Comparative Developmental Physiology: Contributions, Tools, and Trends

Stephen J. Warburton, et al., Editors

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Contributions, Tools, and Trends

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Preface

Aristotle contended that "things are best studied as they come into being." This is not usually the case for the writing of prefaces, which are nearly always the last text to be penned. This one is no exception. The idea for this book arose out of a National Science Foundation funded roundtable, New Directions in Developmental Physiology, held at Glen Rose, Texas, in June 2002. Its own developmental trajectory has been more altricial than precocial and, as such, the resultant book chapters are certainly not a mere recapitulation of the oral presentations given during the workshop. The finished result is far more than the sum of the individual author parts. Many people have been involved, throughout and at critical stages. We are thankful to colleagues who took time out of busy schedules to comment on individual chapters. We also thank Kirk Jensen and Peter Prescott of Oxford University Press for guidance and advice, and the wonderful staff of the Inn on the River in Glen Rose for their warm hospitality and the stunning setting for the workshop. We dedicate this volume to the graduate students, present and future, who will experience the challenge, wonder, and privilege of figuring out just how developing animals work.

The Editors

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Introduction

In early June 2002 a NSF-sponsored roundtable in Glen Rose, Texas, brought together a small group of scientists and their students to spend several days in isolation, discussing the future of a field that seemed both ill defined and nebulous in goals yet also vigorous with intellectual energy—comparative developmental physiology. It was not our goal, as organizers, to try to create a formal new discipline (with the inevitable new journal and unique vocabulary that seems to result from such efforts). Rather our goal was to poke into all the corners of related existing fields as well as the primary field of comparative physiology to learn what futures might exist for such a promising concept as comparative developmental physiology.

We organized the presentations around the concept of the original roundtable, providing equality and the right to speak freely, and arranged for generous time for discussion. Indeed, the organizers pushed the idea even further and intentionally asked speakers to operate outside of their comfort zone and explore provocative new concepts and interrelationships, rather than just hash over published data. In fact, speakers were initially assigned topics on which to speak, and most replied to their tentative assignments with incredulity: "You want me to talk about what?" Yet, to a person, the speakers accepted the challenge and pushed the envelopes.

It was also important for the organizers—and the success of this project—that emerging researchers and students be involved, since they will be the torchbearers in coming years. Often, younger minds are more flexible and are less constrained by ego-driven defensiveness. Karl Lorenz (in *On Aggression*, 1966) probably said it best: "It is a good morning exercise for a research scientist to discard a pet hypothesis every day before breakfast. It keeps him young." It would be less than honest to proclaim that all scientists are enormously willing to truncate pet hypotheses, thus it was our intent

to include young scientists and students to help the rest of us truncate pet ideas and ideals.

Each speaker walked the audience through his or her presentation from the perspective of "Where are we and where are we going?" Each speaker provided unique and provocative ideas, and many more emerged during the lengthy discussions. Although the process of assigning topics was perhaps novel to most speakers, the effect was quite exciting. During the roundtable there was contention, argument, surprise, humor, and—most importantly—respect for nonconventional thought and ideas. Being forced to think about new connections, while annoying if not painful for some of us, resulted in the forging of new ideas that we had hoped for. Some ideas are immediately applicable, whereas others will need much more data collection to begin to bear fruit. Still others require a new *Weltanschauung* (at least new to this area) altogether.

This book, then, represents the distilled essence of many of the roundtable presentations and is intended to challenge and excite the reader. Certainly, the process challenged and excited the participants! While some of us had anticipated speedily putting together our manuscript shortly after the event, the actual discussions and sharing of ideas occurring at the roundtable led to some major restructuring of the papers—some are a metamorphosed version of the original presentations, being updated but also expanded in vision and scope. Some of the chapters contain information on techniques and technologies that will play well in the development of the field, while others are more speculative and focus on paradigms that may come as a surprise in a book on comparative developmental physiology. Yet, all are worth your attention.

