Expanding networks: Signaling components in and a hypothesis for the evolution of metamorphosis

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Synopsis Metamorphosis is a substantial morphological transition between 2 multicellular phases in an organism's life cycle, often marking the passage from a prereproductive to a reproductive life stage. It generally involves major physiological changes and a shift in habitat and feeding mode, and can be subdivided into an extended phase of substantial morphological change and/or remodeling, and a shorter-term phase (for example, marine invertebrate "settlement," insect "adult eclosion," mushroom fruiting body emergence) where the actual habitat shift occurs. Disparate metamorphic taxa differ substantially with respect to when the habitat shift occurs relative to the timing of the major events of morphogenetic change. I will present comparative evidence across a broad taxonomic scope suggesting that longer-term processes (morphogenetic changes) are generally hormonally regulated, whereas nitric oxide (NO) repressive signaling often controls the habitat shift itself. Furthermore, new evidence from echinoids (sea urchins, sand dollars) indicates a direct connection between hormonal and NO signaling during metamorphosis. I incorporate 2 hypotheses for the evolution of metamorphosis—one involving heterochrony, the other involving phenotypic integration and evolutionarily stable configurations (ESCs)—into a network model for metamorphosis in echinoderms (sea urchins, starfish, and their kin). Early indications are that this core regulatory network can be acted upon by natural selection to suit the diverse ecological needs of disparate metamorphic organisms, resulting in evolutionary expansions and contractions in the core network. I briefly speculate on the ways that exposure to xenobiotic pollutants and other compounds might influence successful settlement of juveniles in the wild. Indeed, environmentally regulated life history transitions—such as settlement, metamorphosis, and reproductive maturation—may be developmental periods that are especially sensitive to such pollutants.

Introduction

Metamorphosis has arisen independently numerous (perhaps 8) times in diverse animal taxa (Hadfield 2000), and is also found in fungi, algae, and flowering plants (Bishop, Erezyilmaz, and others 2006). This remarkable example of homoplasy raises several questions. First, and most fundamentally, what is the selective advantage of a metamorphic life history? Conversely, what is different about those organisms (such as roundworms and mammals) that have a simple life history (that is, no metamorphosis)? Why has selection *not* favored metamorphosis in those taxa as well? Or, rather, are there some taxon-specific constraints operating that can account for the evolutionary distribution of groups that lack metamorphosis?

Why metamorphose?

Much has been written about the evolution of complex life cycles and metamorphosis (see Bishop, Erezyilmaz, and others 2006 for definitions) in animals (for example, Strathmann 1993; Wray 1995; Hadfield 2000; Heyland and others 2005). The predominant argument can probably be summarized as follows: selection for specializations at different stages of ontogeny results in a selective conflict and the ability to produce different morphologies at these different stages is the resolution of this conflict. Metamor-phosis, then, is the stage (size, age, season) at which the selective advantage of morphology A ("larva") is outweighed by the selective advantage of morphology B ("juvenile") (Fig. 1). If we further assume that intermediate morphologies between larva and juvenile are selectively inferior to the definitive larval and juvenile morphologies (Strathmann 1993), then what follows is selection for a relatively rapid life history transformation—in a word: metamorphosis.

Such an analysis is useful in certain contexts, and it makes testable predictions as to why some metamorphic taxa—such as gastropod mollusks, ribbon worms, and echinoderms—have much more rapid metamorphic transitions than do other taxa, such as amphibians. Specifically, gastropods, ribbon worms, and echinoderms undergo their "metamorphic

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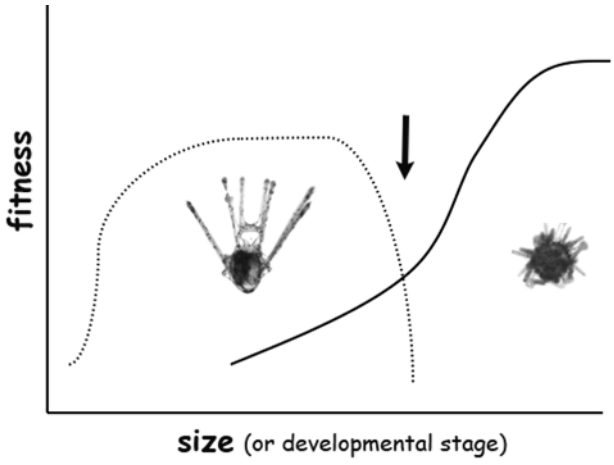

Fig. 1 A hypothesis for the selective advantage of metamorphosis. The dotted line represents the advantage of morphology A (shown here is a sand dollar larva) over a range of sizes; the solid line represents the selective advantage of morphology B (shown here is a sand dollar juvenile). At small sizes (usually <0.5 mm in marine invertebrates; Hadfield 2000), the larva is selectively favored; the juvenile form is favored at larger sizes. The point at which the lines cross (arrow) is the size at which the habitat shift should occur (see Strathmann 1993 for review and important caveats to this simple argument). In taxa with nonfeeding larvae or direct development, the egg size should be the size indicated by the arrow or larger. Eggs of brooders that are provisioned during embryogenesis or later (for example, mammals), however, can be smaller than the arrow. The low fitness at very small sizes for both morphologies indicates a lower limit on egg size, as seen, for example, across marine invertebrate taxa. Note: this is not a formal/mathematical model, so the shapes of the curves are not intended to be strictly accurate, and would certainly vary substantially among taxa.

climax"—or settlement—process with juvenile structures already formed. In gastropods, the shell, foot, and visceral masses are present in the ready-to-settle (that is, competent) larva, and the settling larva simply drops its velar lobes and begins to crawl on the benthos (for example, Hadfield 1978). Similarly, in the feeding larvae of ribbon worms (Nemertea) and echinoderms, the juvenile rudiment forms within the growing larval body as separate entities from the larva, and settlement is essentially a process whereby the larval tissues resorb, and the preformed juvenile everts out of the larval body (see Chia and Burke 1978; Strathmann 1978b; Stricker 1987). In contrast, in amphibians, the metamorphic period is a very gradual process whereby larval tissues are destroyed and resorbed and adult-specific structures are formed. During the intervening period, the individual is, for a time, both a functional larva with gills and a juvenile with lungs. The selective advantage of this ability to temporarily maintain both an aquatic and a terrestrial physiology and morphology may be related to the environmental trigger for metamorphosis in many amphibians: the gradual process of their aquatic habitat drying up (reviewed by Newman 1992). In other words, amphibians may represent an exceptional case where the intermediate form between larva and juvenile is selectively advantageous during the transition from one habitat to another, and hence selection has retained the gradualness of the transition.

Continuing with this thought experiment, one might ask: what did the ancestral life cycle look like in taxa like ribbon worms, echinoderms, mollusks, and others that now have a rapid metamorphic life history? Clearly, a fully functional, free-living larval/immature stage was not simply plugged intact into the life cycle of a direct-developing organism. Likewise, it is not reasonable to assume that a novel, fully functional but distinct adult morphology was merely tacked onto the end of ontogeny in an evolutionary instant. The only reasonable hypothesis is that the ancestral life cycle in metamorphic taxa involved a gradual transition from "larva" to "adult." Therefore, the independent evolution of a rapid metamorphosis must have involved the shortening of this transition, ultimately resulting in the dramatic life cycle transitions present in many modern-day organisms.

The above hypothesis, in its essence, was presented by Pere Alberch in 1989. I reprint here (Fig. 2) a montage of 2 of the figures from Alberch's paper. The implication of this figure is that the metamorphic life history is derived from an ancestral nonmetamorphic life history via a heterochronic shortening of a key group of morphogenetic events leading to the definitive juvenile morphology (see Fig. 2).

Homoplasy and the evolution of metamorphosis

As I described above, metamorphosis across taxa represents a notable example of homoplasy: similarity arising independently in different lineages. To phylogeneticists analyzing character evolution, homoplasy is typically seen as a confounding factor. Indeed, optimizations of character evolution by parsimony are designed to minimize homoplasy in a given dataset (Sanderson and Donoghue 1989). Nevertheless, a closer examination of well-documented cases of homoplasy could shed new and important light onto evolutionary patterns and processes (Wake 1991; Hodin 2000). Towards this end, it is useful to further subdivide homoplasy into parallel and convergent

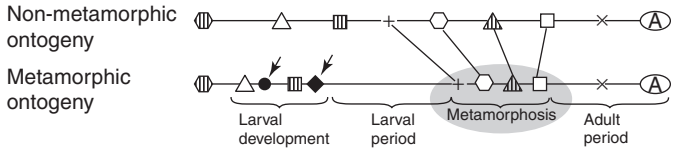

Fig. 2 A hypothesis for the evolution of metamorphosis. The *symbols* represent ontogenetic events (such as the differentiation of limb primordia, or the elaboration of the adult feeding apparatus). According to this hypothesis, 3 key features distinguish the metamorphic from the nonmetamorphic life history: (1) a heterochronic delay in certain ontogenetic events (plus, open hexagon, striped triangle); (2) the acquisition of novel ontogenetic features (filled symbols with arrows) that are only used early in the metamorphic life history (this defines the "larval" morphology as being distinct from the adult); and (3) a heterochronic (*sensu* Gould 1977, 2002; Alberch and others 1979; a change in the relative timing of developmental events) shortening of the progression through a key group of events (gray oval) leading to the juvenile ("adult" here) morphology. Alberch leaves out the destruction of larval specific structures (closed diamond, closed circle), an event that is also characteristic of the metamorphic period. Figure modified with permission from Alberch P, 1989, Development and the evolution of amphibian metamorphosis, In: Splechtna H, Hilgers H, editors, Trends in vertebrate morphology, p 163–73.

evolution. "Parallel evolution," which includes evolutionary reversals, is independent acquisition of similar traits using the same mechanism, for example, similar skeletal changes in independently evolved freshwater stickleback populations (Schluter and others 2004). "Convergence" is independent evolution by different mechanisms, for example, increase in salamander body length in different taxa either by increase in number of vertebrae versus length of individual vertebrae (Wake 1991).

The term "mechanism" needs to be further clarified. I suggest the following: "mechanism" can refer to different levels of organization, depending on the question being asked. One can ask if a mutation in the same "gene" (or indeed the same nucleotide position) is responsible for a case of parallel evolution of anoxic tolerance in independently evolved deep-sea taxa from different ocean basins. Or, instead, the question may concern whether parallel changes in the same "signaling network" might underlie the parallel loss of vision in independently evolved cave-dwelling taxa. Still higher levels of organization might be the mechanism in question when asking if unrelated 6-armed starfish taxa all form their extra limb by modifying the same "morphogenetic" process in similar ways. The reader can undoubtedly think of additional classes and levels of mechanisms, and how they might apply to given instances of homoplasy. The key, I think, is to clearly define what one means by "mechanism" to answer the specific question at hand.

Returning to metamorphosis, one implication of Alberch's metamorphosis hypothesis outlined above (Fig. 2) is that multiple independent examples of the evolution of metamorphosis followed parallel evolutionary paths at least on a superficial level, in that they involve shortening of one phase of the transition between the prereproductive and the reproductive life stages. An additional instance of superficially parallel evolutionary paths in independently evolved metamorphic taxa is that some derived larval forms can be understood as having evolved through "adultation" (appearance of adult features early in ontogeny), as suggested first by Jägersten (1972).

