can be examined by analyzing changes in the genetic composition of populations and species. The raw material for such changes is provided by a panoply of "mutational" events: point mutations, chromosomal rearrangements, gene conversion, mobilization of transposons and changes in methylation patterns (among others). Each of these can modify the environment-specificity of the epigenetic system to produce changes in the developmental reaction norm.

Alternatively, we can study the phenotypic effects accruing from these various causes: the commonly invoked heterochrony (changes in timing or rate of developmental events), canalization (reduction of developmental instability), or less well understood phenomena such as modifications of cell movements or of the plane of cell division, alterations in tissue induction and differentiation patterns, and structural changes in receptors or in allosteric enzymes (Monteiro et al., 1994; Niehrs et al., 1994; Schmidt, 1994; Williams et al., 1994; Wolpert, 1994; Kondo and Asai, 1995).

1. *The Epigenetic System*

Since the discovery that DNA is the "information" molecule that organizes the development and function of living organisms, evolutionary biology has slowly become trapped in the metaphor of the "blue-print". DNA is often regarded as a manual of instructions that details every aspect of biological organization. In light of what we now know about gene expression, this view should clearly be revised.

In order to begin to understand epigenesis, we must first extract its general attributes. We propose that there are fundamentally four: 1) the action of individual genes during development is local, 2) genes interact directly only with small numbers of other genes, 3) there are multiple levels of regulatory control, and 4) the phenotypic outcomes are not predictable from a detailed knowledge of gene action (i.e., there are emergent properties).

The concept of a blue-print is that of a plan to specify or anticipate every possible situation and the response most appropriate to it. However, no molecule can store as much information as needed for the detailed description of the phenotype, including all the biochemical pathways and developmental interactions, and the reactions of all these to the environment. A modular organization, on the other hand, is constructed so as to partition the decision-making process at several partially independent levels, increasing the overall flexibility of

the system. Therefore, what DNA does is to generate products from groups of genes that are characterized by **localized action**, without any direct reference to the overall scheme. The outputs of these numerous local gene actions are controlled by regulatory elements that direct development along alternative pathways depending upon internal or external "environmental" conditions. Selection works on these local gene effects based on their contributions to the final "output" of the program.

The genetic-epigenetic program of development falls into the category of the undecidable; no mathematical treatment will be able to predict exactly what an organism will look like (see, for example, our incomplete understanding of how the phage λ works, even though the complete sequence of its genome has been available for many years: Ptashne, 1992). The combination of local action and loose connectivity of the genetic modules leads to what are then perceived as "emergent properties". The dynamics of genetic algorithms and neural networks are "emergent", in the sense that they are not specified in the lines that make up the program, nor were they necessarily intended by the programmer (e.g., Bains, 1994).

The importance of the environmental component can be appreciated if we consider a particularly popular version of genetic algorithms, a class of programs that "learn" to play games (e.g., chess) more and more efficiently through a series of trial games with opponents of varying skill. The algorithms evolve in different ways, depending on which "environments" the program has been exposed to. Yet, the long-term outcome is remarkably similar: programs capable of playing and winning against highly sophisticated opponents. In a biological context, these disparate evolutionary trajectories can be paralleled with the evolution of the same reaction norm by different genetic-epigenetic systems ("genetic redundancy", "genetic piracy": see below).

Nijhout et al. (1986) applied concepts of localized gene action and complexity theory to biological processes when they investigated a cellular automata model of development and phylogeny. They specified a series of simple genetic rules according to which their "wild type" was constructed, and then mutated some of these rules to examine the effects. There was no way to tell *a priori* what a specific change in the rules was going to do to the relatively simple morphology of the two-dimensional automaton! Mutations causing change in rate of gene action did not cause morphological heterochronies, while some apparently heterochronic alterations in the adult phenotype did not arise from heterochrony at the gene level.

A view of evolution by change of local rules or of the control mechanisms that supervise the interaction among genetic subroutines leads to explanations of several evolutionary phenomena affecting the epigenetic system. The possibility of co-opting some parts of the network of gene interactions for a new and possibly unrelated task is what Roth called 'genetic piracy' (Roth, 1988; Carroll, 1994). Roth cites as an example the evolution of convergent developmental pathways leading to a single control of the formation of hind- and fore-limbs in vertebrates, once completely distinct morphogenetic systems. A somewhat similar phenomenon is genetic redundancy, in which the same phenotype is produced by genetically distinct backgrounds (Goldstein and Holsinger, 1992; Dove, 1993; Thomas, 1993).

2. *Genetic and Epigenetic Modifications of* DRN

Because the developmental reaction norm integrates a complex view of genotype, development, and environment, we view the importance of "mutational" events that generate genetic variation in terms of their effects on the epigenetic system. Therefore, an important issue is what kinds of genetic changes can affect the epigenetic system, and hence the developmental reaction norm? Two fundamental phenomena have been proposed as instrumental in changing the course of development: *heterochrony*, or the change in timing of gene action/developmental events; and *heterotopy*, the change in the place of gene action/developmental events.

