Hormone signaling in evolution and development: a non-model system approach

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Summary

Cooption and modularity are informative concepts in evolutionary developmental biology. Genes function within complex networks that act as modules in development. These modules can then be coopted in various functional and evolutionary contexts. Hormonal signaling, the main focus of this review, has a modular character. By regulating the activities of genes, proteins and other cellular molecules, a hormonal signal can have major effects on physiological and ontogenetic processes within and across tissues over a wide spatial and temporal scale. Because of this property, we argue that hormones are frequently involved in the coordination of life history transitions (LHTs) and their evolution (LHE). Finally, we promote the usefulness of a comparative, non-model system approach towards understanding how hormones function and guide development and evolution, highlighting thyroid hormone function in echinoids as an example. *BioEssays* 27:64–75, 2005.

Introduction

In the ontogenetic transformation from a single-celled zygote to a multicellular, reproductive adult, a tremendous number of complex processes need to be accurately timed and coordinated with one another and the environment. The result is that subsequent life cycle stages are successfully reached in the right condition and at the right place and time. In the majority of animal taxa, such ontogenetic transformations are characterized by a metamorphic life history (see Box 1, Appendix 1). Metamorphosis has evolved only a few times among terrestrial taxa, but many times independently among marine invertebrates.[1,2] One uniting feature of diverse metamorphic life histories across all habitats is their control and coordination by hormones.[3–5] Indeed, hormones regulate and coordinate metamorphic and non-metamorphic life history transitions (LHTs) in animals and non-animals alike. Thus, hormones have been independently coopted in a multitude of diverse organisms as regulators of their LHTs.

With a few notable exceptions [e.g. Bonner,[6] Callery and Elinson,[7] Hanken,[8] Raff,[9] Wray[10]], the concept of life history has been overlooked by most contemporary developmental biologists. This is especially surprising since the holometabolous insects (including *Drosophila melanogaster*) and the amphibians (including *Xenopus laevis*) are well-known as having complex life histories, with distinct larval and adult stages separated by a drastic metamorphosis (Box 1). Marine invertebrates, which include 28 of the approximately 32 animal phyla, are also characterized by diverse and often complex life histories.[1,11] By contrast, nematodes, mice, leeches and zebrafish all lack a drastic metamorphosis. While such non-metamorphic or abbreviated life histories may be preferable for developing laboratory-based ''model systems'', the high degrees of canalization inherent in many such model systems may make results obtained less generalizable than is often assumed.[12] In this way, the intensively studied model systems cannot stand as a proxy for the vast diversity of life histories found throughout the animal and other kingdoms.

In this paper, we propose that the modular nature of hormonal signaling systems predisposes them for their seemingly ubiquitous involvement in organismal LHTs, as well as their cooption in novel developmental pathways. Furthermore, we argue that the evolution of derived life history patterns involves alterations in the coordination of these very same hormonal signaling systems. We stress the indispensability of a comparative, non-model system approach to understanding organismal life histories, and offer our studies on echinoderm metamorphosis as an example. Finally, we introduce a hypothesis that the ancestral source of hormones in many cases was external, in contrast to the general view that hormones are endogenously produced substances.

Cooption and modularity in a comparative context: the emergence of a new EvoDevo paradigm

There has been something of a sea change in the past five years with respect to our general understanding of how development evolves. In the 1990s, comparative molecular and

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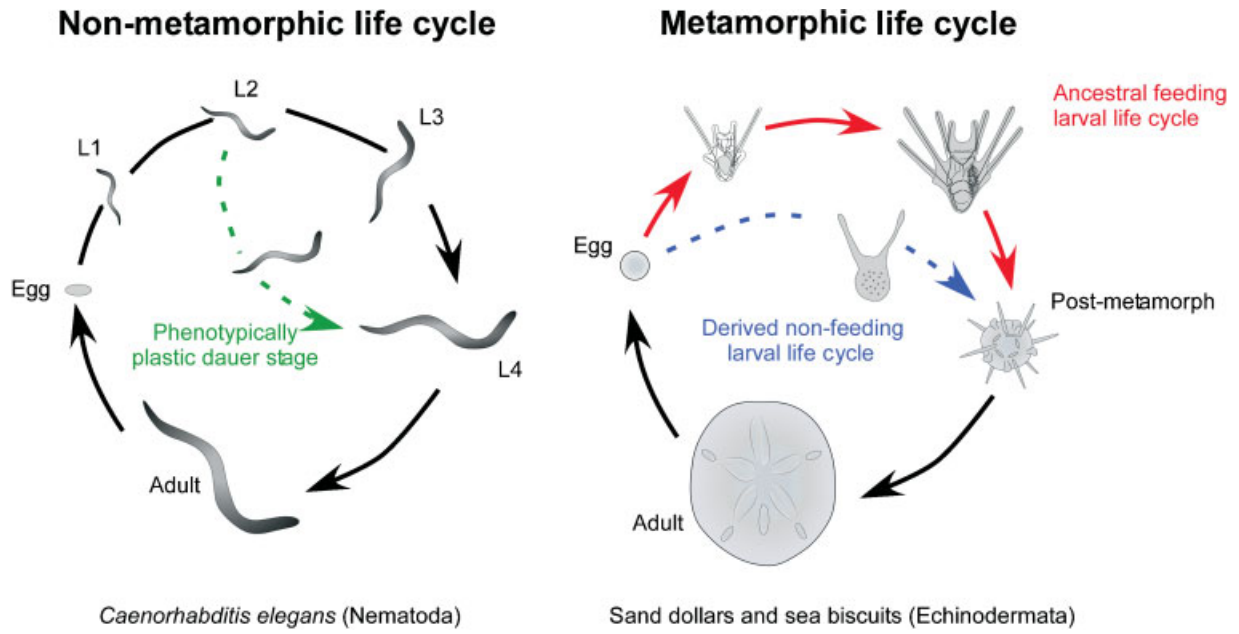
Box 1: Defining metamorphosis

