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# COMPARATIVE DEVELOPMENTAL PHYSIOLOGY: An Interdisciplinary Convergence

Warren Burggren
*Department of Biological Sciences, University of North Texas, Denton, Texas 76203; email: burggren@unt.edu*

Stephen Warburton
*Department of Biology, Northern Arizona University, Flagstaff, Arizona 86011; email: Stephen.Warburton@nau.edu*



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■ **Abstract** Comparative developmental physiology spans genomics to physiological ecology and evolution. Although not a new discipline, comparative developmental physiology's position at the convergence of development, physiology and evolution gives it prominent new significance. The contributions of this discipline may be particularly influential as physiologists expand beyond genomics to a true systems synthesis, integrating molecular through organ function in multiple organ systems. This review considers how developing physiological systems are directed by genes yet respond to environment and how these characteristics both constrain and enable evolution of physiological characters. Experimental approaches and methodologies of comparative developmental physiology include studying event sequences (heterochrony and heterokairy), describing the onset and progression of physiological regulation, exploiting scaling, expanding the list of animal models, using genetic engineering, and capitalizing on new miniaturized technologies for physiological investigation down to the embryonic level. A synthesis of these approaches is likely to generate a more complete understanding of how physiological systems and, indeed, whole animals develop and how populations evolve.

## WHAT IS COMPARATIVE DEVELOPMENTAL PHYSIOLOGY?

Comparative developmental physiology (CDP) is, quite simply, an examination of the comparative physiology of developing animals. Borrowing from the title of Schmidt-Nielsen's (1) wonderful book, *How Animals Work,* we can view CDP as "how developing animals work." Similar to one of its parent disciplines, comparative animal physiology, CDP spans investigations ranging from genomics and proteomics to physiological ecology and evolution. CDP is not a new discipline, and we are not attempting here to provide a comprehensive listing of all studies

falling under the umbrella of this expanding and vibrant discipline. Rather, our goal is to outline the pathway by which CDP has become an interdisciplinary domain, to highlight the current explosion of studies in this area, and to underscore the important contributions that CDP is making and will continue to make toward the ultimate goal of understanding the connection between evolution, development, and physiology.[1]

The roots of CDP go back millennia. Aristotle (384–322 BC) commented on the pulsing red spot in recently laid chicken eggs, an observation oft-repeated, most notably by Vesalius (1514–1564) and Galileo (1564–1642), before the more detailed characterization of bird embryonic heart rate by numerous seventeenth and eighteenth century proto-physiologists. To this day, the 2–3-Hz heart beat (37°C) of the 3 to 4-day old chick embryo holds fascination for all who observe it.

Until fairly recently the questions asked by investigators of CDP were largely descriptive and not unlike those of Aristotle, perhaps reflecting the classic embryological studies that were themselves so highly descriptive in nature. Thus through most of the twentieth century, typical questions might have been, What is the heart rate of larval bullfrog? (2), or, Can neonatal birds thermoregulate? (3). Few and far between were pioneers such as Adolph (4) or Metcalfe (5) who began to ask more sophisticated questions about the regulation and control of physiological systems during the process of animal development.

While answering descriptive physiological questions (and many such important questions still remain unanswered at the organ system/organismal level) is still important, during the last few decades the field of CDP has expanded from these origins to include experimentation and manipulation. Enabled by new, often miniaturized tools for physiological measurements (see below), a fresh generation of mechanism-based questions has emerged. What are the physiological systems for heart rate regulation during development and what controls them? (6–8, 9, 10). How and when do thermoregulatory mechanisms develop in bird embryos? (11–15). Indeed, in recent years the comparative physiological literature has shown an explosion of developmental studies involving experimental manipulation. Currently, development as a crucial "Z axis" (Figure 1) is a seminal theme. This approach elucidates developmental vectors or trajectories that characterize how physiological processes and their control mechanisms change throughout ontogeny (16). Indeed, physiological studies are increasingly looking to developmental perspectives to explain adult physiological traits, to probe phenotypic plasticity in developmental programs, and to resurrect and refine the intersections of evolution, physiology, and development (see below).

The most recent phase in the maturation of CDP has been the rapid expansion of physiological genomics combined with the use of model organisms and genomic tools such as microarrays, genetic engineering, gene knockouts, etc. These interactions are prompting new questions such as, What genes are involved in the

[1]We use the acronym CDP here with some reservation, as acronyms tend to contribute toward the creation of intellectual clusters rather than interdisciplinary gradients.

