CHAPTER 12

Epilogue: Prospects for Research on the Origin and Evolution of Larval Forms

MARVALEE H. WAKE* AND BRIAN K. HALL†

Department of Integrative Biology and Museum of Vertebrate Zoology, University of California, Berkeley, California, †Department of Biology, Dalhousie University, Halifax, Nova Scotia, Canada

Why are there larvae, and how did they come to be? What, indeed, is a larva? How do larvae function—physiologically, ecologically, and as a component of life history? How does larval development differ from and/or contribute to adult form and function? What is the mechanistic basis of the development of larval form and function? What is the interplay of developmental pattern and process with evolutionary pattern and process? The contributors to this volume have clarified and increased our understanding of larval biology, while pointing out a number of areas that remain unresolved. They variously have defined larvae, assumed definitions, or defied definitions. In general, two types of definitions of larvae emerge: one is characterized by morphological attributes that alter during the lifetime of the animal, often dramatically, whereas the other features ecological change during the life history of the animal. Some definitions combine these features. At the same time, several authors point out that the definitions are general ones and that there are exceptions to those generalizations in all major groups of animals. This terminological incongruity is a clue to the variation in larval biology that contributes to the wide range of patterns of evolution discussed in these chapters.

The Origin and Evolution of Larval Forms

The complexity of larvae, their contribution to a species' life history, some of the controls over their development, maintenance, and metamorphosis, their function and behavior, and their ecologies are discussed in various frameworks by the contributors. We appear to be reaching a better understanding of larval biology at several levels, but the investigations that established our understanding have opened many new questions. In fact, the realization and elucidation of new questions, and the reconsideration of some "ancient and honorable" ones in the light of new information, are important contributions of our work, which we hope will stimulate some new research venues as a consequence of what is known and, more importantly, what is not known about the biology of larvae.

An "ancient and honorable" question that is not fully answered, nor even fully grasped, is why larvae evolved at all. The facile answer usually provided is that larvae contribute to the survivorship of species by diversifying both larval and adult life forms and thereby the habitat and resources available. A more interesting question might be why adults evolved. In other words, which came first? Was the reproductive form sexual or not? Did organisms with "larval" features acquire sexual reproduction, or did larvae evolve secondarily to diversify species? Different groups of animals might provide different insights into an answer to these questions. As noted in our Introduction (Chapter 1), Garstang recognized such questions as whether larval and adult evolution occur in parallel, and whether larval evolution is an "escape from specialization" or the achievement of specialization. These and other major questions are not yet answered, but new tools and techniques, data, and analytical and theoretical frameworks provide means of approaching problems of the interplay of development and evolution with new insights. Our contributors provide several examples of new contributions through synthetic approaches.

At the same time, each author either implies or directly states a series of areas that need study or poses questions that are not yet resolved. Virtually all contributors decry the limited numbers of species that have been studied and the relative absence of comparative analysis. Several point out that comparative assessment done in a phylogenetic context is essential to understand the pattern and direction of evolution. Many animal groups for which there are excellent descriptive and even mechanistic studies for individual species are not represented by well-corroborated hypotheses of phylogenetic relationships; understanding of the evolution of such systems remains speculative or intuitive at best. Most authors have found that mechanistic bases for developmental and evolutionary change are poorly known, studied in only a few species, and often of limited generality.

Hickman wrestles with the enormous diversity of invertebrate larvae, especially problems of analysis of their biology, origins, potential contribution to understanding of phylogenetic relationships, and the emerging evidence in the

fossil record that is changing the ways that paleontologists, systematists, and evolutionary biologists view the evolution of major invertebrate groups. She resolves the three conflicting definitions of "larva" (one is based on structure, another on ecology, and the third on morphogenetic mechanisms) in favor of the structural one. Given that a larva is a structural state or series of states—which are transitory and lost at metamorphosis but reflect adaptations for larval life—the structural features allow recognition of larvae and their biology, including ecology, development, and evolution. Further, they permit analysis of the diversity of larval form and structure in terms of characters for phylogenetic analysis. Hickman contends that currently available terminology (and there is a lot of it) and classification vastly underestimate the structural diversity of invertebrate larvae, both at higher taxonomic levels and at those of the individual, population, and species. She points out that inducible traits and phenotypic plasticity can be better addressed in a structural framework. She addresses the question of the origin of larvae by first insisting that questions be framed clearly and then identifying what must be done to effect resolution—largely dependent upon robust hypotheses of relationships. Similarly, the evidence from the fossil record, including first appearances of adults and larval skeletal remains and larval soft anatomy, has new meaning as additional characters are included in phylogenetic analysis. Hickman challenges developmental biologists to incorporate the paleontological evidence into research on developmental mechanisms in order to shed more light on evolutionary history. Her probing questions open several new areas for research on invertebrate larval biology, cast in a structural framework and facilitated by a well-supported phylogenetic hypothesis.