Taken together, the chapters of this book provide exciting suggestions for navigating a field that is both traditional and emergent. For the foreseeable future, it seems certain that much of the research in comparative developmental physiology necessarily will continue to be the collection of basic data, without which broad defining principles cannot be credibly realized or even recognized. This said, it does seem that available data are nearing a critical mass that is beginning to lead to more complex hypotheses, deliberations, and experiments. The position of comparative developmental physiology in the future can either be increasingly constrained by self-imposed views of "what belongs" or it can expand its view to encompass tools, paradigms, and models of most facets of modern biology. Clearly, the practitioners who lay claim to the title of comparative developmental physiologists will ultimately determine the scope of the field, and thus the chapters of this book seem to indicate that it is the expanded view that is becoming the future of developmental physiology.

The early chapters of this volume provide signposts to techniques and approaches that might formerly have seemed out of reach or even extraneous to many of us; these authors have convincingly shown there are many effective and exciting new tools to consider, as well as new collaborations to seek. The exhilaratingly complete work on plasticity of eyespot genetics in butterflies (chapter 4) provides a benchmark for investigating phenomena from the population genetic level to environmental importance. We are introduced to exciting advances in imaging techniques and technologies ideal for embryonic and larval applications (chapter 2). The benefit of combining levels of model systems from species to organism to cell culture in understanding evolutionary patterns is clearly demonstrated in studies on surfactant production (chapter 1). The powerful modeling approach of finite element analysis is elegantly and approachably presented in studies of oxygen flux through amphibian egg masses with some surprisingly nonintuitive

results (chapter 3). Classic questions of scaling metabolism to body size (chapter 6), not totally understood and up to date until now, are shown to be an issue that cannot be divorced from the old-fashioned technique of watching behavior and realizing that development is not always a smooth trajectory, but contains kinks and bends, or plateaus and cliffs. One of the most developed datasets of developmental energetics exists for fish, and this topic is fully explored from several perspectives (chapter 7).

Globally, there is an increasing demand for the application of basic science, and some directions have been effectively delineated in this book. The interaction of medical and comparative physiology is less active than it historically has been, and we are shown the potential for reinvigorating the field in the commonality of questions currently being asked in the medical arena and those in the comparative field (chapter 10). Similarly, applications of developmental inquiries into environmental perturbation and possible remediation are demonstrated to require the inclusion of developmental stages to be truly enlightening (chapter 9).

The increasingly recognized phenomenon of phenotypic plasticity as a concept both in environmental and evolutionary arenas promises to provide application-hungry funding agencies with practical comparative examples in medical concerns such as fetal programming. We are introduced to examples of developmental plasticity from the cellular level to the whole animal as a response to development at altitude and introduction to examples of critical periods of development (chapter 5). Response to development at different environmental temperature is analyzed with respect to genes that may mediate temperature-sensitive responses and the evolutionary consequences of such mediation (chapter 8). Both altitude and temperature are shown to be suitable systems within which to explore sources of nongenetic variation and the potential limits of plasticity.

And for the *digestif*, broad theoretical frameworks are presented to confront preconceived notions in comparative developmental physiology and to challenge investigators to synthesize and integrate across new variables and to pose new paradigms. We are challenged with novel ideas of developing complexity and regulation of nascent systems (chapter 12). Old ideas are reborn with new vigor and exciting promise for understanding evolution, as demonstrated by the physiological application of principles of heterochrony and heterokairy (chapter 13). Finally, the entire thrust of this nascent community is challenged to amplify the power of data collection and tool development by focusing on a few select model organisms, instead of the broader, more traditional comparative approach (chapter 11).

Our intent in this volume was to create a podium from which biologists of all disciplines might see the commonality of this place as an optimal foraging area for collaborative efforts. Certainly the authors who contributed chapters represent only a limited sample of potential grazers, but even with that caveat, the diversity of interests expressed, converging on a single, multiply adjectivized topic, is telling. Comparative developmental physiology is a discipline examining a diversity of organisms as they transform from early single cell to mature reproductive individuals.