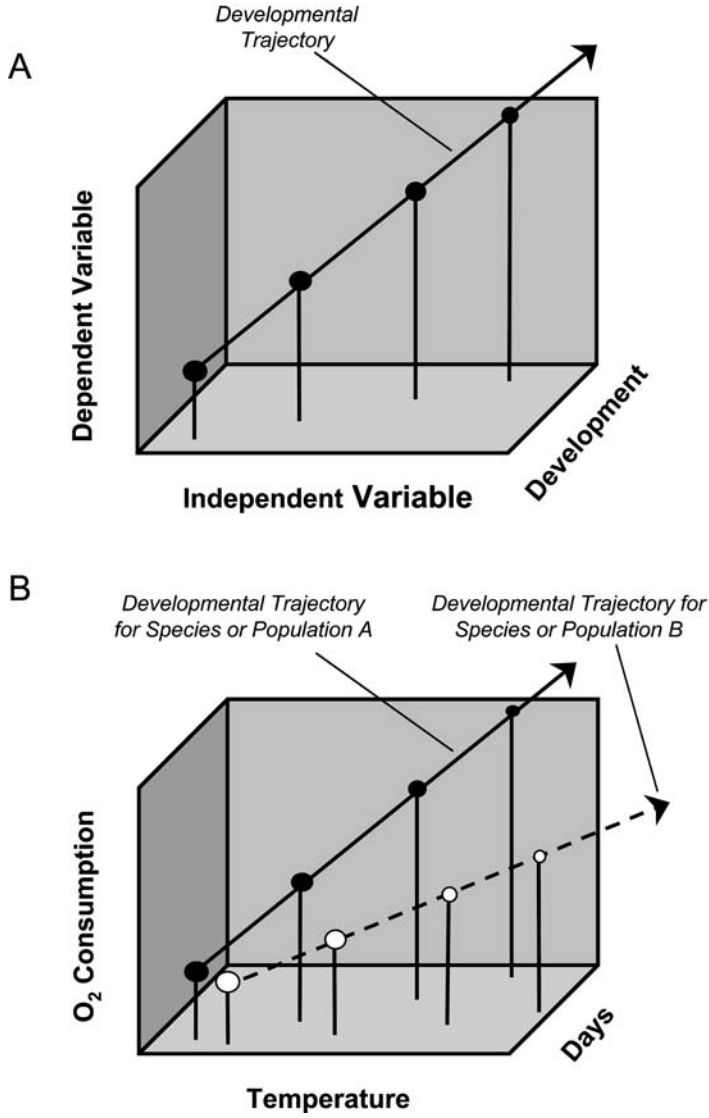


**Figure 1** Developmental trajectories. (*A*) Contemporary studies in comparative developmental physiology often examine the interactions between two variables (X and Y as a function of developmental progression, which forms the third variable or Z axis). The result is a distinct and quantifiable developmental trajectory, depicting the nature of interactions between variables (after Reference 16). (*B*) By comparing multiple species or populations, differences in developmental trajectories become apparent. In this example, a differential response to developmental temperature results in two distinct developmental trajectories for oxygen consumption.

formation of embryonic heart chambers? (17), or, What heat shock proteins are induced, and why, during temperature stress in embryos? (18–19). Full realization of the power of genomics requires the ability to relate gene activation first to proteomics and ultimately back to organismal function in both embryos and adults, thereby allowing a comprehensive systems synthesis from gene to adaptive advantage at the population level. Only with this view is the actual interaction of evolution (genotype) and physiology (phenotype) comprehensible.

In summary, the progression in CDP from past to future can be characterized as

| | | | | |
|---|---|---|---|---|
| Physiological → description | Physiological → mechanism | Genome → | Systems → synthesis | Evolution of characters |

What role does physiology play in the postgenomic steps in this progression? Consider the response of Sydney Brenner, Noble laureate (2002) and champion of genetic approaches using *Caenorhabditis elegans* as an experimental model, when asked to comment on systems biology. He replied ". . ..everybody's running around talking about systems biology and integrative biology. It's nothing new. It's called physiology." (20). Indeed, the integrative and synthetic nature of physiology is becoming increasingly apparent as we try to connect genes, proteins, processes, structures, and evolution.