Heterochrony has always been considered a central process in the evolutionary change of developmental patterns. The word heterochrony was introduced by Haeckel (1875) within the framework of his recapitulation theory, but the modern sense of the word comes from de Beer (1940). An historical as well as technical review of these concepts is to be found in Gould (1977), and updated in McKinney and McNamara (1991). Gould (1977) and Alberch and coworkers (Alberch et al., 1979; Alberch, 1982) simplified de Beer's terminology, proposing a model for the understanding of heterochronic phenomena which—at least potentially—is amenable to a link with genetics (for example see Slatkin, 1987). However, the relationship between phenotypic heterochrony and changes in timing at the level of the gene has rarely been shown to be direct (Raff and Wray, 1989; Wray and Raff, 1991; Bassiri et al., 1992; Conway and Poethig, 1993; Ambros and Moss, 1994; Collazo, 1994). In some cases, heterochronic changes have been shown to be environmentally triggered (with macroevolutionary implications for phenotypic plasticity) such as the studies of diet-induced changes in jaw morphology in cichlids (Meyer, 1987) and paedomorphosis in ambystomid salamanders (e.g., Semlitsch et al., 1990).

Heterotopic phenomena are less commonly reported and less well understood than heterochronic ones. This difference might be due to the fact that the detection of heterotopy requires detailed knowledge of gene action and of its mapping onto phenotypic effects, information that is seldom available to the evolutionary biolo-

gist (see e.g., Simpson et al., 1986; Fa ng et al., 1991; Duboule, 1994). Wray and McClay (1989) reported on the potential importance of changes n the place of gene action toward explaining the adaptive radiation of echinoids in the Early Paleozoic. Analyses of patterns of time and site of gene expression for three proteins in seven echinoid species (for which a rel able phylogeny was available) revealed heterochronic and heterotopic changes for all three proteins.

Many of the mechanisms for modifying epigenetic systems are being uncovered in the intensive research currently underway on the function and evolution of homeotic genes (for recent reviews see Manak and Scott, 1994; Meyerowitz, 1994; Patel, 1994; Carroll, 1995). Work with animal systems (most notably mice, *Drosophila*, and the nematode *Caenorhabditis*) has revealed major areas of homology among the homeobox genes (Manak and Scott, 1994; Salser and Kenyon, 1994; Warren et al., 1994; Sordino et al., 1995). Work with plants such as *Arabidopsis* has indicated the presence of homeotic genes as well (Crone and Lord, 1994; Meyerowitz, 1994; Ray et al., 1994). In addition, alteration of pattern formation (Niehrs et al., 1994; Rivera-Pomar et al., 1995) and modularity/segmentation (Akam et al., 1994a; Patel, 1994), changes in signal transduction (Brown and Hartley, 1994; Patel, 1994), change of function (Hake, 1992), and changes in patterns of cell movement (Salser and Kenyon, 1994) have all been identified.

3. *The Evolutionary Outcome: Selection and Constraint*

The evolution of the developmental reaction norm is ultimately a matter of balance between the selective forces acting on the epigenetic system, and the variability (or lack thereof) of the genetic machinery driving the epigenetic system itself. The limits and preferential routes that are superimposed on selection are collectively known as "constraints". We wish to discuss this concept in some detail in order to achieve a more complete picture of how developmental reaction norms change through evolutionary time.

Biologists have a bewildering selection of "constraints" to choose from: genetic, phylogenetic, mechanical, functional, developmental, selective, ecological, to mention but a few. The literature—and the spirited controversy—about constraints is so vast that any attempt to synthesize the matter is doomed to be both incomplete and a very delicate operation (see e.g., (Antonovics and van Tienderen, 1991; Perrin and Travis, 1992; van Tienderen and Antonovics, 1994). However, we will attempt it anyway.

Although several previous overviews of the concept of constraint have identified both genetic and developmental constraints as distinct from selection (Maynard Smith et al., 1985; Scharloo, 1988; Wagner, 1988; Arnold, 1992; Schwenk, 1995), here we identify only two comprehensive categories of evolutionary forces: **selective pressures** (positive or negative forces) and **genetic/epigenetic constraints** (negative forces) (Figure 9). Evolutionary change (or stasis) results from the outcome of the interaction among the possible components of these two forces. Note that sometimes selection and genetic constraints can act in the same direction: for example, stabilizing selection tends to decrease genetic variation, which in turn will keep the population in the current area of the phenotypic space.

We can use the developmental reaction norm perspective to visualize the process by which selection and constraint define phenotypic space. We have devis-

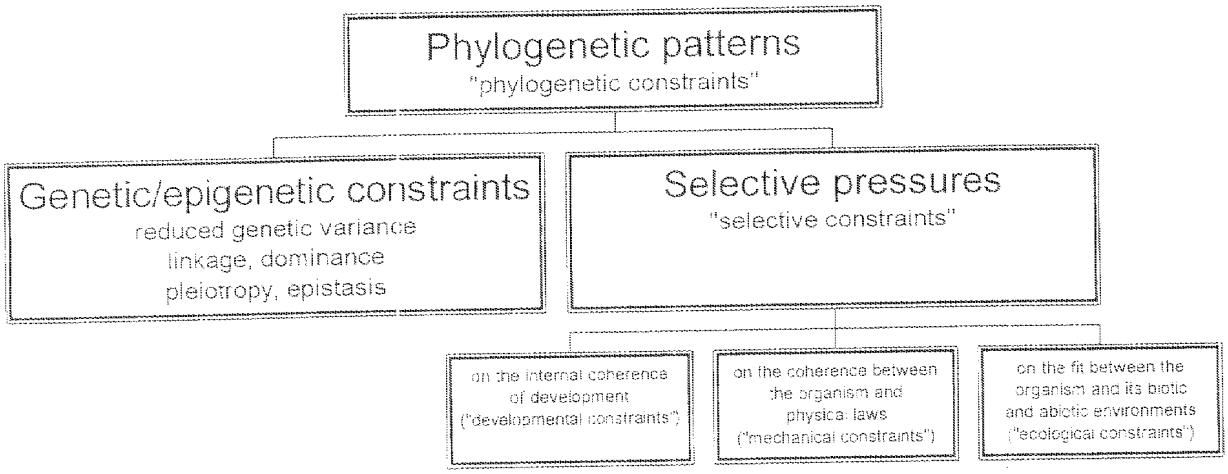

Fig. 9. Genetic/epigenetic constraints and selective pressures. These two categories of evolutionary forces are shown, along with their relationship to some of the former categories of "constraint". Genetic/epigenetic constraints are negative forces, limiting evolutionary movement in phenotypic space, whereas selective pressures may be positive (directional/disruptive selection) or negative (stabilizing selection) forces.