Metamorphosis is an inherently integrative concept, with researchers working in different biological disciplines and with a diversity of taxa, having very different ideas of what is and is not a metamorphosis (e.g. Refs. 2,22,33,50). For the purpose of this review, we define metamorphosis as a period of irreversible, dramatic ontogenetic change from a multicellular, free-living, post-embryonic stage ("larva" in animals) to a multicellular, pre-reproductive adult ("juvenile" in animals). This ontogenetic reorganization, which can take days to months, involves major morphogenetic remodeling with associated cellular and molecular events. Wray[1] provides a list of animal phyla with at least some members that have a metamorphic life history.

In marine invertebrates that undergo a planktonic-to-benthic transition from the larval to the adult form, metamorphosis is also associated with settlement: the change in habitat itself. Thus, settlement is the rapid phase of metamorphosis, occurring in seconds to minutes, which employs neurophysiological rather than transcriptional control mechanisms.[2] "Competence", then, is the stage at which the planktonic form can undergo settlement in response to specific settlement cues.

These definitions of metamorphosis and settlement have implications for various topics in life history evolution in animals and other taxa. Here we list some of the main points, and provide a more detailed discussion in the Appendix. 1) The timing of settlement relative to metamorphosis varies within and among taxa. Metamorphosis does not always precede settlement. 2) Metamorphosis in the derived holometabolous insect clade fits our definition. Eclosion is the insect equivalent of settlement in marine invertebrates. 3) Life history transitions in fish do not always fit our definition of metamorphosis, though the more profound transitions in some groups, such as flounders, do qualify as bona fide metamorphoses. 4) Alternation of generations in plants and many marine algae taxa are not examples of metamorphosis, though many algae undergo a settlement-like process following the dispersive zygote stage.

Our definition of metamorphosis is applicable to certain life stage transitions in non-animal multicellular eukaryotes, such as the mycelium-to-fruiting body transition in some fungi and the crustose-to-thallus transition in some red algae.[83] To our knowledge, no one has ever attempted to determine if hormones similarly regulate these metamorphic transitions in fungi and algae, although a hormonal basis for other ontogenetic processes has been established for fungi.[84,85]



developmental data, predominantly from a fly, a rodent, a roundworm, a fish and a mustard, poured onto the pages of major journals and into the public sequence databases. The preponderance of raw data drove a rapid expansion in the nascent field of evolutionary developmental biology (EvoDevo), and this rapid growth was not without its growing pains. Publications purporting to synthesize data across these disparate animal and plant phyla tended to focus on their similar uses of developmental machinery, generally concluding that such similarities were evidence of shared ancestry (plesiomorphy). However, inherent in this conclusion of shared ancestry is a subtle, self-justification for the model system approach. If arthropods, nematodes and mammals all use the same developmental machinery for the same processes, then any tractable model system would provide the ability to draw conclusions regarding, for example, the nature of a given human disease. While model systems have often proven useful in this regard, the similarities among disparate animals have been frequently overemphasized.[12]

In the closing years of the 20th century, a significantly altered paradigm began to emerge concerning two powerful concepts: cooption and modularity. Neither of these ideas was particularly new,[13,14] but their application to the comparative data sets described above has had a discernible impact upon EvoDevo. A module is an integrated, relatively autonomous subprocesses within a larger process.[15] In this context, we consider hormonal signaling networks to be examples of signaling modules, where the signaling network is the subprocess within the larger ontogenetic process. Such modules have two important features: (1) the networks appear to be robust in the face of perturbation,[16] and (2) given signaling networks show up repeatedly in diverse developmental and evolutionary contexts (reviewed in 17). This second finding, in particular, indicates that the concepts of cooption and modularity are connected, and together provide an account for the aforementioned cross-phylum similarities that so energized the EvoDevo field in the 1990s. More importantly, perhaps, modularity and cooption offer a framework for understanding the nature of diversity: the hallmark of evolutionary change.

Thus, in recent years, the emerging picture of how development evolves has become decidedly more complex. Definitive conclusions concerning, for example, whether or not the ancestor of flies and mice was a segmented creature with a brain, heart, eyes and limbs, have come to seem a bit more elusive. Comparative biologists (e.g. Refs. 18–27) advocate a different approach: by investigating the mechanisms underlying morphological or other differences among more restricted taxonomic groups, one can begin to understand the details of the developmental mechanisms underlying evolution. Such issues are tractable, and may ultimately provide realistic hypotheses regarding larger-scale evolutionary events.