One clear example of adultation involves the independent evolution of the pluteus larva in 2 classes of echinoderms: echinoids (sea urchins, sand dollars) and ophiuroids (brittle stars and basket stars). The pluteus larvae in both of these classes are similar in many ways, most strikingly in the appearance of the skeleton early in ontogeny. Nevertheless, fundamental structural differences in the detailed ways in which the skeleton is formed support the independent evolution of the 2 types of plutei (Hotchkiss 1995; Lacalli 2000). In fact, purely larval skeleton is present in a 3rd class of echinoderm larvae as well: the holothuroids (sea cucumbers) (Pawson 1971). As the presence of a skeleton in adults is a synapomorphy (shared, derived feature) of the phylum as a whole, the independent acquisition of larval skeleton in echinoids and ophiuroids, and to a lesser extent holothuroids, is a clear example of parallel acquisition in skeletal development. But how deep do these parallelisms go? Molecular studies in echinoids have confirmed that the same skeletogenic genes that are active in larvae are also reactivated in the growing juvenile (reviewed in Wilt and others 2003); therefore, in echinoids, larval skeletogenesis can be seen as an example of adultation. Recent comparative studies on larval skeleton formation in the ophiuroid *Ophiocoma wendtii* suggest that the regulatory apparatus that induces larval skeleton in echinoids is also used by ophiuroids (Livingston and Harmon 2006). In other words, the independent adultation of adult skeletal morphogenesis in ophiuroids and echinoids is a case

of parallel evolution at the mechanistic level of the gene regulatory network. In each of these 2 echinoderm classes, it seems that larval skeleton was independently acquired by early activation of the adult skeletogenic network.

The broader question is this: has the independent evolution of metamorphosis across phyla and kingdoms similarly involved parallel acquisition of the signaling systems that underlie metamorphosis? The surprising result of a wide range of recent studies on disparate phyla and even kingdoms is that the answer to this question appears to be yes.

I will begin by giving evidence to support this hypothesis of parallel evolution of metamorphic signaling across phyla and kingdoms. This first part of the paper is divided into 3 sections: (1) evidence for the involvement of hormones in the longer-term phases of disparate metamorphoses; (2) evidence for the role of nitric oxide (NO) and efflux transport in the shorter-term phases of disparate metamorphoses; and (3) new evidence for the connection between hormones and NO during metamorphosis. Next, I will present a network model for metamorphosis and settlement in echinoderms, and I will suggest some ways in which this core network may have been expanded or contracted in different echinoderms with different life history patterns. Finally, I will conclude by suggesting, more broadly, that an expanded and interconnected meshwork of signaling systems not only characterizes the evolution of "rapid" metamorphosis in disparate taxa, but indeed that this process of network expansion is precisely why we observe parallel evolutionary processes in the evolution of metamorphosis across taxa.

Common features in metamorphosis across kingdoms

Metamorphosis as a general phenomenon often includes 2 related but distinct processes or phases: a longer-term morphological change and/or remodeling, and a shorter-term habitat shift. In many of the most familiar metamorphic taxa, the morphological remodeling phase precedes the habitat shift, but several examples of the reverse exist (Chia 1978) (Table 1).

In marine invertebrates, the habitat shift is called "settlement." Still, many studies confound the terms "settlement" and "metamorphosis" [see Chia (1978) for a clear distinction]. Furthermore, it has been argued that the rapidity of the changes that occur at settlement in many marine invertebrates justifies distinguishing marine invertebrate metamorphosis from seemingly similar processes in terrestrial taxa, such as insects and amphibians (Hadfield 2000; Hadfield and others 2001). As I will attempt to demonstrate here, the deep mechanistic similarities (parallelisms) among metamorphoses in terrestrial and marine taxa justify the use of the same terminology. Indeed, I also advocate the inclusion of certain cases of nonanimal life history transitions as *bona fide* metamorphoses.

Feature #1: Morphological remodeling/change and the role of hormones

Why are hormones a key feature of metamorphosis across taxa?

One fundamental aspect of metamorphosis is what can be called a "discontinuous" change in morphology. I use the term discontinuous to distinguish metamorphic change from the allometric/isometric growth of body parts that characterizes general ontogeny in all organisms (D'Arcy Thompson 1917; see also Maslakova's definition of metamorphosis in Bishop, Erezyilmaz and others 2006). Furthermore, metamorphic morphogenesis proceeds from one postembryonic stage to another, for example, larval morphogenesis is neither metamorphic in indirect developers, nor is juvenile morphogenesis in direct developers (terms *sensu* McEdward and Janies 1997). In animals, the cellular events underlying metamorphic morphogenesis often involve cell death as well as differentiation of new structures by proliferation from undifferentiated cells and/or by cellular or tissue remodeling. In plants, fungi, and algae, however, differentiation of new structures at metamorphosis (flower, fruiting body, thallus) can only occur through proliferation; their rigid cell walls do not allow cellular movements or changes in shape of the cell. Finally, while cell death has not been examined during algal metamorphosis (see Santelices and Alvorado 2006), cell death does occur at various stages during flowering in plants and fruiting in mushrooms (Greenberg 1996; Moore 2003).

The destruction and differentiation of cells and tissues that occurs across the organism at metamorphosis is not a haphazard series of disconnected events. Indeed, the various morphogenetic processes that unfold over time—days to weeks or longer, depending on the organism—are carefully orchestrated. The correct sequence and temporal progression of events is critical in order to accomplish the major morphological makeover that occurs at metamorphosis. How is this temporal coordination established? Although the answer is only known for a

Table 1 Broad comparison of patterns of metamorphosis across phyla and kingdoms

Taxon	Morphological remodeling/change	Habitat shift	Most morphological change precedes habitat shift Class I	Habitat shift precedes most morphological change Class II	Overlapping or simultaneous Class III
Porifera (sponges)	Specialized larva to juvenile	Plankon to benthos		X	
Cnidaria	Planula to polyp	Plankton to benthos		X	
Cnidaria: Scyphozoa (strobilation) and Cnidaria: Hydrozoa (medusa budding)	Polyp to jellyfish	Benthos to plankton	X		
Platyhelminthes (flatworms)	Specialized larva to juvenile	(Usually) plankton to benthos		X	
Nemertea (ribbon worms)	Trochophore/pilidium to juvenile worm	Plankton to benthos	X		
Polychaete Annelida	Nectochaete to juvenile worm	(Usually) Plankton to benthos	X		X
Epitokous polychaetes (for example, some eunicids, syllids, and nereids) Annelida	Benthic worm to swimming epitoke	Benthos to plankton	X		
Mollusca: Gastropoda and Bivalvia	Veliger to snail or bivalve	Plankton to benthos	X		
holometabolous Insecta (Arthropoda)	Larva/pupa to adult	Terrestrial to aerial	X		
Insecta: Odonata and Ephemeroptera (dragonflies, damselflies, mayflies)	Aquatic larva to winged adult	Aquatic to terrestrial/aerial	X		
Barnacles (Arthropoda: Cirripedia)	Cyprid to juvenile	Plankton to benthos		X	
Articulate Brachiopoda (*Terebratulina* and *Terebratalia*—Class II; *Waltonia*—Class III)	Specialized larva to lophophorate juvenile	Plankton to benthos		X	X
Brachiopoda: Linguliformea (for example, *Lingula, Discinisca*)	Specialized larva to lophophorate juvenile	Plankton to benthos	X		
Brachiopoda: Craniiformea (for example, *Crania*)	Specialized larva to lophophorate juvenile	Plankton to benthos			X?
Phoronida	Actinotroch to lophophorate juvenile	Plankton to benthos			X
Bryozoa (moss animals) (marine taxa)	Specialized larva to lophophorate juvenile	Plankton to benthos		X	
Bryozoa (moss animals) (freshwater taxa, Class Phylactolaemata; for example, *Plumatella*)	Specialized larva to lophophorate juvenile	Plankton to benthos	X		
Sipuncula (peanut worms) (most genera)	Pelagosphera to juvenile	Plankton to benthos		X[a]	
Sipuncula (peanut worms) (Phascolion and Phascolopsis)	Trochophore to juvenile	Plankton to benthos		X	
Echinodermata (Asteroida, Echinoida, Ophiuroida, and Holothuroida)	Bilateral auricularia/ pluteus larva to pentameral juvenile	Plankton to benthos	X		

Taxon	Morphological remodeling/change	Habitat shift	Most morphological change precedes habitat shift Class I	Habitat shift precedes most morphological change Class II	Overlapping or simultaneous Class III
Echinodermata (Crinoida)	Bilateral nonfeeding larva to pentameral juvenile	Plankton to benthos		X	
Hemichordata (acorn worms)	Tornaria to juvenile worm	Plankton to benthos			X
Solitary sea squirts (Chordata: Tunicata)	Tadpole to juvenile	Plankton to benthos		X	
Compound/colonial sea squirts (Chordata: Tunicata)	Tadpole to juvenile	Plankton to benthos	X		
Lamprey (Chordata: Agnatha: Petromyzontiformes)	Larva to juvenile	Infaunal to limnetic			X
Salmon (Chordata: Teleostei: Salmoniformes)	Fry to smolt	Freshwater to saltwater	X		
Eel (Chordata: Teleostei: Anguilliformes)	Larva to juvenile	Saltwater to freshwater	X		
Eel (Chordata: Teleostei: Anguilliformes)	Juvenile to adult	Freshwater to saltwater	X		
Flatfish (Chordata: Teleostei: Pleuronectiformes)	Larva to juvenile	Plankton to benthos	X		
Frogs, toads, salamanders (Chordata: Amphibia)	Tadpole to juvenile	Aquatic to terrestrial			X
Hymenomycetous fungi (mushrooms)	Mycelium to fruiting body	Subterranean to above-ground	X		
Angiospermous (flowering) plants	Vegetative to flowering	Usually none[b]	X		
Rhodophyta (red algae), for example, Phyllophoraceae	Crustose to erect thallus	Benthos to superbenthos[c]		X	
Chromalveolata: Phaeophyceae (brown algae), for example, Ralfsiaceae	Crustose to erect thallus?	Benthos to superbenthos[c]		X	