ed a scenario in Figure 10 (for the hypothetical determination of digit number) moving from the level of the gene through the epigenetic process to the phenotypic state. The character has a genetic component and if there is only a single allele for a gene, a genetic constraint exists. In our example there are multiple genes with multiple alleles contributing to the expression of a continuous character, cell number. The epigenetic rules provide for a symmetrical bifurcation of cells when the cell number exceeds 300, converting the continuous cell number distribution into a discrete pattern of 1, 2, 4, 8 ... digits. In this example, we have also set limits to cell number through natural selection—combinations of alleles that result in <125 or >1000 cells are lethal due to disruption of the developmental system, restricting digit production to 1 through 4.

Although an analysis based on the fitness function suggests that 2, 3 or 4 digits would be equally fit, the epigenetic rule results in a pattern of phenotypic expression (i.e., 2 or 4, but not 3 digits) that would be recognized as a 'developmental' constraint. By examining the developmental reaction norm, we can see that this pattern results from the epigenetic rule of bifurcation of the cell mass that we specified (an *epigenetic* constraint). Thus expression of different sets of alleles at the lower level (i.e. cell number) is controlled in turn by

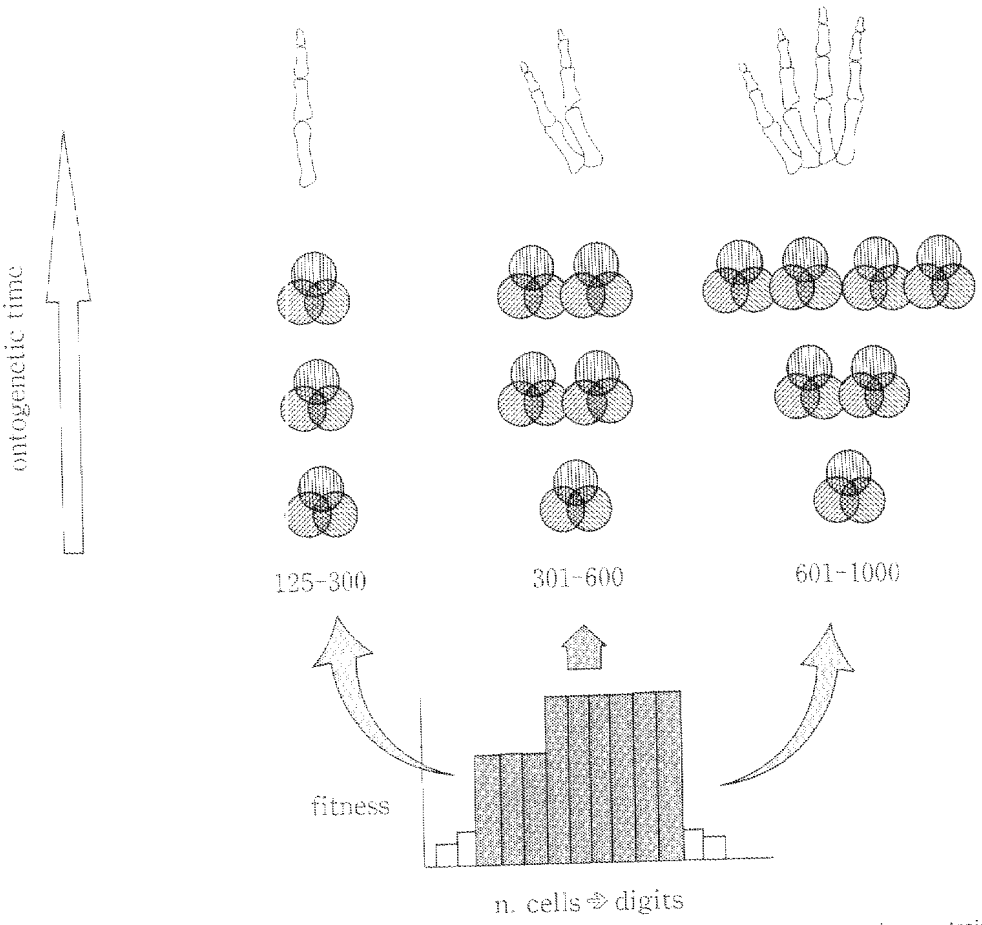

Fig. 10. A developmental reaction norm view of the relationship between genetic/epigenetic constraint and natural selection. The lower diagram delineates the relationship between initial cell number and fitness: cell numbers below 125 or above 1000 (open boxes) are lethal, those between these thresholds (shaded boxes) produce 1, 2 or 4 digits. Organisms with a single digit are less fit than those with 2 or 4 digits. The upper diagram depicts the operation of the epigenetic system during ontogeny. The fate of cell masses of different sizes is determined by an epigenetic rule specifying an equal bifurcation of cell masses greater than 300 cells: masses less than 301 cells do not bifurcate and a single digit eventually results, masses between 301 and 600 cells bifurcate once leading to formation of two digits, and cell masses between 601 and 1000 cells bifurcate a second time with four digits resulting. Given the specified epigenetic rules, there is no way to produce three digits, even though such an organism would presumably have equivalent fitness to the two and four digit morphs (see text for further discussion).

the action of other genes at a higher level (bifurcation rule), resulting in an example of epistasis. However, it becomes clear that if we shift our focus to that higher level of the hierarchy, the observed pattern of 1, 2 or 4 digits results from a *genetic* constraint: there is no allelic variation at the gene loci determining the bifurcation rule. To get three toes, we could envision a rule that might allow a trifurcation, or one that results in an unequal division of the cell mass.