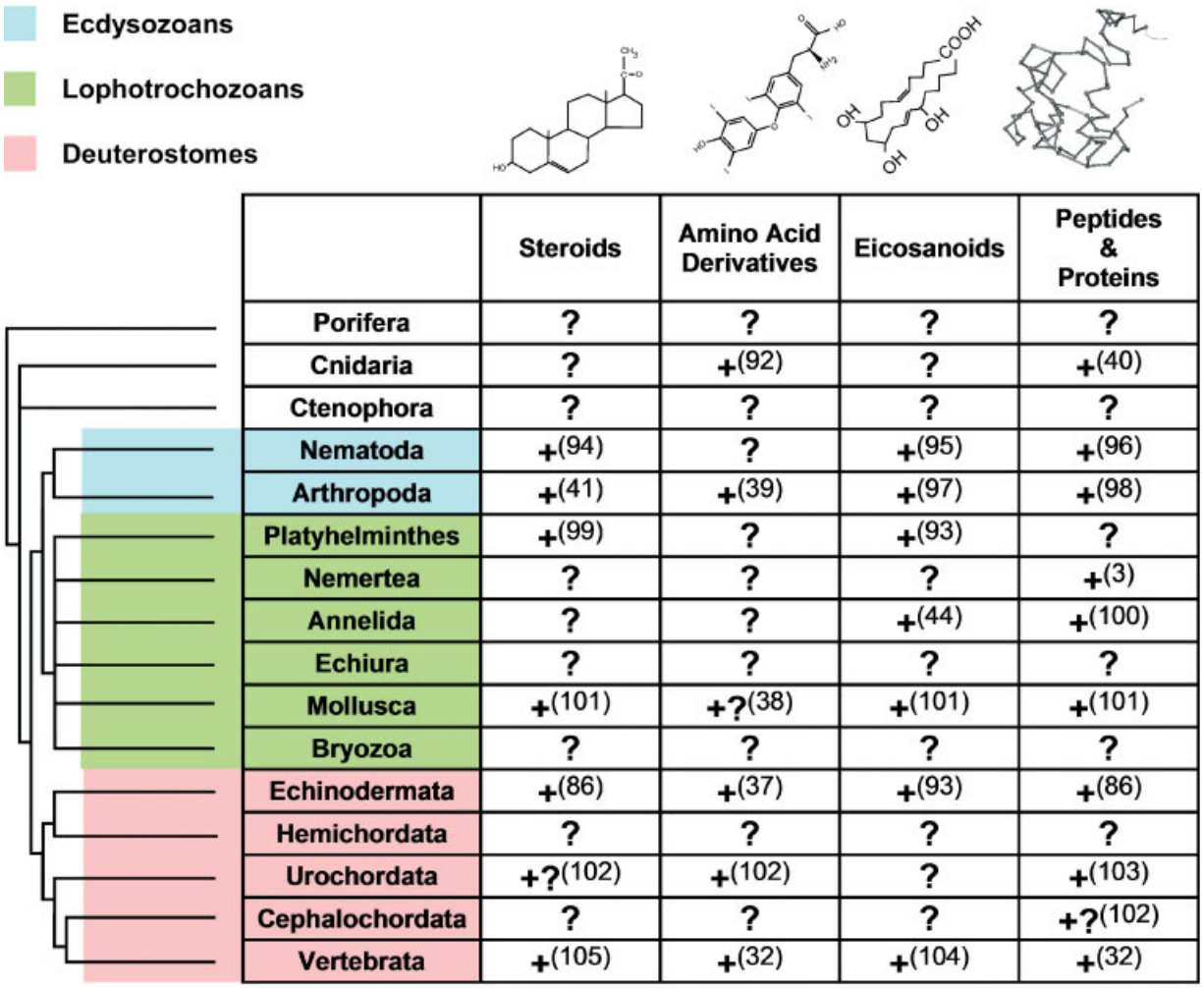
Does this approach invalidate the use of model systems? Not at all! The intensively studied model systems are crucial for identifying developmental modules and for developing techniques to study gene function.[24] But model systems have a critical limitation: alone, they do not allow for evolutionary conclusions. Thus, we strongly advocate a comparative, non-model system approach to the study of organismal development in general, and their life histories in particular. Evolutionary patterns in the hormonal regulation of ancestral and derived LHTs across kingdoms makes them an ideal case study for this emerging EvoDevo paradigm of modularity and cooption in a comparative context.

Hormonal signaling networks: modules coopted for LHTs

Hormones control and coordinate complex physiological and developmental processes in plants, animals and fungi, such as growth, differentiation reproduction and homeostasis. In plants, a diversity of hormones regulate signaling systems, developmental processes and life history transitions. Examples include the role of ABA (abscisic acid) in seed dormancy and the induction of flowering by sucrose and cytokinins (reviewed in 25). In animals, hormones either signal through cell surface receptors or intracellular nuclear receptors (NRs, reviewed in Refs. 26,27). NRs are unique in that they act as transcriptional regulators that coordinate intra- and extracellular signals. This property allows them to act as nodes in the complex regulatory networks that play crucial roles in development and homeostasis.[28] Genes that act within such hormonal signaling networks can influence multiple developmental and cellular processes, and have been shown to underlie trade-offs and other correlations in life history parameters (e.g.[5,29–31]).

Such diverse ontogenetic usages of hormones, as well as their central position in a wide variety of cellular signaling systems, are consistent with the view of hormonal signaling networks as modules, coopted in different lineages for evolutionarily independent cases of LHTs. The central role of hormones in organismal life cycles was most clearly advocated by Matsuda,[3] who presented an immense and invaluable synthesis of comparative data concerning the function and occurrence of hormones with respect to animal LHTs. In Fig. 1, we provide an updated version of Matsuda's 1987 data set, emphasizing the role of hormones as regulatory modules in a broad array of animal LHTs. Below, we highlight specific examples that provide strong evidence for the independent cooption of hormonal signaling in LHTs in various animal taxa.