One exciting new trend is the merger of contemporary physiology, genomics, and evolutionary-developmental biology into a previously unrecognized interdisciplinary zone of CDP. From this novel perspective we can better understand how physiological systems develop, how they respond to the environment, and how changes in these systems contribute to the fitness of the animal at each developmental stage.

## POSITIONING COMPARATIVE DEVELOPMENTAL PHYSIOLOGY WITHIN BIOLOGY

The disciplines of evolution, physiology, and developmental biology have all helped define the current state of CDP. Their intersection produces fertile interdisciplinary zones where integration is likely to be highly productive (Figure 2). Evolutionary and developmental biology, or "evo-devo," is of escalating significance in elucidating mechanisms linking evolution and development (21–23). Evolutionary physiology, another expanding interdisciplinary zone, is contributing to our understanding of how physiology evolves and how physiology enables and constrains evolution (24–26). The third zone, developmental physiology, helps us understand how structure-function linkages develop from embryonic to adult forms, as well as how physiology plays a permissive role in development. CDP, the nexus of these three zones, is an interdisciplinary crossroads. By explicitly integrating physiology, development, and evolution, CDP provides a unique outlook that enriches our understanding of evolution. This is especially true for

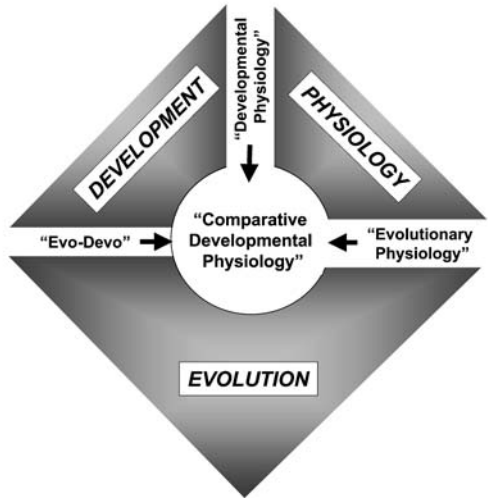


**Figure 2** The position of comparative developmental physiology vis-à-vis three major biological disciplines: evolution, physiology, and developmental biology. These disciplines share overlapping zones of integration comprising developmental physiology, evolutionary physiology, and evo-devo. The intersection of these three zones creates a biological crossroads where we are likely to achieve a considerably improved understanding of biology.



understanding the evolution of physiology, which has traditionally received relatively little attention from evolutionary biologists (see 27). Clearly, CDP is playing an increasingly important role in organismal biology. What are the current themes being explored within this burgeoning discipline?

## CURRENT THEMES IN COMPARATIVE DEVELOPMENTAL PHYSIOLOGY

Modern studies in CDP are an interdisciplinary fusion of traditional comparative physiology and developmental biology, embryology, and evolutionary biology. Contemporary areas of concentration in CDP are generally (and somewhat arbitrarily) divisible into

- determining how physiological systems apportion regulatory responsibility during their establishment in the developing animal;
- determining how developing physiological systems respond to changing environmental conditions;

- understanding how differences in physiological capacity of developing animals contribute to differences in fitness and, hence, in the evolution of animals; and
- understanding developmental constraints on evolution.

## Apportioning Regulatory Responsibility

The transformation of a single cell to a multicellular organism requires the transfer of physiological regulation from the cell to shared or autocratic "governance" involving an overarching regulatory system (79). Simply put, any system whose function depends on neural or hormonal integration is limited until the nervous system or appropriate endocrine system has developed functionality. The onset of the regulation of cardiac rhythm is among the most-studied system in this regard (for reviews see 10, 29–33). Early in development, regulation of contractile frequency resides in individual cardiomyocytes. However, as gap junctions begin to connect cardiomyocytes, pacemaker cells come to regulate the cardiac frequency as a whole. Still later, endocrine regulation becomes functional and can modulate pacemakers and then, finally, neural regulation assumes a dominant role. This changing of control modality from local or simple to more remote and/or more complex may also occur in other organ systems. In an amphibian, ventilatory regulation may initially depend upon a simple pacemaker, but later, complex oscillating circuits emerge to dominant the system (34). In the rat gut, modulation of enteric activity via interstitial cells of Cajal is not fully in place at birth and must mature during postnatal life (35). Is the pattern of development from single cell to cellular pacemaker networks indicated in these examples a hallmark of development in all complex animals, where networks cannot exist until there are sufficiently differentiated cells to create such a association? Are these patterns of regulation conserved throughout evolution? This is an important, but largely unexplored sub-genre of CDP.

