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Modeling In Evodevo: How To Integrate Development, Evolution, And Ecology

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Evolutionary developmental biology, or EvoDevo, integrates perspectives from evolutionary and developmental biology, and increasingly also from ecology, to understand patterns and processes of phenotypic evolution. Initially progress in EvoDevo was driven mainly by conceptual approaches, beginning with such ideas as "burden" and "developmental constraint," designed to account for the specific and limited trajectories of morphological change. Then the discovery of a small number of highly conserved developmental genes, foremost the *Hox* genes, gave rise to notions such as the "genetic toolkit of development" (Carroll et al., 2005). These concepts were generally well received, as evidenced by scientific and popular pronouncements of "success" and of a "new" or a "completed synthesis." But in the meantime an increasingly vocal chorus of critical voices emerged, questioning to what extent the reality of EvoDevo has lived up to its promise, and even whether such a synthesis of evolutionary and developmental biology is at all possible. Of greatest concern has been the lack of genuine models for EvoDevo, abstract and organismal, that integrate diverse perspectives in ways that could make them a reference point for EvoDevo explanations.

In contrast, transmission genetics had Mendel's rules as an abstract formalism and *Drosophila* as its iconic model system. The Modern Synthesis had the theory of allopatric speciation and local adaptation represented by, for instance, Sewall Wright's adaptive landscapes and any number of examples of adaptive radiation, such as Darwin's finches and the cichlids of the East African lakes. There are also examples of close links between empirical case studies and formal models relating to both evolutionary and developmental biology, such as the Hymenoptera for inclusive fitness, or *Hydra* for reaction-diffusion models and gradients of determining morphogenetic factors. None of the current concepts and research programs in EvoDevo, however, have reached the degree of cohesion that is characteristic of representative models in other domains. In this chapter we first explore the history of and the conceptual difficulties in modeling development *and* evolution, paying special attention to the kinds of model systems used in different explanatory contexts. Next, we address

the question of modeling EvoDevo, both in its conceptual approaches and in its potential new model systems.

Modeling Evolution *and* Development

Evolutionary ideas have been closely linked to embryological phenomena ever since both captured the imagination of researchers in the early nineteenth century. Since these temporal processes were difficult to observe, models—both theoretical and material—were crucial to the development of these scientific enterprises. In embryology, practical demands led to the study of a small number of organisms, such as the frog, the chick, and, after the establishment of marine stations in the later decades of the century, marine invertebrates. Painstaking observations allowed the reconstruction of embryological stages, and comparative studies led to the formulation of embryological "laws" and the theory of recapitulation. The latter took on a particular meaning after Darwin's work paved the way for a general acceptance of the evolutionary history of organisms.

A phylogenetic perspective provided the first context for modeling development and evolution when developmental sequences were interpreted as a window into the evolutionary past of organisms. Embryological observations became the basis for reconstructing genealogical relationships. Homologies between characters were modeled as a sequence of morphological transformations, while embryological and comparative data provided the evidence supporting these reconstructions.

These attempts at reconstructing phylogeny left many dissatisfied. A younger generation of researchers was especially concerned with the high degree of ambiguity in those fundamentally historical models. Headed by Hans Driesch and Wilhelm Roux, they had as their alternative goal understanding development mechanistically as a way to uncover the causal connections between different embryological stages and structures. The resulting new science of *Entwicklungsmechanik* provided a different context for modeling development. The first models, such as those of Wilhelm His, were inspired by mechanical principles of folding, bending, and differential growth of tissues and cell layers (Hopwood, 2000). These models became more physiological when phenomena such as regulation and differentiation (specialization, division of labor, etc.) rose in prominence.

The physiological paradigm, in contrast to the historical perspective, also emphasized experimental intervention, which required cultivating model organisms suitable for these tasks. The chick, amphibians (frogs and salamanders), flatworms, and, to some extent, sea urchins were especially suited for experimental manipulations such as transplantation and selective destruction of embryological tissues. Experiments in transplantation gave rise to a biochemical approach when researchers tried to uncover the chemical nature of signals that had the power to shape the organism, such

as the chemical nature of the organizer. The biochemical orientation and the new technologies that came with it again changed the ways development was modeled. Tissue and cell cultures greatly aided in the search for the chemical determinants of development and became a new model for these processes.

While a combination of developmental mechanics and physiology was used to model the development of individual organisms, new trends in evolutionary biology were focusing on variation and its genetic basis. In the study of phenotypic variation the emphasis shifted very early to the behavior of those (abstract) factors—genes—that could be correlated with the observed patterns of phenotypic inheritance. Even though development as well as the environment played a crucial role in conceptualizing genes—as in Johannsen's definitions of genotype and phenotype (Johannsen, 1911) or in Woltereck's idea of a reaction norm (Woltereck, 1909)—the *Drosophila* model system soon privileged a genetic account of phenotypic variation. It is widely known how this model then became the basis for the Modern Synthesis in evolutionary biology and how it was transformed by the molecular revolution in biology after World War II.

The privileging of genetics can also be seen in the standard seven model organisms of developmental biology: the fruit fly *Drosophila melanogaster*; the nematode *Caenorhabditis elegans*, the mouse *Mus musculis*, the frog *Xenopus laevis*, the zebra fish *Danio rerio*, the chick *Gallus gallus*, and the mustard *Arabidopsis thaliana*. Except for the chick, all of these systems are especially suitable for genetic approaches.

