CHAPTER FOUR

Extreme phenotypic divergence and the evolution of development

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Contents

Abstract

As analyses of developmental mechanisms extend to ever more species, it becomes important to understand not just what is conserved or altered during evolution, but why. Closely related species that exhibit extreme phenotypic divergence can be uniquely informative in this regard. A case in point is the sea urchin genus *Heliocidaris*, which contains species that recently evolved a life history involving nonfeeding larvae following nearly half a billion years of prior evolution with feeding larvae. The resulting shift in selective regimes produced rapid and surprisingly extensive changes in developmental mechanisms that are otherwise highly conserved among echinoderm species. The magnitude and extent of these changes challenges the notion that conservation of early development in echinoderms is largely due to internal constraints that prohibit modification and instead suggests that natural selection

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actively maintains stability of inherently malleable trait developmental mechanisms over immense time periods. Knowing how and why natural selection changed during the evolution of nonfeeding larvae can also reveal why developmental mechanisms do and do not change in particular ways.

1. Introduction

Developmental mechanisms, like any other set of functionally integrated traits, are continuously shaped by evolutionary processes. While technological advances have enabled detailed dissection of developmental mechanisms in a rapidly expanding range of interesting species, understanding *why* particular features of development change or are conserved remains a challenge. To be sure, a growing set of examples demonstrates that natural selection can alter specific features of development to produce adaptations in organismal traits (e.g., Chan et al., 2010; Gompel, Prud'homme, Wittkopp, Kassner, & Carroll, 2005; Hines et al., 2011). But these cases of positive selection (a new genotype eventually replaces the original one because it increases fitness), is just one way that evolutionary processes act on traits, and almost certainly not the normal mode. Negative selection (the elimination of unfavorable mutations) and drift (fixation of new mutations by chance) are likely to be far more pervasive influences on the evolution of developmental mechanisms, just as they are on traits more generally.

Inferring the relative influences of drift, negative selection, and positive selection is challenging for any trait. Yet this information is essential for understanding why traits do and do not change during the course of evolution. For instance, early embryonic patterning mechanisms are often highly conserved within major clades of animals. This broad conservation is sometimes interpreted as the result of internal constraint, the inability to change a particular developmental process without severely disrupting other ones. Such constraints are thus viewed as the product of unavoidable strong negative selection against any trait variation. To the extent that this interpretation is correct, it implies that some developmental mechanisms are impervious to drift and positive selection. But is that actually the case and how can one test such a claim?

Fortunately, the history of life contains a great variety of natural experiments where evolutionary processes have remained constant or shifted in ways that expose how developmental mechanisms respond. This chapter illustrates how comparative analyses can leverage such natural experiments

to understand the relative influences of drift, negative selection, and positive selection on developmental mechanisms, as well as how these influences change over time and among lineages. The focus is on the sea urchin genus *Heliocidaris*, within which an ancient life history strategy has recently changed, triggering a cascade of profound changes in a surprisingly broad range of organismal traits. Due to its phylogenetic context and evolutionary history, *Heliocidaris* provides a window into how natural selection shapes the evolution of developmental mechanisms through its influence on organismal traits.

2. Studying developmental evolution

2.1 Evolutionary context matters

Clades do not evolve in a vacuum. Understanding the distribution of developmental features among extant species, as with any other kind of trait, requires knowing something about the evolutionary history of that clade. The importance of phylogenetic and temporal context is widely appreciated: an accurate understanding of phylogenetic relationships is essential for reconstructing the polarity of trait changes and for identifying any reversals and parallel changes, while divergence times reveal the duration of conservation and rates of change in traits of interest.

Information about evolutionary mechanisms is an equally important, but perhaps less widely appreciated, aspect of context. Drift and natural selection operate continuously but they do not do so in a uniform manner. Instead, the influence of these two fundamental processes is in a constant state of flux. At a microevolutionary scale, the vast majority of mutations are context-dependent: their trait consequences, impact on fitness, and probability of eventual fixation will all differ depending on genetic background, population size, and biotic and abiotic environment. As these factors change over time, what was once a beneficial trait can become deleterious and vice-versa. Knowing something about how drift and natural selection may have changed over time and along different lineages within a clade of organisms is essential for understanding the distribution of traits in extant species.

2.2 Canonical approaches in evo-devo

From the perspective of evolutionary context, two general approaches dominate studies of the evolution of developmental mechanisms. The first

involves comparing species that are phylogenetically separated by tens or hundreds of millions of years (MY) of evolution (Fig. 1A). In the vast majority of such cases, it is possible to identify evolutionary differences in both developmental processes and in organismal traits such as anatomy, physiology, or behavior. While this means that there are plenty of interesting evolutionary differences to examine, the large evolutionary distance poses practical problems. It is often challenging to relate specific developmental features to specific organismal traits, simply because so many changes have evolved since the species last shared a common ancestor and these can obscure the genetic and mechanistic basis for traits of interest. While it is often possible to identify orthologous genes across even deep phylogenetic distances, the same is generally not true of regulatory elements, which diverge rapidly and often contain more structural rearrangements. This is problematic because the genetic basis for trait evolution often includes a substantial component of non-coding mutations that influence interactions between transcription factors and regulatory elements.

The second common approach to studying the evolution of development involves comparing closely related species, with divergence times generally <10 MY (Fig. 1B). In these cases, the practical problems just mentioned are typically much less severe. Identifying homologous traits and orthologous genes is generally straightforward, and the orthology of individual regulatory elements can often be established with confidence based on a combination of sequence and synteny. In addition, closely related species can sometimes be hybridized, allowing for genetic analyses. There is, however, an important disadvantage that comes with examining closely related species: organismal traits are typically very similar and development is often even more so. Thus, while more regulatory interactions within the noncoding genome are open to direct computational and experimental analyses, the few differences that exist in development are generally subtle and less interesting than the ones that are evident across deeper phylogenetic divides.

These complementary strengths and weaknesses have naturally led to different questions being addressed at the two evolutionary scales. Comparisons among distantly related species have tended to focus on identification of conserved features, particularly in embryos; differences are often interpreted in relationship to major morphological differences such as body plans or central nervous system organization. Comparisons among closely related species, in contrast, more commonly examine functional change in the noncoding genome or utilize genetic approaches to understand differences in development; when organismal traits are examined, they

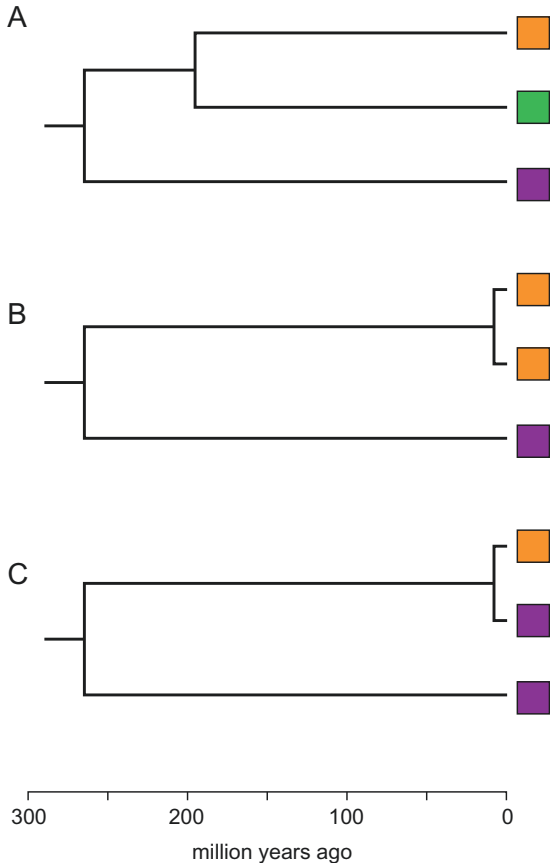

Fig. 1 Contexts for studying the evolution of development. Divergence times and trait diversity determine what can be learned from comparisons of developmental mechanisms among species. (A) Distantly related species with highly divergent organismal traits (different colored boxes) generally reveal numerous underlying differences in development. In such cases, however, it can be challenging to relate specific differences in development to specific organismal trait differences due to the lengthy divergence times separating species. (B) Closely related species generally reveal far fewer and more subtle differences in organismal traits (orange boxes) and the same is true of development. On the other hand, the few differences that do exist are more tractable to direct comparison due to the recent divergence. (C) Cases of extreme recent phenotypic divergence following long prior conservation (orange versus purple boxes) combine the advantages of the previous two evolutionary contexts, providing several practical advantages and a useful framework for understanding how evolutionary mechanisms shape developmental evolution (see text).

are typically ones that tend to evolve quickly and are derived from post-embryonic development, such as pigmentation or quantitative changes in morphology.