## Response of Developing Systems to Environment

Molecular biologists have emphasized the primacy of genes in the function and development of the organism. Yet, the environment also plays an important role in phenotype determination. Evolutionary biologists use the term phenotypic plasticity for this ability of a single genotype to produce multiple phenotypes. Phenotypic plasticity can be adaptive, maladaptive, or neutral (36–38). The spectrum of phenotypes possible within a given genotype is the reaction norm. Environmental influence on phenotype can appear as both acclimatization and fetal programming. Acclimitization during development has the same definition as when used in reference to adult animals, that is, typically adaptive alteration in characteristics upon exposure to a new, natural environment (e.g., 11, 12, 40, 41). Importantly, acclimitization can reverse or animals can deacclimitize. However, developing animals have the additional capacity (or limitation) permanently to alter adult form and function when perturbing conditions occur during critical windows of

development. Examples include blood pressure in animals with reduced glomerular number (42), sound production in midshipman fish (43), olfaction in queenless honeybees (44), and the embryonic determination of glomerular number or coronary geometry (45). Such permanent alterations in the normal developmental trajectory, independent of genotype, are typically termed fetal programming in the medical literature (46, 47), but perhaps should be re-named ontogenetic programming to avoid taxonomic chauvinism (e.g., larva in addition to fetus). The history of studying developmental plasticity is long. Examples include alterations in isoforms of myosin heavy chain expression in carp (48); complex motor patterns in feeding in amphibians, which change during metamorphosis and with diet (49); vascular development during hypoxia in chick embryos, in which vascular lumen diameter and developed ventricular pressure decrease upon incubation in hypoxia (50); and gill surface and hematopoiesis in larval bullfrogs, which hypoxia enhances and hyperoxia inhibits (51, 52). These and numerous other examples have provided much information on developmental plasticity in response to environmental cues.

Regarding mechanisms of evolutionary change, we speculate that in some cases genetic mechanisms of sub-adult organisms have evolved to adjust their host's internal physiological state and so program an appropriate adult morph, and that environmental variation can sometimes override this programming. This suggests that alterations in climate, geography, salinity, etc. may have important effects on speciation. This area is in its infancy but holds exciting possibilities.

## Developmental Fitness and Evolutionary Consequences

Comparative physiology has a long-standing relationship with evolutionary biology, and vice versa (Figure 2). Understanding how specific physiological traits allow an organism, population, or species to survive and reproduce better has been a mainstay of comparative physiology almost since its inception. In general, the focus has been on adaptive traits of adult organisms, the rationale being that these are the reproductive individuals. Overlooked is that in many species, the majority of mortality (and thus selection) occurs long before reproductive capacity is realized. Dramatic examples of this abound in both vertebrates and invertebrates that exhibit "r selection," release of thousands to millions of offspring into an unpredictable environment, of which few survive (see 53 for examples and references). Consequently, selection may be stronger on immature forms (embryos, larvae, fetuses, or juveniles), than on adults (see 54), which is a largely neglected area of emphasis for physiological investigation. Potentially, adult forms are at the mercy of juvenile "bottlenecks" in physiological options. Changes in developmental trajectory early in ontogeny, while advantageous then, may yield permanent alterations that are disadvantageous to adults. Suddenly, the notion of tradeoffs emerges, and the importance of life history studies becomes obvious in integrating physiology and evolution. If so, the numerous offspring that some species produce may be an evolutionary necessity to produce those much smaller numbers of offspring that are fit as both juveniles and adults. Perhaps the the adult phenotype is so important that potentially adaptive responses early in ontogeny are "prohibited," and

thus sheer numbers are instead required to overcome nonadaptive or maladaptive stages. This connection between embryonic or larval requirements and adult requirements may be direct (i.e., fetal programming) or may be result of correlated selection or genetic correlation (55).