But these trends are only one side of the history of modeling development and evolution. Important parallel developments included the physiological genetics of Richard Goldschmidt, who conceived of the gene as something far less corpuscular and more dynamic (i.e., physiological; Goldschmidt, 1927), and Alfred Kühn's physiological developmental genetics (Kühn, 1941; Laubichler and Rheinberger, 2004). The latter was based on a concept of interlocking genetic and physiological systems that we would now call gene cascades, reaction chains, and substrate chains that interact to produce a phenotypic effect. Goldschmidt and Kühn worked with different model systems—*Lymanntria* and *Ephestia*, respectively—that were less suited for genetic analysis but much better for physiological and biochemical studies.

Besides Kühn and Goldschmidt, Hans Przibram at the Vienna Vivarium emphasized an integrated approach to modeling development and evolution in the early twentieth century. Using a variety of organisms, the work at the Vivarium Institute headed by Przibram emphasized the study of the whole life cycle of organisms and organism-environment interactions. This included studies of regeneration as a model for normal development, endocrinology, experimental evolution, and the study of reaction norms. But—and this is particularly interesting in the context of the present volume—Prizbram was also keen on the integration of experimental and theoretical work (e.g., Przibram, 1923). The latter included attempts to mathematically model

developmental processes and a major push for a quantitative orientation within biology. Paul Weiss and Ludwig von Bertalanffy, who later championed theoretical and mathematical approaches in biology, such as the field concept and the idea of a general systems theory, were connected to Przibram and the Vienna Vivarium.

It seems, then, that one reason for the small number of model systems in current developmental biology has to do with the alliance between developmental biology and genetics. When developmental biology was more of a "physiological science," it had different models—newts, sea urchins, ranid frogs, ambystomid salamanders, slime molds, flatworms, and chicks. As it became a "genetic science," the environment was relegated to a smaller role. Part of the explanation for the difference lies in the fact that a predictable, controlled genetic analysis depends on a model organism whose phenotype is not significantly controlled by the environment.

As Sonia Sultan (2003) points out, "Neo-Darwinian botanists were often quite frustrated in their attempts to discern genetically based local adaptations through this 'environmental noise,'" and this led them to overlook the adaptive nature of developmental plasticity. On the zoological side of developmental biology, the desire to link developmental biology with genetics (and the desire to breed the animals easily) led to adoption of a limited number of model species selected for the lack of significant environmental contributions to the phenotype (Bolker, 1995).

In this context it is interesting to read the preface to volume 1 of *Current Topics in Developmental Biology* (1966), which was written by Joshua Lederberg, who was a geneticist, not an embryologist. He proposed that if developmental biology were going to make progress, it required a model organism such as *E. coli* B. He suggested two such models: the mouse (as a surrogate for humans) and "some very simple system like a rotifer or nematode." Lederberg was prophetic; most of today's model organisms in developmental biology are favored for reasons similar to those he suggested.

Today EvoDevo and its subdiscipline, ecological developmental biology, both assert that the canonical model systems are starting points for evolutionary and developmental investigations, but that they may give a biased view of nature. They are good starting points because countless efforts have led to a detailed understanding of these systems; they may be biased because they often represent derived and specialized lineages that may not be suitable for questions about the *evolution* of morphological novelties and patterns of phenotypic evolution. First, these animals can give the erroneous impression that everything needed to form the embryo is within the fertilized egg. Second, in the laboratory these animals may not provide adequate explanations for the way animals develop in the wild. Tadpoles in the wild, for example, may look different from tadpoles reared in aquaria because their phenotype develops, in part, from cues emanating from competitors and predators absent in the laboratory Environmental chemicals that are harmless in the laboratory may be

dangerous to developing organisms in the wild (Colburn et al., 1996; Hayes et al., 2003; Relyea and Mills, 2003). Third, as Neff and Rine (2006) noted, "Model organisms have become a 'comfort zone' for biologists, luring them away from investigating questions that cannot be answered with any of the existing models." And fourth, the organisms used for modeling a particular phenomenon may be idiosyncratic.

Species can be defined as those organisms which develop in a particular way, using particular molecules and processes. Thus, the development of a single organism, by this definition, cannot circumscribe the development of its clade. Most arthropods probably do not use a gradient of Bicoid to form their head, even though this gradient is remarkably important for *Drosophila*. Most amphibians probably do not form their mesoderm as *Xenopus* does, even though *Xenopus* is a model for mesoderm formation. The mouse is a good starting point for studying other mammals, but mammalian development has diverged enormously, and certainly beyond what one murine species expresses (see Benirschke, 2006). David and Marilyn Kirk (2004) have spent their professional lives sorting out the ways that *Volvox carteri* distinguishes germ line from soma and have come to the conclusion that "*Volvox carteri* is an excellent model for other *Volvox carteris*," because most other *Volvox* species do this important act in different ways.

The problems of suitable model systems for EvoDevo are reflected in a workshop document published by the National Science Foundation of the United States (NSF, 2005). This booklet specifically relates to the influence of model systems in directing the course of developmental research.

> Developmental biologists have, for many years, focused their efforts to understand ontogeny by selecting a few model organisms that are genetically tractable, and that are appropriate to the study of fundamental processes of development at the genetic, molecular, and cellular levels. These efforts have led to a detailed understanding of the genetic mechanisms that are involved in the control of developmental events. Many of the findings that have emerged from this work have proven remarkably transferable among the models studied. Developmental biologists have relied on model systems with relatively little but controllable genetic variation. Consequently they have typically not studied the way developmental mechanisms differ among species, nor the variance in mechanism among individuals due to normal variation in genetic and environmental factors. Some developmental biologists have recently begun to expand their studies to include non-model species for understanding aspects of developmental processes not reflected in the models. Still others are interested in illuminating the breadth or limitations of the generalizations discovered in the model systems. Recent developments in genomic approaches have facilitated this move away from the few genetically tractable model systems.