The future of comparative developmental physiology is now. The reductionist approach using model systems has led to a greater understanding of cellular machinery but not to the integrated function of the whole organism. It is becoming increasingly apparent that the sum of an organism's parts (genes, mRNA, protein, etc.) is greater than the whole. Yes, genes appear to be conserved between organisms, but a fly will never swim and a zebrafish will never fly, and thus conservation of genes does not equate to

parallel developmental environment or even conservation of developmental patterns. Evolution has provided comparative developmental physiologists with a diverse array of tools (biological diversity) with which to investigate a broad range of questions that are critical for our understanding of how life works. This does not just include the basic nuts and bolts of cellular mechanisms but the integrated functional whole, from the mechanistic level to behavior within and between organisms. This union of traditional developmental systems with the breadth of comparative physiology and evolutionary theory holds forth the promise of new insights into the *Grundstoff* of evolutionary processes.

COMPARATIVE DEVELOPMENTAL PHYSIOLOGY

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Pulmonary Surfactant, Cell Culture, and Tissue Regeneration as Models for Understanding the Evolution of Developmental Physiology

CHRISTOPHER B. DANIELS
SANDRA ORGEIG

Introduction

Recently there have been substantial advances in our understanding of the ontogeny of the control and regulation of developmental processes in animals. It is clear that a particular system is generally composed of similar cells and tissue types and often demonstrates a similar overall structural pattern across a range of vertebrate groups. In many cases, it is the same groups of genes that control the morphology, biochemistry, and development of a structure. However, the function of these structures can differ dramatically between species. Therefore, a subset of often highly conserved genes can create spectacular phenotypic diversity. How has this functional diversity evolved, and how do evolutionary forces control the developmental processes to create the functional diversity in anatomical structures? Hence, the big question in developmental biology (especially from an evolutionary viewpoint) is: "If the genes controlling development are conserved, then how do these systems demonstrate such phenotypic diversity?"

Developmental plasticity can be associated with changing environmental conditions during development, resulting in changes to morphology, physiology, or behavior. This developmental plasticity arises because of the presence of critical windows during ontogeny when the external environment influences the expression of a subset of genes involved in development, leading to alternative phenotypes. Such developmental windows are particularly demonstrable in oviparous and viviparous animals as there is a finite period of time in which to complete the "race to be ready for life" at the conclusion of the developmental period. It is therefore reasonable to hypothesize that for animals developing in ovo or in utero:

1. Developmental processes are likely to be conserved and regulated by homologous genes.
2. Endogenous and exogenous (environmental) cues will induce developmental plasticity that may affect timing, function, and structure.
3. The timing of the development of individual tissues and physiological functions can vary between species.

If so, then the selection forces creating these differences can be determined and the answers to these hypotheses will provide the evolutionary story behind development.

However, often the systems in which we are most interested do not lend themselves to identifying evolutionary processes and developmental patterns, because they are so variable within the groups of interest. For example, lungs have different evolutionary origins and are structurally and functionally diverse, despite being created by highly conserved genes. Here we discuss the issues relating to choosing a system and a group of species to examine the evolution of the development of a process and how two approaches new to evolutionary biology may provide dramatic insights into developmental physiology.

What Systems Do We Look For?

When examining the evolution of any particular physiological system, it is important that it exists within all the species under examination, at least in some stage during the life cycle. In fact the system need not develop (e.g., lungs in plethodontid salamanders), but the genetic code must be present. How a system is inhibited from developing can be as interesting as the developmental process itself. Second, the system tested must be conserved in general structure, so that it can be identified, isolated, and examined. Third, the system must be amenable to experimentation and demonstrate significant phenotypic plasticity in response to genetic or environmental manipulation. For many of our studies we have chosen the pulmonary surfactant system, because it fulfills these criteria. Furthermore, the surfactant system has the additional advantage that it is well studied in adults and embryos, particularly of mammalian species, because of its clinical importance, thereby providing a substantial body of background data.