I here classify each taxon into 1 of 3 groups: those in which the major morphogenetic events of metamorphosis precede the habitat shift, such as in adult eclosion in holometabolous insects (Class I); those in which the shift in habitat precedes most of the major morphogenetic events, such as the planula to polyp transition in cnidarians (Class II); and those in which the habitat shift occurs somewhere in the middle of the process of morphogenetic change, as in amphibians (Class III). Although, in most cases, the classifications that I give are rather consistent within each listed taxon, exceptions certainly exist. For example, the evolutionary loss of feeding larvae, which occurred independently multiple times in many of the taxa listed above, often involves substantial heterochronic change in metamorphic patterns. Here I have based the classifications on the presumed ancestral developmental pattern for the group in question (for example, development through a feeding larva in annelids and nemertines; tadpole larva in sea squirts). There are several unicellular forms that undergo life cycle transitions remarkably similar to those outlined here, including some bacteria, such as *Caulobacter*, and ciliate suctorians (for example, Poindexter 1971). I have excluded them from this table (perhaps unfairly) based upon my definition of metamorphosis being restricted to multicellular forms. Nevertheless, it will be very interesting to examine the mechanisms underlying such metamorphic-like life cycle transitions in unicellular organisms. [a]Note that Rice advocates the idea that there are 2 metamorphoses in the typical sipunculan life cycle, one from the trocophore to the pelagosphera stage, and then, subsequently to the juvenile stage (for example, Rice 1978). [b]A habitat shift is a key feature in all of the groups listed, except possibly the angiosperms (flowering plants). However, if recent phylogenetic evidence is proven correct, the first angiosperms were aquatic plants. Thus, flowering may have originally involved an aquatic to aerial transition, as it often does in modern aquatic angiosperms. Furthermore, in many wind-pollinated plants (the ancestral mode of pollination in angiosperms), flower development involves a major vertical growth of the apical meristem in preparation for flowering. This differential growth allows the pollen to be more efficiently carried away from the plant by the wind, and might be considered a change in subhabitat. Also, in general, the concept of metamorphosis may apply better to annuals then to perennials, since the transitions in the former are essentially irreversible, as is the case in most animal metamorphoses (but see Reitzel and others 2006). [c]Change in habitat classification for red and brown algae *sensu* Santelices and Alvorado (2006). References for the information in this table (mainly reviews) are Chia and Rice (1978); Rice (1978); Highnam (1981); Dring and Lüning (1983); Strathmann (1987); Youson (1988); Giese and colleagues (1991); Murray and Dixon (1992); Kües (2000); Andries (2001); Denver and colleagues (2002); Degnan and Degnan (2006).

small subset of metamorphic taxa, hormones are involved in each of these examples.

The most famous and best-studied cases are from the holometabolous insects, which include beetles, bees, butterflies, and flies. In these insects, 2 major classes of morphogenetic hormones, the ecdysteroids and the sesquiterpenoid juvenile hormones (JHs), regulate all the manifold and profound morphological changes that occur between the worm-like larva and the winged adult (see Nijhout 1994; Truman and Riddiford 2002; Flatt and others 2005 for review). Likewise, the morphological transformation from larva to frog/salamander in amphibians is orchestrated by prolactin and the thyroid hormones (THs) (reviewed by Denver and others 2002). Interestingly, recent evidence suggests that THs function similarly during metamorphosis in solitary sea squirts (Chordata: Tunicata: Patricolo and others 2001; Davidson and others 2004; D'Agati and Cammarata 2006) and sea stars and sea urchins (Echinodermata; reviewed by Heyland and others 2005), as well as possibly abalone (Mollusca: Fukazawa and others 2001). In scyphozoans (Cnidaria), too, TH's or their precursors are involved in the metamorphic-like strobilation process: the transition from benthic polyp to pelagic jellyfish (Spangenberg 1974; Berking and others 2005). An unidentified "head hormone" regulates the metamorphic-like epitoky process in some annelids (reviewed by Andries 2001), and there are indications of a JH-like metamorphic hormone function in the more typical metamorphic process in other annelids (Biggers and Laufer 1999). In fact, this JH-like molecule could actually be TH or a metabolite (see Flatt and others 2006). In plants, the metamorphic vegetative-to-flowering transition is regulated by the hormone "florigen," whose molecular identity may have finally been discovered (Ayre and Turgeon 2004; Parcy 2005).

[Note that the convention thus far has been to refer to these nonvertebrate hormones as "thyroid hormones" based on chemical similarity, despite the fact that, with the possible exception of tunicates, there is little evidence that nonvertebrates have a homolog of the vertebrate thyroid gland.]

It has been suggested (Hadfield 2000; Hadfield and others 2001) that hormones in metamorphosis are a specific adaptation in terrestrial metamorphic taxa (insects and amphibians), and may be related to their larger size at, and slower rate of, metamorphosis than in their marine invertebrate counterparts. Still, the data presented by Hadfield and colleagues (2001) to support this generalization are worthy of reconsideration. Those authors state, for example, that insect "metamorphosis is slow. . .4–5 days for small dipterans; and up to weeks or months for large Lepidoptera" (p 1125). The long pupal period for many Lepidoptera (moths and butterflies) is certainly related to their seasonality. Many species are univoltine, and the longest part of their life cycle can be spent in pupal diapause, where development is arrested (reviewed in Ramaswamy and others 1997). Multivoltine Lepidoptera can have a much shorter pupal period, for example, 3 days at 27°C for the diamondback moth *Plutella dylostella* (Ho 1965). As for the dipterans (flies and their relatives), the cited 4–5 days is not at all the lower limit. Depending on developmental temperature, pupal development in dipterans such as mosquitos and midges can be as fast as 24 h (for example, Cuda and others 2002). Considering the degree of morphological change inherent in producing a winged fly from a maggot, 24 h is fast, and certainly within the range of rapid metamorphosis cited by Hadfield and colleagues. Indeed, many of the most rapidly metamorphosing marine taxa cited by Hadfield and colleagues have, by comparison with dipterans, much more subtle morphological change occurring at the habitat transition, as is the case with most cnidarians and gastropods. Furthermore, although these authors cite ascidians as having metamorphic rates of ">30 min," such rapid metamorphic rates are only found in some highly adultated colonial and social species. In these cases, the branchial basket, gut, siphons, heart, and other tissues are completely developed, such that the sole event required to transform from a planktonic to a benthic habitat is the loss of the tail. Solitary ascidian species, by contrast, take much longer after settlement to complete metamorphosis to the feeding stage—from days to a week or more (Cloney 1987).

Still, this entire discussion of "metamorphic rates" itself is wrought with difficulties. Hadfield and colleagues (2001) stated that in "most" marine invertebrate taxa, metamorphosis begins at settlement. It seems, for example, that they do not consider the extensive juvenile morphogenesis that occurs before settlement (indeed before release of the brooded larvae from the mother) in colonial ascidians to be part of metamorphosis. Nevertheless, they would apparently consider the clearly homologous processes of juvenile morphogenesis that occur *after* settlement in solitary ascidians to be part of metamorphosis. Thus, the concept of rate of metamorphosis, when applied across taxa, needs to be qualified by the timing and extent of the changes occurring relative to the moment of irreversible commitment to transform. When considered broadly, nonanimals, animals, and even marine invertebrates exhibit an extreme range of variation in the rates and timing of metamorphic

events, a degree of variation that presumably matches the diversity in selective forces that shape their life cycles.

Therefore, I propose that metamorphosis begins with the differentiation of juvenile-specific structures, as opposed to those structures that are either larval-specific or shared between the larval and juvenile stage. [For comparative purposes outside of animals—and for those cases in animals where metamorphosis does not involve a larval–juvenile transition (such as epitoky in some annelids, as well as hypermetamorphoses in some insects and parasitic flatworms; see Table 1 for other examples)—the terms "larva" and "juvenile" can be substituted with the non-specific terms I used earlier: "morphology A" and "morphology B."]

In this conception, metamorphosis in sea urchins begins with the invagination of the echinus rudiment on the left side of the larvae, and ends when the juvenile begins to feed. As a result, this process can take weeks or longer to complete. The same could be said, for example, for juvenile morphogenesis in nemertines, colonial ascidians, and mollusks: lengthy processes that are mostly complete at settlement. Finally, Hadfield and colleagues (2001, p 1125) state that in marine invertebrate metamorphosis, "formation of most juvenile structures precedes destruction of larval-specific structures." The comparative data I present in Table 1, however, shows that this is not true for several marine invertebrate taxa (Class II taxa in Table 1).

So, with this perspective in mind, when I hypothesize (as others have previously; for example, Matsuda 1987) that hormones play a key role in metamorphosis across taxa, I am referring specifically to the longer-term morphogenetic changes that can occur either before or after (or coincident with) the habitat shift (see also Chia 1978). For example, in heavily adultated insects, in which the imaginal discs (primordia of the adult appendages) begin to proliferate and differentiate early in larval development, hormones regulate their precocious development (reviewed by Truman and Riddiford 2002). Likewise, in echinoderms (Class I in Table 1), THs function during the latter half of larval development, during which time juvenile morphogenesis is occurring. The same pattern continues to hold at a different developmental stage in solitary ascidians: Davidson and colleagues (2004) reported that THs only influenced the postsettlement metamorphic events of juvenile morphogenesis in the solitary ascidian *Boltenia villosa* (Class II in Table 1). Interestingly, similar experiments with the solitary ascidian *Ciona intestinalis* demonstrated TH effects both on settlement and postsettlement metamorphic events in this species (Patricolo and others 1981, 2001; D'Agati and Cammarata 2006). As Davidson and colleagues (2004) pointed out, *C. intestinalis* development is adultated relative to *B. villosa*: *C. intestinalis* larvae settle with some degree of juvenile morphogenesis underway (that is, they are more "Class III-like"). Therefore, the differences in timing of juvenile morphogenesis in the 2 species may account for the observed stage-specific differences in TH effects, a hypothesis that can be more fully tested with additional comparative data on other sea squirt species. As for amphibians, most of the comparable morphogenetic processes overlap with the habitat shift (Class III in Table 1), and hormones regulate morphogenetic processes that occur before, during, and after their "metamorphic climax" period (see Denver and others 2002). That metamorphic climax corresponds to the habitat shift in amphibians is evidenced by the onset of lung functioning and the degeneration of the gills during that period (Burggren and West 1982).

Evolving roles of hormones in derived life cycles

Inherent in many of the examples I cited above are cases in which the roles of hormones have changed along with modifications in life history patterns within metamorphic taxa. Here I will discuss 2 disparate animal taxa that certainly evolved metamorphosis independently: insects and echinoderms. Similar patterns are obvious in other groups, most famously amphibians, as has been discussed in detail elsewhere (see Denver and others 2002 for review).

Case 1: Hormones and heterochronies in insect metamorphosis

Insects represent a unique case among animals: there is near unanimity among entomologists that complete metamorphosis evolved once in the common ancestor of holometabolous insects, a robust monophyletic grouping of insects that include the Hymenoptera (bees, wasps, ants), Diptera (flies, mosquitos), Lepidoptera (moths, butterflies), Coleoptera (beetles, weevils), Neuroptera (lacewings, ant lions), and several less well-known orders. There are few other examples where one can point so confidently to the evolutionary origin of metamorphosis (but see Reitzel and others 2006). The key synapomorphy (shared, derived feature) of the holometabolous insects that essentially defines complete metamorphosis among insects is the presence of a distinct pupal stage intervening between the last larval stage and the adult. What is perhaps less appreciated is that the route from

larva to adult varies quite substantially among the holometabolous insects. For example, the canonical developmental pattern in holometabolous insects (exemplified by the vinegar "fruit" fly *Drosophila melanogaster*) is that the adult appendages arise from ectodermal invaginations called "imaginal discs" (from the term "imago" (Latin), meaning "adult insect") that arise in the embryo, grow throughout the larval stages, and evert to take on their final form within the pupa. Nevertheless, as the cladistic analysis of Truman and Riddiford (1999) clearly showed, this pattern of early formation of imaginal discs is actually a derived (adultated) developmental pattern among holometabolous insects that almost certainly arose in parallel at least 6 independent times within various orders.