The authors go on to contrast this with the physiological approach.

> Animal physiologists, by contrast, have been reluctant to adopt the use of a relatively small number of model species. This is in part because the physiological principles that bind the subscience cohesively, such as regulation and control of the functions required for normal operation, are known to differ between species. Thus, animal physiologists have employed a

broad array of study systems, each selected for its suitability to address a specific physiological mechanism. Interestingly, some investigators have recently advocated the adoption of model systems that are genetically tractable as a means to approach questions about the genetics and evolution of physiological mechanisms, and as a means to leverage financial support of genomic approaches, which remain costly.

The NSF then calls for an integrative developmental biology that would synthesize the methodologies, analytical tools, and conceptual approaches of these two disciplines. EvoDevo, if it is to avoid the conceptual pitfalls mentioned above, also needs to adopt a similar perspective and, indeed, has already taken the first steps in this direction. Various potential new model systems are being explored, and traditional model systems are being retooled to fit EvoDevo-type questions. In addition, a number of systematic accounts of EvoDevo call for a closer integration of mathematical models, which have traditionally been the domain of evolutionary biology, with the mechanistic perspective of developmental biology. In particular, the multiple layers of epigenetic control of development that have recently been uncovered have the potential to dramatically transform the traditional abstractions of population and quantitative genetics. They provide us with a much richer understanding of the molecular mechanisms of development, one that also transcends the traditional gene-environment dichotomy. Below we will first systematically discuss seven clusters of EvoDevo concepts, and their associated questions and research approaches, and then explore how these are reflected in new model organisms and model systems for EvoDevo.

Models in EvoDevo

Approaches

Despite recent efforts to consolidate the field, EvoDevo is still a pluralistic discipline, as is illustrated by the different approaches taken in its research programs. In part these reflect the different disciplinary origins of its practitioners (in either developmental genetics or evolutionary biology, for instance), but they are also a consequence of the number of different questions that fall within the purview of EvoDevo. Following Müller (2007), we distinguish seven types of questions, each characterized by its own set of organizing concepts, models, and explanatory strategies.

Origin of Developmental Systems The first premise of EvoDevo is that phenotypic evolution is a consequence of changes in the developmental systems of organisms. These developmental systems are, of course, themselves a product of evolution, and thus subject to evolutionary dynamics. As with phenotypic transformations more generally, we can distinguish between gradual modifications of developmental systems and the more complicated problem of their origin. The latter is tied to such

questions as life cycle evolution (Bonner, 1974), the role of cell lineage competition in structuring early developmental processes and the evolution of individuality (Buss, 1987), and the origin of generic forms as a consequence of an interaction of basic physical laws with self-reproducing biological materials such as cells (Newman, 1992, 1994).

These questions point to complex interactions between physical processes and constraints, and the developmental systems that incorporate and then stabilize these processes. Conceptually, these questions are connected to the difficult problems of emergence and major transitions. Due to the likely rarity of these events, we depend on a few select model systems for theoretical and empirical studies. We also rely on heuristic models that allow us to explore possible scenarios in the origination of developmental systems (Müller and Newman, 2003; Newman et al., 2006). Explanations of the origin of developmental systems thus depend on a combination of material, heuristic, and theoretical models.

Evolution of the Developmental Repertoire Once developmental systems are established, their subsequent modification provides the foundation for further phenotypic evolution. The basic architecture and transformations of these developmental systems are thus the subjects of intense study within EvoDevo. Comparative studies reveal that developmental systems have a highly conserved architecture that is based on a small number of elements and their combinatorial transformations. The most important features of developmental systems are their modular organization (Schlosser and Wagner, 2004; Callebaut and Rasskin-Gutman, 2005), the hierarchy of regulatory pathways and networks (Wilkins, 2002; Davidson 2006), the conservation of regulatory genes and the evolution of *cis-regulatory* elements (Carroll et al., 2005; Davidson, 2006), the duplication and further deployment of regulatory genes (Holland, 1999), and the co-option of existing modules into new tasks (True and Carroll, 2002).

Taken together, these elements of the evolution of developmental systems establish a conceptual model for the genetic basis of phenotypic evolution. Within this broad vision several heuristic concepts and additional models have emerged, such as the idea of a "genetic toolkit for development," the reconstruction of the basic features of the ancestor of all bilaterally symmetrical organisms, and the "Urbilateria," or the idea of an hourglass model of development passing through a conserved "phylotypic stage." These heuristic conceptual models guide empirical research in important ways, and they also provide a starting point for theoretical models and formal treatments, such as the analysis of network properties of regulatory gene networks and general features in the evolution of signaling pathways.

Evolutionary Modification of Developmental Processes Next to evolutionary transformations of the developmental repertoire and their implications for phenotypic

evolution, certain features of developmental processes also allow modifications of phenotypes. Morphologists and developmental biologists have long noticed how changes in timing of certain events during development can result in often dramatically altered phenotypes. They described these phenomena as heterochrony (Haeckel, 1866; de Beer, 1930; Gould 1977; McKinney and McNamara, 1991). Building on these classical observations and interpretations, developmental biologists have more recently analyzed the underlying developmental mechanisms and described additional elements of morphogenesis, such as morphoregulation (Edelman, 1986, 1988), ontogenetic repatterning (Wake and Roth, 1989), and dissociability (Needham, 1933; Raff, 1996). All these studies have led to a revival of morphogenesis as a topic of evolutionary research. Studies in morphogenesis also have traditionally been at the vanguard of theoretical modeling, and the recent focus on EvoDevo has initiated renewed interest in this area.