Thyroid hormones (TH) regulate a variety of functions such as growth, development and metabolism. In addition, increasing levels of the thyroid hormones (TH) T3 and T4 promote metamorphosis in amphibians, via binding to nuclear hormone receptors (reviewed in Ref. 32). In contrast, jawless



	Steroids	Amino Acid Derivatives	Eicosanoids	Peptides & Proteins
Porifera	?	?	?	?
Cnidaria	?	+(92)	?	+(40)
Ctenophora	?	?	?	?
Nematoda	+(94)	?	+(95)	+(96)
Arthropoda	+(41)	+(39)	+(97)	+(98)
Platyhelminthes	+(99)	?	+(93)	?
Nemertea	?	?	?	+(3)
Annelida	?	?	+(44)	+(100)
Echiura	?	?	?	?
Mollusca	+(101)	+?(38)	+(101)	+(101)
Bryozoa	?	?	?	?
Echinodermata	+(86)	+(37)	+(93)	+(86)
Hemichordata	?	?	?	?
Urochordata	+?(102)	+(102)	?	+(103)
Cephalochordata	?	?	?	+?(102)
Vertebrata	+(105)	+(32)	+(104)	+(32)

Figure 1. Wide-spread involvement of hormones in life history transitions (LHTs). "+" indicates that this class of hormones has been demonstrated to be involved in a LHT [hatching, larval transitions, resting stages (such as dauer and diapause), metamorphosis, settlement, migration, attainment of reproductive maturity and ovulation/oviposition]. "?" indicates that such a role has not been demonstrated for that phylum. "+?" indicates preliminary evidence for such a role. Note that the four large hormone families considered here do not encompass all animal hormones involved in LHTs; certain hormones were omitted for simplicity. Also, hormones belonging to the same family can be synthesized by vastly different biochemical pathways. Furthermore, the original sources of animal hormones might have been plants/algae in many cases, and could well have been independently derived from plants/algae in distinct lineages. Finally, different taxa use similar hormones to regulate totally different LHTs (such as steroids in molluscan reproduction and arthropod molting) and, in some cases, the same hormone has been coopted multiple times to regulate independently evolved metamorphoses (TH is the prime example; see the text). For these reasons, the reader should not interpret the presence of a similar class of hormones in related animal taxa to be sufficient evidence for common ancestry of particular LHT functions and their hormonal control. (data for phylogeny from Ref. 91; numbers in table refer to references).

fish (lampreys) require a critical time period during which TH is absent in order to undergo the metamorphic transition[33] (see also Appendix 1). In light of these opposite roles, as well as the substantial evolutionary distance between amphibians and lampreys, this finding provides strong evidence for an independent acquisition of TH control of metamorphosis in these two taxa (and more transitions are probable within chordates; see below). Indeed, recent data from two other deuterostome phyla suggests additional instances of independent co-option of TH function in metamorphosis: echinoderms (see below) and sea squirts (Urochordata). TH synthesis inhibitors block post-settlement juvenile morphogenesis in the sea squirt *Boltenia villosa*,[34] and thyroxine has been localized and measured in larvae of the sea squirt *Ciona intestinalis*.[35] Also, a putative nuclear receptor for T3 in urochordates has been identified in vivo, which has a similar

affinity (Kd) to those found in other chordates, but with low maximal binding capacity.[36] Still, chemically similar ligands, such as insect and vertebrate steroids, are known bind distantly related NRs. This latter finding not only emphasizes the role of NRs as true evolutionary modules that were recruited for a broad spectrum of biological functions, but also should cause us to pause, for example, in making *a priori* assumptions concerning the exact nature of any invertebrate TH receptor.

TH-like function has also been reported from other invertebrate taxa (reviewed in Ref. 37), including a possible role in abalone metamorphosis,[38] as well as interactions with the juvenile hormone (JH) signaling system in insects.[39] Given the function of TH across the bilaterians, the question of how ''deep'' this particular hormone's function goes is an interesting one from an evolutionary perspective. In cnidarians, a basal animal phylum that includes sea anemones, jellyfish and hydroids, TH has been reported to influence the transition from benthic polyp to pelagic ephyra in the jellyfish *Aurelia*. This jellyfish can apparently synthesize thyroid hormone precursors (mono- and dityrosine: T1 and T2), although not thyroid hormone *sensu strictu* (T3 and T4). But experiments with various cnidarians have failed to support a role for hormones in metamorphosis from the planula to the polyp stage, though a settlement-inducing neuropeptide in the hydroid *Hydractinia* has been identified from neurosensory cells at the anterior pole of competent larvae.[40]

Thyroid hormones are, of course, not the only hormones involved in animal LHTs. Two classes of hormones are major regulators of developmental transitions in insects: the sesquiterpene juvenile hormones (JH) and the ecdysteroids. Their roles in diverse insect taxa indicate multiple cooptions for the regulation of various LHTs. In the completely metamorphosing holometabolous insects, such as bees, beetles and butterflies, ecdysteroids are specialized beyond their prototypical role in molting as coordinators of the metamorphic transition itself. Furthermore, these same hormones regulate reproductive maturation in insects (reviewed in Ref. 41). Still, there is good evidence for independent cooption of JH and ecdysteroids for unique roles in various insect taxa. For example, while JH promotes the synthesis and oocyte uptake of yolk proteins, or vitellogenesis, in many insect taxa, JH represses vitellogenesis in others, such as in the sweet potato weevil *Cylas formicarius*,[42] (reviewed in Ref. 43). The roles of ecdysteroids in insect reproduction are more variable still (reviewed in 43). Furthermore, in social insects, such as ants and honeybees, JH has been coopted for regulating caste determination, and its reproductive roles have been reduced or lost (reviewed in Ref. 41). In sum, the comparative data from insects demonstrates multiple cooptions of JH and ecdysteroids for taxon-specific LHTs.

A unique class of cooption of hormonal mechanisms is found in some cestodes (Platyhelminthes) as well as a diversity of arthropod parasites, which utilize the hormones of their host to regulate their own LHTs (reviewed in Refs. 3,41; discussed further below). Another derived, hormonally regulated life history transition is epitoky, a benthic-to-pelagic transformation that occurs during sexual maturation in some marine annelids. Careful experimental manipulations done by Durchon and later Hauenschild revealed that an unidentified head hormonal factor is involved in this unique metamorphic process (reviewed in Ref. 44).