Another synapomorphy of the holometabolous insects is the key metamorphic functions of the 2 major classes of insect morphogenetic hormones: the ecdysteroids and the sesquiterpenoid JHs. In holometabolous insects of the ancestral type (that is, late formation and proliferation of imaginal tissue), the high titers of circulating JH in the larval stages suppress imaginal growth. As JH levels drop in the final larval stage, these tissues start to invaginate from the ectoderm and proliferate (see Truman and Riddiford 1999, 2002). In contrast, in those taxa with early imaginal disc formation, such as *D. melanogaster* and the wax moth *Galleria mellonella*, the imaginal tissues proliferate and begin to differentiate in a high JH environment. How is this possible? The answer is not known for most insects, but data from *G. mellonella* (Reddy and others 1980) suggest that selective metabolism of JH by esterases in the wing disc tissue may be one mechanism by which adultation in imaginal disc development is accomplished in insects. Truman and Riddiford (1999) suggest that changes in tissue-specific JH receptor expression patterns could be another mechanism. Such changes in the tissue-specific expression of hormone receptors seem to be related to the evolution of an even more extreme life history shift in holometabolous insects: the independent acquisition of larval reproduction (also called paedogenesis, a type of loss of metamorphosis) in 2 separate clades of gall midges (Diptera: Cecidomyiidae) (Hodin and Riddiford 2000).

Thus, the evolution of metamorphosis in insects has involved several of the features that I propose to be common among metamorphic taxa in general: (1) the manifold morphogenetic changes are under the orchestration of hormones; (2) evolutionary patterns within metamorphic taxa can involve a wide range of heterochronic alterations, from adultation to the evolutionary loss of metamorphosis; and (3) such subtle and dramatic heterochronic changes involve alterations in the morphogenetic hormones that regulate metamorphosis. What is the evidence that such features of metamorphosis apply to noninsect taxa as well?

Hormones as metamorphic regulators across phyla and kingdoms

Relative to the numbers of animal and nonanimal taxa with a metamorphic life history (see Table 1), the numbers of taxa in which the mechanisms underlying metamorphic morphogenesis have been investigated is quite limited. Nevertheless, in all such well-studied metamorphic taxa, morphogenetic hormones are utilized as overall regulators of the morphogenetic processes (reviewed by Heyland and others 2005). Well studied noninsect examples are amphibians, metamorphic fish, such as flounders and lamprey, and flowering in plants. If we include epitoky in annelids as metamorphosis, an as yet to be identified hormone is involved in this example as well (Hauenschild 1960; reviewed by Andries 2001). More recently, metamorphosis in tunicates (Patricolo and others 2001; Davidson and others 2004; D'Agati and Cammarata 2006) and echinoderms (reviewed by Heyland and others 2005) has also been shown to be under morphogenetic hormonal control.

Surprisingly, many of the aforementioned examples (all the vertebrate cases, tunicates, and echinoderms, and possibly abalone) involve acquisition of the same hormone as a metamorphic regulator: TH. In addition, evidence from amphibians and echinoderms suggest that derived life history patterns within these metamorphic taxa, such as loss of the feeding larval stage, involve alterations in hormonal regulation (reviewed by Denver and others 2002; Heyland and others 2005), as is the case for insects as well (for example, Hodin and Riddiford 2000). Below, I focus on the echinoderms, reviewing published studies, and presenting some new data on the role of THs in those species with derived life histories.

Case 2: THs and the development and evolution of echinoderm metamorphic patterns

The canonical life history in echinoderms is development through a bilateral feeding larva, with a subsequent drastic metamorphosis to the pentameral adult. The majority of described echinoderm species actually have nonfeeding (either planktonic or brooded) development (data compiled from McEdward and Miner 2001; with the caveat that its generally easier to judge feeding/nonfeeding developmental mode in brooders than it is in broadcast spawners,

leading to a possibly skewed sample in favor of those species with nonfeeding larval development). Nevertheless, the idea that a feeding larva is ancestral for the echinoderms is supported by (1) similarities across echinoderm classes in detailed morphological aspects of their feeding larvae, such as the convoluted ciliated band, the location and shape of the mouth, and the L-shaped gut; (2) shared feeding mode by upstream capture and by local reversal of ciliary beat; (3) the observation that many of these same morphological features and feeding behaviors are also found in the feeding larvae of hemichordates, sister taxon (Cameron and others 2000) to the echinoderms; (4) the presumably vestigial feeding larval features found in many nonfeeding echinoderm larvae, such as the pluteus arms in the larvae of the gutless sand dollar, *Peronella japonica* (Okazaki and Dan 1954); and (5) the greater general likelihood of convergent loss rather than convergent gain of similar structures (see also Strathmann 1974, 1978a).

Thus, if we accept the predominant opinion that feeding larval development is ancestral for the echinoderms, then nonfeeding development must have arisen numerous times independently in each of the extant echinoderm classes (although as few as 1 time in crinoids, where all known species have nonfeeding larvae; McEdward and Miner 2001) In this way, the Echinodermata represent fertile ground for investigating modifications in the utilization of hormones in metamorphic transitions. Specifically, we can test the hypothesis that hormones are especially useful as regulators of drastic metamorphoses; derived taxa with more subtle (that is, "more direct") metamorphic progressions may rely correspondingly less on hormones to complete their life cycles.

THs *sensu stricto* are 2 tyrosines with 3 (triiodotyrosine; T3) or 4 (thyroxine; T4) iodines attached. The enzyme in vertebrates that is responsible for linking the 2 tyrosines, as well as attaching the iodines, is thyroperoxidase (reviewed by Heyland, Price and others 2006). Orthologs of thyroperoxidase have now been isolated both from tunicates and echinoderms (see D'Agati and Cammarata 2006; Heyland, Price and others 2006), and the expression profiles in each phylum are consistent with a function in TH synthesis. Specific inhibitors of thyroperoxidase (such as thiourea) have proven useful for investigating echinoderm TH functions, as I will describe below.

The functions of these hormones in vertebrates are diverse, including regulating growth, metabolism, and temperature. In those vertebrates with a metamorphic life history (amphibians, some bony fish, and lamprey), THs have additional functions in regulating their metamorphic processes (see Youson 1988, 1997, 2003; Power and others 2001; Denver and others 2002). Similar metamorphic TH effects on echinoderm larvae, in biologically significant (nanomolar) doses, have now been shown for 3 classes spanning 12 families, including species with feeding and nonfeeding development: Echinoidea (sea urchins, sea biscuits, and sand dollars) (Chino and others 1994; Suyemitsu and others 1997; Johnson 1998; Saito and others 1998; Hodin and others 2001; Heyland 2004; Heyland and Hodin 2004; Heyland and others 2004; Bishop, Huggett and others 2006; Heyland, Reitzel and others 2006; A Heyland, J Hodin and T Capo, unpublished data; J Hodin and M Martindale, unpublished data, the present study); Asteroidea (sea stars) (Johnson and Cartwright 1996; A Heyland and J Hodin, unpublished data); and Ophiuroidea (brittle stars) (A Heyland and J Hodin, unpublished data).

Interestingly, the source of hormone for feeding echinoderm larvae appears to be predominantly via the unicellular planktonic algae that the larvae consume. Such algae are known to actually contain *bona fide* T4 and other related metabolites (Chino 1994; Heyland 2004). We recently reviewed the roles of, and evidence for, TH effects on echinoderms (Heyland and others 2005). In general, TH treatment results in shorter development time to the juvenile stage, and the resultant juveniles are smaller than controls, but otherwise morphologically indistinguishable, as judged by spine size, type, and number (Heyland and others 2004). Experiments with large-egged, obligatorily feeding larvae of the sand dollar *Leodia sexisperforata* indicate that TH treatment in the absence of food is sufficient to support development through metamorphosis and settlement to the juvenile (Heyland and others 2004). Thus, as originally hypothesized by Leland Johnson (1997), TH appears to be related in some direct way to attaining competence to respond to settlement cues, a topic to which I will return later in the paper.

If ingested TH is necessary—and in some cases even sufficient—for feeding echinoderm larvae to complete metamorphosis to the juvenile stage, then what about nonfeeding larvae? The nonfeeding planktonic larvae of the sand dollar *P. japonica* (Suyemitsu and others 1997; Saito and others 1998) and the brooded larvae of the lamp urchin *Cassidulus caribbearum* (Fig. 3) apparently synthesize all their required THs endogenously. Similarly, larvae of the sea biscuit *Clypeaster rosaceous*, which have plutei but can complete metamorphosis if starved, can also synthesize all their necessary THs endogenously (Heyland 2004; Heyland, Reitzel and others 2006).