Environment-Development Interaction Phenotypic plasticity is one of the more obvious examples of how environment and genes interact to yield developmental programs with patterns of variation that can be continuous or discontinuous. The environment is often thought of as only or mainly abiotic factors, as in the way temperature can affect wing color and pattern in butterflies in a case we will describe shortly. But environment also includes other organisms, as illustrated in the way that the density and size distribution of conspecifics affects expression of cannibalism in salamanders (Collins et al., 1993). Gene-environment interactions may cause individuals to vary in physiology, morphology, or behavior, which can also yield intraspecific variation in birth and death rates that affect demography. Density, size distribution, and gene frequencies can then feed back on the development of individuals. Finally, other species acting as competitors and predators will help shape development across a range of reaction norms.

As we come to understand the complexity of the genome, it will be possible to model these systems using a vision that goes beyond a one gene-one trait perspective. Modern molecular techniques, especially the rapidity at which sequencing is now possible, are opening opportunities to study diverse model systems chosen especially for their usefulness in answering leading ecological and evolutionary questions. An especially interesting development in the last decades of the twentieth century was the increasing appreciation by evolutionary ecologists that modeling population dynamics required an incorporation of evolutionary principles. Among the factors that drove this conceptual transition was an appreciation of the convergence of ecological and evolutionary times (Collins, 1986). More recently, Hairston et al. (2005) concluded that "to understand the temporal dynamics in ecological processes it is crucial to consider the extent to which the attributes of the system under investigation are simultaneously changing as a result of rapid evolution."

All of this means that diverse model organisms that vary intraspecifically in fundamental traits, such as the capacity to metamorphose or not in salamanders, and the presence or absence of wings in insects, can be analyzed with the aim of understanding the gene-environment interactions surrounding traits indicative of key evolutionary transitions. Understanding these transitions is a central goal of EvoDevo.

Phenotypic Variation Heritable phenotypic variation is the basis for evolution. Analyzing patterns of phenotypic variation has, therefore, been a prime concern of evolutionary biologists ever since Darwin (1859) and Bateson (1894). More recently the role of developmental processes in both enabling and constraining phenotypic variation has become a major part of EvoDevo research. The main problems in this context are:

1. The observation that not all thinkable phenotypic variants are indeed possible—a fact captured by the concept of constraints, such as developmental constraints (Alberch, 1982; Maynard Smith et al., 1985) or physical, functional, and architectural constraints (see chapters 5 and 14 in this volume)
2. The idea that development can act as a buffer that filters out both genetic and environmental variation and perturbations (Katz et al., 1981)
3. The suggestion that the developmental system can bias the expression of underlying (molecular) genetic variation in such a way that the resulting phenotypic variation might appear directed—a concept termed developmental drive (Arthur, 2001)
4. The realization that the specific structures of the genetic and developmental systems are crucially important for the future capacity of species to evolve (Wagner and Altenberg, 1996; Kirschner and Gerhart, 1998).

Many of these heuristic concepts gave rise to analytical models that in different ways have become the theoretical foundation of EvoDevo.

Phenotypic Innovation Explaining phenotypic innovation is a central goal for EvoDevo (Müller and Wagner, 1991; Love, 2003). The question of how new structures and behaviors arise during evolution has been the main challenge for evolutionary biologists since Darwin. From the very beginning, development has played an important part in these explanations. Within his generalized law of recapitulation and its mechanism of terminal addition (new structures are added on at the end of developmental sequences), Haeckel already allowed for exceptions. These "caenogenetic" features represented adaptations of the developing embryo to internal and external conditions, and were therefore a consequence of development. More recently, EvoDevo researchers have focused on developmental side effects (Müller, 1990), epigenetic causation (Newman and Müller, 2000), altered *cis-regulatory* interactions

(Carroll et al., 2005; Davidson, 2006), developmental exaptation (Chipman, 2001), and environmental induction (West-Eberhard, 2003) in their efforts to develop a mechanistic model for the origin of novel phenotypes during development and evolution. So far the focus on innovation has generated heuristic and functional models (see, e.g., Müller and Newman, 2006). A main challenge for EvoDevo is therefore to embed these models within a formal and analytic account of morphological evolution.

Genetic and Epigenetic Fixation EvoDevo is based on the assumption that development is a central feature of all explanations of morphological evolution. Over the last few decades a number of heuristic and analytical models have emerged that have helped to make this general statement more concrete by showing exactly how development is reflected in specific features of the genetic and epigenetic systems. These concepts all focus on the systemic effects of the developmental, genetic, and, increasingly, environmental contexts on the expression of morphological and behavioral traits. They include Waddington and Whyte's idea of internal selection pressures in analogy to external selection (Waddington, 1953; Whyte, 1965); several models of canalization (Waddington, 1942; Wagner et al., 1997; Wilkins, 2003) that are also connected to the concept of developmental constraints, Riedl's (1978) concept of burden as a measurement for the limitations on future variability imposed by a highly integrated developmental system; Wimsatt's (1986) related formulation of generative entrenchment; Waddington's (1956) concept of genetic assimilation as an account of how selection can eventually lead to the cooption of favorable environmentally induced variation; several ideas related to the emergence of the hierarchical organization of developmental systems (Riedl, 1978; Buss, 1987; Salazar-Ciudad et al., 2001). New findings related to the molecular details of epigenetics (Stillman and Stewart, 2005) already provide the kind of experimental data that will help turn these concepts into functional and analytical models of the genetic and epigenetic bases of morphological evolution.