An Example: The Pulmonary Surfactant System

Pulmonary surfactant is a complex mixture of phospholipids (PL), neutral lipids (NL), particularly cholesterol (Chol), and proteins, which lines the inner lung of all vertebrates and regulates the surface tension at the air–liquid interface (Veldhuizen et al. 1998). Surfactant is stored in lamellar bodies, which consist of a dense proteinaceous core with lipid bilayers arranged in parallel, stacked lamellae surrounded by a limiting membrane. After the lamellar bodies have been released into the fluid lining of the alveolar space (the hypophase), they swell as they hydrate and unravel into a highly characteristic cross-hatched form of surfactant termed tubular myelin. It is this structure that supplies the lipids to create the surface film, which regulates the surface tension of the air–liquid interface of the lung (Goerke 1998).

The ability to lower and vary surface tension with changing surface area is attributed to the interactions between the disaturated phospholipids (DSP), particularly dipalmitoyl phosphatidylcholine (DPPC), the unsaturated phospholipids (USP), and cholesterol.

The DSP consist of a hydrophilic headgroup, immersed in the hypophase, and two fully saturated fatty acid "tails" that are hydrophobic and extend into the airspace. Because of the saturated nature of the fatty acids, the DSP molecules are capable of being compressed tightly under dynamic compression (e.g., during expiration). In this state they exclude water molecules from the air–liquid interface, thereby dramatically lowering surface tension (Veldhuizen et al. 1998). Furthermore, upon film cycling, which occurs through progressive inspiration–expiration cycles, the surfactant film becomes enriched in DSP. This may occur through a combination of selective adsorption of DSP molecules, aided by the surfactant proteins, and/or by the selective elimination or "squeeze-out" of the less surface-active lipids, for example, USP and Chol (Possmayer et al. 2001). However, because DSP molecules have a high phase transition temperature, that is, the temperature at which they change state from a solid gel to a fluid liquid-crystalline state, the film will exist in a solid gel state at biological temperatures. For example, DPPC has a phase transition temperature of 41°C (Goerke and Clements 1985). In order for the surface film to be spreadable, the transition temperature of the mixture has to be lowered, which can be achieved by the addition of Chol and/or USP. Hence, upon expansion of the lung, these fluidizing molecules are recruited into the surface film to promote respreading (Possmayer 2004).

It is clear, therefore, that the body temperature of an animal is likely to profoundly influence the lipid composition of pulmonary surfactant. We have discovered that the amount of DSP as a percentage of total PL (%DSP/PL) in surfactant has increased throughout the evolution of the vertebrates from the air-breathing fish, lungfish, amphibians, reptiles, birds, and mammals (Daniels et al. 1995a; figure 1.1). The amount of Chol relative to total PL (Chol/PL), however, demonstrates the opposite trend, with the air-breathing fish and the primitive dipnoan, the Australian lungfish (*Neoceratodus forsteri*), having 3-fold greater amounts than all the other vertebrate groups (Daniels et al. 1995a; figure 1.2). These opposite trends in Chol/PL and DSP/PL result in a very dramatic pattern for the Chol/DSP ratio (Orgeig and Daniels 2001; figure 1.3). The fish and *N. forsteri* with their relatively simple bag-like lungs have a Chol/DSP ratio up to an order of magnitude greater than the reptiles and mammals. The amphibians and the derived dipnoans, the African and South American lungfish (*Protopterus annectens* and *Lepidosiren paradoxa*, respectively), have intermediate levels of Chol relative to DSP, that is, the ratio is approximately double that of the reptiles and mammals (Daniels et al. 1998; Orgeig and Daniels 2001). Differences among terrestrial groups in the composition of surfactant probably reflect the temperature-dependent fluidity of surfactant phospholipids and the need to maintain homeoviscosity. Hence, the ectotherms with their relatively lower body temperatures (~20°C) contain greater levels of Chol, in order to maintain their surfactant mixture in a fluid and spreadable state. Conversely, it is only the most heliothermic reptiles and the endothermic birds and mammals that are capable of tolerating a high %DSP/PL of 40–50% (Daniels et al. 1995a).