These examples support the hypothesis of Matsuda and others (see reviews in Refs. 45 and 46) that hormones are critical regulators of LHTs in disparate taxa, and are modular in that these hormonal signaling systems are coopted in disparate lineages as regulators of independently evolved LHTs. The best way to test this hypothesis directly is to investigate hormonal signaling in derived LHTs within restricted taxonomic groups in a phylogenetic context.

Hormones and life history evolution (LHE)

Matsuda[3] hypothesized that hormones are also major players in LHE in a broad array of animal groups. This proposal seems eminently sensible: evolutionary alterations in organismal life histories must involve a radical reorganization of the mechanisms underlying the LHTs themselves (see also Ref. 5). As we have reviewed above, hormones coordinate and orchestrate LHTs in a wide variety of taxa; therefore, LHE likely involves modifications in the production, regulation and/or tissue-specific response of these very same hormones. Comparative studies have uncovered examples of each of these mechanisms of LHE, and we will review several such cases here.

Evolutionary alterations in organismal life histories have been profitably described in terms of heterochronies: changes in the relative timing of developmental events.[47,48] For example, neoteny, which has evolved multiple times independently in various salamanders from metamorphic ancestors, can be described in terms of alterations in the relative timing of sexual and somatic differentiation.[48] Specifically, metamorphosis is blocked, and sexual maturation proceeds within an otherwise larval morphology. This metamorphic block has different physiological causes in different taxa (reviewed in Ref. 49). In one case (e.g. the tiger salamander *Ambystoma tigrinum*), a hypothalamic neurohormone in the thyroid axis is non-functional, in another (e.g. the axolotl *A. mexicanum*) TH is not produced, while a third neotenic taxon (the mudpuppy *Necturus maculosus*) has apparently lost a functional TH receptor (TR). These examples clearly support the notion that LHE, of necessity, involves modifications in hormonal signaling, albeit distinct (i.e. convergent) modifications in different taxa.[4]

The second major heterochrony underlying LHE in amphibians is the evolution of direct development, where metamorphosis is skipped (but see Ref. 50), and little froglets hatch

directly from their egg masses. In this case, the heterochrony involves early activation of adult development within the embryo. This precocious adult development is correlated with precocious TH synthesis as well as early upregulation of TRs in the Puerto Rican tree frog *Eleutherodactylus coqui*.[8,50,51]

Truman and Riddiford[52] provide recent support for Burlese's 1913 hypothesis on the evolution of metamorphosis in the derived holometabolous insect clade. This hypothesis links three life history stages of the non-metamorphosing hemimetabolous insects, pro-nymph, nymph and adult, to three holometabolous life history stages, namely larva, pupa and adult. Truman and Riddiford provide a scenario for such an evolutionary transition via major heterochronic shifts in metamorphic hormones and the tissue-specific responses to these hormones via their cellular receptors. Recent evidence suggests that the Broad Complex (BR-C) of zinc finger proteins may have occupied a central position in this evolutionary scenario. These proteins, which are direct transcriptional targets of ecdysteroid receptor signaling, are by-and-large metamorphosis-specific cellular factors in holometabolous insects: they induce precocious adult development when activated early, and block metamorphosis when their function is removed. Intriguingly, interference with BR-C signaling during the nymph stages of the hemimetabolous milkweed bug *Oncopeltus fasciatus* results in a molt to a larger version of the same nymph stage. In this way, BR-C proteins have analogous functions in hemimetabolous nymphal and holometabolous pupal development, in support of the Burlese hypothesis.[53]

Alterations in the timing of expression of the hormone receptor signaling network also underlie the independent evolution of larval reproduction (paedogenesis: ovarian maturation in the larval stage) in two species of fungus-eating cecidomyiid gall midges (Insecta: Diptera). Here the heterochrony is facultative. Under plentiful food conditions ovarian development is activated early, correlated both with a rise in ecdysteroids[54] and early appearance, specifically in the ovarian cells, of the two NR proteins [Ecdysone Receptor (EcR) and Ultraspiracle (USP)] that constitute the functional ecdysteroid receptor.[55] When the food quality is poor, the ovarian expression of these proteins is down-regulated, ovarian development is delayed, and the midges proceed through metamorphosis, hatch and disperse to find a new fungal patch.

Life history evolution in some plants is associated with similar hormonal alterations. For example, mangroves are a polyphyletic assemblage of coastal plants: they come from sixteen distinct plant families, and are often more closely related to upland, non-mangrove taxa than they are to other mangroves. Of these sixteen distinct experiments in mangrove evolution, vivipary (where the embryos lack a seed dormancy stage) has evolved six times independently. The presence of the plant hormone abcissic acid (ABA) is known to induce seed dormancy in a wide variety of plants. In independently evolved mangroves from four of the six viviparous families, embryonic ABA levels are reduced relative to both non-mangrove outgroups and one non-viviparous mangrove species (*Sonneratia alba*) (reviewed in Ref. 56). Other viviparous plants that live in predictably moist environments (the amazonian cocoa *Theobroma cacao*, the english oak *Quercus robur*, the southeast Asian heavy hopea tree *Hopea odorata* and the east Asian red machilus *Machilus thunbergii*) are also characterized by low embryonic ABA levels (reviewed in Ref. 56). Thus, reduced embryonic ABA levels have evolved in parallel with vivipary in a wide variety of desiccation-intolerant plants.