Model Systems and Model Organisms

An important part of modeling EvoDevo is the selection of adequate model organisms. In selecting model organisms, two sets of demands must be met—the pragmatic considerations of housing, breeding, and easy manipulation, and theoretical considerations related to whether or not a model organism is representative of the phenomenon in question. These two demands sometimes conflict. While easy manipulability facilitates experimental work and the standardization of results that ensures the quality and comparability of the experimental data, the issue of to what degree model organisms exemplify a scientific problem is tied to the question of whether it is possible to develop a more general model based on work done with one or a few selected

organisms. This problem is even more acute in the context of EvoDevo (cf. Metscher and Ahlberg, 1999).

As mentioned above, the seven basic model systems of developmental biology were selected because of their easy manipulability and, except originally for the chick, also because they are well suited for research within the genetic paradigm in developmental biology. However, not all of them are particularly useful for Evo-Devo questions, since most of these organisms are highly derived and specialized, and thus not suited for modeling major phenotypic transformations or any of the other questions that are being asked in EvoDevo. It is by now almost universally accepted that addressing these problems requires new model systems. This is, in a sense, an interesting phenomenon since EvoDevo was originally characterized by its revolt against model systems (Bolker, 1995; Bolker and Raff, 1996). One of the earliest and by now well-known EvoDevo model systems was developed by Rudy Raff and coworkers, who used sea urchins of the genus *Erythrogramma* to study the differences between larval and direct development in closely related species (e.g., Raff and Wray, 1989). Now, as EvoDevo becomes stabilized around additional, specific questions, new model systems are emerging.

Each of the seven clusters of EvoDevo concepts and their associated theoretical, experimental, and empirical research strategies attracts new investigators, who introduce new model systems and organisms into EvoDevo, as well as the "repositioning" of traditional model systems such as zebra fish and *Drosophila*. These become increasingly employed for the discovery of specific differences within lineages by studying altered gene expression through evolution. The zebra fish, for instance, is seen as a useful source of genes through which the evolution of specific piscine lineages might be studied (Webb and Schilling, 2006). The dog *Canis familiaris* and the three-spined stickleback fish are also considered model systems for studying altered gene expression during evolution. The latter two are new model systems, specifically developed in the context of EvoDevo to identify genes in which small changes can make large phenotypic differences.

Other new and nontraditional organisms are the cnidarian *Nematostella*, as a model system for looking at the origins of the bilateria, and the dung beetle *Onthophagus*, which is proposed as a model system for studying the evolution and the properties of developmental plasticity. More recently there has also been a push to use social insects as a new model system for EvoDevo, especially for studying the origins of morphological, physiological, and behavioral novelties. In this regard it is important to note, as Gilbert did at a recent conference in Paris, that "model systems" in EvoDevo are not merely "model organisms." Rather, these systems include the organism plus their historical or ecological context. Here we will briefly introduce a few of these new model organisms and model systems, and discuss their significance for EvoDevo.

The Dog Model System The title of the Neff and Rine (2006) essay in *Cell* says it best: "A Fetching Model System." As recognized by Darwin (1859), artificial selection is a mode of evolution wherein harsh selective pressures imposed by human selection and mating regimes can rapidly change the appearance of an organism. There are now over 200 recognized breeds (and about 1000 local breeds) of dogs, each derived from *Canis lupus*, the wolf, starting about 135,000 years ago (Vila et al., 1997).

Dog breeding, write Neff and Rine, "has been an ongoing experiment in the rapid evolution of form and function." Moreover, the completion of the canine genome has made *Canis familiaris* "genetically tractable and poised to offer insights into evolution, development, and behavior." These authors point out that while null mutations, such as those readily produced in the mouse, can tell you about how a system breaks down, such mutations are not usually relevant for understanding natural diversity or evolution. In dogs, however, you have remarkable diversity of functioning systems. Dogs can differ fiftyfold in mass and have behaviors ranging from completely docile to overtly vicious. Moreover, these differences are heritable. There are not only breed-specific temperaments, but even dog breeds that perform the same behaviors (such as herding) differently from one another. The variation that dogs have is very different from the variation produced in the laboratory using caged mice. Studying this normal and enormous variation is critical if one wishes to study evolution or, for that matter, brain function.

The central argument for the dog as model system for EvoDevo is that it has enormous variation; that these variants are functional, not pathological; and that this variation occurs within the same species. Stockard (1941), and more recently others (Gilbert, 1991), have pointed out that differences in dog snouts represent remarkable changes in the migration and proliferation rates of cranial neural crest cells. Now that the canine genome is complete (Lindblad-Toh et al., 2005), it is hoped that comparisons can be made. There are over 50 million pedigreed dogs in the United States alone, and there should be plenty of molecular variations to map. The goal is to elucidate the genetic variations that underlie the different morphologies, embryologies, and behaviors that define each breed.

Such studies have already started. Fondon and Garner (2004) and Caburet et al. (2005) have shown that length variation of tandem repeats in protein-coding regions of developmental genes are associated with morphological changes in dog breeds. For instance, the Great Pyrenees breed is characterized by bilateral polydactyly of the first digit, which correlates with a deletion of seventeen repeats of a Pro/Gly sequence in the *Alx-4* gene. The deletion characterizes the breed, and a single Great Pyrenees dog without this polydactylous condition was homozygous for the full length of the *Alx-4* allele that characterizes all nonpolydactylous breeds. Similarly, repeat variation in the *Runx-2* gene is correlated with craniofacial depth. The gene

is homologous to human *TCOF*, mutations of which cause the Treacher-Collins syndrome of facial shape anomalies, and shows variants highly associated with head depth in dogs (Haworth et al., 2001).