In addition to the lipids, pulmonary surfactant also contains four surfactant-specific proteins, termed surfactant protein A (SP-A), SP-B, SP-C, and SP-D (Haagsman and Diemel 2001). We recently used the surfactant proteins to determine that surfactant had a single evolutionary origin that predated the evolution of the vertebrates. We demonstrated that an SP-A-like protein is present in surfactant from all vertebrate classes, even from goldfish swimbladders (Sullivan et al. 1998; figure 1.4). Furthermore, the ultrastructural characteristics of pulmonary surfactant (e.g., type II cells, lamellar bodies,

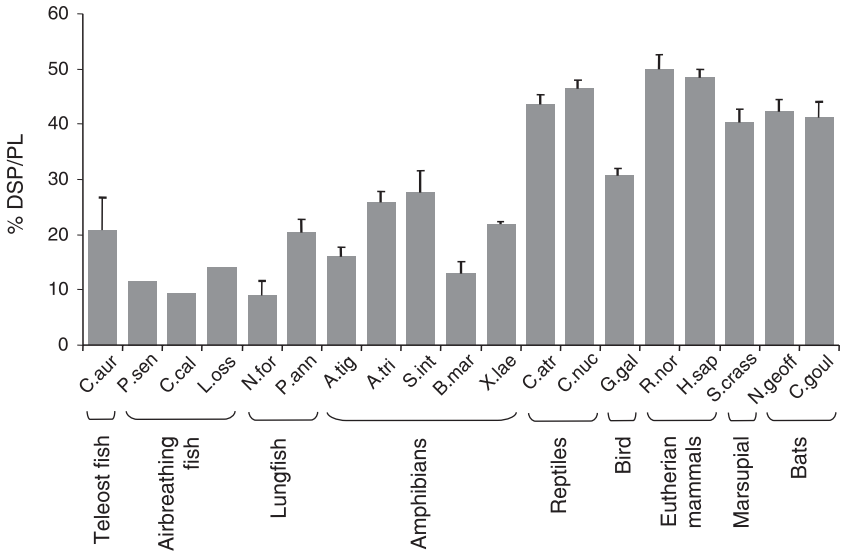

Figure 1.1 Relationship between disaturated phospholipid and total phospholipid during the evolution of the vertebrates. The %DSP/PL is presented as the mean ± SE of a teleost fish, the goldfish *Carassius auratus* (C.aur) (Daniels and Skinner 1994); the air-breathing Actinopterygiian fish *Polypterus senegalensis* (P.sen), *Calamoicthys calabaricus* (C.cal), and *Lepisosteus osseus* (L.oss) (Smits et al. 1994); the Australian and African lungfish *Neoceratodus forsteri* (N.for) and *Protopterus annectens* (P.ann) (Orgeig and Daniels 1995); the tiger salamander *Ambystoma tigrinum* (A.tig) (Orgeig et al. 1994); the amphibians *Amphiuma tridactylum* (A.tri), *Siren intermedia* (S.int), *Bufo marinus* (B.mar), and *Xenopus laevis* (X.lae) (Daniels et al. 1994); the rattlesnake *Crotalus atrox* (C.atr) (Daniels et al. 1995b); the lizard *Ctenophorus nuchalis* (C.nuc) (Daniels et al. 1990); the chicken *Gallus gallus* (G.gal) (Johnston et al. 2000); the rat *Rattus norvegicus* (R.nor) (Orgeig et al. 1995); the human (H.sap) (Doyle et al. 1994); the fat-tailed dunnart *Sminthopsis crassicaudata* (S.crass) (Langman et al. 1996); the microchiropteran bats *Nyctophilus geoffroyi* (N.geoff) (Slocombe et al. 2000) and *Chalinolobus gouldii* (C.goul) (Codd et al. 2000). The lizard, the dunnart, and the bats were at their warm-active body temperature (33–37°C). (Figure reproduced from Orgeig et al. 2003 with permission from CSIRO Publishing.)

tubular myelin) are conserved across a large range of vertebrate species (Daniels and Orgeig 2001), and have even been described in an invertebrate, the pulmonate snail (Daniels et al. 1999). Thus, surfactant from nonmammalian vertebrates would appear to be produced, stored, and released in a similar manner to mammalian surfactant. Moreover, the system predated the evolution of lungs (Daniels et al. 2004).