The process of building and breaking constraints through evolutionary time results from the continuous interplay between the expressed phenotypes and the agents of natural selection, and the subsequent feedback onto the developmental system. The phenotypes we observe are a snapshot depicting the current developmental reaction norm. As new alleles arise, their pleiotropic and epistatic effects must be integrated with the existing genetic architecture—selection will favor those with higher fitness and these may become established in the population. However, because there may be a number of possible alleles at any given locus that could be successfully integrated, the actual identity of the new allele (or the particular locus) will help determine the success or failure of later opportunities for "tinkering". This is the historical contingency (arising from the random appearance of new alleles) that has been referred to as "phylogenetic constraint". Thus, access to some portions of phenotypic space will be prohibited sometimes directly by selection, sometimes by genetic/epigenetic constraint, and often by the constraints resulting from previous episodes of selection, drift or chance mutational events.

From the point of view of this paper, the important aspect to consider is that because phenotypic evolution results from modifications of the developmental reaction norm of the genetic-epigenetic system, then selection and constraints in particular environments can have ramifications for the entire reaction norm, and not only the phenotype produced in the focus environment (*contra* Via, 1987, 1994). This implies that in order to produce a realistic representation of an evolutionary trajectory, we need an understanding of the range and frequency of actual environments in which evolution occurs, and a description of the environment-specific selective pressures and constraints.

Conclusions

1. The developmental norm of reaction is the object of selection. We see the phenotype of an organism as characterized by the potentiality of its genotype to express a series of developmental trajectories, depending on the particular set of environmental conditions to which the individual is exposed. This view stems from three basic considerations. First, the common focus on adult stages may lead to a restricted understanding of evolutionary potentials. The origin of the observed differences in adult phenotypes can only be understood by tracing their ontogenetic trajectories. In some cases, similarities in adult phenotypes may not be due to the same developmental pathways (Jones, 1993; Pigliucci and Schlichting, 1995; Pigliucci et al., 1996). From what we know about gene expression and organization, major phenotypic effects can be obtained by altering either the time or the place of gene action. Therefore, heterochrony and heterotopy of gene action represent major ways to alter ontogenetic trajectories.

Second, an organism cannot be considered independently from its environment. To discuss phenotypes without the context of particular and ecologically relevant environment(s) neglects a crucial aspect of phenotypic complexity. Third, organisms are harmonious ensembles of traits, and character correlations and integration must play a fundamental role in our theories of how selection and constraints shape phenotypic evolution.

We explicitly integrate these three components of the phenotype in our concept of the developmental reaction norm. Evolution proceeds from a dialectical interaction between organisms and environments through ontogeny.

2. The innate complexity of genetic systems necessarily leads to emergent properties, usually encompassed by the term "epigenesis". We consider misleading the common metaphor of genes encoding the "blueprint" for the phenotype. More realistically, genes represent a series of subroutines that interact with each other, initiating and locally controlling the events that unfold during ontogeny. In principle, knowledge of the mechanistic details of what each subroutine does and how it interacts with others, does not allow prediction of what the final phenotype will be. As a consequence, the phenotypic effects of altering the rules (i.e., mutation) are also unpredictable. Conversely, it is also not possible to predict the genetic basis of a particular phenotypic change.

Epigenesis then, is the deterministic, yet "undecidable", series of ontogenetic events resulting from the interaction of numerous local genetic systems (as opposed to a global central control).

3. Both the control of the development of complex phenotypes and the capability of these to respond to environmental variability (internal or external) requires a system of balanced regulatory interactions. Both surveys of current knowledge of the molecular mechanisms of gene regulation (Schlichting and Pigliucci, 1995a; Pigliucci, 1996), and models of gene networks based on complexity theory point to the occurrence and advantages of intermediate and localized levels of regulation (Bak and Chen, 1991). Systems that are

very tightly regulated are prone to instability (so-called "complexity catastrophes"). On the other hand, classical Fisherian models of independent genes additively acting on the phenotype lack the connectivity that is necessary to readily move within the adaptive landscape, and show the tendency of settling on local rather the global optima.

We have arrived at this view of the phenotype by combining our different vantage points of evolutionary morphology, the evolution of development, and ecological genetics. These disparate viewpoints have made us receptive to the argument that development was left out of the grand synthesis of the '30s and '40s, and cognizant of the importance of a view of phenotypes from a reaction norm perspective. Thus, the move toward combining ontogeny and reaction norms was a natural progression. The difficulties inherent in combining all aspects in an experimental program are obvious. However, even if all components are not explicitly examined, we believe that it is just as important to openly maintain an awareness of the potential contributions of the environment, the interactions among traits, and the possible roles of other ontogenetic stages, when assessing phenotypic expression and evolution.