The *Nematostella* Model System *Nematostella vectensis*, the starlet sea anemone, is a cnidarian that represents a basal phylum. Moreover, its proponents argue that it represents two of the most fundamental transitions in animal evolution: the origin of bilateral symmetry and the origin of the mesoderm. A *Nematostella* Web site (http://www.nematostella.org) claims: "The starlet sea anemone, *Nematostella vectensis*, is becoming an increasingly important model system for the study of development, evolution, genomics, reproductive biology, and ecology." When Martindale and colleagues (2004) introduced *Nematostella* as a model system for the study of triploblasty, they proposed it for the reasons traditionally used to justify such a designation: simplicity, ability to be cultured, large number of embryos, availability of embryos all year, and rapid development of the embryo:

> *Nematostella* has many practical advantages as a developmental model, including a simple body plan and a simple life history. It is a hardy species, easy to culture (Hand and Uhlinger, 1992) and will spawn readily throughout the year under laboratory conditions (Fritzenwanker and Technau, 2002; Hand and Uhlinger, 1992). Sexes are separate and fertilized embryos develop rapidly to juvenile adults bearing four tentacles. (Martindale et al., 2004:2464)

To demonstrate the usefulness of *Nematostella* as a model organism for looking at the origins of triploblasty and bilateral symmetry, Martindale and his colleagues showed that they have the typical bilateral body plan (common to vertebrates and insects), but in a rudimentary form. Thus, the genes for dorsal-ventral polarity (*BMP* and *chordin*) are found, but they appear to be playing slightly different roles than in the more highly specialized bilaterians (Matus et al., 2006); the genes used in insects and vertebrates for germ-cell specification are found there, too, but seem to be playing more roles than expected (Extavour et al., 2005).

The finding in *Nematostella* of many of the transcription factor families known to be critical in the development of contemporary insects and vertebrates gives further reasons to look at this organism as an example of an organism that is ancestral to all the major lineages of the animal domain (Magie et al., 2005). In 2004 Finnerty and colleagues showed that *Nematostella* uses homologous genes to achieve bilateral symmetry by means of staggered *Hox* gene expression along the primary body axis. They suggested, therefore, that bilateral symmetry arose before the evolutionary split of Cnidaria and Bilateria. Thus, bilateral symmetry can first be seen in the Cnidarians; moreover, so can mesoderm. Not only are there muscle cells among the cnidarians, but these cells are expressing the "mesodermal genes" that characterize mesodermal specification in insects and vertebrates (Martindale et al., 2004). Basal

metazoa, such as *Nematostella*, will thus be invaluable model systems for understanding earliest events in the evolution of higher animals (Martindale, 2005).

Martindale continues that the field needs these models to place renewed emphasis on the functional interactions of complex gene regulatory pathways in a phylogenetic context so that scientists can "unravel the legacy of morphological complexity that is seen in the animals of today." This is echoed by another laboratory that emphasizes the importance of *Nematostella* over other basal organism models:

> In recent years, a handful of model systems from the basal metazoan phylum Cnidaria have emerged to challenge long-held views on the evolution of animal complexity. The most-recent, and in many ways most-promising addition to this group is the starlet sea anemone, *Nematostella vectensis*. The remarkable amenability of this species to laboratory manipulation has already made it a productive system for exploring cnidarian development, and a proliferation of molecular and genomic tools, including the currently ongoing *Nematostella* genome project, further enhances the promise of this species. In addition, the facility with which *Nematostella* populations can be investigated within their natural ecological context suggests that this model may be profitably expanded to address important questions in molecular and evolutionary ecology. (Darling et al., 2005:211)

The Dung Beetle Model System A major change in developmental biology since the mid-1990s is the recognition that the environment plays an instructive role in producing phenotypes. Polyphenisms, norms of reaction, and developmental symbioses, long a part of ecology, are now increasingly seen as being part of developmental biology. What had been a province of exceptions is becoming the rule, as mammalian gut development has been found to be symbiotically regulated, and as evolutionarily cued epigenetic methylation is seen to alter DNA in numerous animals, including mammals (see Gilbert, 2004).

The question then becomes how best to study ecological developmental biology, or EcoDevo (Gilbert, 2001; Hall et al., 2004), and the multiple effects of environmental factors on regular development. Again, the selection of an appropriate model system proves crucial. First, one needs an animal with a readily identifiable suite of traits that change consistently with the environment. Two claimants for such an EcoDevo model system have recently come forward. The first, from Paul Brakefield's laboratory, is the Malawian butterfly *Bicyclus anynana* (see Beldade et al., 2002, 2005). In this butterfly, temperature helps determine the phenotype. During the cool, dry season the butterfly walks among the leaf litter and its cryptic brown coloration protects it. During hot, moist months, the butterfly flies, and its eyespots protect it from insect predators (Lyytinen et al., 2004). *Bicyclus* thus provides an excellent system for looking at phenotypic plasticity.

Moreover, by combining forces with Sean Carroll's developmental genetics laboratory, Brakefield's group has begun to uncover the molecular mechanism for this plasticity (Brakefield et al., 1996). A temperature rise causes an increase in the ecdysone

hormone during a particular stage of larval development, and this hormone sustains expression of the *Distalless* gene in the presumptive eyespots of the imaginal wing disk. The *Distalless* protein activates a series of transcription factors that initiate color development throughout the wing spot in a concentric manner. The ability to transform the butterfly by molecular means, study its physiology, monitor its development, and analyze its ecology and evolutionary biology makes this a particularly exciting species to follow. Beldade and colleagues (2005) remarked, "This system provides the potential for a fully integrated study of the evolutionary and developmental processes underlying diversity in morphology," although it might be more cautious to restrict this claim to essentially two-dimensional color patterns.