2.3 A third approach: Extreme biology

Although less common, a third approach effectively combines the strengths and minimizes the weaknesses of the two just discussed. This third approach leverages extreme phenotypic divergence among very closely related species following a long prior period of conservation (Fig. 1C). The tree of life contains many such cases, where an abrupt shift in natural selection has driven dramatic adaptive change within a lineage over a relatively short period of time. A familiar example is our own evolutionary history: climate change drove our ancestors out of the forest and onto the savanna where climate, resources, predators, and diseases all differed enormously. The resulting shifts in natural selection rapidly altered our anatomy, physiology, cognition, behavior, and life history in profound ways. Yet our genome remains highly similar to that of chimpanzees. This genomic similarity has allowed detailed analyses of coding and noncoding loci and identification of numerous individual mutations that alter our development in ways that can be directly related to specific organismal traits of interest (e.g., Boyd et al., 2015; Gokhman et al., 2021; Prabhakar et al., 2008).

This and other cases of recent and extreme phenotypic divergence provide valuable windows into how developmental mechanisms respond to large shifts in selective regimes. Practical advantages associated with examining any set of closely related species were mentioned earlier. In addition to these are two advantages specifically associated with extreme trait divergence. Both derive from the fact that most organismal traits are likely to remain similar between closely related species, while the few that have diverged to a great degree are more likely to be the product of the same recent change in natural selection. This simplifies the challenging process of linking a particular change in development with a specific organismal trait. It also means that careful examination can reveal potentially co-adapted suites of organismal traits that might not be obvious from first principles.

These are all practical advantages. But there is also an important intellectual advantage of studying closely related species with extreme trait divergence, namely the ability to understand how evolutionary mechanisms shape development. Why do some regulatory interactions persist over

enormous spans of time, while others change rapidly or frequently? Why do some changes in development produce changes in organismal traits while others do not? And why are some features of development evolutionarily variable within one clade but not another?

Cases of recent and rapid trait divergence can provide answers to these questions because they generally involve a change in selective regimes that is both large in magnitude and can be related to specific organismal traits. For example, panda bears are the product of a recent evolutionary shift in diet from carnivory to herbivory with extreme specialization on bamboo, a plant of low nutritional value. Diverse organismal traits have responded to this enormous shift in selective pressures, including anatomical specialization in the hands, an elongated digestive tract, low fecundity, and (less obviously) pseudogenization of a specific taste receptor (Hu et al., 2017; Wei et al., 2012). It is plausible to attribute most of these trait changes directly to the shift in diet, in part because they are not found in related species that retain the ancestral condition of carnivory, and in part because they make sense in terms of known functional demands imposed by shift in selective regimes. Further, because the change in diet was recent, most organismal trait changes are likely a direct consequence of selection for herbivory rather than functions unrelated to diet.

Stated more generally, if natural selection has recently operated in very different ways on two closely species, a larger fraction of the overall trait differences that distinguish those species will be the product of that particular difference in natural selection than would be the case if it had happened in the distant past. The reason is that major shifts in natural selection are rare but when they do occur, they can have rapid and profound effects on a diversity of what are normally evolutionarily conservative traits. This means that trait differences between species will initially be enriched for those caused by the major shift in selection but that over extended time these will be diluted by subsequent, unrelated trait changes. Importantly, the same holds for the underlying developmental changes that produce the altered organismal traits. This approach can be applied to cases where: (1) there has been a major change in natural selection, (2) closely related species represent the ancestral and derived conditions, and (3) the cause of the change in natural selection is clearly defined.

Life history shifts are particularly attractive cases of extreme phenotypic divergence for several reasons. First, the changes in natural selection are well defined. A mature body of theory and extensive empirical data provide insights into the fitness costs and pleiotropic consequences of evolutionary

changes in key life history traits such as maternal investment and fecundity (Flatt & Heyland, 2011; Stearns, 1992). If one of these traits is altered by natural selection, it is often possible to predict which other traits will be affected and how they will respond. Second, parallel changes in life history have evolved on multiple occasions. It is therefore possible to leverage the power of replicate evolutionary transitions to help distinguish causal changes in developmental from incidental ones. Third, and perhaps less obviously, life history shifts can have a major impact on developmental mechanisms, including those that operate during embryogenesis. The reasons for this impact are discussed below, but the important point here is simply that a life history shift acts as a natural "perturbation experiment" on development, revealing features of development that are capable of evolving in adaptive ways.

3. Life history and the evolution of development

3.1 Life history evolution in echinoderms

Echinoderms have evolved a remarkable diversity of life histories. Most species produce many small eggs that develop into a morphologically complex feeding larva that consumes phytoplankton for several weeks before gaining sufficient mass to complete metamorphosis (Emlet, McEdward, & Strathmann, 1987; McEdward & Janies, 1997). Although larval feeding (planktotrophy) allows for production of many small eggs, mortality is severe during the larval phase, with estimates on the order of 10–20% per day (Morgan, 1995). Thus, while a single female may shed 10^4–10^6 eggs, only a tiny fraction of these survive the rigors of the planktonic phase to successfully complete metamorphosis. On numerous occasions, echinoderms have exchanged this high fecundity, high mortality strategy for lower fecundity and lower mortality (Emlet et al., 1987; McEdward & Janies, 1997). These species produce many fewer but much larger, nutrient-rich eggs that develop into nonfeeding larvae (lecithotrophy) that typically reach metamorphosis much more quickly than related planktotrophs. This shift in developmental mode involves a classic life history trade-off between maternal investment per egg and the overall number of eggs produced, with attendant changes in stage-specific mortality.

Phylogenetic analyses indicate that planktotrophy is the ancestral life history within echinoderms (McEdward & Miner, 2001; Strathmann, 1988; Wray, 1996) (Fig. 2). Lecithotrophy has evolved on dozens of separate occasions across a wide range of groups (Emlet et al., 1987; McEdward & Miner, 2001). Although the precise reasons for the switch from

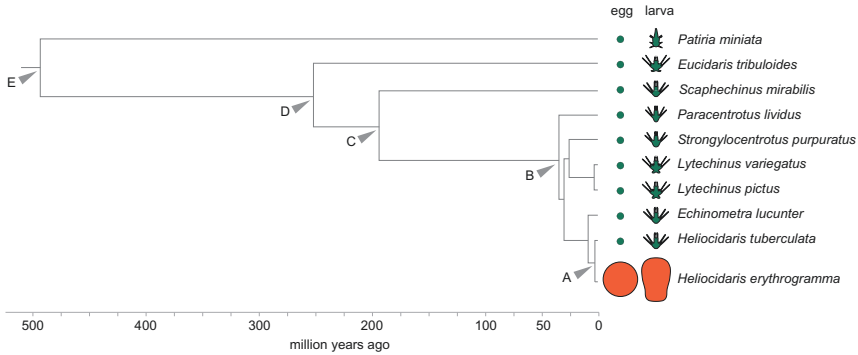

Fig. 2 Evolution of life history and dGRNs within echinoderms. Most echinoderms produce small (~90–120 μm diameter) eggs that develop into feeding larvae (green), but a few have independently evolved to produced large (~250–2000 μm diameter) eggs that produce nonfeeding larvae (orange). Eggs and larvae are shown approximately to scale. Feeding is the ancestral state and has persisted in many lineages for nearly half a billion years. The genus *Heliocidaris* contains species representing the ancestral and derived state. Because these species diverged just a few million years ago (arrowhead **A**), the shift in life history must have evolved quite recently relative to prior conservation of the ancestral state. The dGRN that patterns the early embryo to produce a feeding larva has been studied in multiple echinoderm species, including those shown here. In camarodont sea urchins with the ancestral life history, nearly all tested regulatory interactions remain intact, implying conservation of ~40 MY (arrowhead **B**). The dGRNs of more distantly related sea urchins contain some but not all of these interactions, indicating conservation of specific interactions for ~190 or ~250 MY (arrowheads **C** and **D**). In other echinoderm groups such as sea stars and sea cucumbers a few interactions appear to be conserved although many differ (arrowhead **E**). Differing degrees of evolutionary conservation among the dGRNs of these species provide a useful context for understanding how evolutionary mechanisms act to preserve and alter specific developmental mechanisms.

planktotrophy to lecithotrophy are not well understood, nonfeeding development is likely favored when phytoplankton is not a reliable source of energy for larvae (Emlet et al., 1987; Marshall, Krug, Kupriyanova, Byrne, & Emlet, 2012). What is clear is that in many cases, lecithotrophy has evolved quite recently in relation to the long prior history of planktotrophy (Hart, 2002; Hart, Abt, & Emlet, 2011; Hart, Byrne, & Smith, 1997; Jeffery, Emlet, & Littlewood, 2003; Smith, Boom, & Raff, 1990) (Fig. 2).