Hanken, in his discussion of amphibian larvae in development and evolution, emphasizes that homology of larvae remains unresolved. Further, variable and inconsistent terminology hinders syntheses of information about development, including interpretations of heterochrony. The embryonic derivation of larval and adult features is not well-studied for any species. Conservatism of cranial development generally is accepted, but there are huge gaps in our knowledge—the assumption of conservatism is based on the study of a handful of frogs, information about salamanders is limited, and there is less about caecilians. For example, there is no detailed information for any species of amphibian about the contribution of neural crest to cranial development, comparable to that available for certain amniotes. Hanken notes that the evolution of larvae through the modification of developmental patterns, especially the timing of events, is well-known, but that the mechanistic bases of the modifications are poorly known. He states that more attention should be paid to early embryology, for perturbations of early ontogeny relevant to later development are not well-documented. Finally, the loss of larvae in lineages for which a biphasic life history is ancestral deserves more attention in terms

of developmental patterns, especially mechanisms, as does the recruitment of larval features to perform new functions in embryos and fetuses.

Webb asks what drives larval specializations and how specializations are selected. She notes the need for synthesis of data about morphology, growth, development, and ecology. The data are distributed in a number of pure and applied subfields of biology, often with nonoverlapping participants. Fish larvae are highly diverse—they can be extremely different from adults or rather similar, and they can metamorphose gradually or abruptly. The mechanistic basis for these differences is not understood. Webb stresses the multiple sensory systems possessed by larval fishes and their interaction to effect the ontogeny of behavior, which is another area of study often discussed, but for which there are few data about mechanisms of interaction and ontogenetic change. She strongly states the case for the need for more experimental study of development in comparative and phylogenetic contexts, asking for an interdisciplinary approach to larval biology that ranges from shipboard collaborations to that among laboratories and museums with different perspectives.

Hart and Wray extend a challenge to those who consider heterochrony an "explanation" of pattern and process in development and evolution. They find that heterochrony is *not* an important generative process in the origin and evolution of larvae, but that it arises as a result of epigenetic interactions and, therefore, is a consequence, not a process. They suggest that an alternative research program to that of larval ecology might be feasible, but that it would be limited to "smaller scale variation in morphological development of species with invariant order of developmental events."

Chris Rose's thesis is that complex life histories *produce* specialized larval forms. He, too, recognizes that few species (of amphibians, but this applies to all groups) have been studied in terms of mechanistic versus descriptive development and that there is a significant need for mechanistic research in a phylogenetic context. He develops his concern by discussing the pattern of endocrine control of amphibian development and the apparent conservatism of the few hormones involved, coupled with the essential modification of timing of events. The mechanistic bases for differentiation of patterns among species are not known. He posits that only a phylogenetic approach will allow resolution of the causation of developmental and evolutionary change and allow an understanding of the evolution of diversity of form and function.

In contrast, Fred Nijhout illustrates that whereas postembryonic development in insects is controlled by hormones and developmental transformation involves only a few hormones that have broad activity, embryonic development involves cascades of genetic activation, with no extrinsic or hormonal control. He suggests that morphogenetic field size is a key to this distinction. As the organism increases in size, the fields must operate under "local control;" hormones are a mechanism that provides long-range signals that coordinate

development. Nijhout poses the contrast between postembryonic development, in which hormones operate in an all-or-none matter, and adult metabolism, in which hormones operate in terms of threshold levels and control. He recommends two major research arenas: (1) a better understanding of the ways that environmental cues are transduced by hormones during short windows of sensitivity and (2) an examination of the "modular control" of development, whereby body parts can develop, and potentially evolve, independently, thereby generating great organismal diversity.