Another eco-devo model is the dung beetle *Onthophagus* and its fascinating structural and behavioral polyphenisms. Male dung beetles can be separated into two distinct classes. The large males have head horns, while small males have rudimentary horns or are hornless. Horn length varies allometrically with body length, resulting in a bimodal distribution of horn sizes. Up to a particular body size, the males are essentially hornless. Then, after they reach this threshold, the horn grows much faster than the body. Body size is determined primarily by the amount and quality of the dung provided to the larva by its mother. When a larva runs out of food, it metamorphoses into an adult (Emlen, 1994, 1996). The regulation of horn size by food is achieved through the prepupal endocrine system, wherein ecdysone and juvenile hormone cooperate to stimulate horn growth (Emlen and Nijhout, 2001).

The hornless and horned males have very different sexual strategies (Emlen, 1997; Nijhout, 2003). The horned males defend tunnels that are dug by the females and use their horns to fight other males who want access to these females. The male with the longer horns wins. The hornless males would seem to be at a reproductive disadvantage; but not only are the horns polyphenic, so is a behavior. Instead of fighting, the hornless males either try to sneak past a defending male or dig their own tunnels into the tunnels of the females. This polyphenism results in divergent selection: large males benefit from large horns (they helps them win combats), while small males benefit from the smallest possible horns (because horns get in the way of digging and sneaking).

Thus, the polyphenism in dung beetles involves the coordination of both morphological structures and behavioral strategies by the endocrine system. But for a male dung beetle, body and attitude are due largely to the amount of dung left by the mother. Emlen (2000) sees a reciprocal relationship wherein the study of beetle development contributes to EvoDevo and EvoDevo contributes to the study of beetle development.

In principle, understanding how development affects the expression of morphological traits should explain the evolution of those traits. However, empirical studies demonstrating an immediate relevance of development for understanding evolution in natural populations are rare

because most population biologists do not study the developmental mechanisms regulating the expression of their traits of interest. One trait in which to examine this question is in the horns of beetles. The behavior associated with horns, the evolution of horns, and the development of horns have been explored for the same two species; consequently, it is now possible to integrate the results from these studies and to explore how knowledge regarding the mechanism of horn development influences our understanding of beetle horn evolution. (Emlen, 2000:403)

Thus, the dung beetle might be a model system for looking at the evolution of developmental plasticity (of both form and behavior), the consequences of developmental plasticity, and the hormonal mediation by which such plasticity is regulated via environmental factors.

The Vertebrate Limb Model The amenability of embryonic limbs to experimental manipulation and the extensive fossil record of limb skeletal patterns have for a long time inspired EvoDevo perspectives on the vertebrate limb (Hinchliffe and Johnson, 1980). Mostly these models are concerned with the pattern of skeletal elements that arises in a proximodistal sequence from localized accumulations of prechondrogenic cells in embryonic limb buds. Early EvoDevo models (that would account not merely for embryonic patterning but also for its taxon-specific evolutionary modification) were based on physical processes of cell association and the macroscopic properties of growing skeletogenic tissue masses (Oster et al., 1985; Shubin and Alberch, 1986). In these types of models the evolution of skeletal patterns would occur through modulations of spatial or temporal aspects of macroscopic events of skeletogenic tissue organization (Shubin and Alberch, 1986; Müller, 1991; Hinchliffe, 2002).

The growing understanding of the molecular basis of limb development has led to the identification of some of the key genes that are associated with skeletal patterning, and consequently the models of limb evolution have shifted to relating gene expression patterns in the development of extant limbs to the evolution of the skeletal patterns (e.g., Izpisua-Belmonte et al., 1991; Tabin, 1992; Sordino and Duboule, 1996). Genetic pattern-based models of limb evolution rely explicitly or tacitly on a hierarchical developmental program notion in which gene activity affects the ''positional information'' provided to individual cells during the cell condensation process (Wolpert, 1969, 1989) via candidate molecules that generate a putative coordinate system along the limb's three Cartesian axes.

This spirit of positional identity also underlies recent limb models that propose a stepwise subdivision of broad initial expression domains to produce the proximodistal array of skeletal elements (Richardson et al., 2004). The limb pattern and its evolutionary modification would thus be a direct consequence of feed-foreward gene-gene interactions that specify skeletogenic patterns without relevant contributions of other developmental parameters. As a consequence, such ''informational

models'' require a high level of regulatory intricacy to generate patterns of any complexity. Position-specific promoters (Stanojevic et al., 1991) or ''smart genes'' (Davidson, 1990) are invoked to explain such intricacy in other systems, and major innovations are thought to arise from shifts of gene expression domains in the early limb bud mesenchyme (Sordino et al., 1995; Wagner and Chiu, 2001).

A different class of models is based on the self-organizational properties of cell and tissue masses in a confined developmental space. Such ''generic models'' of limb development give priority to the capacity of precartilage mesenchymal tissues to autonomously generate regularly spaced skeletogenic accretion centers (Newman and Frisch, 1979; Newman, 1996). These kinds of patterning processes are based on a core set of self-organizing cellular and molecular processes. Gene regulatory evolution is here thought to capture, stabilize, and refine the tissue interactions that produce generic initial forms (Newman and Müller, 2005). *In silico* modeling and simulation of a minimal set of self-organizing interactions within limb budlike geometries are shown to generate patterns that correspond to the natural limb patterns (Hentschel et al., 2004). These models are generative in the sense that they include a mechanistic account for the origin of first patterns and for later skeletal innovation. Daeschler and collegues (2006) describe well-preserved pectoral appendages in recent finds of Late Devonian sarcopterygian fish that exhibit a transitional morphological and functional stage between fins and limbs, suggesting a generative rather than an informational mode of skeletogenic pattern evolution.