3.2 Developmental evolution within the ancestral life history

This phylogenetic framework provides a useful context for interpreting the evolution of development within echinoderms. Comparative studies have used as a framework the well-defined developmental gene regulatory

networks (dGRN) of sea urchins (Angerer et al., 2000; Ben-Tabou de-Leon, Su, Lin, Li, & Davidson, 2013; Davidson et al., 2002a; Davidson et al., 2002b; Materna, Ransick, Li, & Davidson, 2013; Oliveri, Tu, & Davidson, 2008; Peter & Davidson, 2011; Sethi, Angerer, & Angerer, 2009; Sethi, Wikramanayake, Angerer, Range, & Angerer, 2012; Su et al., 2009).

Initial studies identified a "kernel" of specific dGRN interactions conserved between a sea urchin and a sea star, as well as adjacent interactions within the dGRN that are not conserved (Hinman & Davidson, 2007; Hinman, Nguyen, Cameron, & Davidson, 2003). Subsequent studies extended these findings to sea cucumbers and brittle stars and to different parts of the dGRN (Dylus, Czarkwiani, Blowes, Elphick, & Oliveri, 2018; McCauley, Weideman, & Hinman, 2010; McCauley, Wright, Exner, Kitazawa, & Hinman, 2012). These studies provided some of the first clear examples from any group of animals for conservation of specific dGRN interactions across very deep time scales, as the fossil record provides direct evidence for divergence among these groups of echinoderms nearly 500 MY ago (Smith, 1988).

The interactions that establish the primary signaling center of the embryo have been studied across a range of time scales, providing an unusually detailed view of evolutionary conservation and change in some of the earliest patterning events of the embryo (Dylus et al., 2018; Erkenbrack, 2016; Erkenbrack & Davidson, 2015; Erkenbrack, Davidson, & Peter, 2018; Thompson et al., 2015; Thompson et al., 2017; Yamazaki et al., 2020; Yamazaki, Kidachi, & Minokawa, 2012; Yamazaki, Kidachi, Yamaguchi, & Minokawa, 2014). These studies have revealed the stepwise evolutionary assembly of a novel set of regulatory interactions that specify the skeletogenic cell fate. This has involved the recruitment two different transcription factors, Pmar1 and HesC, into the very early zygotic portion of the dGRN at two different times during the evolutionary history of sea urchins, the first >250 MY ago and the second between 250 and 190 MY ago. These evolutionary "rewiring" events are remarkable in that they involve the earliest cell fate decisions and are involved in establishing the primary signaling center of the sea urchin embryo.

Sea urchins belonging to the group Camarodonta have been particularly well studied because a variety of species are easily collected in different parts of the world. These species diverged on the order of 30–40 MY ago, providing a window into much shorter time scales than those mentioned above. Developmental transcriptomes are highly conserved among

these species (Gildor & Ben-Tabou de-Leon, 2015; Israel et al., 2016; Malik, Gildor, Sher, Layous, & Ben-Tabou de-Leon, 2017; Massri et al., 2021). Numerous regulatory interactions within the dGRN have been experimentally tested among species, and nearly all are conserved (reviewed in McClay, 2011).

Overall, these studies suggest that the sea urchin dGRN is generally well conserved, with a subset of key interactions intact over time scales of hundreds of MY and most interactions present across time scales of tens of MY. However, all species examined in the studies just discussed have the ancestral life history: small eggs, high fecundity, and a complex feeding larva. What happens to the dGRN in species with derived life histories?

3.3 Rapid evolution of development within *Heliocidaris*

The sea urchin genus *Heliocidaris* includes species that encompass both feeding and nonfeeding development despite having diverged <10 MY ago (Hart et al., 2011; Smith et al., 1990; Williams & Anderson, 1975) (Fig. 2). *H. tuberculata* and *H. crassispina* represent the ancestral condition, with small eggs that produce a complex feeding larva that takes ~2–4 weeks to reach metamorphosis, depending on food availability. *H. erythrogramma*, in contrast, produces much larger eggs and swimming nonfeeding larvae that reach metamorphosis in just 3.5 days. *H. tuberculata* and *H. erythrogramma* are by far the most intensively studied species in the genus, especially regarding developmental evolution and are thus the focus of what follows. It is worth noting, however, that two other species, *H. bajulus* and *H. australiae*, also produce large eggs but that their nonfeeding larvae are externally brooded by their mother rather than developing in the much more dangerous environment of the plankton. A sixth species, *H. robertsi,* has recently been described but its life history remains unknown.

At first glance, the developmental basis for switching from planktotrophy to lecithotrophy might seem limited to oogenesis. Certainly, increased provisioning of energy-dense molecules during oogenesis is necessary, and it has long been evident that the eggs of lecithotrophs are generally much larger than those of planktotrophs (Emlet et al., 1987; McEdward & Morgan, 2001; Vance, 1973). But it is equally clear that increased maternal provisioning triggers a cascade of secondary effects that are remarkable for their breadth and depth, since traits have changed in parallel ways in echinoderms that independently evolved lecithotrophy (McEdward & Miner, 2001; Wray, 1996). These are manifest within *Heliocidaris* as striking

changes in larval morphology, physiology, behavior (Raff, 1992; Williams & Anderson, 1975). The larvae of *H. tuberculata* and *H. erythrogramma* are so dissimilar that it can initially be difficult to believe they belong to such closely related species.

Indeed, morphological differences are present throughout premetamorphic development (Fig. 3). Subsequent studies have revealed a surprising range of changes in developmental mechanisms, including: greatly accelerated breaking of left-right asymmetry (Henry & Raff, 1990; Henry, Wray, & Raff, 1990), delayed cell fate specification (Wray & Raff, 1989, 1990), and several dramatic changes in early morphogenesis (Henry, Wray, & Raff, 1991; Smith, Collins, & Raff, 2009; Williams & Anderson, 1975; Wray & Raff, 1991). Underpinning these modifications in development are numerous changes in the timing and location of gene expression, including genes that play critical roles within the dGRN (Israel et al., 2016; Kauffman & Raff, 2003; Smith, Turner, & Raff, 2008; Wilson, Andrews, Rudolf Turner, & Raff, 2005).

3.4 Life history switches as natural perturbation experiments

It is these numerous and often dramatic secondary consequences, rather than simply producing a larger egg, that make the evolution of lecithotrophy in *Heliocidaris* a case of extreme biology. But why did so many changes in organismal traits evolve? One possibility is that increased maternal provisioning directly alters many other traits, perhaps by changing the physical properties of cells or by changing which metabolites are present. While such effects may be real, the majority of secondary trait changes are more likely to have evolved in response to the intense rate of mortality in the plankton, which imposes very strong selection to minimize the time taken to reach metamorphosis (McEdward & Miner, 2001; Vance, 1973; Wray, 1996). Even the eggs of planktotrophs contain more than twice as much maternally deposited energy stores than needed to complete the pre-feeding phase of development (Bertram, Phillips, & Strathmann, 2009). This suggests that ecological demands, not developmental ones, set egg size for planktotrophs.