Acknowledgements We thank the many people who have given their time to reading drafts of this time or for extensive discussions of various points: Jennifer Butler, Phil dilorio, Charles Henry, Kent Holsinger, Rick Miller, Courtney Murren, Sean Rice, and Günter Wagner. They do not necessarily agree with all we have written here (in fact, the authors have been hard-pressed to reach consensus on some of these issues). CDS wishes to single out the intestinally-challenged anonymous reviewer who encouraged us to "concentrate on doing less, better." MP was supported by NSF Dissertation improvement grant DEB91-22762, CDS by NSF DEB92-20593, and CSJ by NSF DEB94-08208.

References

Akam, M., Averhof, M., Castelli-Gair, J., Dawes, R., Falciani, F. and Ferrier, D. 1994a. The evolving roles of Hox genes in arthropods. Development, Supplement: 209–215.

———, Holland, P., Ingham, P. and Wray, G. 1994b. The Evolution of Developmental Mechanisms. The Company of Biologists, Cambridge.

Alberch, P. 1982. Developmental constraints in evolutionary processes. *In:* Bonner, J.T. (ed.), Evolution and development, 313–332. Springer-Verlag, New York.

———, Gould, S.J., Oster, G. and Wake, D. 1979. Size and shape in ontogeny and phylogeny. Paleobiology **5**: 296–317.

Ambros, V. and Moss, E.G. 1994. Heterochronic genes and the temporal control of *C. elegans* development. Trends in Genetics **10**: 123–127.

Andersson, S., and Shaw, R.G. 1994. Phenotypic plasticity in *Crepis tectorum* (Asteraceae): genetic correlations across light regimens. Heredity, **72**: 113–125.

Antonovics, J. and van Tienderen, P.H. 1991. Ontoecogenophyloconstraints? The chaos of constraint terminology. Trends in Ecology and Evolution **6**: 166–168.

Arnold, S.J. 1992. Constraints on phenotypic evolution. Am. Nat. 140, Supplement: S85-S107.

Arthur, W. 1988. A Theory of the Evolution of Development. John Wiley & Sons, New York.

Atchley, W.R. 1984. Ontogeny, timing of development, and genetic variance-covariance structure. Am. Nat. **123**: 519–540.

——— and Hall, B.K. 1991. A model for development and evolution of complex morphological structures. Bio. Rev., **66**: 101–157.

Bains, S. 1994. Even a robot cricket always gets her mate. Science **266**: 1809.

Bak, P., and Chen, K. 1991. Self-organized criticality. Sci. Amer. January: 46–53.

Barton, N.H. and Turelli, M. 1989. Evolutionary quantitative genetics: how little do we know? Ann. Rev. Genet. **23**: 337–370.

Bassiri, A., Irish, E.E. and Poethig, R.S. 1992. Heterochronic effects of *Teopod 2* on the growth and photosensitivity of the Maize shoot. Plant Cell **4**: 497–504.

Bonser, S.P., and Aarssen, L.W. 1994. Plastic allometry in young sugar maple (*Acer saccharum*): adaptive responses to light availability. Am. J. Bot. **81**: 400–406.

Bradshaw, A.D. 1965. Evolutionary significance of phenotypic plasticity in plants. Adv. Genet. **13**: 115–155.

Brown, N.H., and Hartley, D.A. 1994. Exploring signalling pathways. Nature **370**: 414–415.

Carroll, S.B. 1994. Developmental regulatory mechanisms in the evolution of insect diversity. Development, Supplement: 217–223.

———. 1995. Homeotic genes and the evolution of arthropods and chordates. Nature **376**: 479–485.

Charlesworth, B. 1990. Optimization models, quantitative genetics, and mutation. Evolution **44**: 520–538.

Cheplick, G.P. 1995. Life history trade-offs in *Amphibromus scabrivalvis* (Poacaeae): allocation to clonal growth, storage, and cleistogamous reproduction. Am. J. Bot. **82**: 621–629.

Cheverud, J.M. 1982. Relationships among ontogenetic, static, and evolutionary allometry. Am. J. Phys. Anthropol. **59**: 139–149.

Cock, A.G. 1966. Genetical aspects of metrical growth and form in animals. Quart. Rev. Biol. **41**: 131–190.

Collazo, A. 1994. Molecular heterochrony in the pattern of fibronectin expression during gastrulation in amphibians. Evolution **48**: 2037–2045.

Conway, L.J., and Poethig, R.S. 1993. Heterochrony in plant development. Dev. Biol. **4**: 65–72.

Cowley, D.E. and Atchley, W.R. 1992. Quantitative genetic models for development, epigenetic selection, and phenotypic evolution. Evolution **46**: 495–518.

Creighton, G.K. and Strauss, R.E. 1986. Comparative patterns of growth and development in cricetine rodents and the evolution of ontogeny. Evolution **40**: 94–106.

Crone, W. and Lord, E.M. 1994. Floral organ initiation and development in a wild-type *Arabidopsis thaliana* (Brassicaceae) and in the organ identity mutants *apetala2–1* and *agamous-1*. Can. J. Bot. **72**: 384–401.