The predictive and heuristic roles of informational models and of generic models in limb EvoDevo differ to a great extent. Whereas the former suggest that we need to provide an ever more detailed account of all gene regulatory interactions in limb development, and hence need to continue with the genetic dissection of the limb system, the latter suggests a program that explores the rules of cell and tissue organization in limb development. These rules could explain the generation of similar patterns from developmental processes that have quite different genetic and molecular underpinnings.

Furthermore, in the informational models nearly all changes of pattern are equally possible and the continued evolutionary identity of individual skeletal elements (homology) disappears (Müller, 2003). In the generic models not all changes are equally possible, and the identity of elements is maintained. Here the history of morphogenetic structure itself is a determinant of possible evolutionary change.

The Mammalian Tooth Model In recent years, the development and the evolution of the mammalian tooth has become a major focus of convergence for paleontology and development. Since enamel is far more durable than ordinary bone, teeth often remain after all the bones have decayed. Indeed, tooth morphology has been critical to mammalian ecology and classification. At the same time, the teeth represent

a circumscribed developmental module that can be studied in its molecular and morphogenetic aspects without much interference from/with other parts of the embryo. Changes in the cusp pattern of molars is seen to be especially important in allowing the radiation of mammals into new ecological niches. The question, then, is what developmental mechanism allows the mammalian molars to change their form so rapidly.

Jukka Jernvall and colleagues (Jernvall et al., 2000; Salazar-Ciudad and Jernvall, 2002) pioneered a computer-based approach to phenotype production using geographic information systems (GIS) to map gene expression patterns in incipient tooth buds. These studies have shown that specific gene expression patterns forecast the exact locations of the tooth cusps and that the differences between the molars of mice and voles are predicated on differences in gene expression.

The tooth system is particularly suited for modeling EvoDevo processes. Salazar-Ciudad and Jernvall (2004) correlated the morphogenetic kinetics with known paracrine factor properties and distributions. Small changes in a gene network, working through the interactions of the *BMP* and *Shh* proteins, are seen as crucial. *Shh* and *FGF*s (produced by the enamel knot signaling center of the developing tooth bud) inhibit *BMP* production, while *BMP* production stimulates both the production of more *BMP*s and the synthesis of its inhibitors, the *FGF*s and *Shh* proteins. In the model this generates regions of activators (*BMP*s) that block epithelial proliferation, and regions of inhibitors (*FGF*s, *Shh*) that block *BMP* synthesis and independently stimulate mesenchymal proliferation. The result is a pattern of gene activity that changes as the shape of the tooth changes, and vice versa.

Several kinds of EvoDevo predictions can be derived from such a combined molecular and morphogenetic model. Since the kinetic parameters change while the tooth is forming, this gives a great amount of flexibility to the developing system, allowing it to change at relatively rapid rates during evolution. The diverging shapes of mouse and vole molars may have resulted from very small changes in the initial molecular and topological conditions. And the model also predicts that some types of teeth are more likely to evolve in certain ways and that certain shapes are more likely to arise than others. This morphogenetic potentiality conforms significantly to the patterns observed in mammalian tooth evolution (Jernvall et al., 2000; Salazar-Ciudad and Jernvall, 2002).

Perspectives for EvoDevo Modeling

In discussing modeling strategies within EvoDevo, we painted a broad picture and touched upon several different dimensions of modeling discussed in this volume. Here, as a conclusion, we will sketch some of the trends within current EvoDevo

that best illustrate the importance of modeling for the theoretical synthesis of the discipline.

A productive interaction between experimental research and heuristic models characterizes current EvoDevo research. The results of empirical and comparative studies, such as correlations of gene expression patterns with morphological transformations, give rise to heuristic models of the genetic control of development and of regulatory evolution. They also produce more theoretical models that emphasize general aspects of the evolution of developmental systems, such as the roles of gene duplication and regulatory evolution. So far, these models are mostly diagrammatic and functional; very few analytical and predictive models exist within EvoDevo. In part this is because of the disciplinary bias of current EvoDevo research, which is dominated to some degree by developmental genetics and is less guided by evolutionary theory. Insofar as evolutionary models do exist—those that deal with the problem of evolvability, the role of epistatic and epigenetic effects, canalization, or generative entrenchment—they provide a more analytical and predictive framework for what its practitioners sometimes call "developmental evolution" (Wagner, 2000)

In general, EvoDevo has the same problems as any other field of current biology—it has an overabundance of data and very few genuine theoretical principles. One function of models in EvoDevo is thus to organize and visualize large amounts of data, such as those concerning gene expression patterns in developing systems. This involves a huge amount of computational modeling and data mining, aided by hypotheses and concepts about relevant connections and links between different data sets. This theoretical biology aspect of EvoDevo will become an increasingly important tool in the future development of the discipline (Müller, 2005; Laubichler et al., 2005).

We started our analysis on a cautionary note: Unless EvoDevo develops models that integrate empirical and theoretical studies while capturing the essential features of an EvoDevo explanation, it will not live up to its promise as a new synthesis of development and evolution. Such genuine models would combine theoretical, material, and heuristic dimensions of modeling biology (cf. chapter 1 in this volume). We have argued that some developments in EvoDevo represent steps in this direction. There is now an active push for new EvoDevo model organisms, specifically chosen for the study of genuine EvoDevo questions, such as the role of genotype–environment interactions and plasticity, or the role of particular genes in morphological and behavioral differences. This is an important start. In a subsequent step the information extracted from these model organisms will need to be embedded within a framework of analytical and theoretical models that connect the specific empirical details with general processes of development and evolution. The future of EvoDevo, like that of any other discipline, will depend on the successful integration of its material and theoretical models.

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