## Developmental Constraints on Evolution?

For many years evo-devo, the interdisciplinary zone between development and evolution, languished as a footnote in embryology texts. During the last decade, evo-devo has undergone a renaissance (56, 57). Yet, one aspect that has persisted from evo-devo's embryological roots is the extensive focus on morphological (but rarely physiological) traits to draw evolutionary conclusions (see 27 for earlier commentary on this phenomenon). Presumably, this arises from the relative ease with which morphological alterations are observable both through the fossil record and through experimental genetic manipulations. The consequence of this history of morphological observation is that physiology is rarely mentioned in dicussions of the future directions in evo-devo. Nielsen (58) echoes this morphocentric view in his endnote to *Animal Evolution: Interrelationships of the Living Phyla*, to wit, "Evolutionary developmental biology shows great potential for phylogenetic work, and I hope that there will be close collaboration between morphologists and the 'evo-devo' people." This overly restrictive view apparently is reinforced by the conventional hierarchal view of organismal plasticity, with behavior being most plastic (and thus least useful for evolutionary studies), physiology being less plastic but still inconveniently so, and anatomy being the least plastic, most stable and allegedly the most useful to study (27). Yet, highly inflexible physiological traits are numerous: For example, mammalian blood pressure and ventricular wall tension is predictable and almost invariant regardless of animal size (59), and respiratory frequency and heart rate are highly predictable based on animal size (60). Nonetheless, relatively few attempts have been made to use physiological characters, with evolutionary biologists arguing that physiological invariants (e.g., blood pressure) may be due to evolutionary constraints on anatomy, which are simply mirrored in the physiology of the systems. We would be the first to agree that anatomy puts limits on physiology. However, most physiologists would view the causal relationship between anatomy and physiology as far more tenuous than would anatomists, based on the common observations of large physiological differences enabled by almost undetectable gross anatomical changes. As an extreme example, consider the crab *Scopimera inflata*, where a reduction in the thickness of the chitin covering the meral segments of the walking legs turns these appendages from strictly locomotory organs into respiratory organs (61). Thus relatively trivial anatomical changes in structures can lead to profound reassignment of their physiological function (27, 62)!

The pattern of ontogenetic change in heart rate and metabolism during development in vertebrates is another example of a heritable physiological trait previously overlooked because of the assumption that physiological variation equals physiological plasticity. These studies, which have included birds (63), mammals

(64), and amphibians (65), have revealed that complex changes in heart rate and metabolism during development are highly correlated between sibling groups sharing a common genetic heritage.These findings suggest that even subtle, apparently random, physiological variations may be genetically predetermined.

Given the nongenetic input to developmental programs and the identifiable genetic components of physiology variables, CDP provides an invaluable vantage point from which to examine limitations on evolutionary processes. The study of developmental limitations or constraints on evolution gives us considerable insight into subjects as diverse as life history cycles, heterochrony, and heterokairy. Developmental constraints have been postulated to account for unique traits such as the foramen of Pannizzi in crocodilian reptiles (66). Altering cardiac hemodynamics alters cardiovascular structure (67, 68), and altering cardiovascular structure may alter hemodynamics (28, 68, 69) in the adult. Transgenic or mutant animals certainly provide useful information regarding gross malformations, but the diversity of natural species provides "evolutionary feasibility studies" (27). Studies of the earliest chordate cardiovascular systems or the metabolism of the smallest, fastest living mammals, bats and shrews, provide models of extreme organisms. Investigation of their developmental pathways may reveal constraints on evolutionary options, based not on anatomy or phylogenetic constraints, but on physiological limitations.

The obvious importance to animal survival of physiological function during development should be sufficient to promote the inclusion of physiological characteristics in evolutionary analyses. This may be especially true of comparative studies given the multitude of species available and the relative ease with which developing systems can be perturbed physiologically. Hopefully, as more genetic underpinnings of physiology are elucidated, physiology will be become a full partner in the evo-devo paradigm.



## Selected Experimental Approaches and Methodologies of Comparative Developmental Physiology

The field of physiology provides a key point of continuity and connection between cellular/molecular and ecological/evolutionary organization levels. As discussed earlier, CDP occupies a clear crossroads linking evolutionary biology, physiology, and developmental biology. Not surprisingly, the experimental approaches and methodologies employed by comparative developmental physiologists encompass most of contemporary biology. However, here we highlight a few such approaches, and emphasize how the integration of common approaches from different fields can yield distinctive new insights.

### Scaling and Development

For more than 50 years comparative physiologists have argued about the significance of scaling parameters that relate physiological functions such as heart rate or metabolic rate to anatomical features such as body weight or surface area.