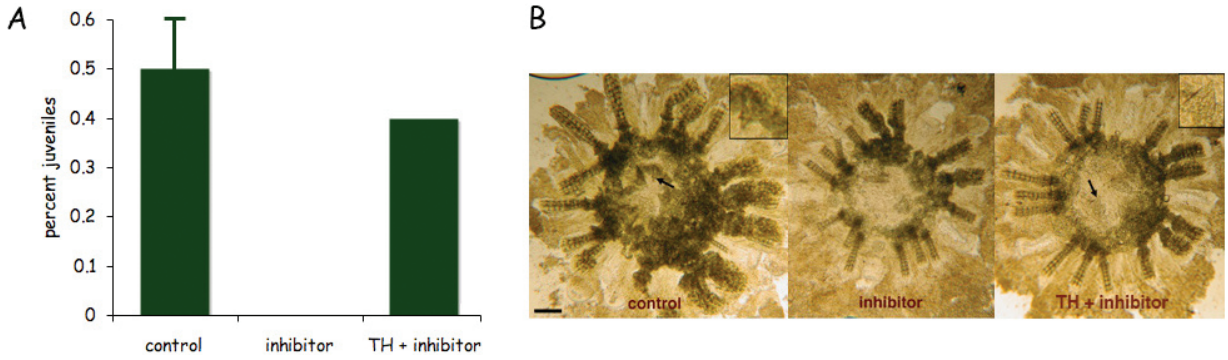

Fig. 3 Brooded larvae of the lamp urchin (Echinoidea: Cassidulidae) *C. caribbearum* synthesize TH endogenously. This evidence comes from studies with the thyroperoxidase (TH synthesis) inhibitor thiourea. Larvae at 4 days after fertilization (23–28°C), at which point they had visible "pluteus" arms (see Fig. 3C in Gladfelter 1978), were reared in a 6-well plate, 5 larvae/well (assigned randomly, 12 ml volume), 2 replicates each of control (UV treated 1 μM filtered seawater—"UVFSW"), inhibitor (1 mM thiourea in UVFSW), and TH + inhibitor (1 mM thiourea + 0.1 nM thyroxine in UVFSW) treatments. I changed their water (and added chemicals as appropriate) every 2 days. (A) I scored larvae 9 days after fertilization (day 5 of the treatment) as either prejuveniles or juveniles: the latter had emergent and mobile tube feet clearly visible in a dissecting microscope. Error bars are standard errors. These results indicate that 1 mM thiourea causes a metamorphic delay ($P = 0.012$), rescuable by 0.1 nM thyroxine ($P = 0.029$). (B) Four days later, I killed the juveniles by compressing them under a cover slip and took pictures of their developing juvenile skeleton. Control larvae had more extensive skeletal growth than did larvae treated with 1 mM thiourea ("inhibitor"). Addition of 0.1 nM thyroxine ("TH + inhibitor") partially rescued this effect. The arrows indicate the tooth pyramids, an example of the advanced skeletal growth in the control and TH + inhibitor juveniles (the pyramids indicated by the arrows are also shown at greater magnification in the insets). I did not observe this structure in any of the control larvae/juveniles on this day. Scale bar = 0.1 mm. Additional methods: I collected 25 adults (many with broods) by snorkeling in ~3 m of water in Spring Bay, Virgin Gorda, BVI, on 13 November 2001. I maintained adults in a tupperware container in their native sand and pure, aerated seawater (collected in Spring Bay), with water changes every 6 h or so during air transit to Miami, at which time I reared them at the Rosentiel Marine Laboratory's hatchery (University of Miami, Virginia Key, FL, USA), in their native sand and running UVFSW, in individual 4 in diameter PVC pipe flow-through chambers with 400 μM Nitex mesh hot-glued to the bottom end. I checked them daily for new broods. The embryos from the described experiment were from 3 females that spawned on 5 December 2001, while they were together in a single finger bowl after I had checked them for broods, and 2 females that spawned after I placed them in finger bowls in the sun for 30 min on the same day. I immediately and gently aspirated the embryos off of the 5 mothers and reared the larvae together in untreated, washed 6-well plates (~10 larvae/well) with 12 ml UVFSW/well and water changes every 1–2 days. For details regarding chemicals, see Heyland and Hodin (2004). I took the photomicrographs using a Zeiss compound microscope with an attached Nikon CoolPix E990 digital camera, and processed the images with Adobe Photoshop. Results were compared pairwise by a Mann–Whitney nonparametric test using SPSS 11.

These data, in combination with the results described above for *L. sexiesperforata*, suggest that the independent derivation of nonfeeding development from feeding ancestors involves the upregulation and/or acquisition of the ability to synthesize THs. In other words, echinoderms represent another apparent example in which evolutionary alterations in metamorphic life history patterns involve changes in hormonal regulation.

One implication of these comparative data is that TH is involved in regulating the progression of metamorphic change in feeding as well as nonfeeding development. The data with *C. caribbearum* (Fig. 3) suggests, further, that brooded larvae also utilize internally synthesized TH as a metamorphic regulator. Nevertheless, *C. caribbearum* is somewhat of a special case: I noticed that their brooded nonfeeding larvae are ciliated and can swim (not shown), although they are normally retained among the spines on the aboral surface of their mother until they are functional juveniles (Gladfelter 1978; my personal observations). This ability to swim suggests a possible dispersal mechanism—perhaps in severe circumstances, such as a storm, or the death of their mother—and indicates that their metamorphic timing may not be a simple "clock-like" developmental progression.

An example of an echinoderm brooder that is much less likely to disperse as a larva is the 6-armed starfish *Leptasterias hexactis*. These broods are maintained by the mother below the oral surface, and adhere together so strongly that it is indeed impossible to separate the larvae without destroying them. I fortuitously discovered that if their oocytes are removed by dissection at maturity (but before they spawn and begin to brood), then they are fertilizable and viable *in vitro*. [Chia (1968) reported that all

of his attempts at *in vitro* fertilization resulted in developmental arrest before the blastula stage. I noticed that sperm concentrations need to be extremely low to avoid polyspermy, which may have been the cause of the developmental arrest in Chia's experiments.]

In this way, the embryos can be kept apart, so as not to adhere to one another, and are thus amenable to experimental study. The resultant larvae do not swim, and develop normally through metamorphosis (personal observations).

I thus performed a similar study with *L. hexactis* as described above for *C. caribbearum*, and saw no effects either of TH treatment or of the thyroperoxidase (TH synthesis) inhibitor thiourea on metamorphic progression in *L. hexactis* (Fig. 4). Therefore, in "extreme" cases of brooded development, where metamorphosis is relatively subtle, hormonal regulation of the progression may be unnecessary, and may have therefore been lost. Alternatively, there could be differences in asteroids and echinoids in the degree to which TH signaling is involved in non-feeding and/or brooded metamorphic development. Additional comparative data with brooded taxa from the various echinoderm classes would allow us to test such hypotheses further. Nevertheless,

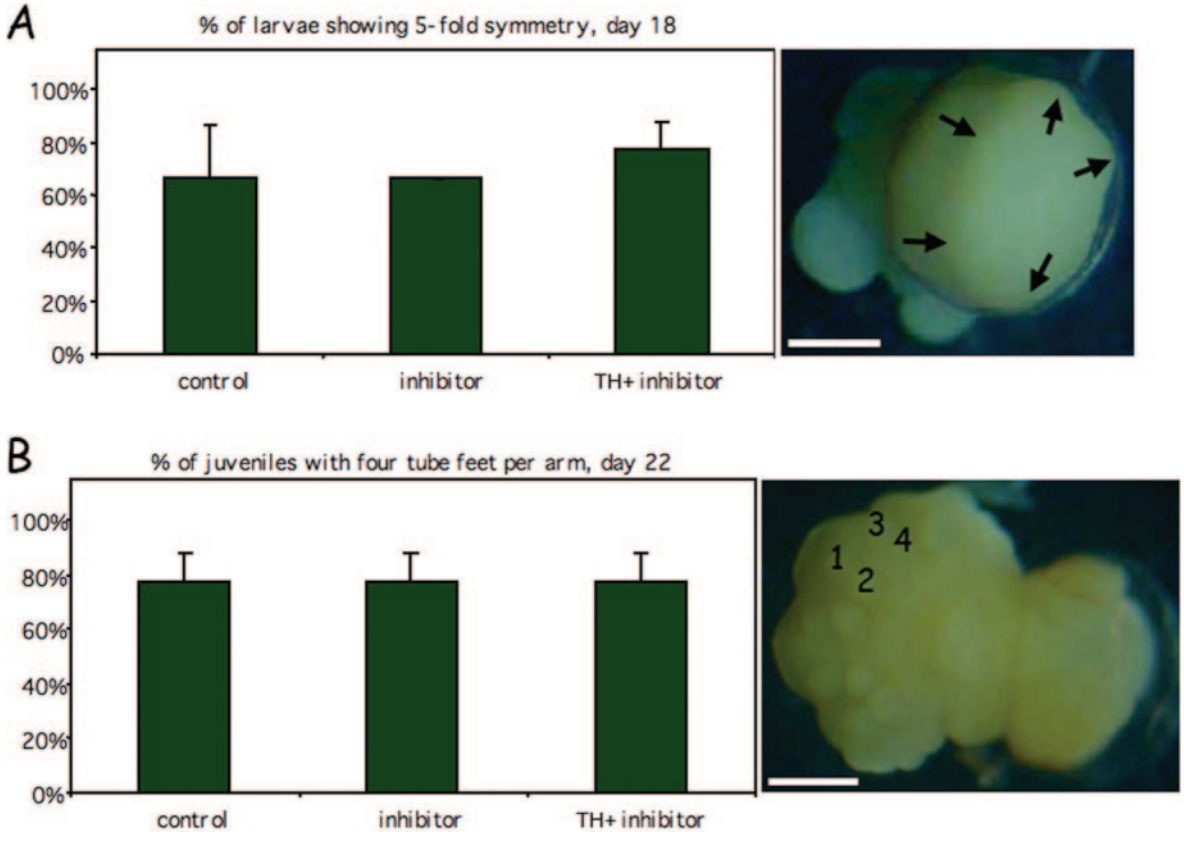

Fig. 4 Brooded larvae of the 6-armed starfish *L. hexactis* apparently do not synthesize THs, and show no clear effects of exogenous TH. I randomly distributed 27 full-sibling, recently hatched embryos (day 15) into 1 of 3 treatments in 6-well plates (10 ml/well, 3 replicates/treatment, 3 embryos/replicate). Treatments as follows: control [0.1 μM UV-treated filtered seawater (UVFSW2)]; inhibitor (1 mM thiuorea in UVFSW2); and TH + inhibitor (1 mM thiourea + 1 nM thyroxine in UVFSW2). I changed water and chemicals every 2 days, at which time I scored embryos/larvae in a dissecting scope for visible metamorphic features, including the appearance of 5-fold symmetry [panel (A), 18 days after fertilization; arrows in the picture at right point to the bumps on the surface of the juvenile ectoderm indicating the appearance of 5-fold symmetry] and the numbers of tube feet on each of the 5 arms [panel (B), 22 days after fertilization; in the picture at right, this juvenile has 4 visible tube feet per arm, numbered]. Here, I present representative data showing no detectable differences among any of the treatments in the scored metamorphic events; these patterns extended for the duration of the experiment (from day 15 to day 26 after fertilization). Error bars are standard errors. Scale bars are 0.1 mm. Developmental temperature: 14°C. Experiment was from April 2004, conducted at Hopkins Marine Station, Pacific Grove, CA, USA (I also collected the adult stars there in the high intertidal zone). I took the photomicrographs using a Zeiss dissecting microscope with an attached Nikon CoolPix E995 digital camera, and processed the images with Adobe Photoshop. Results were compared pairwise by a Mann–Whitney nonparametric test using SPSS 11.

this difference in TH regulation in *L. hexactis* is noteworthy, and I will discuss it again at some length near the end of the paper.

Feature #2: Habitat shift

As stated above, one key component of metamorphosis in many marine invertebrates is a shift in habitat from the plankton to the benthos. This habitat shift is, not surprisingly, often accompanied by profound changes in feeding mode, community composition, organismal physiology, and attendant morphological change (see Chia and Rice 1978). As a result, the habitat shift itself is, for good reason, often considered the defining moment of metamorphosis. Still, as the information in Table 1 demonstrates, the relationship between the shift in habitat and the major morphological changes varies considerably across taxa. For this reason, I like to consider the habitat shift as 1 critical phase of metamorphosis (see also Chia 1978).

In marine invertebrates, the planktonic (larval) form tends to be the dispersive phase of the life cycle, while the benthic (adult) form is typically less mobile. Metamorphoses in other taxa, however, do not necessarily follow this pattern. In holometabolous insects, the habitat shift takes place at adult eclosion: when the winged form emerges from the specialized "pupa" stage. In this case the typical habitat shift is from terrestrial (larva/pupa, less mobile or nonmobile) to aerial (adult, highly mobile). In mushrooms, the transformation of vegetative mycelium into a fruiting body is generally followed by a shift in habitat from beneath to above the earth's surface. That this transformation involves a true habitat shift is apparent from the special cellular adaptations that fungi use to break the surface tension from their moist, mycelial environment, and emerge into the air (Wösten and others 1999). A similar habitat shift occurs in certain red algae that undergo a transition from a crustose (encrusting) stage to an erect thallus stage (see Santelices and Alvorado 2006, this issue). In the latter 2 examples, neither life stage—premetamorphic or postmetamorphic—is truly mobile.