de Beer, G.R. 1940. Embryos and Ancestors. Clarendon

Press, Oxford.
de Jong, G. 1995. Phenotypic plasticity as a product of selection in a variable environment. Am. Nat. **145**: 493–512.
Diggle, P.K. 1991a. Labile sex expression in andromonoecious *Solanum hirtum*: floral morphogenesis and sex determination. Am. J. Bot. **78**: 377–393.
———. 1991b. Labile sex expression in andromonoecious *Solanum hirtum*: sources of variation in mature floral structure. Can. J. Bot. **69**: 2033–2043.
———. 1993. Developmental plasticity, genetic variation, and the evolution of andromonoecy in *Solanum hirtum* (Solanaceae). Am. J. Bot. **80**: 967–973.
———. 1994. The expression of andromonoecy in *Solanum hirtum* (Solanaceae): phenotypic plasticity and ontogenetic contingency. Am. J. Bot. **81**: 1354–1365.
Dove, W.F. 1993. The gene, the polygene, and the genome. Genetics, **134**: 999–1002.
Dragavtsev, V.A., and N.V. Utemisheva, N.V. 1975. Ontogenetic variability of statistical genetic parameters in plant populations. I. Theory of the problem. Genetika **11**: 128–140.
Duboule, D. 1994. Temporal colinearity and the phylotypic progression: a basis for the stability of a vertebrate Bauplan and the evolution of morphologies through heterochrony. Development, Supplement: 135–142.
Fang, X., Wu, C. and Brennan, M.D. 1991. Complexity in evolved regulatory variation for alcohol dehydrogenase genes in Hawaiian *Drosophila*. J. Mol. Evol. **32**: 220–226.
Goldschmidt, R.B. 1940. The Material Basis of Evolution. Yale University Press, New Haven.
Goldstein, D.B., and Holsinger, K.E. 1992. Maintenance of polygenic variation in spatially structured populations—roles for local mating and genetic redundancy. Evolution **46**: 412–429.
Gottlieb, G. 1992. Individual Development and Evolution: the Genesis of Novel Behavior. Oxford University Press, New York.
Gould, S.J. 1966. Allometry and size in ontogeny and phylogeny. Biol. Rev. **41**: 587–640.
Gould, S.J. 1977. Ontogeny and Phylogeny. Belknap Press, Cambridge.
Gupta, A.P., and Lewontin, R.C. 1982. A study of reaction norms in natural populations of *Drosophila pseudoobscura*. Evolution **36**: 934–948.
Haeckel, E. 1875. Die Gastraea und die Eifurchung der Thiere. Jena Z. Naturwiss. **9**: 402–508.
Hake, S. 1992. Unraveling the knots in plant development. Trends in Genetics **8**: 109–114.
Hakkarainen, H., and Korpimaki, E. 1995. Contrasting phenotypic correlations in food provision of male Tengman's owls (*Aegolius funereus*) in a temporally heterogeneous environment. Evol. Ecol. **9**: 30–37.
Hall, B.K. 1992. Evolutionary Developmental Biology. Chapman and Hall.
Houle, D. 1991. Genetic covariance of fitness correlates: what genetic correlations are made of and why it matters. Evolution **45**: 630–648.
Huxley, J.S. 1932. Problems of Relative Growth. MacVeagh, London.
Johannsen, W. 1911. The genotype conception of heredity. Am. Nat. **45**: 129–159.
Jolicoeur, P. 1989. A simplified model for bivariate complex allometry. J. Theor. Biol. **140**: 41–49.
Jones, C.S. 1992. Comparative ontogeny of a wild cucurbit and its derived cultivar. Evolution **46**: 1827–1847.
———. 1993. Heterochrony and heteroblastic leaf development in two subspecies of *Cucurbita argyrosperma* (Cucurbitaceae). Am. J. Bot. **80**: 778–795.
———. 1995. Does shade prolong juvenile development? A morphological analysis of leaf shape changes in *Cucurbita argyrosperma* subsp. *sororia* (Cucurbitaceae). Am. J. Bot. **82**: 346–359.
Kellogg, E.A. 1990. Ontogenetic studies of florets in *Poa* (Gramineae): allometry and heterochrony. Evolution **44**: 1978–1989.
Klingenberg, C.P., and Spence, J.R. 1993. Heterochrony and allometry: lessons from the water strider genus *Limnoporus*. Evolution **47**: 1834–1853.
——— and Zimmermann, M. 1992. Static, ontogenetic, and evolutionary allometry: a multivariate comparison in nine species of water striders. Am. Nat. **140**: 601–620.
Kondo, S., and Asai, R. 1995. A reaction-diffusion wave on the skin of the marine angelfish *Pomacanthus*. Nature **376**: 765–768.
Lande, R. 1979. Quantitative genetic analysis of multivariate evolution, applied to brain: body size allometry. Evolution **33**: 402–416.
Lechowicz, M.J., and Blais, P.A. 1988. Assessing the contributions of multiple interacting traits to plant reproductive success: environmental dependence. J. Evol. Biol. **1**: 255–273.
Manak, J.R., and Scott, M.P. 1994. A class act: conservation of homeodomain protein functions. Development Supplement: 61–71.
Matthies, D. 1990. Plasticity of reproductive components at different stages of development in the annual plant *Thlaspi arvense* L. Oecologia **83**: 105–116.
Maynard Smith, J., Burian, R., Kauffman, S., Alberch, P., Campbell, J., Goodwin, B., Lande, R., Raup, D. and Wolpert, L. 1985. Developmental constraints and evolution. Quart. Rev. Biol. **60**: 265–287.
McKinney, M.L. and McNamara, K.J. 1991. Heterochrony: the Evolution of Ontogeny. Plenum Press, New York.
Meyer, A. 1987. Phenotypic plasticity and heterochrony in *Cichlasoma managuense* (Pisces, Cichlidae) and their implications for speciation in Cichlid fishes. Evolution **41**: 1357–1369.
Meyerowitz, E.M. 1994. Flower development and evolution: new answers and new questions. Proc. Natl. Acad. Sci. USA **91**: 5735–5737.
Miller, T.E., Winn, A.A. and Schemske, D.W. 1994. The effects of density and spatial distribution on selection for emergence time in *Prunella vulgaris* (Lamiaceae). Am. J. Bot. **81**: 1–6.
Monteiro, A.F., Brakefield, P.M. and French, V. 1994. The evolutionary genetics and developmental basis of wing pattern variation in the butterfly *Bicyclus anynana*. Evolution **48**: 1147–1157.
Neyfakh, A.A. and Hartl, D.L. 1993. Genetic control of the rate of embryonic development: selection for faster development at elevated temperatures. Evolution **47**: 1625–1631.
Niehrs, C., Steinbesser, H. and Robertis, E.M.D. 1994. Mesodermal patterning by a gradient of the vertebrate homeobox gene *goosecoid*. Science **263**: 817–820.