Ironically, whereas most agree that body size and metabolism display a highly conserved relationship, there is less agreement as to what constitutes the specific nature of that relationship (70, 71). There are highly conserved scaling relationships of individual organ systems across phylogenetic groups as well (59, 72). Across phylogenetic groups, adult organisms conform to well-known and rigid scaling laws, in which adults of a particular body size have a predictable heart rate, metabolic rate, etc. The numerical parameters describing these systems have generated intense interest both for their evolutionary implications of optimality but also as a means to explore the underlying biological mechanisms (73, 74). Moreover, the study of these mechanisms and pathways may contribute to the explanation of the highly conserved scaling relationships. Thus, for example, whereas it may be advantageous to devote a constant percentage of body weight to heart size, we know little of the developmental mechanisms or signaling pathways that result in this conformity. These contentious issues are largely unexplored in the field of physical signals and transduction mechanisms. It may well be that identification of the complex network of developmental cues and cellular responses will answer the questions of scaling parameters with more accuracy than has assessing end results in adult animals. Importantly, advances in this field will reveal which physiological variables are linked and which are independent, if indeed any are. If parameters are invariant, is there a genetic basis (or a genetic constraint or other type of constraint)?

## Event Sequences in Physiological Development

One major focus in CDP is the determination of the order of developmental events and whether that order is genetically fixed or plastic (see 9 for examples). That the sequence of key events during development can vary (i.e., heterochrony) is, of course, an old notion (e.g., 75–78). An example of physiological heterochrony is in the differences among vertebrate species in the relative timing and sequence of onset of vagal cardiac control, chemoreflexes, and baroreflexes (29). However, recent papers have argued for the application of heterochrony only to the changes over evolutionary time between species. This argument reserves the term heterokairy for the naturally occurring and experimentally inducible changes in the onset and timing of events within a population between individuals during a single life span (9, 79). For example, the adult metabolic response to hypoxia in the brine shrimp *Artemia* typically occurs simultaneously with segmentation when animals are reared in air-saturated seawater (80). Yet, when reared under conditions of chronic hypoxia, heterokairy is evident because the onset of respiratory regulation in this crustacean now occurs earlier in a sequence of developmental events, i.e., before segmentation.

Even as heterochrony provides insights into physiological evolution, the new conceptual framework of heterokairy provides insights into physiological phenotypic plasticity. Concomitant with these new insights is the requirement for new ways of thinking about development timing and rates, especially when body temperature is a potential variable during development (81).

It is ironic that, despite our earlier protestations about the morphocentric view of evolution, staging in physiological studies is still carried out using anatomically based staging schemes (e.g., Hamilton-Hamburger for chick embryos, Nieuwkoop and Faber for *Xenopus*). Future studies would do well to determine how morphological and physiological plasticity map onto each other during the development of a single animal. That is, are the developmental critical windows of the same width and position for both physiological and anatomical events? Must one consider separate physiological and anatomical heterochronies? If so, what are the ecological and evolutionary implications?



## Using Animal Models: Establishing Universal Mechanisms and/or Learning from Diversity

The use of model organisms, historically a mainstay of physiology, has never been more essential to making both pragmatic and conceptual advances in physiology (e.g., 82–86). Numerous discoveries have resulted from focused, persistent investigation of the fruit fly (*Drosophila*), the zebrafish (*Danio rerio*), the chicken (*Gallus gallus*), the nematode worm (*Caenorhabditis elegans*), the mouse (*Mus musculus*), or plants such as *Arabidopsis*. In some instances, however, model organisms have emerged simply because as a species they were the first to be investigated in a particular context—and not because they were best-suited for such investigation. As more and more information was collected, they became wonderful models simply because so much was known about them— a form of self-fulfilling prophecy (87).

When the level of examination is at the molecular or cellular level, cells are cells, and thus the lessons learned from animal models are typically broadly applicable (e.g., the role of Hox genes or the influence of fate mapping on structure/function relationships). However, as molecular and cellular biologists begin to ask broader questions of physiology, ecology, and evolution, some investigators remained focused on the model organisms with which they are familiar. Such models may not be truly representative of a larger taxon, nor represent the full extent of organismal diversity needed to understand ecological and evolutionary relationships (87). Moreover, study of model organisms needs to be informed by an understanding of the conditions in which these species develop in nature. An example of the caution that needs to be exterted when studying model organisms is the use of *C. elegans* and its many relevant mutants to study adaptation and acclimation to hypoxia-induced metabolic-suspended animation. While exciting genomic and proteomic information on hypoxic adaptation is emerging (e.g., 88, 89), often unappreciated is that *C. elegans* evolved in a highly hypoxic soil environment (one also rich in nitric oxide), and that cultures maintained under control laboratory conditions might be more appropriately viewed as continual hyperoxic exposure of this species. Another example is the zebrafish. Many researchers' knowledge of the ecology, life history, and evolution of this important model is summed by the opening sentence of Westerfield's (90) in his widely distributed *The Zebrafish*