As expected, a profound and generally irreversible (but see Reitzel and others 2006, papers presented at meetings) shift in habitat must be carefully coordinated with reliable environmental indicators, or severe consequences would follow. For a marine invertebrate larva looking for a place to settle, the larva must be able to receive and process environmental information that indicates an appropriate site. Such coordination of the habitat shift with environmental signals extends to all well-studied metamorphic taxa listed in Table 1. In amphibians, crowding, pond drying, and the presence of predators are all well-described signals that initiate the change in habitat that occurs at metamorphosis (Newman 1992). Similarly, adult eclosion in insects is often regulated by day-length, temperature, or other environmental stimuli. For example, the vibrations indicating the presence of a potential host trigger adult eclosion in some fleas (Marshall 1981). The highly specific seasonality in appearance of fruiting bodies of different mushroom species points to environmental signals that stimulate fungal metamorphosis (Kües 2000). Indeed, fungus cultivators are well aware of the different conditions (humidity, temperature, light) that initiate fruiting in diverse fungi (for example, Stamets 2005). As for red algae, the specific environmental signals that signal the crustose-to-thallus transition are not well described, but the limited available evidence suggests their existence in this group as well (see Dring and Lüning 1983; Murray and Dixon 1992).

Parallel evolution of NO signaling in metamorphic habitat shifts?

The specific natural cues that promote settlement vary widely across species, even very closely related species. Such a pattern is best described in marine invertebrate taxa, as in the response to coral effluent in the coral-eating nudibranch *Phestilla sibogae*, a riboflavin degradation product in the solitary ascidian *Halocynthia roretzi*, a peptide released by conspecific adults in the sand dollar *Dendraster excentricus*, and coralline algae as in the coral *Acropora millepora* (see Hadfield and Paul 2001 for review). Since there is clearly strong selection for the utilization of accurate settlement cues, the fact that the particular cues vary widely among species is hardly surprising.

What is perhaps more surprising, though, is that at least a subset of the internal signaling events that lie downstream of cue reception show striking similarities across phyla and even across kingdoms. In particular, the use of NO/cyclic-guanosine monophosphate (cGMP) signaling as a repressor of settlement appears to be a common feature in sea urchins (Echinodermata), sea squirts (Chordata: Tunicata), and a gastropod (Mollusca) (see Bishop and Brandhorst 2003 for review). Furthermore, NO signaling is involved in metamorphic transitions in fungi (see Georgiou and others 2006) and endogenous NO signaling also represses the prereproductive to reproductive (vegetative to flowering) transition in the mustard *Arabidopsis thaliana* (He and others 2004).

Bishop and Brandhorst (2003) offer 2 possible explanations for these remarkable similarities in divergent taxa. First, they propose that NO repression might be a general eukaryotic mechanism for delaying reproduction. Since settlement is generally the point of transition between a prereproductive and a reproductive life stage, this first hypothesis suggests that the similarities in NO regulation of settlement across taxa are elaborations of a more deeply conserved NO repression of reproductive maturity. The second hypothesis is that there is something special about the NO signaling system that makes it suitable for maintaining repression of morphogenetic processes. Therefore, the second hypothesis is that NO repression of settlement across kingdoms is a clear example of parallel evolution.

Recent data point in the direction of parallel evolution as the explanation for NO involvement in these taxonomically diverse, settlement-like processes. For example, in the Eastern mud snail *Ilyanassa obsoleta*, NO is a potent repressor of settlement (Leise and others 2001). In constrast, in the coral-eating nudibranch *P. sibogae* (C. Bishop, personal communication) and the queen conch *Strombus gigas* (A. Boettcher, personal communication), NO is not a potent settlement repressor. These differences in the involvement of NO signaling in settlement in these 3 disparate mollusks parallel the specificity of their settlement cues: the nudibranch and conch have highly specific settlement cues associated with their obligate juvenile food source (*Porites* coral and nursery algae such as *Laurencia poitei*, respectively). In contrast, the mud snail appears to have a less specific settlement cue: intertidal mudflat effluent. The consequence of this lower specificity can be seen dramatically by the robust ability of *I. obsoleta* to invade and establish on the west coast of North America (for example, Race 1982).

As hypothesized by Bishop, Huggett and colleagues (2006), NO "repression" of settlement may be selectively advantageous in organisms that use a wide range of possible settlement inducers as a way of preventing accidental, precocious, or otherwise inappropriate settlement. On the other hand, taxa with more specific settlement cues may effectively and efficiently rely on a positive "inductive" mechanism to regulate settlement. These data suggest that the utility of NO as a repressor of settlement depends on the precise ecological requirements of the settling larva. Such a scenario points to homoplasy (parallel evolution) rather than to homology of NO utilization in settlement within mollusks, and thus across broader taxonomic scales as well.

Coping with the external environment: settlement, protection, and pollutants

Another commonality among marine (or aquatic) organisms with a settlement phase in their life cycle is that inherent in the change in habitat is an exposure to a novel physical and chemical environment. In particular, a planktonic larva settling to the sea floor would be expected to face exposure to particular environmental chemicals (such as waste products from microbial degradation occurring in benthic sediments) that had not been encountered previously by the larva. How can organisms prepare for unanticipated chemical exposure? There is a cellular mechanism, shared by prokaryotes and eukaryotes, that deals with such situations: multixenobiotic resistance (MXR) efflux transport. These transporters, also known in the health science field as multidrug resistance (MDR) transporters, are ABC-family membrane proteins that rid the cells of a broad range of lipophilic compounds (see Smital and others 2004 for review). Our preliminary evidence (J Hodin, A Hamdoun, and DL Epel, unpublished data) suggests that life stage transitions in echinoderms—such as fertilization (Hamdoun and others 2004), hatching, larval feeding, and settlement—are accompanied by changes in the activity of these transporters. These data support the notion that organisms preemptively protect themselves from novel chemical exposure as they change habitats.

In my neo-Alberchian conception of metamorphosis outlined above (and elaborated below), I assume that cellular signaling systems that are used during the metamorphic transition are likely to have become mechanistically integrated with previously unrelated metamorphic signaling components during the evolution of a more extreme metamorphosis. This notion led me to ask the following question: does efflux transport have a function in the settlement process itself? In other words, if one were to perturb efflux transport activity, would the result be interference with normal settlement?

Thankfully, we have a broad range of efflux transport inhibitors (competitors, steric inhibitors, those of unknown mechanism), varying in specificity, to address this question (see Smital and others 2004). Therefore, I applied various transport inhibitors to precompetent and competent echinoderm larvae to ask if transport-inhibited larvae fail to respond to settlement cues, or if such larvae actually settle inappropriately.

Most of the transport inhibitors that I have tried (MK571, cylclosporin A, verapamil, reversin) had no obvious effect on settlement: inhibited larvae

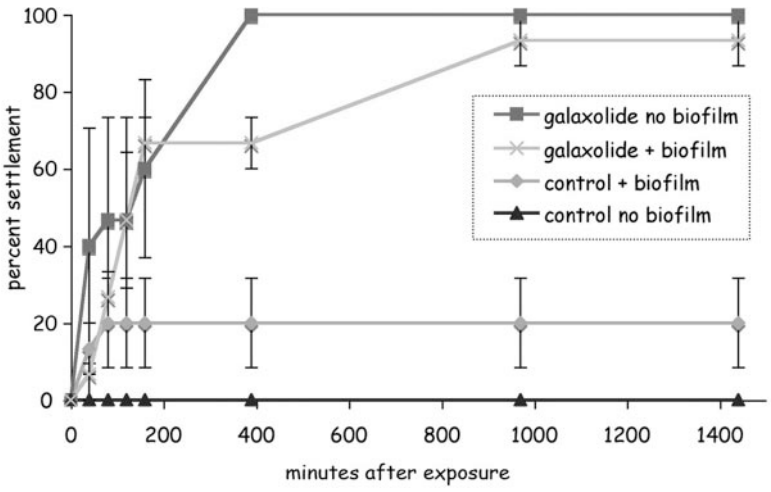

Fig. 5 Precompetent larvae of the purple sea urchin *S. purpuratus* settle when exposed to the synthetic musk galaxolide (HHCB, International Flavors and Fragrances, Inc.), whether or not a natural settlement cue is present. These full-sib larvae were 3 months old at the time of exposure. I had fed them on a combination of *Isochrysis galbana, Rhodomonas lens* and *Nannochloropsis* sp. (3:2:1 cells/μl), with water changes every 2–3 days. The culture was gently stirred using a motor-driven stirring apparatus (Strathmann 1987) at 14°C. The experiment was conducted in 12-well plates, 5 larvae/well, 3 replicates/treatment. Larvae were randomly assigned to treatment conditions as follows: control [0.005% DMSO in 0.1 μM UV-treated filtered seawater (UVFSW2)], galaxolide (5 μM galaxolide in 0.005% DMSO in UVFSW2), no biofilm (washed, untreated 12-well plate), biofilm (4 day incubation of 12-well plate in sea table with many adult *S. purpuratus*). I had chosen these larvae because they appeared competent to settle, but their tepid response to biofilm (control + biofilm) suggests that ~80% of the chosen larvae were precompetent. That these larvae were largely precompetent was apparent by the short or absent spines in some of the musk-induced juveniles. Experiment conducted at Hopkins Marine Station, October 2005. Urchins were from a colony maintained in sea tables at Hopkins Marine Station, originally collected from various locations in southern California (thus, the precise collection locality of the 2 parents in this study is unknown). Spawning was by standard KCl method (see Strathmann 1987). Error bars are standard errors.

responded like controls (data not shown). However, 1 class of compounds that I tested—synthetic musks—efficiently activated settlement both in the absence of settlement cues and in precompetent larvae (Fig. 5).

Synthetic musks, comprising 2 classes of chemicals (polycyclic musks and nitromusks), are human-made fragrances found in colognes, perfumes, soaps, detergents, and other personal care products. These compounds are produced in large quantities (perhaps 5000 or more metric tons/year), are highly persistant, accumulate in organismal tissues, and increase in concentration at higher trophic levels (that is, they biomagnify much like DDT; see references in Luckenbach and Epel 2005). Recently, Luckenbach and Epel (2005) demonstrated that synthetic musks are also potent inhibitors of efflux transport in the mussel *Mytilus californianus* in micromolar or lower concentrations (which approach tissue concentrations in mussels in somewhat polluted areas). Similar concentrations of musks result in precocious settlement in the absence of settlement inducers in the sea urchins *Strongylocentrotus purpuratus, S. droebachiensis, Lytechinus pictus* and the sand dollar *D. excentricus* (for example, Fig. 5). Juveniles can survive and grow for at least 2 months after musk-induced settlement (which is as long as I have kept them), suggesting that the settlement response is not merely a toxic effect. Indeed, musks induce stereotyped behavioral responses associated with settlement in *L. pictus* (C Bishop and J Hodin, unpublished data), providing further evidence against a nonspecific effect of musks on induction of settlement.