During the evolution of lecithotrophy, maternally supplied energy stores increase to a level where feeding is no longer necessary. At this point, a sudden shift takes place in selective regimes. The complex feeding apparatus that is widely conserved among planktotrophic larvae no longer provides a clear benefit. This is not a situation where a structure simply becomes

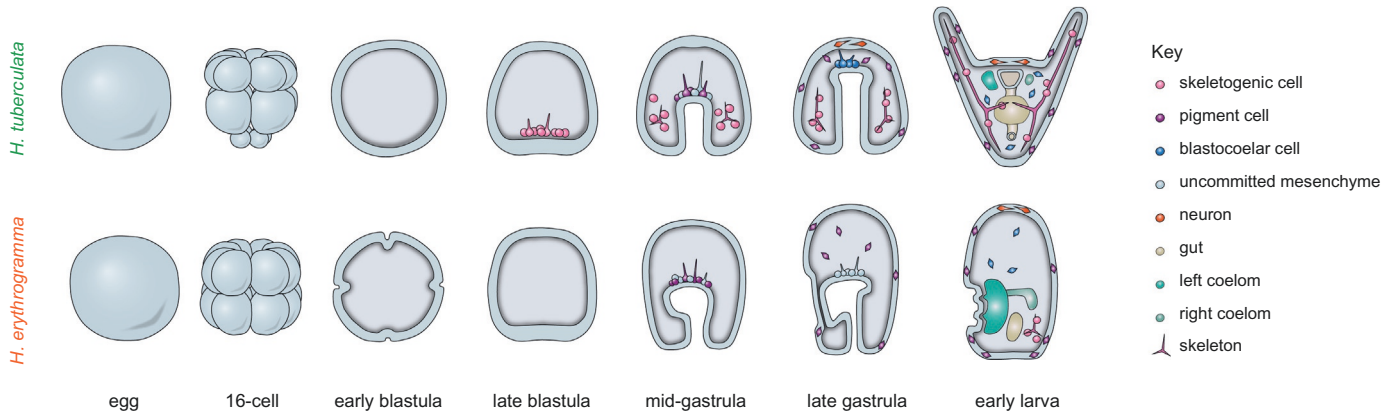

Fig. 3 Morphological changes in early development within *Heliocidaris*. Not to scale; see Fig. 2 for scale. The embryos and larvae of *H. tuberculata* resemble those of other sea urchins that produce feeding larvae. Those of *H. erythrogramma* show several notable differences that hint at underlying changes in developmental mechanisms (see text for references). One of the earliest manifestations is the geometry of cleavage divisions. In the ancestral state two successive asymmetric divisions are mechanistically tied to specification of two cell fates (germ line and skeletogenic mesenchyme) and simultaneously establish the primary signaling center of the embryo. Cleavage divisions are all equal in *H. erythrogramma*, suggesting a delay in these processes. Differences in the morphology of blastula stage embryos are likely related to the large lipid stores of maternally deposited lipids into the eggs of *H. erythrogramma*, which is exocytosed into the blastocoel following cleavage producing a "wrinkled" epithelium. In the ancestral state, the skeletogenic cell precursors ingress as a distinct population prior to gastrulation, but these cells ingress during gastrulation along with other mesenchymal cell lineages in *H. erythrogramma*. Gastrulation itself is left-right symmetric in the ancestral state, but a strong expansion of the left side of the archenteron begins mid-way through gastrulation in *H. erythrogramma*, marking the precocious growth of the left coelomic pouch. By the end of gastrulation in *H. erythrogramma*, interactions between the left coelom and overlying ectoderm establish the adult rudiment, long before this takes place in the ancestral state. The adult rudiment in *H. erythrogramma* rapidly undergoes morphogenesis to form the pentaradial juvenile body plan and central nervous system, reaching competency in ~3.5 days rather than the 2–4 weeks this requires in species with feeding larvae. In contrast, production of the larval skeleton is delayed in *H. erythrogramma*, and the skeleton is comparatively small and simplified. These changes are all very recent in relation to their prior conservation among species with feeding larvae. Importantly, many have evolved in parallel in other echinoderm species with nonfeeding development, underscoring the extent of changes to development that accompany this shift in life history.

useless; feeding structures are also suddenly very costly in terms of increased mortality since it takes time and energy to build them. Selection now actively favors any change in development that reduces the time taken to reach metamorphosis without compromising its successful completion. Importantly, greatly reduced fecundity is an inevitable consequence of much higher maternal provisioning per egg, which makes mortality in the plankton an even more acute problem for species with nonfeeding larval development.

Thus, a massive shift in selective pressures occurs abruptly with the loss of larval feeding. The most obvious consequence is an enormous decrease in the time to metamorphosis from 3 to 4 weeks to just 3–4 days. This has been achieved in *Heliocidaris* in part by eliminating structures that are no longer needed for larval feeding (Emlet, 1995; Williams & Anderson, 1975) and in part by speeding up some developmental processes (Henry et al., 1990; Henry & Raff, 1990). As discussed next, however, additional changes in development point to more profound changes even in otherwise highly conserved developmental mechanisms. These changes illustrate how knowing something about changes in evolutionary mechanisms can deepen our understanding of why changes in development do and do not evolve as well as what role they play in adaptation.

4. Evolution of developmental processes within *Heliocidaris*

4.1 Evolution of maternal provisioning

Egg volume is closely tied to life history in many clades of marine invertebrates, with the eggs of lecithotrophs typically one or even two orders of magnitude larger in volume than those of related planktotrophs (Emlet et al., 1987; Herrera, McWeeney, & McEdward, 1996). Measurements of energy content across species reveals a roughly linearly relationship with volume (Jaeckle, 1995; McEdward & Morgan, 2001). Evolutionary increases in egg size thus appear to be directly tied to increasing energy content rather than some other factor such as the need to increase surface area. This higher level of maternal investment produces a classic life history trade-off between brood size and investment per egg: for a given amount of energy a species can make many small eggs that each has a low chance of survival or fewer large eggs that each has a higher chance of survival. While the theory behind such life history trade-offs is well established

(Flatt & Heyland, 2011; Stearns, 1992; Vance, 1973), the molecular and genetic basis for the evolution of egg size and provisioning has received far less attention.

Heliocidaris has become one of the most thoroughly studied cases of the evolution of maternal provisioning, with direct comparisons of energetics, biochemical composition, cell structure, and molecular processes. Histological comparisons indicate that oogenesis is initially similar in the two *Heliocidaris* species, resulting in cells ~95 μm in diameter (Byrne et al., 1999; Laegdsgaard, Byrne, & Anderson, 1991). Uniquely in the lecithotroph, however, a second phase of oogenesis occurs during which massive amounts of lipid and protein are deposited and immature oocytes expand to ~430 μm in diameter (Byrne et al., 1999). Although lipid droplets are present in mature oocytes of both species, this novel second phase of oogenesis in *H. erythrogramma* produces lipid droplets that are much larger than those in the planktotroph (*H.t.* = 0.84 μm vs *H.e.* = 5.43 μm mean diameter). During embryogenesis in *H. erythrogramma*, much of this lipid is exocytosed into the blastocoel (Henry et al., 1991), a process that may be necessary for reducing cell size or achieving mechanical properties needed for epithelium formation and morphogenesis. Mature oocytes of the two species differ by an astonishing 1000-fold in volume (*H.t.* = 0.31 nL vs *H.e.* = 41.63 nL) (Laegdsgaard et al., 1991; Williams & Anderson, 1975) and in dry mass (*H.t.* = 0.1 μg vs *H.e.* >11 μg) (HoeghGuldberg & Emlet, 1997). These numbers highlight the massive changes in maternal provisioning that have accompanied the evolution of lecithotrophy within *Heliocidaris*.

Size comparisons do not, however, tell the full story. The eggs of the lecithotroph are not simply larger versions of those produced by the planktotroph but instead show substantial differences in biochemical composition. This is immediately obvious from the fact that eggs of planktotrophs are negatively buoyant while those of *H. erythrogramma* are positively buoyant, a density difference based primarily in lipid content. Initial work using thin plate chromatography revealed quantitative differences in the absolute abundance of broad categories of lipids present in the eggs of the two *Heliocidaris* species (Byrne et al., 1999; Byrne et al., 2008; Villinski, Villinski, Byrne, & Raff, 2002). Mass spectrometry, which can identify specific molecules, reveals that these recent evolutionary changes in lipid composition are complex, with several-fold changes in the relative abundance of dozens of distinct diacylglyerols, diacylglyerol ethers, wax esters, and cholesterol esters (Davidson et al., 2019).