*Book*, "Zebrafish are available at pet stores throughout the world." In an action that flouts their natural thermal evolutionary history, these fish are typically bred and reared at 28.5°C, even though this industry-standard rearing temperature is much closer to the upper rather than lower lethal limit for this fish. Indeed, zebrafish prove more fecund at 25°C, more toward the middle of their thermal range (91).

Both deeply understood model organisms and less well understood but diverse animals deserve study. Consider the study of the ontogeny of cardiovascular regulation in bird embryos. Because bird embryos developed in a self-contained egg, they have long been favored animals for investigating how vertebrate cardiovascular physiological regulation unfolds during development. In this regard, the embryos of the chicken *Gallus gallus* have been the basis for seemingly well-established conclusions. Yet, our recent comparisons of more exotic avian species (such as the emu *Dromiceius novaehollandiae)* with the chick embryo reveal profound differences in the developmental patterns of cardiovascular control between the two species. For example, in developmental patterns reflective of physiological heterochrony, the cardiac vagal tone, chemoreflexive cardiovascular control, and baroreflexes all develop much later in the emu than in the chicken (29). Moreover, these physiological landmarks in emu (chemoreflexes, baroreflexes vagal tone) appear in the exact opposite order in the chicken (vagal tone, baroreflexes, chemoreflexes). Of course, this is only a two-species approach not supported as yet by a more rigorous, cladistic approach, and the question remains as to which of these two species is the more representative of birds (if there is indeed a representative bird). Yet, such data do question the generality of the extensive physiological data available for the chicken but few other birds. Thus, ironically, we investigate an exotic species to calibrate and learn more about a model species! Indeed, the focus on animal models probably slowed our understanding of physiological evolution. We advocate a systematic investigation of other fishes, nematodes, etc. patterned after studies on model organisms, to learn how many of the physiological findings for model animals such as the zebrafish or *C. elegans* are generalizable. Given the importance of Genbank in elucidating phylogenies of genes, consider the impact that could result from a corresponding database of physiological variables from a variety of animals.

Finally, the choice of species for CDP studies depends in part on how early one chooses to look in the overall development process. The earlier the point of investigation, the greater is the interspecific similarity in emerging physiological properties, and the more useful is any given organism as a general physiological model (16). For example, blood pressure, blood flow, and peripheral resistance during the first few days of convective blood flow are similar in the embryos of *Gallus gallus* (92–94), *Xenopus* (95–98), and *Danio rerio* (99, 100). Thus any of these models, when examined early in development, might be equally useful in determining how vertebrate circulations begin their function. Of course, the late bird embryo with a four-chambered heart might tell us little about the larval fish with two-chambered heart. At what time during ontogeny a species ceases to serve as a

general developmental model depends upon the system being investigated and the questions being answered, but clearly a heavily comparative approach is effective and warranted at least in understanding early developmental stages of vertebrates.

## Technologies for Investigating Comparative Developmental Physiology

Investigations in CDP now span molecules to populations, and, not surprisingly, experimental tools are drawn from all levels. Our intent here is to highlight only a few of the many approaches that have been particularly useful in CDP and to then direct the reader to additional sources on these topics.



MINIATURIZATION Immature animals are relatively small and embryos sometimes microscopic. Consequently, the relentless drive toward electro-mechanical miniaturization has been a boon to CDP. In some cases, rather astonishing miniaturization of conventional technologies for blood pressure, flow, pH, blood gases, etc. have occurred, allowing unprecedented measurements and insights into physiological function in early development (see 87, 101–103). Perhaps one of the most graphic examples is that of micropressure systems. Using a glass microelectrode with a 2–5 $\mu$m diameter tip inserted into a vessel or cardiac chamber, high-frequency response pressure measurements can be made in embryos weighing only milligrams (96, 98–101). Microelectrodes that measure gases and ions have, of course, been available for some time (104, 105).