Metamorphosis in fish, as we and others[33] have defined it (Box 1, Appendix 1), is relatively rare. Thus, the presence of a metamorphic life history in vertebrates in general is most likely secondarily derived from an ancestral life history characterized by a more gradual LHT (i.e. Refs. 2,57). Brown[58] demonstrated that TH influences a limited subset of the ontogenetic changes that occur during the major LHT in the zebrafish *Danio rerio*. By contrast, the more dramatic metamorphic alterations in flounders, eels, lamprey and salmon are under greater relative TH control. With these differences in mind, we propose a modification to Alberch's[59] definition of metamorphosis. In ancestral taxa with less drastic metamorphoses, hormones regulate only a subset of the morphogenetic remodeling events. In derived taxa with more drastic metamorphoses, the hormones not only directly regulate a greater subset of these morphogenetic processes, but come to be coopted as general orchestrators of the overall metamorphic transition: metamorphosis cannot proceed in the absence of the hormonal signal. In this conception, zebrafish typify the ancestral condition, while flounders, lamprey, eels and salmon represent independently derived examples of TH regulation of metamorphosis. Truman and Riddiford[52] have offered a similar account for insect evolution, as we outlined above. Still, more comparative work—particularly on ''outgroup'' fish with gradual LHTs—is required in order to fully evaluate this modification of Alberch's metamorphosis hypothesis.

In summary, a careful interspecific comparison of reasonably closely related species allows the conclusion that shifts in timing of hormonal release and/or the cellular response to hormones in the target tissues are intimately associated with the evolution of derived life history strategies. Next we will focus on another independent instance of the involvement of thyroid hormone in modified life histories: the evolutionary loss of larval feeding in echinoderms.

Echinoderm life history evolution: a case study

There are five extant classes within the phylum Echinodermata: Echinoidea (sea urchins and sand dollars), Asteroidea (starfish), Ophiuroidea (brittle stars and basket stars),

Holothuroidea (sea cucumbers), and Crinoidea (feather stars and sea lilies). Most studied echinoderms have a free swimming larval stage that disperses from the parental location, undergoes metamorphosis and settles to the benthos as a pre-reproductive juvenile. A minority of echinoderms brood their offspring. Those echinoderms with dispersing larvae can be further subdivided into those that feed as larvae and those that do not (reviewed in Ref. 60).

The similarities in morphology and feeding biology among the disparate larvae of echinoderms, and even their sister group the hemichordates, suggests that the ancestral mode (plesiomorphy) of development in the echinoderms is development via a feeding larva, and that non-feeding development has evolved independently on many occasions[1,11] (see Fig. 2 for echinoids). Much has been written regarding the ecological and evolutionary consequences of feeding versus non-feeding larval development,[1,11,61] including trade offs between egg size and egg number, differences in survival in the plankton, limitations to dispersal, as well as differences in juvenile growth and mortality in feeding and non-feeding taxa. Still, the developmental mechanisms involved in the multiple evolutionary transitions from feeding to non-feeding have remained obscure. While egg size can be a strong predictor of developmental mode in echinoderms and other taxa, in some cases, such as the echinoids, this correlation breaks down, suggesting that there are other factors involved as well (see Refs. 61,62). Recent work on the mechanisms of metamorphosis in echinoderms has shed some new light on this problem.

Several authors have now demonstrated that in the Asteroidea and Echinoidea, at least, thyroid hormones (THs) are important regulators of the metamorphic transition.[62–68] Application of excess TH to feeding larvae results in accelerated development towards the juvenile stage, while inhibitors of TH synthesis result in a metamorphic delay. Importantly, feeding echinoid larvae depend on an external source of hormone, the planktonic algae that they consume as larvae, in order to undergo the metamorphic transition. This finding of an exogenous source for a crucial morphogenetic hormone is not without precedent, and reminds us that hormones should not be thought of as purely endogenous chemical messengers (see below).

The involvement of thyroid hormones in echinoderm metamorphosis is clearly an additional example of independent cooption of a hormonal mechanism underlying LHTs. Strikingly, the hormone is once again TH. But do alterations in

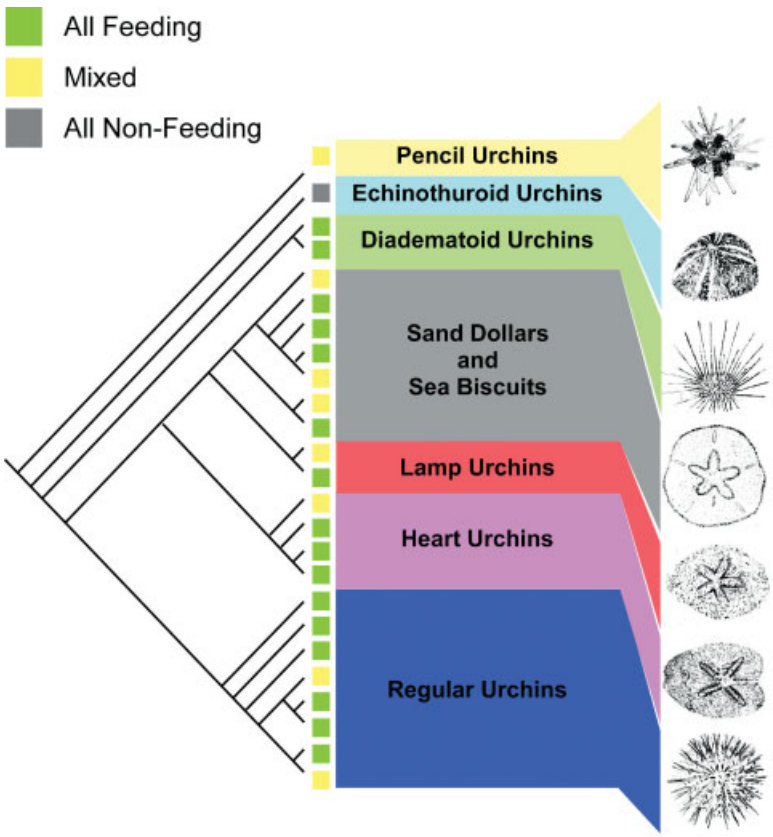


Figure 2. Life history strategies in echinoids (Echinodermata). It is generally accepted that a feeding mode of development is ancestral for the echinoids,[1,11] but non-feeding development has certainly evolved many times independently in the group.[106] These multiple independent evolutionary transitions provide fertile ground for studying the mechanisms underlying LHTs and LHE. Phylogeny modified from Littlewood and Smith.[107] Life history characters compiled from McEdward and Miner.[60]