Somewhat surprisingly, triacylgylcerols, which function as energy storage molecules in many organisms including planktotrophic sea urchin larvae (Byrne et al., 2008; Byrne & Sewell, 2019a), are actually present at lower relative abundance in the lecithotroph (Davidson et al., 2019). Another unexpected finding is that stores of diacylglyerol ethers, the most abundant class of maternally provisioned lipids in *H. erythrogramma*, remain almost intact at the end of larval development, suggesting that they are used primarily during or after metamorphosis (Byrne & Sewell, 2019a; Davidson et al., 2019). Consistent with this hypothesis, transcription of alkylglycerol mono-oxygenase, the enzyme that cleaves the ether linkage of diacylglycerol ethers, is low in planktotrophs but in the lecithotroph its expression rises 10-fold in larvae (Davidson et al., 2019).

Additional compositional differences are evident in the proteome. In sea urchins, the major yolk protein is synthesized in the intestine and transported to the ovary, where it is taken up by nutritive phagocytes and formed into granules that are then deposited into immature oocytes (Shyu, Raff, & Blumenthal, 1986). This process takes place in both *Heliocidaris* species (Byrne et al., 1999). Unexpectedly, however, major yolk protein is present at ~30-fold lower levels in mature eggs of the lecithotroph relative to other proteins (Davidson et al., 2019), indicating a reduced reliance on protein as an energy source in *H. erythrogramma*. Instead, glycolytic enzymes and other components of carbohydrate metabolism are present at elevated levels, perhaps signaling a greater dependence on carbohydrates for energy (Davidson et al., 2019).

Together, these results indicate that changes in maternal provisioning during the evolution of lecithotrophy in *Heliocidaris* involved far more than simply increasing the level of existing energy stores. Although direct experimental evidence is lacking, changes appear to have evolved in the primary energy sources that power embryonic and larval development in *H. erythrogramma*, with a likely reduced reliance on major yolk protein and greater dependance on specific classes of lipids and perhaps carbohydrates. Additional changes include the evolution of a qualitatively distinct phase of oogenesis, the deposition of substantial amounts of a specific class of lipid that is not drawn down until metamorphosis, and changes in cellular structure in the oocyte and during later development.

The finding that the eggs of *H. erythrogramma* contain more energy-rich molecules than those of *H. tuberculate* was expected; that so many other aspects of developmental metabolism changed during the origin of lecithotrophy was not. Why such extensive modifications? A combination

of factors suggests that most of the changes are directly tied to the life history switch itself. The two *Heliocidaris* species are not only closely related phylogenetically but occupy similar habitats and have broadly overlapping bathymetric and geographic ranges, which together makes it unlikely that major changes in developmental physiology represent adaptations to distinct physical or biotic environments. In addition, ancestral features of developmental physiology present in *H. tuberculata* are broadly conserved among other planktotrophs, while at least some of the recently evolved features in *H. erythrogramma* are present in an independently evolved lecithotroph, *Holopneustes purpurascens* (Byrne et al., 1999; Byrne & Sewell, 2019b; Villinski et al., 2002).

4.2 Evolution of developmental gene expression

The evolution of transcriptional regulation is another area of research where closely related species with highly divergent life histories can provide valuable information. Gene expression profiles during early development are typically highly similar among closely related species and the differences that do exist are dominated by subtle quantitative changes. This is true among camarodont sea urchins (McClay, 2011; Gildor & Ben-Tabou de-Leon, 2015; Israel et al., 2016; Malik et al., 2017). In contrast, the extreme phenotypic divergence in *Heliocidaris* was accompanied, and likely caused in part by, evolutionary changes in the expression of thousands of genes during early development (Israel et al., 2016). This large set of cases provides statistical power to discern general properties of evolutionary change in gene expression and to test specific hypotheses about the evolution of life histories.

But how best to do this? An important first step is to infer which expression changes occurred on the branch leading to *H. erythrogramma* and might therefore be directly or indirectly related to the evolution of lecithotrophy, and which occurred on the other branch leading to *H. tuberculata* and therefore likely are not. The conventional way to do this is with reference to an outgroup species, in this case *Lytechinus variegatus*, which can be used to polarize changes within *Heliocidaris* based on parsimony (Fig. 4A). The second challenge is how to handle differences in degree of expression divergence. The conventional approach to analyzing evolutionary changes in transcriptomes treats all expression differences as equivalent, classifying the expression of each gene as either "conserved" or "differentially expressed"; it then seeks to identify characteristics of differentially expressed

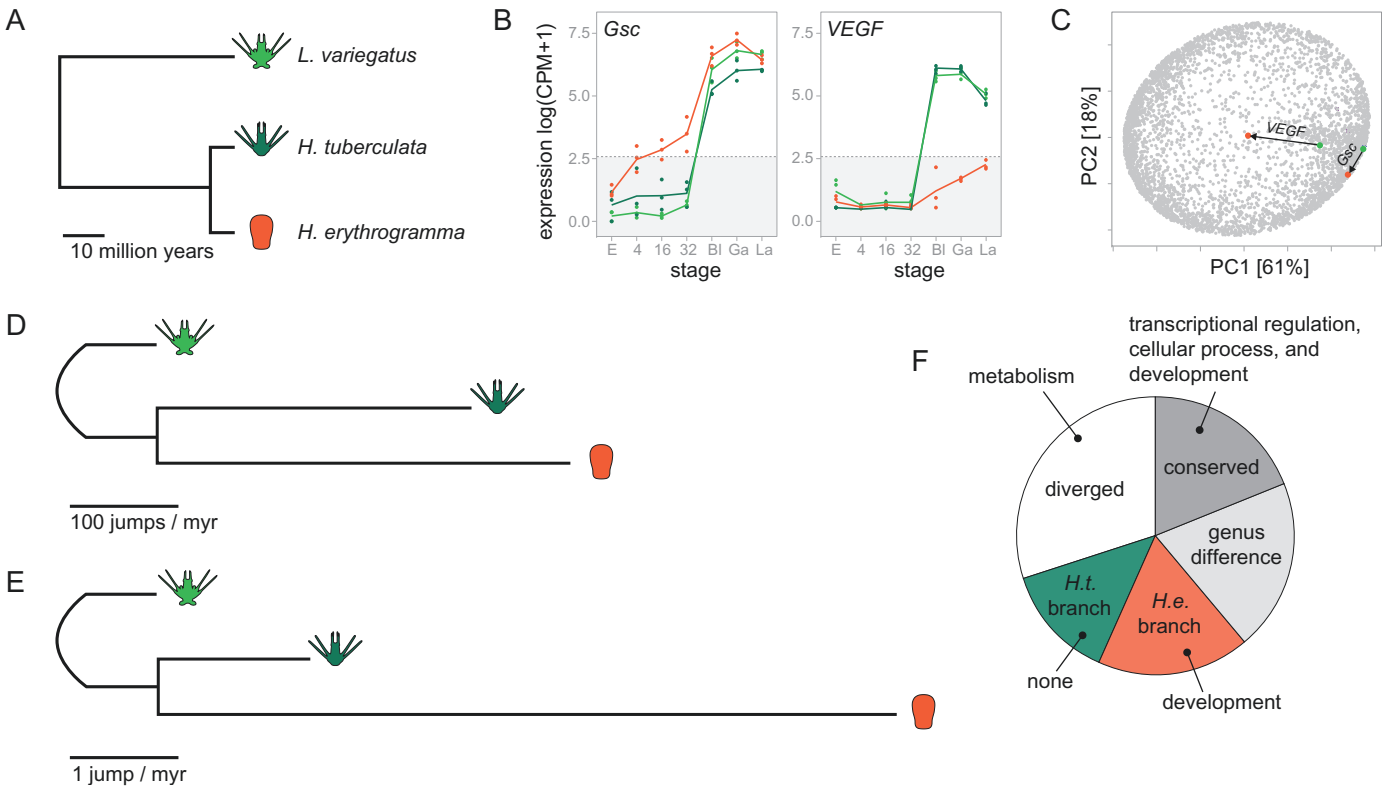

Fig. 4 See figure legend on opposite page.

genes that contrast with the remainder of the transcriptome, typically in terms of an enrichment of ontological terms such as molecular function.