The primary selection pressure for the evolution of lungs was probably aquatic hypoxia (Perry 1989). The ancestral bony vertebrate was most likely lunged and inhabited warm stagnant pools and gulped air to gain sufficient oxygen. Lungs developed as outpouchings of the foregut (Perry et al. 2001). The cells that produce surfactant and contain SP-A have been located in the gut of many animals, including man (Bourbon and Chailley-Heu 2001; Engle and Alpers 2001). In the gut, surfactant may be important in controlling fluid–fluid interactions between liquids of different viscosities

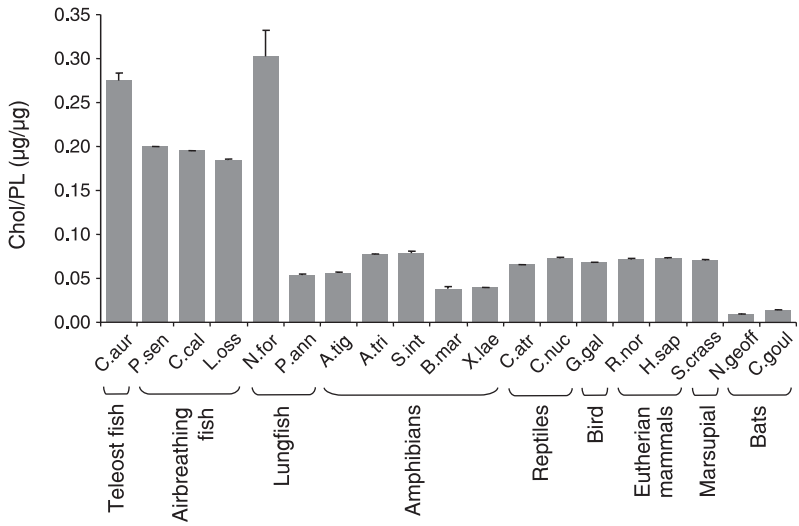

Figure 1.2 Relationship between cholesterol and total phospholipid in pulmonary surfactant during the evolution of the vertebrates. The Chol/PL ratio is expressed as mean ± SE (μg/μg). All abbreviations, data sources, and other details as for figure 1.1. (Figure reproduced from Orgeig et al. 2003 with permission from CSIRO Publishing.)

(in particular, the mucus and serous fluid layers). The surfactant-secreting cells were presumably recruited by the air-filled outpouchings of the gut and the surfactant took on its current surface tension-controlling functions. In this way, the surfactant system both predated the evolution of lungs and was crucial for the evolution of air breathing (Daniels et al. 2004). Surfactant was also crucial for the next three of the major evolutionary steps for the vertebrates:

1. The separation of the Actinopterygiian (bony) fish from the Sarcopterygiia (lungfish) and the tetrapods (land-dwelling vertebrates).
2. The land–water transition.
3. Changes in body temperature, particularly the general increase from cold ectotherms to warm heliotherms and endotherms.

Hence, the ultrastructure and the lipid and protein components of pulmonary surfactant are highly conserved across the vertebrate groups. Surfactant properties appear to have coadapted with temperature, but not lung structure, and generally do not show lineage-specific effects. The fact that surfactant composition demonstrates subtle but important differences that transcend the phylogenetic groupings, but are attributable to specific selection pressures (e.g., temperature), makes this an ideal system in which to explore evolutionary processes in respiratory physiology.

Undertaking Evolutionary Studies into Developmental Physiology

In evolutionary physiology, it is important to study as many species as possible, which differ widely from each other in phylogeny and/or birthing strategies. As there has to be a