The emergence of nanotechnology is likely to provide additional experimental tools with unimagined possibilities for CDP. As just one example, "smart dust" is being developed to provide detailed three-dimensional environmental assessments. In this emerging technology, microscopic silicon-based sensors made up of such dust are sprinkled over an environment. The particles are then interrogated with a laser beam (e.g., from an overflying aircraft), and the reflected beam is modified in way that encodes information on variables such as pressure, humidity, temperature, or oxygen levels (106, 107). One can imagine in the near future being able to inject nanotechnology-derived microscopic sensors into near-transparent embryos, and then, using laser interrogation, derive a three-dimensional assessment of internal physico-chemical variables of physiological interest.

IMAGING Embryos are not only microscopic, but they are often translucent or transparent. Thus there has also been an explosion of approaches using nonintrusive optically based techniques for measurement of physiological variables, techniques collectively termed optophysiology (108). Cardiac output, blood oxygenation, blood flow distribution, and other physiological variables are now commonly measured through such optical techniques (see 101, 103, 108–111). After introduction of various dyes or indicators or even using substances intrinsic to muscle, physiologists now optically derive localized tissue $PO_2$ (112) and track muscle cell excitation and contraction (13, 113). By keying in on the profound

spectral shifts in hemoglobin as it changes oxygenation state, in vivo changes in blood oxygen transport can be determined in real time (108, 111). The advent of multiphoton confocal microscopy (also called nonlinear microscopy) allows in vivo imaging with greater penetration then conventional laser confocal microscopy and with less radical by-product production (114, 115).

GENETIC ENGINEERING Genetic knockouts in zebrafish, mice, *C. elegans*, and other model species are being widely exploited to gain insight into the assembly of fully functional physiological systems (e.g., 84, 116–122). Indeed, the utility of such approaches has led some to argue that screening studies to "see what is out there" should replace hypothesis-driven research (45)! Yet, the limited number of knockout models available in non-model animals makes it difficult to use a comparative approach to probe the complexity of evolutionary constraints and possibilities. Thus expansions of knockouts beyond the conventional models are to be encouraged for the promise they hold.

One area where CDP can contribute greatly to developmentally directed genomic studies with mutants or knockouts is to expand the scope of some of these studies beyond an analytical approach that seems drawn from traditional toxicology: Do knockout animals die or survive? A more sophisticated question, yielding a more illuminating answer, might be, How well do they survive? or even, What did they die from? Incorporating the techniques and approaches of CDP, namely, quantifying physiological performance and ultimately fitness, into genomic/proteomic studies should prove extremely useful in documenting and understanding the myriads of important but nonlethal effects induced by genetic engineering.

## Unanswered Questions in Comparative Developmental Physiology

CDP appears bound for increasing emphasis and significance in evolutionary biology. The specific future of CDP is difficult to predict, but by virtue of this field's position at a biological crossroads (Figure 2), it seems certain to involve enhanced collaboration between physiologists, evolutionists, and developmental biologists. These collaborations will allow us to address key questions (and practical implications), such as

- Do genes or environments make species? (e.g., how much of the variation in phenotype is genetic and how much is environmentally induced?)
- How straightforward is physiological evolution? (e.g., does the evolution from species A to B require more anatomical changes or more physiological changes?)
- Are current, popular animal models most appropriate for advancing developmental physiology? (e.g., should we focus on any one model, or is it important to maintain and explore diversity?)

- Are the basic tenets of developmental physiology overarching across all or most taxa? (e.g., will collaborations between animal and plant biologists provide useful insights to either?)
- What is the role of developmental programming in the ultimate phenotype? (e.g., what are the critical physiological windows, and are they moveable by adaptation or acclimation?)
- How fixed in development are traditional ontogenetic events? (e.g., are developmental landmarks locked in place, or have we just not tried to move them?)
- How interdependent are physiological systems during development? (e.g., when do physiological systems begin to interact and influence each other during the course of development?)
- Does a physiological system have the same function throughout development? (e.g., are there major changes in responsibility of physiological systems as the animal matures and potentially even changes environment?)
- What are the origins of scaling constants? (e.g., how do size and immaturity interrelate in a developmental context?)
- How important is the study of the complete developmental continuum? (e.g., is the aging process a natural extension of development, and can we learn about evolution from its study?)

Transitions in CDP and the fields from which it is formed (Figure 2) will continue. Driven by new forms of collaboration, conceptual advancements both within and outside the field, and by improvements in technology, perhaps the single safe prediction is that additional insights into the critical physiological underpinnings of evolutionary processes will only accelerate during the decade to come.



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