In the case of the two *Heliocidaris* species, however, most genes show an expression difference at some point during development (Israel et al., 2016). Further, the degree of expression divergence varies considerably among genes. For instance, *Gsc* shows a fairly minor change in timing of expression, while *VEGF* shows a more substantial difference (Fig. 4B). Both genes are differentially expressed with the change on the *H. erythrogramma* branch, but the latter is more likely to have a trait consequence because transcript abundance remains very low throughout early development (note that the y-axis is a log scale and the gray zone indicates very low expression). In cases of extreme trait divergence, transcriptomes may be so extensively altered that simply counting cases of differential expression obscures the contribution of more substantive changes in gene expression.

A solution is to quantify evolutionary changes in the shape of the temporal profile of expression within the coordinates of a principal components analysis (PCA) (Israel et al., 2016) (Fig. 4C). The expression profiles of all genes in all three species are plotted in "expression space" and the distance the expression profile of a particular gene moves between species provides a measure of the magnitude of change in a temporal profile. This metric provides an unbiased way to distinguish relatively subtle, but statistically

Fig. 4 Evolutionary changes in gene expression within *Heliocidaris*. (A) A comparative analysis of transcriptomes during development in three species of sea urchins reveals that many changes in gene expression evolved within the genus *Heliocidaris* (Israel et al., 2016); figures shown here are based on data from that study). *Lytechinus variegatus* was used as an outgroup to polarize changes within *Heliocidaris* to a specific branch. (B) The majority of statistically significant expression differences between the two species are relatively minor in magnitude (e.g., *Gsc*). A few, however, show much more divergent expression, particularly when the temporal expression profiles are compared (e.g., *VEGF*). (C) An "expression profile space" can be created based on dimensional reduction of expression profiles from all genes for all three species. This allows for an objective measure of the magnitude of evolutionary differences in gene expression profiles between species. This approach allows one to identify genes whose expression shape differ the most substantially between the two *Heliocidaris* species (called 'jumps' in expression space; see text). (D) When the transcriptome as a whole is considered (10,882 transcripts), the number of jumps is similar on the two branches. (F) Gene set enrichment analysis of genes reveals distinct patterns of conservation and change among functional categories of genes. (E) In contrast to the transcriptome as whole, dGRN genes (95 transcripts) show a strong asymmetry between branches, with most large changes in expression profiles on the branch where nonfeeding development evolved.

supported, differences from more extensive evolutionary changes in gene expression. For instance, the distance between points for the two *Heliocidaris* species for *Gsc* is smaller than that for *VEGF* (Fig. 4C), confirming intuition based on their expression profiles.

These differences can be explored by subsetting the largest changes in expression profiles and mapping them onto an evolutionary tree where branch length is proportional to the number of changes. When this is done for the transcriptome as a whole, the branches are nearly symmetrical, with the *H. erythrogramma* branch slightly longer (Fig. 4D). This asymmetry is much stronger, however, when the comparison is limited to dGRN genes (Fig. 4E). Returning to the entire transcriptome, gene set enrichment analyses show that developmental genes are among those most likely to have conserved expression (Fig. 4F). Notably, however, developmental genes are also enriched for change on the branch leading to *H. erythrogramma*. In contrast, no functional category is enriched for change in expression on the branch leading to *H. tuberculata*. These observations collectively suggest that developmental genes generally have more conserved expression than the transcriptome as a whole but are also more likely to show large changes in expression during the life history shift. This in turn suggests that changes in the expression of several dGRN genes are directly or indirectly related to the evolution of lecithotrophy.

The location of expression during development also changed for many genes during the evolution of lecithotrophy within *Heliocidaris* genes (Byrne et al., 2015; Byrne et al., 2018; Ferkowicz & Raff, 2001; Haag & Raff, 1998; Koop et al., 2017; Love & Raff, 2006; Parks, Parr, Chin, Leaf, & Raff, 1988; Wilson et al., 2005). These studies reveal an unexpected evolutionary reconfiguration of expression territories for several genes within the larval ectoderm. In planktotrophs, the ectoderm of the early larva is composed of four territories each demarcated by the expression of multiple genes: large aboral and oral regions composed of squamous epithelium, a closed loop of columnar cells that make up the ciliated band that separates them, and a relatively small neurogenic territory at the site of the former animal pole. In *H. erythrogramma*, the ciliated band and neurogenic ectodermal territories are evident, although the former is shifted in location (Emlet, 1995; Williams & Anderson, 1975). However, conserved markers of the oral and aboral ectoderm are not expressed in mutually exclusive territories: instead of two large and homogenous regions, the larval ectoderm is a patchwork of several smaller and distinct expression domains for several genes encoding transcription factors (Byrne et al., 2015; Byrne et al., 2018;

Haag & Raff, 1998; Koop et al., 2017). These more subdivided ectodermal gene expression territories in *H. erythrogramma* appear to be related to the accelerated formation of the adult rudiment, a point discussed later.

Gene expression comparisons among species reinforce the idea that altered natural selection is responsible for some of the largest changes in the expression of developmental regulators see in *H. erythrogramma*. Contrasting expression among a variety of species representing the ancestral and derived stages can reveal how natural selection shapes expression profiles during long periods of conservation and how that influence changes during life history switches.

4.3 Genetics of evolutionary change in transcriptional regulation

Heliocidaris offers another useful property for studying the evolution of gene expression, namely the ability to generate interspecies hybrids (Raff et al., 1999). Hybrids are useful because they can reveal the genetic basis for trait differences, including gene expression. For any gene that is expressed at different levels among species, hybrids can provide two kinds of genetic information known as inheritance mode and regulatory mode (Wittkopp, Haerum, & Clark, 2008a, 2008b). Inheritance mode concerns dominance effects, namely whether expression in hybrids resembles that of one or the other parent (simple dominance), an intermediate value (co-dominance), or lies outside the range defined by the parents (over-dominance and under-dominance). Regulatory mode, in contrast, provides insight into where in the genome causal mutations affecting gene expression reside in relation to a differentially expressed gene: *cis* effects are due to nearby mutations (for instance, within an enhancer), while *trans* effects are due to mutations elsewhere in the genome (for instance in the structure or expression of a transcription factor) (Fig. 5).

Measuring the genetic architecture underlying evolutionary differences in gene expression can reveal how natural selection operates on mechanisms of transcriptional regulation. Early studies revealed that both *cis* and *trans* effects are common contributors to expression differences within and between species (Crowley et al., 2015; Wittkopp et al., 2008b), indicating that multiple molecular mechanisms contribute to expression divergence. This is significant, because a mutation in *cis* is likely to directly influence the expression of one or at most a few local genes, while a mutation in *trans* could directly influence the expression of many genes, potentially including all the targets of the regulatory molecule in question. Another important