Raff's examination of cell lineages in the development and evolution of larvae also reveals gaps in our knowledge and new questions to be explored. If relatively few cleavages are needed to generate the number of cells that establish lineages, what generates diversity? Is pattern conservation due to developmental constraints, or are features relatively unconstrained but limited for other reasons? The causal basis of cell lineage modification begs for comparative studies. One approach that Raff recommends is an examination of the way that selection maintains suites of larval characters (e.g., for feeding). Rapid evolution of cell lineages and gene expression can occur when selection is relaxed. Such studies, in a phylogenetic context, have the potential to elucidate mechanism.

Nagy and Grbić provide an innovative approach to the analysis of cell lineages in invertebrates, using insects as their model. They note that the concept of invariant cell lineage "can sometimes be a red herring;" developmental mechanisms can evolve while the cell lineage pattern does not change. The syncytial early development of insects and their patterning via gradients that then establish domains of gene expression as the embryo cellularizes suggest that at least some embryonic parts are lineage-independent. Nagy and Grbić make a conceptual leap as they realize that the linkage of gene expression domains and developmental fate is well-established in several model animals and recommend that "groups of cells under similar genetic control" be substituted for "differentiating cell lineages" in order to bring new coherence to the study of gene networks and cellular differentiation. They recommend that comparison of groups of cells that share common gene expression patterns be analyzed comparatively and in a phylogenetic framework in order to understand the origin and evolution of insect larvae. They provide lucid examples of this approach, including analyses of life-history evolution, origins of larvae, and modification of adult form, and find that such phenomena as insertions of novel stages into life histories, dissociation of developmental features, and the rearrangement of gene circuits that generate new morphologies have occurred repeatedly in the evolution of insect larvae. The concept of "groups of cells that express the same gene networks" as an analogue for the standard concept of cell lineage promises a new approach to comparative analysis of mechanisms and new principles underlying morphogenesis.

Sanderson and Kupferberg's discussion of aquatic larval feeding in fishes and amphibians further illustrates the need for synthesis of developmental, functional, and ecological information in an evolutionary framework. They propose that several research directions would be profitable. For larval fishes, examination of the function of ontogenetic changes in proportions and spatial relations of cranial structures, and the connections between functional elements, would yield a better understanding of larval development and diversification within and across lineages. For larval amphibians, the role of diet quality in morphogenesis and the ability of larvae to select foods that induce changes in morphogenesis are virtually unexplored; some data exist for frogs, but effectively there are none for salamanders or caecilians. The more general questions of the ontogenetic changes in sources of nutrition and means of its acquisition and the role of habitat segregation in feeding require more study as well.

Greene, too, advocates the integration of studies of genetics, development, and environmental effects in order to understand the evolutionary causes and consequences of phenotypic variation. He points out that we have an incomplete understanding of phenotypic evolution and asks a series of questions: How do developmental events and timing influence the range of possible phenotypes? How do developmental trade-offs in resource allocation influence possible phenotypic and developmental outcomes? Do developmental processes impose genetic constraints? Developmental events precede selection events for which they are required—how does the decoupling of development and selection occur? How do ecological interactions between the organism and its environment influence phenotypic evolution? How do patterns of phenotypic variation influence patterns and modes of speciation and macroevolutionary patterns?

In summary, questions and problems for new research on the origin, function, and evolution of larvae abound. These questions and problems pertain to the biology of larvae in general and specifically for different lineages of animals, to different processes of regulation of development, to different structural and functional attributes, and to different responses to environments. Old questions remain unresolved, and new questions constantly arise as consequences of investigation. New techniques and new theoretical constructs permit new insights. More species must be studied, within and across lineages of animals, in order to assess the generality of current conclusions. Cross-disciplinary syntheses of data and approaches will provide more profound answers to questions of larval biology. A comparative approach in a phylogenetic context is most likely to provide answers to questions of mechanism and causation of the evolution of larvae and their development, structure, and function. The research presented by the contributors to this volume and their new options for study indicate that investigation of the origin and evolution of larval forms is a field of study as lively and engaging today as it was for Balfour and Garstang nearly a century ago.