Nijhout, H.F., Wray, G.A., Kremen, C. and Teragawa, C.K. 1986. Ontogeny, phylogeny and evolution of form: an algorithmic approach. Syst. Zool. **35**: 445–457.

Patel, N.H. 1994. Developmental evolution: insights from studies of insect segmentation. Science **266**: 581–590.

Perrin, N. and Travis, J. 1992. On the use of constraints in evolutionary biology and some allergic reactions to them. Funct. Ecol. **6**: 361–363.

Pigliucci, M. 1996. How organisms respond to environmental change: from phenotypes to molecules (and *vice versa*). Trends in Ecology and Evolution **11**: 168–173

———, dilorio, P.J. and Schlichting, C.D. 1996. Plasticity of ontogenetic trajectories, phenological and reproductive traits in *Lobelia cardinalis* and *L. siphilitica*. in review.

——— and C.D. Schlichting. 1995. Ontogenetic reaction norms in *Lobelia siphilitica* (Lobeliaceae): response to shading. Ecology **76**: 2134–2144.

———, Whitton, J. and Schlichting, C.D. 1995. Reaction norms of *Arabidopsis*.I. Plasticity of characters and correlations across water, nutrient and light gradients. J. Evol. Biol. **8**: 421–438.

Platenkamp, G.A.J., and Shaw, R.G. 1992. Environmental and genetic constraints on adaptive population differentiation in *Anthoxanthum odoratum*. Evolution **46**: 341–352.

Ptashne, M. 1992. A Genetic Switch: Phage Lambda and Higher Organisms. 2nd ed. Blackwell Scientific. Cambridge.

Radinsky, L. 1984. Ontogeny and phylogeny in horse skull evolution. Evolution **38**: 1–15.

Raff, R.A., and Wray, G.A. 1989. Heterochrony: developmental mechanisms and evolutionary results. J. Evol. Biol. **2**: 409–434.

———, Wray, G.A. and Henry, J.J. 1991. Implications of radical evolutionary changes in early development for concepts of developmental constraint. *In:* Warren, L. and Koprowski, H. (ed.), New Perspectives on Evolution 189–207. Wiley-Liss, New York.

Ray, A., Robinson-Beers, K., Ray, S., Baker, S.C., Lang, L.D., Preuss, D., Milligan, S.B. and Gasser, C.S. 1994. *Arabidopsis* floral homeotic gene BELL (*BEL1*) controls ovule development through negative regulation of AGAMOUS gene (*AG*). Proc. Natl. Acad. Sci. USA **91**: 5761–5765.

Rivera-Pomar, R., Lu, X., Perrimon, N., Taubert, H. and Jackle, H. 1995. Activation of posterior gap gene expression in the *Drosophila* blastoderm. Nature **376**: 253–256.

Roff, D.A. 1994. Optimality modeling and quantitative genetics: a comparison of the two approaches. *In:* Boake, C.R.B. (ed.), Quantitative Genetic Studies of Behavioral Evolution, 49–66. The University of Chicago Press, Chicago.

Rollo, C.D. 1994. Phenotypes: their Epigenetics, Ecology and Evolution. Chapman and Hall, London.

Roth, V.L. 1988. The biological basis of homology. *In:* Humphries, C.J. (ed.), Ontogeny and Systematics, pp. 1–26. Columbia University Press, New York, NY.

Salser, S.J., and Kenyon, C. 1994. Patterning *C. elegans*: homeotic cluster genes, cell fates and cell migrations. Trends in Genetics **10**: 159–164.

Scharloo, W. 1988. Selection on morphological patterns. *In:* Jong, G.d. (ed.), Population Genetics and Evolution, 230–250. Springer-Verlag, Berlin.

Schlichting, C.D. 1986. The evolution of phenotypic plasticity in plants. Annu. Rev. Ecol. Syst. **17**: 667–693.

———, 1989a. Phenotypic integration and environmental change. BioScice **39**: 460–464.

———, 1989b. Phenotypic plasticity in *Phlox*. II. Plasticity of character correlations. Oecologia **78**: 496–501.

——— and Pigliucci, M. 1995a. Gene regulation, quantitative genetics, and the evolution of reaction norms. Evol. Ecol. **9**: 154–168.

——— and ———, 1995b. Lost in phenotypic space: environment dependent morphology in *Phlox drummondii* (Polemoniaceae). Int. J. Plant Sci. **156**: 542–546.

Schmalhausen, I.I. 1949. Factors of Evolution. Blakiston, Philadelphia.

Schmid, B., and Dolt, C. 1994. Effects of maternal and paternal environment and genotype on offspring phenotype in *Solidago altissima* L. Evolution **48**: 1525–1549.

Schmidt, K. 1994. A puzzle: how similar signals yield different effects. Science **266**: 566–567.