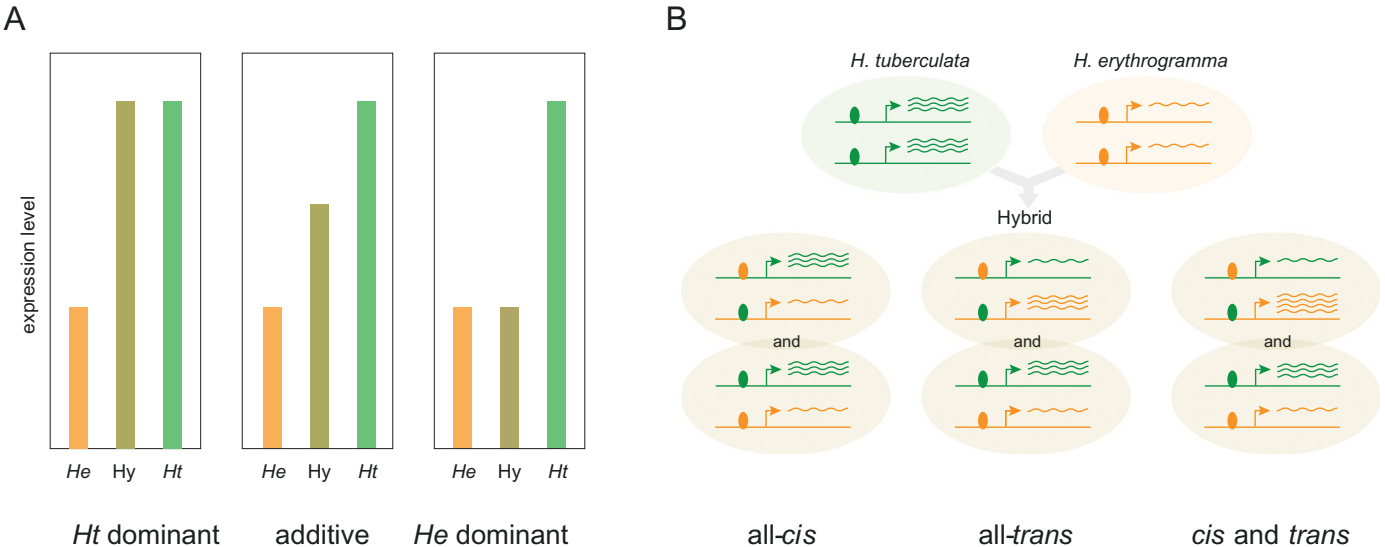

Fig. 5 Inference of genetic basis for evolutionary changes in gene expression. Inheritance mode captures dominance effects on transcript abundance and can be measured in a straightforward way: the expression of a gene is considered additive (or co-dominant) if its level in hybrids is the average of that in two species, and dominant if close to the value of one species or the other. Regulatory mode is concerned with whether the genetic basis for an expression difference is located near the gene of interest or elsewhere in the genome. This inference relies on the ability to identify which chromosome an mRNA was transcribed from based on sequence differences between the two species. If a gene is transcribed at different rates from the maternal and paternal chromosomes (called allelic imbalance), the genetic basis must lie nearby (*cis* effect). This inference is based on the fact that both chromosomes are exposed to the same set of regulatory macromolecules in hybrid nuclei, so allelic imbalance cannot be due to a difference in the presence, level, or activity of a regulatory molecule between species (*trans* effect). Conversely, allelic balance in hybrids indicates that the evolutionary difference must be the product of such a *trans* effect. See Wittkopp et al. (2008a, 2008b) for additional details.

discovery was that many, if not most, *cis* effects in enhancers are tissue- or cell type-specific in their effects, while mutations in core promoters are more likely to have broad effects (Kundaje et al., 2015; Thurman et al., 2012). Together these studies suggest that mutations with *cis* effects and those within enhancers are less likely to be pleiotropic than those with trans effects or within core promoters. This matters, because evolutionary theory suggests mutations with lower pleiotropy are more likely to contribute to adaptation.

Most published studies that have used hybrids to measure genetic effects on evolutionary differences in transcript abundance have considered adult tissues and cell types. Work with *Heliocidaris* hybrids was among the first to measure genetic effects on gene expression during early development and to contrast such effects across multiple stages of the life cycle (Wang et al., 2020). Maternal transcripts are numerically dominant and maternal genetic effects far more common than paternal effects in the early embryo, when zygotic transcription is just beginning. This is to be expected, based on the enormous stores of maternally synthesized mRNA and protein deposited into the egg. Later in development, maternal and paternal transcripts are nearly equivalent and the same is true of dominance effects. This shift reflects the progressive activation of the zygotic transcription from both sets of chromosomes that eventually replaces the entire pool of maternal mRNAs. Interestingly, even some of the earliest paternal transcripts appear to have an almost immediate effect on transcription of both maternal and paternal genes, an inference that comes from cases where the maternal gene is activated earlier in hybrids than in *H. erythrogramma* and the effect is paternal dominant.

A more significant result to emerge from analysis of hybrid transcriptomes is that the genetic basis for differential expression can change during development. For example, a gene may be differentially expressed due to *cis* effects alone in blastulae but due to *cis* and *trans* effects in larvae. At an even more basic level, a gene may be differentially expressed at one stage but not at others. In the case of *Heliocidaris*, ~3/4 of all differentially expressed genes show some change in genetic basis during early development, even when comparing just three stages. This finding has broad implications for understanding the evolution of gene expression, since it implies generally low pleiotropy, namely that many mutations capable of influencing developmental gene expression do so only at some stages. If it is generally the case that mutations that alter a gene's expression at one stage of development frequently do not influence its expression at other stages, then it

seems likely that natural selection can in some cases fine-tune a given gene's expression in a larva (for example) independently from that in an embryo or an adult. This possibility was first proposed based on the finding that multiple regulatory elements influencing the expression of a single gene during development often have modular and largely independent effects (Stern, 2010; Wray et al., 2003), but until recently direct empirical evidence has been lacking.

An unexpected outcome of analyzing transcriptomic data from hybrids is that the maternal-to-zygotic transition (MZT) is delayed in *H. erythrogramma* (Wang et al., 2020). Limited data exist regarding the evolution of the MZT, with most information coming from distantly related species where direct comparisons are confounded as discussed earlier (Section 2.2. The best-studied case among closely related species involves the genus *Drosophila*, and reveals tight evolutionarily conservation in the timing of the MZT (Cartwright & Lott, 2020). Those species, however, also share conserved morphology and life history mode, making it difficult to know why the timing of the MZT has not changed. One possibility is internal constraint, meaning that the timing cannot be altered without disrupting some other process, for instance because that process depends directly on when MZT takes place. A very different possibility is stabilizing selection, meaning that natural selection actively maintains the timing because it happens to be advantageous given external factors, but if those external factors change the timing could be altered in response. The case of *Heliocidaris* demonstrates that, at least in some circumstances, natural selection can shift when the MZT occurs. In this particular case, the delay may allow partitioning of the enormous lipid stores into the blastocoel and simultaneously additional cell divisions to reduce cytoplasmic volume to more typical levels prior to beginning the MZT.

4.4 Evolution of dGRNs and organismal traits

Ultimately, it is the way that an organism interacts with its environment that influences which changes in gene expression and gene regulatory networks persist and which ones change. This is true at the scale of a population, an entire species, and a clade of species. The dGRN for planktotrophic sea urchins provides a powerful framework for investigating how evolutionary changes in gene expression contribute to the evolution of organismal traits. Because in many cases we know what a particular protein does in the ancestral state, it is possible to interpret changes in its expression in *H. erythrogramma*.

An example is *msp130*, which encodes a large glycoprotein specific to skeletogenic cells that is involved in Ca2+ uptake (Anstrom, Chin, Leaf, Parks, & Raff, 1987; Karakostis et al., 2016). In the ancestral condition, *msp130* is expressed around the time that the precursors of the skeletogenic cells begin to undergo ingression, an epithelial-to-mesenchymal transition (Anstrom et al., 1987). Although there are some minor differences in timing of expression among species with planktotrophic larvae (Wray & McClay, 1989), expression is delayed by several hours in *H. erythrogramma* (Parks et al., 1988). Several other components of the "biomineralization toolkit" of sea urchins (Karakostis et al., 2016) also show and delay or reduction in overall expression in *H. erythrogramma* (Israel et al., 2016). These evolutionary changes in timing of expression for several important effector genes of the larval skeleton parallel, and may be causally related to, a similarly large delay in the ingression of the skeletogenic cells and in production of the skeleton itself (Emlet, 1995; Williams & Anderson, 1975).

Another example is *gsc*, which encodes a transcription factor that in the ancestral condition is involved in regional specification of the larval ectoderm. In planktotrophs, *gsc* is initially expressed in about half of the ectoderm during gastrulation then shrinks to the presumptive oral ectoderm territory and finally into a ring around that territory (Angerer et al., 2001). In *H. erythrogramma*, *gsc* expression begins in a similarly broad domain but soon shrinks to the left side of the embryo where the adult rudiment later forms (Wilson et al., 2005). This change in location is part of an extensive rearrangement of spatial expression domains for ectodermal patterning genes in *H. erythrogramma* (Byrne et al., 2015; Byrne et al., 2018; Haag & Raff, 1998; Koop et al., 2017) and which are likely related to the greatly accelerated development of its adult rudiment (Williams & Anderson, 1975).