I have confirmed that musks are inhibitors of efflux transport in sea urchin larvae using the calcein-AM method described by Hamdoun and colleagues (2004) for sea urchin embryos (data not shown). Indeed, those musk compounds (both polycyclic musks and nitromusks) that are the most potent settlement inducers also show the greatest degree of transport inhibition by the calcein-AM method. However, the fact that none of the other tested inhibitors showed settlement effects appears to argue against efflux transport as the explanation for the observed effect of these musks on settlement. Interestingly, musks seem to only inhibit transport effectively in echinoid larvae, but not in their embryos (data not shown).

This finding raises the possibility that musks inhibit a specific subset of transporters only found in later developmental stages, thus possibly accounting for the negative settlement data from other known transport inhibitors. Recently, I have obtained preliminary evidence that caulerpenyne, a toxic compound from the invasive green alga *Caulerpa taxifolia*, induces settlement in a manner very similar to that of musks (and at comparable concentrations; data not shown). Furthermore, my preliminary evidence suggests that caulerpenyne is also an efflux-transport inhibitor in echinoid embryos and larvae (calcein-AM method, data not shown).

Clarifying the possible role of efflux transport in echinoderm settlement clearly awaits further study. Nevertheless, these results raise the possibility not only that this highly conserved cellular defense mechanism may be involved in settlement processes across taxa, but also that certain human pollutants (such as musks and other efflux inhibitors, for example, some pesticides) may be having unrecognized impacts on life stage transitions in aquatic organisms (Kurelec 1997). An extreme scenario is that polluted areas may be actually attracting certain planktonic larvae to settle in these totally inappropriate locations. We are currently designing experiments to test such possibilities.

These results with natural toxins from invasive species and pollutants have an additional ecological implication. Life stage transitions—such as fertilization, metamorphosis, settlement, and reproductive maturation—may be especially sensitive periods to environmental toxins and pollutants. This seems likely since such life history transitions are characterized by extensive communication with the external environment (Hatle 2003). As such, conservative toxicological studies should probably evaluate the effects of relevant compounds on organismal life stage transitions; currently, toxicological studies focus mainly on effects within a given life stage, such as embryo or adult.

Feature #3: The morphological change at metamorphosis is connected to the habitat shift. But how?

It is not surprising that a shift in habitat is often connected to a change in morphology: new habitats present new challenges for organisms, thus providing potent selective pressures for a change in morphology (as well as behavior) as the organism shifts between habitats (see also Fig. 1 and legend). Let us consider the case of a swimming planktonic marine invertebrate larva seeking a place to settle, and changing into a deposit-feeding, benthic adult. The larva needs to maintain locomotory structures and the sensory apparatus used to find an appropriate settlement site, after which these structures are no longer required. Furthermore, the juvenile will need a major remaking of its feeding mechanics and body structure in order to effectively exploit the postsettlement habitat.

Alberch (1989) realized that the manifold events occurring in and around the time of the habitat shift represent an evolutionary compression of developmental sequences into a shortened window of time (see Fig. 2). As I have outlined above, the metamorphic events to which Alberch referred are known to be regulated—across wide phylogenetic distances—by 2 classes of signaling molecules: hormones in the case of the morphogenetic changes, and NO in the case of the shift in habitat.

Here I would like to add a corollary to Alberch's hypothesis, an addendum that will include our current understanding of metamorphosis and settlement in the phenomenological conception proposed by Alberch. This corollary depends upon the following assumption: when signaling molecules from diverse signaling systems coincide in space and time, the result will be an integration of the signaling components into a single, cross-regulatory signaling architecture. Although the concept of integration has not, to my knowledge, been specifically considered in the context of metamorphosis in the past, the relevance seems apparent.

Phenotypic integration and evolutionarily stable configurations: A hypothesis for how metamorphic networks expand in parallel

The concept of phenotypic integration has recently been considered in some detail as leading to what has been termed an "evolutionarily stable configuration" (ESC; Wagner and Schwenk 2000; Schwenk and Wagner 2001). Key components of an ESC are as follows: (1) strong functional and anatomical relationships among component parts; (2) selection for this integration of parts is internal, in that the selection pressure for maintaining the ESC is intrinsic to organismal function; (3) the ESC remains intact across a range of environments; (4) since origin and escape from ESCs are presumed to be relatively rare, they should be found in large clades (high taxonomic levels) or large parts of it; that is, the distribution should not be phylogenetically haphazard; and (5) variation in the ESC is possible within certain limits—in this way, ESCs are hierarchically organized in ways that permit variation in subprocesses while maintaining the functionality of the entire system.

Specifically with respect to the ESC concept, metamorphosis shares the features of having (1) strong functional and anatomical connectivity; (2) presumed selection for coordination of the various subprocesses; (3) functional integrity in a range of environments (for example, marine invertebrate larvae need to be able to accomplish the transition despite variations in environmental conditions such as temperature, currents, and wave action, larval food, complex cocktails of environmental chemicals; some fungi (Georgiou and others 2006) and amphibians (Newman 1992) complete metamorphosis during particularly stressful conditions, such as habitat drying; some plants flower in response to day-length cues despite variation in other climatic conditions; (4) metamorphosis is a dominant feature of higher level taxonomy; and (5) variation in the metamorphic ESC, as in the loss of larval feeding, still maintains elements of the core metamorphic network (as in hormonal regulation of nonfeeding larval development in echinoids; see above).

Therefore, I hypothesize that metamorphoses in various unrelated taxa are examples of ESCs. To evaluate this hypothesis, let us reexamine Alberch's figure with this assumption of integration of units in mind (see Fig. 2). The symbols referring to the kinds of events that happen at metamorphosis (plus, open hexagon, striped triangle, and open square) as well as those that he does not show (the destruction of the larval specific structures—closed diamond and closed circle) are each regulated by a unique (if overlapping) set of signaling processes, including growth factors, tissue-specific transcriptional regulators, cell-death machinery, possibly efflux transport, and so on. The compression of these events into a relatively short developmental time (shaded oval in Fig. 2) implies that these signaling events are taking place simultaneously. If the organisms in question were infinitely modular, then one would not expect any interactions among these different ontogenetic processes. We know, however, that this is not the case. Metamorphosis, like embryonic development, necessitates tight coordination among diverse ontogenetic processes. Or, put another way, to insure the fidelity of development, the various processes that occur during ontogeny need to be carefully coordinated, both spatially and temporally. The result is selection for integration in the signaling components underlying these diverse processes thereby maintaining a stable output of ontogeny (for example, van Dassow and others 2000). Furthermore, the more temporally and spatially overlapped the ontogenetic processes are, the more integration one would predict to be apparent.

Therefore, the more "dramatic" the morphogenetic change that occurs at metamorphosis, the more substantial is the overlap one would expect among these various ontogenetic and signaling processes, resulting in a more integrated network of interacting components. In fact, we have a rather good idea of one of the key factors that maintains this integration during metamorphic change: hormone action (reviewed in Heyland and others 2005). The best-studied cases here are amphibians and insects, but data from plants and marine invertebrates suggest a similar function for hormones in maintaining integration of the ontogenetic processes occurring during metamorphosis (see Feature #2 section, above).

In the case of insects, comparative data on the hormonal regulation of metamorphosis by JHs and ecdysteroids suggest 2 features of hormones related to this concept of integration. First, tissue-specific differences in the presence of specific hormone receptors and/or in localized hormone metabolism can account for how one broad hormonal peak can coordinate the wide diversity of morphogenetic events that occur in and around the metamorphic period (Truman and others 1994; Hodin and Riddiford 1998, 2000). Second, such localized effects can account for observed evolutionary differences in the timing or progression of certain metamorphic events, while maintaining integration of the overall ontogenetic process.

Recent evidence suggests that a further level of integration during metamorphosis is apparent in the direct interaction among the signaling systems regulating morphogenetic change and those controlling the habitat shift. Specifically, 2 studies have now shown a direct interaction between THs and NO signaling during metamorphosis. The first example is from amphibians, in which the habitat shift is rather gradual. Still "metamorphic climax" in anurans (frogs and toads) is generally defined as the time when the tadpole tail is shortened and removed by programmed cell death. This morphological change corresponds quite well with the aquatic-to-terrestrial transition, so I will here consider it to be the analog of "settlement" in marine invertebrates. This process of tail shortening has been known for a long time to be stimulated by high circulating TH levels (reviewed in Denver and others 2002). More recently, Kashiwagi and colleagues (1999) showed that TH promotes tail shortening via activation of NO synthase (NOS) in the leopard frog *Rana pipiens*.

The second example of a direct interaction between metamorphic TH and NO signaling comes from data reported in this symposium by Bishop, Huggett and colleagues (2006). As others and we have shown

previously (and as I outlined above), THs accelerate metamorphosis in echinoderms in a manner analogous to the canonical TH effects on amphibians. Also discussed above, Bishop and Brandhorst (2001, 2003) demonstrated that NO and cGMP signaling represses settlement in the banded sea urchin *L. pictus*. Bishop, Huggett and colleagues (2006) have shown that TH is directly antagonistic to NO signaling, as evidenced by (1) TH-induced decreases in the NOS-immunopositive neuronal arborization thought to be responsible for regulating settlement; and (2) TH-induced settlement in response to subthreshold level pharmacological NO inhibition, in the absence of a cue (controls did not settle in response to this NO concentration).

Interestingly, these 2 examples (amphibians and echinoderms) represent opposing effects of TH on NO signaling. I suggest that this finding provides additional support for 2 ideas: (1) that TH-regulated metamorphosis evolved independently in these taxa; and (2) that the evolution of more and more extreme metamorphic patterns (see Fig. 2 and explanation) are characterized by increasing integration in underlying signaling pathways, although the specifics of the integrated network would be predicted to be different in independently evolved metamorphoses.

Holometabolous insects represent a parallel case, where pupal development is the longer-term morphogenetic stage of metamorphosis while adult eclosion is the rapid habitat shift. The morphogenetic hormone 20-hydroxyecdysone (20-E) is known to orchestrate pupal development, and falling levels of 20-E result in activation of adult eclosion via cGMP (but apparently not NO) activity (Gammie and Truman 1999). [Interestingly, there is a direct, antagonistic interaction between ecdysteroids and NO repression during insect metamorphosis, but apparently not at eclosion. Instead, such an interaction regulates adult eye morphogenesis in the tobacco hornworm *Manduca sexta* (Champlin and Truman 2000).]

Here, then, is another independent case where similar signaling systems are integrated in their cross regulation of analogous phases of metamorphosis. I hypothesize that such integration will be found in other disparate cases of metamorphosis as well. Specifically, I hypothesize a functional connection between florigen and NO signaling in plant flowering as well as TH and NO signaling in sea squirt (Chordata: Tunicata) metamorphosis and settlement. In sea squirts, though, a critical variation would be to investigate the nature of this connection in colonial and social species with mostly presettlement morphogenesis, in addition to solitary species that undergo mainly postsettlement morphogenesis (Cloney 1987). Comparative studies in other metamorphic taxa—invertebrate, noninvertebrate, and nonanimal—will help determine exactly how widespread these parallels are in the signaling architecture underlying metamorphoses across taxa.