TH regulation underlie echinoderm LHE as well? Recent comparative data suggest that the answer is yes: the sea biscuit *Clypeaster rosaceus* and the sand dollar *Peronella japonica*, two echinoid taxa in which non-feeding larvae have evolved independently from feeding ancestors, can apparently synthesize all of their required hormones endogenously (Refs. 65,66 and Heyland unpublished data). Furthermore, we have recently shown that TH application is sufficient to change the developmental mode of a sand dollar (*Leodia sexiesperforata*) from an obligatorily feeding larva to one that can complete metamorphosis and settle in the absence of any food.[108] This result represents the first such successful experimental alteration in developmental mode in echinoderms, and suggests that the upregulation of internal TH synthesis is a necessary preadaptation for the evolutionary loss of larval feeding in echinoderms.

If this pre-adaptation scenario is correct, it indicates that different echinoderm taxa might differ in their abilities to synthesize the hormone internally, and that such differences may account for trends in the evolutionary loss of larval feeding in echinoderms. For example, although it has been hypothesized that there are at least 20 independent losses of larval feeding in the class Echinoidea alone,[69] the distribution of non-feeding taxa does not appear to be random (Fig. 2). The sand dollars and sea biscuits (order Clypeasteroida) have multiple independent transitions from feeding to non-feeding, while the Diadematoida have no known non-feeding larvae (see Fig. 2). To fully test the above pre-adaptation hypothesis, we need to analyze several taxa in which non-feeding larvae have arisen independently. If the loss of larval feeding in a given clade is always associated with an evolutionary increase in the capacity for internal hormone synthesis in planktotrophic outgroups, then this would indicate that increased internal TH synthesis is a precondition for the evolutionary loss of larval feeding.[62,68] Alternatively, internal TH synthesis in larvae could be ancestral for echinoids, and may have been lost in taxa such as the regular urchins and the Diadematoida. Comparative studies of the TH synthesis pathways in a variety of echinoids could allow us to distinguish between these possibilities. The robust basal position of the pencil urchins (family Cidaroida; see Fig. 2) makes them a particularly important taxon for such studies.

Such a broad comparative analysis would yield more profound implications than simply for our understanding of life history evolution in echinoderms. As one additional indication of the critical involvement of hormones in life history evolution, it would support our contention that evolutionary alterations in metamorphic life histories involve alterations in the regulation of metamorphic hormones.

Furthermore, the study of TH-related function in echinoderms elucidates another important point that may have far-reaching consequences for our understanding of hormonal functions in multicellular organisms: hormones need not be synthesized endogenously, but can originate from exogenous sources and be obtained by animals through feeding.[37]

Signaling molecules from exogenous sources: a new hypothesis for the origin of hormonal signaling in animals

Plant steroids and terpenes are widespread and ancient, and function as insect feeding deterrents in many cases.[70,71] Indeed, insects cannot synthesize ecdysteroids without first obtaining an external source of sterols through feeding.[41] These findings raise the intriguing possibility that ancient insects first used plant-derived chemicals as modulators of LHTs, and only later evolved the ability to internally synthesize particular hormonal compounds, such as the sesquiterpenoid juvenile hormones.[43] The evolution of thyroid hormone (TH) function in animals could have followed a similar route. The ancestral function of thyroid hormone could have been as feeding deterrents in algae and/or plants,[37] and the signaling functions in animals might, therefore, have been acquired secondarily, perhaps even through horizontal transfer from their hosts or other co-associated microbes with more ancient relationships with the host.[72] This situation could create positive selection to supplement exogenous supplies by endogenous synthesis, thus lessening the animal's dependence on feeding for these newly acquired signaling functions.

A comparative analysis of thyroid hormone synthesis capacity in basal chordates provides some tantalizing evidence supporting this theory. The endostyle as it occurs in urochordates, cephalochordates and lampreys is a good candidate for a homolog of the vertebrate thyroid[73–75] (but see Ref. 76). One obvious morphological feature of the endostyle is its proximity to the digestive system. In fact, in urochordates and cephalochordates, the endostyle retains the function of a feeding organ with at least one band of ciliated cells.[75] In lampreys, the endostyle acts in many respects more like an exocrine than an endocrine gland: it secretes endogenously synthesized T4 into the alimentary canal, where it can be reabsorbed by the gut wall and then converted into T3.[77] Results from amphibians further suggest that tadpoles have the ability to absorb TH-like compounds from their nutrition, subsequently leading to an acceleration of development to metamorphosis.[78] As described above, echinoderm larvae also obtain THs from their diet of planktonic algae,[63] ultimately resulting in their attainment of metamorphic competence.[62]

Furthermore, ingested THs seem to be the signal for phenotypic plasticity in both sand dollar[62] and spadefoot toad[78] larvae. These findings suggest that the effect of ingested TH on larval morphogenesis could have been an ancestral mechanism through which TH was coopted, in both the amphibian and echinoderm lineages, as a stimulator of metamorphosis. Similar instances of cooption are found in many vertebrate parasites, including some flatworms and

arthropods, which synchronize their life cycles with those of their hosts by responding to their host's hormones (reviewed in Refs. 3,41). Finally, rotifers (*Asplanchna* spp.) utilize an ingested vitamin E metabolite (α-tocopherol, a terpenoid) to regulate their parthenogenetic-to-sexual LHT and associated morphogenetic alterations.[79]

A full evaluation of this exogenous origin of hormones hypothesis awaits more detailed comparative biochemistry of hormonal signaling systems in a diverse array of animals and their plant and algal host taxa. Still, these varied examples of dietary and other exogenous hormone sources, which can be considered instances of what Schultz[72] refers to as "phylogenetic espionage", should cause us to reconsider the commonly understood definition of hormones as strictly endogenously synthesized signaling molecules.