Schwenk, K. 1995. A utilitarian approach to evolutionary constraint. Zoology **98**: 252–261.

Semlitsch, R.D., Harris, R.N. and Wilbur, H.M. 1990. Paedomorphosis in *Ambystoma talpoideum*: maintenance of population variation and alternative life-history pathways. Evolution **44**: 1604–1613.

Simpson, J., van Montagu, M. and Hererra-Estrella, L. 1986. Photosynthesis-associated gene families: differences in response to tissue-specific and environmental factors. Science **233**: 34–38.

Slatkin, M. 1987. Quantitative genetics of heterochrony. Evolution **41**: 799–811.

Smith-Gill, S.J. 1983. Developmental plasticity: developmental conversion versus phenotypic modulation. Am. Zool. **23**: 47–55.

Sordino, P., van der Hoeven, F. and Duboule, D. 1995. *Hox* gene expression in teleost fins and the origin of vertebrate digits. Nature **375**: 678–681.

Stearns, S.C. 1982. The role of development in the evolution of life histories. *In:* Bonner, J.T. (ed.), Evolution and Development, 237–258. Springer-Verlag, New York.

———, 1983. The interface of life-history evolution, whole-organism ontogeny and quantitative genetics. Am. Zool. **23**: 1–125.

———, 1989. The evolutionary significance of phenotypic plasticity. BioScience **39**: 436–445.

———, de Jong, G. and Newman, B. 1991. The effects of phenotypic plasticity on genetic correlations. Trends in Ecology and Evolution 6: 122–126.

Stebbins Jr., G.L. 1950. Variation and Evolution in Flowering Plants. Columbia University Press, New York.

Strauss, R.E. 1990. Heterochronic variation in the developmental timing of cranial ossifications in Poeciliid fishes (Cyprinodontiformes). Evolution **44**: 1558–1567.

Sultan, S.E. 1987. Evolutionary implications of phenotypic plasticity in plants. Evol. Biol. **21**: 127–178.

Thomas, J.H. 1993. Thinking about genetic redundancy. Trends in Genetics **9**: 395–399.

Thompson, D.B. 1992. Consumption rates and the evolution of diet-induced plasticity in the head morphology of *Melanoplus femurrubrum* (Orthoptera, Acrididae). Oecologia **89**: 204–213.

Turelli, M. 1988. Phenotypic evolution, constant covariances, and the maintenance of additive variance. Evolution **42**: 1342–1347.

Van Tienderen, P.H. and Antonovics, J. 1994. Constraints in

evolution: on the baby and the bath water. Func. Ecol. **8**: 139–140.
Via, S. 1987. Genetic constraints on the evolution of phenotypic plasticity. *In:* Loeschcke, V. (ed.), Genetic Constraints on Adaptive Evolution, 47–71. Springer-Verlag, Berlin.
———, 1994. The evolution of phenotypic plasticity: what do we really know? *In*: Real, L. (ed.), Ecological Genetics, 35–57. Princeton University Press, Princeton.
Waddington, C.H. 1942. Canalization of development and the inheritance of acquired characters. Nature **150**: 563–565.
———, 1952. Selection of the genetic basis for an acquired character. Nature **169**: 278.
———, 1953. Genetic assimilation of an acquired character. Evolution **7**: 118–126.
———, 1957. The Strategy of the Genes. Allen & Unwin, London.
———, 1959. Canalization of development and genetic assimilation of acquired characters. Nature **183**: 1654–1655.
———, 1961. Genetic assimilation. Adv. Genet. **10**: 257–290.
Wagner, G.P. 1988. The significance of developmental constraints for phenotypic evolution by natural selection. *In:* Jong, G.d. (ed.), Population Genetics and Evolution, 222–229. Springer-Verlag, Berlin.
Warren, R.W., Nagy, L., Selegue, J., Gates, J. and Carroll, S. 1994. Evolution of homeotic gene regulation and function in flies and butterflies. Nature **372**: 458–461.
West-Eberhard, M.J. 1989. Phenotypic plasticity and the origins of diversity. Ann. Rev. of Ecol. Syst. **20**: 249–278.
Williams, J.A., Paddock, S.W., Vorwerk, K. and Carroll, S.B. 1994. Organization of wing formation and induction of a wing-patterning gene at the dorsal/ventral compartment boundary. Nature **368**: 299–305.
Windig, J.J. 1994. Genetic correlations and reaction norms in wing pattern of the tropical butterfly *Bicyclus anynana*. Heredity **73**: 459–470.
Wolpert, L. 1994. Do we understand development? Science **266**: 571–572.
Woltereck, R. 1909. Weiterer experimentelle Untersuchungen über Artveranderung, Speziell über das Wessen Quantitätiver Artunterschiede bei Daphniden. Versuchung Deutsch Zool. Gesell., **19**: 110–172.
Wray, G.A. 1992. The evolution of larval morphology during the post-Paleozoic radiation of echinoids. Paleobiology **18**: 258–287.
———, 1995. Punctuated evolution of embryos. Science **267**: 1115–1116.
——— and McClay, D.R. 1989. Molecular heterochronies and heterotopies in early echinoid development. Evolution **43**: 803–813.
——— and Raff, R.R. 1991. The evolution of developmental strategy in marine invertebrates. Trends in Ecology and Evolution **6**: 45–50.
Zelditch, M.L. 1988. Ontogenetic variation in patterns of phenotypic integration in the laboratory rat. Evolution **42**: 28–41.

Received May 1, 1996. Accepted August 30, 1996.