Hybrids provide more specific information about the genetics of trait evolution. Fertilizing eggs of *H. erythrogramma* with sperm of *H. tuberculata* results in a striking rescue of ancestral anatomical traits: in hybrids the larval skeleton is enlarged and supports arms that protrude from the body, the ciliated band is repositioned, and both a mouth and anus are present (Raff et al., 1999). These rescued larval traits suggest that the paternal genome is transcribed in hybrids, a prediction that is directly confirmed by analysis of transcriptomes (Wang et al., 2020). But how does transcription of paternal genes result in rescue of anatomical features that were lost during the evolution of lecithotrophy? A clue comes from differences in the genetic basis for expression in effector genes of morphogenesis and their regulators that operate earlier in the dGRN.

This contrast can be seen in the portion of the dGRN associated with the larval skeleton. Early genes encode transcription factors that specify the skeletogenic cell fate and later activate a large set of effector genes that encode proteins involved in calcium transport, cell fusion, and structural components of the biomineral matrix (Shashikant, Khor, & Ettensohn, 2018). Most genes in this portion of the dGRN have reduced or delayed expression in *H. erythrogramma*, likely related to the delayed and reduced larval skeleton in this species. Expression differences in effector genes are largely based in *trans* and are maternally dominant, while expression differences in the transcription factors that regulate them are based on a mix of *cis* and *trans* effects. This suggests that it is changes in the expression of the transcription factors and not changes in the *cis*-regulatory elements of effector genes that is responsible for delayed expression of the proteins that actually construct the skeleton (Wang et al., 2020). The same seems to be true of the portion of the dGRN that builds the larval mouth.

One interpretation is that coordinated evolutionary changes in the expression of a large set of effector genes is simpler to achieve by altering the expression of a few regulators rather than many effectors. If this interpretation is true, it may be a general property of evolutionary changes in gene expression that influence temporal changes in morphogenesis.

5. Conclusions

Species with highly divergent organismal phenotypes are natural "perturbation experiments" that can provide valuable information about why developmental mechanisms do or do not change during the course of evolution. The switch from feeding to nonfeeding larval development that evolved within the genus *Heliocidaris* is a particularly useful case because the changes in selective pressures are well understood. In the ancestral state larvae experience stabilizing selection to maintain a highly efficient feeding apparatus and associated behaviors that result in rapid growth based on exogenous food sources. The physiology of embryos and larvae is likewise tuned to initially utilize the carbon sources deposited into the egg and, once feeding begins, those provided by consuming a variety of unicellular algae. The optimized nature of these structures, behaviors, and physiology is apparent from their long-term evolutionary conservation across hundreds of millions of years of evolution. In the derived state, the selective landscape abruptly shifts in multiple ways. Structures and behaviors associated with feeding become detrimental to survival due to the strong selective pressure to reach

metamorphosis as quickly as possible, while physiology must be tuned to utilize very different sources of energy. These altered organismal traits are the product of changes in developmental processes which are in turn the product of extensive changes in gene expression.

A striking feature of *Heliocidaris* is the extent and magnitude of change in additional developmental traits that are otherwise broadly conserved among echinoderms (Fig. 6). An important implication is that broad evolutionary conservation of a developmental mechanism does not necessarily indicate that it can no longer evolve due to internal constraint. The rapid modification of these traits within *Heliocidaris* reveals that they instead remain readily evolvable under appropriate circumstances. Further, most of these changes seem unlikely to be the product of neutral processes, such as developmental systems drift (True & Haag, 2001), but instead are most plausibly interpreted as adaptive changes that are the indirect product of a highly provisioned egg and the resulting shift in selection for very rapid pre-metamorphic development.

If these developmental traits remain evolvable, something must keep them in a highly conserved state for tens or hundreds of MY in species with feeding larvae. Over such immense time scales, neutral processes will eventually modify any traits that can change without a fitness cost. The absence of change thus suggests a persistent and powerful role for stabilizing selection in what we observe as conservation of development among species. But selection for what? Planktotrophy is the only known organismal trait that is both shared by the dozens of species that retain the ancestral developmental features and absent from the species where those same developmental features have changed. This suggests that natural selection actively maintains numerous, specific intermolecular interactions in embryos over vast stretches of time precisely because they advantage a life history involving feeding larvae. It also suggests that when the selective landscape changes following the loss of larval feeding, many of those features remain sufficiently evolvable that they can respond to directional selection towards a new adaptive state.

Replicate life history transitions provide an opportunity to extend these findings and test their generality. Sea urchins representing independently evolved cases of lecithotrophy all show substantial acceleration in premetamorphic development, the degree of acceleration varies, possibly reflecting differences in time since larval feeding was lost or the influence of maternal brooding (Hart et al., 2011; McEdward & Miner, 2001; Raff & Byrne, 2006). Replicate cases of lecithotrophy also show parallel

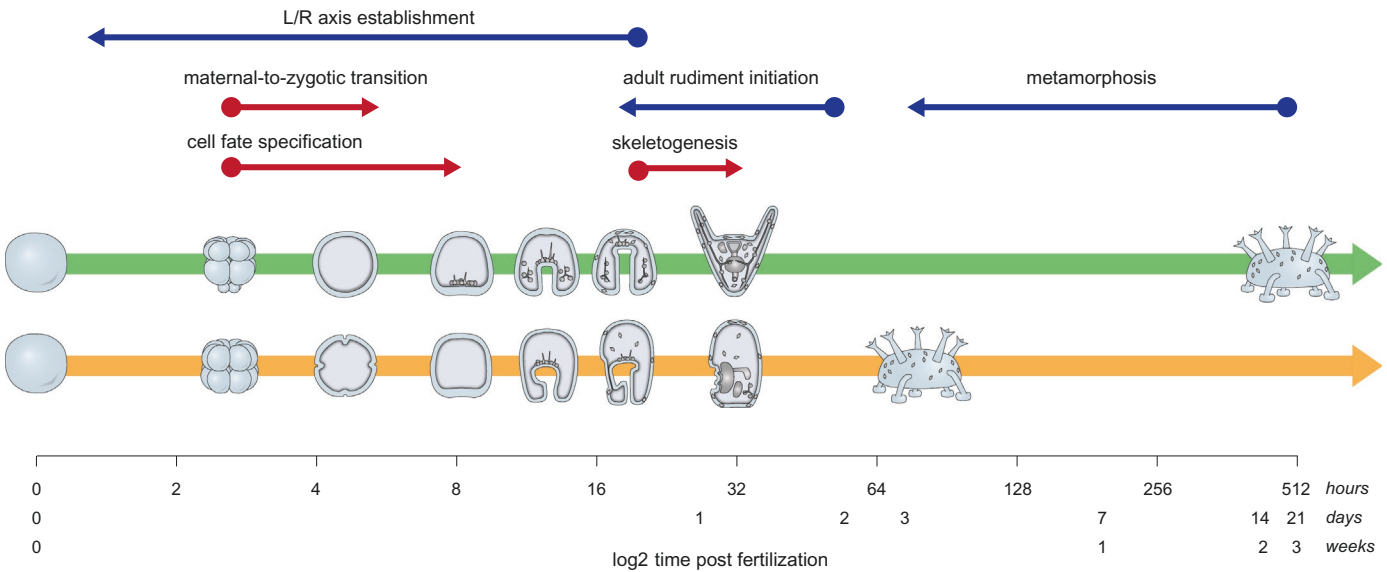

Fig. 6 Complex shifts in developmental timing. The evolution of nonfeeding larval development in *Heliocidaris* has been accompanied by an enormous reduction in the time to reach metamorphosis, from several weeks to 3.5 days (note the log2 scale). As might be expected, many features of development are accelerated (*blue arrows*). Some, however, are actually delayed (*red arrows*). The latter are likely secondary consequences that follow from the primary drivers in the life history shift, namely the need for increased maternal provisioning and the need to reach metamorphosis as quickly as possible.

developmental changes in egg composition, cleavage geometry, and larval morphology (Byrne & Sewell, 2019b; Wray, 1996), hinting at underlying changes in regulatory interactions within dGRNs. The growing set of high-quality genomes and availability of tools for experimentally manipulating gene function in echinoderms provides an exciting opportunity to examine the degree to which parallel instances of natural selection produce parallel changes in specific molecular processes during development.

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