Conclusion

Hormones coordinate life history transitions in a wide variety of animal and non-animal taxa, and similar hormones have been repeatedly coopted in independently evolved life history transitions in disparate taxa (an example of homoplasy). Derived life histories, such as vivipary in plants and the evolutionary loss of larval feeding in echinoderm larvae involve alterations in these same hormones. We suggest that it is the modular nature of hormonal signaling systems that predisposes them to be used in these diverse developmental and evolutionary contexts, and propose a broadly comparative, non-model system approach to illuminate evolutionary patterns in organismal ontogenies.

What should one look for in choosing a taxon for such a comparative approach? We propose the following: (1) choose a restricted taxon, whose members show substantial diversity with respect to the mechanism in question, (2) have a phylogenetic hypothesis for the taxon, and (3) be able to obtain offspring from several different members of the taxon, to perform experimental manipulations and to make descriptive observations. Other useful features are: (4) taxa with good representation in the fossil record, (5) taxa that include a close relative whose genome is being analyzed, (6) embryos that can be manipulated and observed while alive, and (7) short generation times, allowing genetic manipulations and selection experiments.

Our example non-model taxon is the echinoderms, a group that satisfies 6 of these 7 key features. The exception is feature 7, as typical echinoderms have generation times well in excess of one year; however, embryos from several different sea urchin species have proven to be amenable to modern gene knockout and knock-down techniques (e.g. Ref. 80). Still, we do not wish to dwell on the echinoderms; we advocate the application of this comparative approach to a wide diversity of organisms, and not merely to "satellite systems" sensu Rudel and Sommer.[81] In particular, such a comparative approach should be extended to include all of the multicellular kingdoms[82] (plants, animals, fungi, brown algae and red algae), including those in which multicellularity has evolved independently on more than one occasion. While studies of model systems will continue to provide an ever-expanding collection of molecular and cellular techniques to study organismal ontogenies, it is only through such a widespread application of the comparative approach in an ecological context that we will deprovincialize developmental biology, and thus gain a fuller understanding of the mechanisms of evolution.

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Appendix: Settlement and metamorphosis across kingdoms

Settlement and metamorphosis in marine invertebrates are clearly related, though mechanistically distinct, processes. As we have shown, metamorphosis generally involves the activation and repression of batteries of target genes, and is under the global regulation of morphogenetic hormones. Settlement, by contrast, is much more rapid, and the control is neurophysiological rather than transcriptional.

The timing of settlement relative to the longer-term ontogenetic remodeling characterizing metamorphosis varies widely among animal taxa. For example, in echinoderms and colonial sea squirts, the juvenile form has developed within the larval body, so settlement reveals the essentially fully formed adult body plan. By contrast, bryozoans and solitary sea squirts, for example, settle before the major events of metamorphic remodeling have occurred, so a substantial post-settlement process of juvenile morphogenesis precedes the appearance of a recognizable adult body plan.[86] Thus the relative timing of settlement and juvenile morphogenesis underlie important differences in life history trajectories in disparate animal taxa.[83]

The derived metamorphosis in holometabolous insects (wasps, flies, moths etc.) follows a remarkably similar pattern to that described above for metamorphosis and settlement in marine invertebrates (c.f. Ref. 2). In this group of insects, pupal development, like marine invertebrate metamorphosis, is the longer-term, morphogenetic remodeling period under hormonal/transcriptional control, while eclosion, like settlement, is

the neurophysiologically regulated, rapid change in habitat (terrestrial to aerial). Indeed, both eclosion in insects and settlement in various marine invertebrates involve intracellular cyclic nucleotide second messengers (reviewed in 43), a clear example of parallel evolution.

There is an ongoing debate about whether fish as a whole are metamorphic.[33,87] Based on our definition given above, we would not consider changes occurring between immature stages and adult life in fish to always be examples of metamorphosis. Still, substantial morphological, ecological and physiological changes do occur in some groups. The larval pattern of neural-crest-derived pigment cells changes dramatically into the more complex adult pattern during zebrafish development.[22] One of the obvious characteristics of summer flounder "metamorphosis" is the migration of the right eye to the left side of the head, as well as the transition from a primarily cartilaginous to an ossified skull, and a shift in habitat. Eel (*Anguilla*) and salmon life histories are characterized by major migrations of pre-adults. Such migrations are accompanied by a multitude of physiological and metabolic changes that allow the animals to shift from fresh-water to salt water. The striking finding is that the physiological and morphological changes characterizing all of these independently evolved instances of fish metamorphoses are controlled by TH, and substantial alterations in TR expression levels have also been reported.[88,89]

Finally, we do not consider alternations of generations in plants and algae to be examples of metamorphosis; these are, rather, reproductive LHTs that pass through an intervening zygote stage. However, many marine algae undergo a process analogous to settlement following their zygotic dispersal stage. In addition, although we define metamorphosis as including only multicellular forms, some single-celled organisms undergo similar LHTs. Indeed, the asymmetrically dividing bacterium *Caulobacter crescentus* attaches to the substrate and transforms from the swarming to the stalked stage in a strikingly parallel fashion to settlement and metamorphosis as we have defined them (reviewed recently in Ref. 90). The physiological control mechanisms underlying these settlement-like processes are virtually unknown, and are important topics for future comparative studies.

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