One key question to be addressed by such comparative studies, both within and across taxonomic groups, is the following: how can we reconcile the substantial evolutionary flexibility in the identity and specificity of settlement cues with the more tightly conserved metamorphic process itself? I suggest that a detailed understanding of the network of interacting components underlying disparate metamorphoses will be a precondition for addressing this question. I conclude this paper with a sketch of what such a network model might look like for echinoderms with different life history patterns.

Expanding (and contracting) networks

Based upon the published and unpublished data for echinoderms outlined above, I outline the following network model for the signaling systems known or hypothesized to be involved in settlement and metamorphosis (Fig. 6). Most of these data come from work on echinoids (sand dollars, sea biscuits, and sea urchins), along with some comparative data on THs in asteroids (sea stars). Evidence for the inhibitory effect of THs on NO signaling is reported by Bishop, Huggett and colleagues (2006).

We here consider 2 life history characters: feeding mode (planktotrophy or lecithotrophy; see the legend of Fig. 6 for definitions) and specificity of the cue for settlement (specialist or generalist). Together, these 2 characters, each with 2 states, total 4 metamorphic types (Fig. 6A–D).

One example of a planktotrophic specialist (Fig. 6A) is the sand dollar *D. excentricus*, which has a feeding larva that settles in response to sand conditioned with adult sand dollars. Unpublished results of C. Bishop (personal communication) suggest a reduced function for NO/cGMP in *D. excentricus* settlement, but the results of Heyland and Hodin (2004) and Bishop, Huggett and colleagues (2006) indicate that TH increases the likelihood of spontaneous settlement in this species. A likely example of a planktotrophic generalist (Fig. 6B) is the banded sea urchin *L. pictus*, with its robust NO repressive network having been well characterized (Bishop and Brandhorst 2001, 2003). A plausible example of a lecithotrophic specialist (Fig. 6C) is the nonfeeding larva of the Australian thickened sea urchin *Holopneustes purparascens*, which settles only in

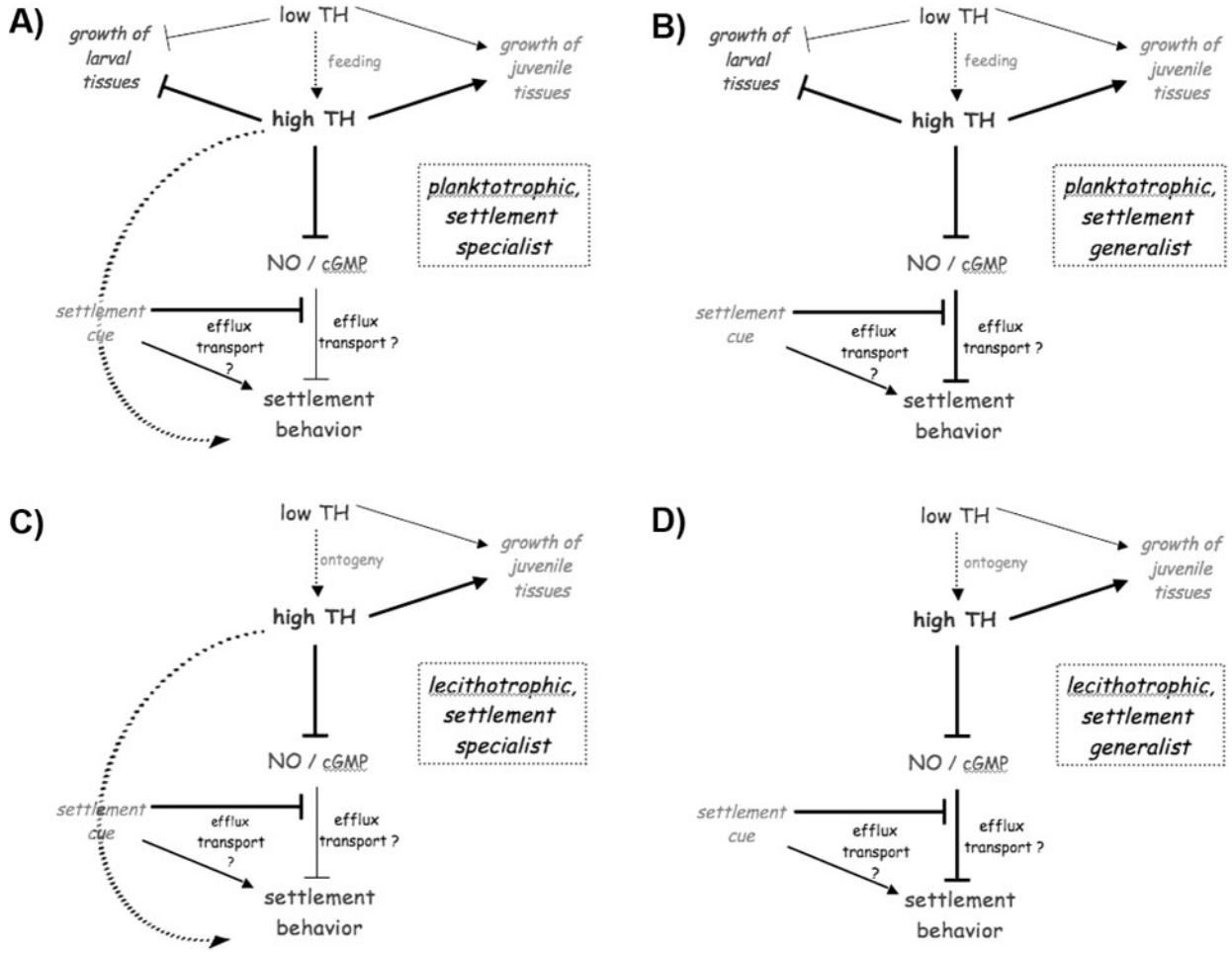

Fig. 6 Hypothesis for a metamorphic/settlement network in echinoderms. The specifics are based upon some data (most of it described herein, as well as by Bishop, Huggett and others 2006; Heyland and others 2005) and some speculation. In this network model, the strength of the connection between the different network elements is indicated by the darkness of the lines; arrows indicate positive (stimulatory) interactions, blunt ends indicate repressive interactions. The dotted curved line in A and C is meant to indicate 2 things for settlement specialists: (1) that TH seems to be connected to the attainment of competence and hence the ability to respond directly to settlement cues (without a major NO repressive function); and (2) that experimental augmentation of TH results in overloading the system and thus activating spontaneous settlement. The settlement cues themselves often vary widely, even across quite closely related taxa. One aspect of the functioning of this network that remains to be clarified is how evolutionary changes in these settlement cues are incorporated into an otherwise seemingly stable core metamorphic network. In this figure, I introduce 2 typical terms in invertebrate biology. "Planktototrophy" is roughly equivalent to "feeding larval development," and is strictly defined as the inability to complete metamorphosis without exogenous food. "Lecithotrophy," then, is defined as the ability to complete metamorphosis and settle in the total absence of food (definitions *sensu* McEdward and Janies 1997). Note that some "feeding larvae" (such as in the heart urchin *Brisaster lattifrons*) (Hart 1996) are lecithotrophic by this definition; these larvae are also known as "facultative planktotrophs," "facultative feeders," or "functional lecithotrophs." The distinction between planktotrophy and lecithotrophy is important for these network models, since functional lecithotrophy indicates the ability to synthesize all necessary THs endogenously (see text). I developed this model in collaboration with Cory Bishop; we first presented it as an unofficial poster entry at the 2005 Society for Integrative and Comparative Biology meetings in San Diego, CA, USA.

response to a histamine from its host alga (Williamson and others 2000; Swanson and others 2004). The lamp urchin *C. caribbearum* (see the text and Fig. 3) seems to be a good example of a lecithotrophic generalist (Fig. 6D), since their larvae will readily complete metamorphosis in clean dishes with no sand. Not included in the figure are the "extreme brooded" larvae of some echinoderms, such as *L. hexactis* (see above). This species is apparently not dependent on THs to make a functional juvenile (see Fig. 4), and thus seems to be a rare example of an "escape" from its ESC (*sensu* Wagner and Schwenk 2000, see below).

Adopting an evolutionary view of these hypothesized metamorphic signaling networks will help

illustrate what I intend to indicate with the title of this paper: "expanding and contracting networks." As Alberch (1989) hypothesized (see Fig. 2), the origin of metamorphosis in various taxa is most reasonably thought of as a compression of morphogenetic events into a small developmental window. Incorporating Wagner and Schwenk's (2000) concept of ESCs leads me to imagine the origin of metamorphosis as being associated with an expanding network of integrated signaling systems. As the metamorphic process gets more extreme, the components of, and connections within, the network continue to expand. The core network, then, persists throughout metamorphic clades, allowing certain variations while still maintaining the integrity of the overall process. Furthermore, the number and strength of interacting components could contract under certain selective scenarios (such as in the derived evolution of nonfeeding larval development). Under extreme conditions, such as a holobenthic brooding life history and/or holobenthic encapsulated direct development, the network could dissolve as the taxon escapes from the metamorphic ESC.

The example I presented of such a dissolution—the metamorphic network in the brooding sea star *L. hexactis*—warrants some special consideration. When the morphogenetic program is simplified such that the larval form is reduced to a mere "phantom" (*sensu* Okazaki and Dan 1954), then the ontogenetic program can begin to escape from its ESC via contraction of the network, and ultimately release of hormonal regulatory control. The reverse also appears to be true: namely, that the expansion of networks during the evolution of more rapid and profound metamorphosis requires the regulatory control of diverse cellular and morphogenetic processes that hormonal signaling provides so well (for example, Nijhout 1994).

Furthermore, when the life cycle dictates a significant shift in habitat, selection repeatedly favors a situation in which the postmetamorphic form is rapidly revealed upon entry into the new habitat. Such a pattern is seen in the multiple independent examples of marine invertebrate metamorphosis (I previously mentioned ribbon worms, mollusks, and echinoderms), as well as adult eclosion in holometabolous insects, fruiting in mushrooms, and flowering in plants. Such a binary process that needs simultaneously to be responsive to environmental cues, and be faithfully executed despite substantial environmental variation, is ideally suited to utilize NO repressive signaling (Bishop and Brandhorst 2003). The process of integration towards an ESC involves establishing enhanced connectivity between, and among, the hormonal and NO regulatory subsystems into an expanded, integrated, stable network. This, then, is a plausible explanation for the parallel evolution of hormonal and NO signaling at metamorphosis in disparate animal and nonanimal taxa.

Detailed examinations into TH, NO, efflux transport, and other signaling pathways in echinoderms with a range of metamorphic patterns will allow us to evaluate the accuracy of this vision of an expanding and contracting ESC metamorphic network. Nevertheless, the true test of this concept will come from broadly comparative studies beyond the Echinodermata: namely, investigations into the signaling networks within several disparate metamorphic taxa that show comparable variations in life history patterns (see also Heyland and Moroz 2006, papers presented at meetings). This approach must be thoroughly integrative, involving genomics, cell biology, physiology, classic developmental biology, genetics, and ecology in a comparative evolutionary context. Such broad integration is both the challenge and the promise of twenty-first century biology.

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