Investigating the Immune System and Colonialism in Sea Urchins

by

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Department of Biology
Duke University

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Gregory Wray

Dissertation submitted in partial fulfillment of
the requirements for the degree of Doctor
of Philosophy in the Department of
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ABSTRACT

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Abstract

Sea urchins are relegated as background or non-playable characters in Western Science and Western culture on a daily basis. This dissertation shines a spotlight on my non-human relatives and gives readers a chance to learn about the sciences and cultures that sea urchin species play a role in. Along with offering research within the realms of development biology and immunology, there are pieces relevant to Science & Society and general audiences blended throughout the chapters. I address two major problems in this work. The first problem is determining how the complex immune system of the *Lytechinus variegatus* larva develops and reacts to injury. The second, is uncovering what colonial practices have led to the sea urchin research climate today.

The first chapter of this dissertation offers a broad Introduction to sea urchins. Moving away from a "cold" or impersonal description, you'll learn about the diversity of sea urchins that move about the world's oceans every day. Topics include what urchin's eat, their etymological origin, their influence on historical and contemporary cultures, what some taste like, and other ranging topics. Parallel to these topics, this chapter contains basic biology on the adult and larval sea urchin, and the development of the embryo's mesenchyme cell populations, which will be the foci of multiple chapters.

Moving into wet lab research, chapter 2 provides the methods used in molecular, cellular, and embryological experiments. The major novel research method is an injury assay where we are able crush or "squish" *L. variegatus* larvae, causing a reproducible and quick immune cell response. Chapter 3 involves a review and recommendations on how Western Science developmental biology researchers think about and approach studying the larva's mesenchyme. Using these new practices, we were able to uncover tissue specific candidate markers in silico, by cloning, and through expression data.

Chapter 4 is the characterization of the immune-related cytokine family, the macrophage migration inhibitory factors (MIFs) in sea urchins. Utilizing newly published genomes, we were able to provide a fuller description of MIF gene duplications, and confidently clone select MIFs and perturb candidate regulators. The following chapter addresses the conundrum of wound healing and the immune system's role in the process. By crushing *L. variegatus* larvae, we have been able to describe and characterize roles of pigment and blastocoelar cells in epithelial and skeletal wound repair. Our conclusions for the respective chapters are, the MIF family has gone through a major gene duplication, and are now able to perform tissue specific and potentially redundant roles in immune cell function; and pigment cells and blastocoelar cell networks are activated quickly in injuries and can remodel tissues in the larvae. Part of chapter 4 will be included in a McClay lab publication on EMT (epithelial-mesenchyme transition) and a publication for chapter 5 is in preparation.

The Science & Society, and penultimate chapter of this dissertation takes a deeper look into why Western Scientists study sea urchin, doing so through the lens of colonialism. In outlining personal kinship, obligations, and an interdisciplinary lens, I am able to name the colonial intentions of marine stations through their justification and use of invertebrate species in research. For contemporary examples, I name general instances of colonial research methods and propose select ways to promote anti- and decolonial practices in sea urchin science that move beyond metaphors.

This project, and the questions it answers and generates moves away from myths of Western Science such as neutrality, and away from a spiritless resource for academic consumption. Along with learning about the immune system, cytokines, and injury response from my non-human, sea urchin relatives, we can also begin to address broad and systemic problems that are faced in sea urchin science and culture. My goal, and what should be thought about early in reading this work, is to learn from, appreciate, reciprocate, respect, and co-create knowledge with sea urchins in a way that doesn't harm the Land.

Dedication

This thesis is dedicated to all my past, present, and future Indigenous kin that survived, did not survive, or are going through an academic system that wasn't built for us.

This thesis is also dedicated to my late, non-human boy – Naruto LaBelle.

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1. Introduction

1.1 Introduction to sea urchins

Sea urchin species are present throughout all of the world's oceans, foraging for food, gnawing at rocks, swimming through the water column (if you're a larva), protecting themselves and fellow invertebrates from predators, and being collected by peoples for food and by scientists for research (Deep Look, 2016; Gibbens, 2017; Metaxas, 2020; Russell et al., 2018). From the perspective of human societies, our sea urchin relatives are mesmerizing, slow-moving and spikey creatures that are food for us and for our other non-human relatives. They are grazers that keep algae in balance, and landscapers that slowly modify the substrate around them. From the point of view of Western scientists, sea urchins, especially their embryos and larvae, teach us about the intricacies of cell biology, development, regeneration, evolution, and a myriad of other topics in different fields of research (McClay, 2011; Narvaez et al., 2020; Raff, 1987).

Sea urchins are a 'classical' model in the field of developmental biology, and have been heavy contributors to its predecessor field of embryology. Over a century ago, Western researchers observed and experimented on the relatively large eggs, or oocytes, of these echinoderms to gain key insights into cellular biology, embryogenesis, and fertilization (Colonna, 2014; Müller, 1846). Western scientists scattered throughout the world today continue to learn from sea urchin embryos about evolution, developmental gene regulation, cell-to-cell signaling, and single cell development and differentiation

(Ettensohn, 2020; Massri et al., 2021; McClay, 2011; Nesbit et al., 2019; Slota et al., 2019; Zuch et al., 2019). This dissertation builds upon this knowledge and aims to inform Western scientists and communities that have close relationships with sea urchins about novel discoveries in a sea urchin larva's developing, constructive, and reparative mesenchyme — historically called the primary and secondary mesenchyme (Cheng et al., 2014; Sharma & Ettensohn, 2011).

Readers in and outside of Western Science may ask, 'Why sea urchins?' Common responses may be, 'Sea urchins are deuterostomes and so are we!' or 'They produce a large number of embryos and their larvae are optically clear!' (Jacobson, 2000; Pierce and Stanley, 2017). These answers may be sufficient for the asker, but the question itself brings to light critical conundrums in Western Science and society (S&S). This discourse includes, why were sea urchins studied to begin with and why there is an anthropocentric or humanistic focus in Western S&S. Through academic work, internships, and teaching experience, I have been able to zero in on these conundrums and provide an analysis of the roots of these questions and their implications for settler-colonialism in Western S&S. The introduction and sixth chapter of this dissertation explores an Indigenous Science and Technology Studies (STS), anticolonial and decolonial perspective to the research fields of sea urchins and developmental biology. This Indigenous STS work will serve as the foundation for a manuscript for a nonfiction book on anticolonial research with non-human relatives.

1.2 An overview of sea urchins

1.2.1 Habitats, species diversity, and adult & larval anatomies

Sea urchins are echinoderms, a name given to organisms in the phylum Echinodermata, and they are present exclusively in marine waters (Pierce and Stanley, 2017). Their habitats range from the tropical and arctic, the shallow shorelines and the deepsea, and rocky terrains to beneath the sands (Keesing, 2020; Lawrence, 2020; Metaxas, 2020; Stevenson and Kroh, 2020). To flourish in these diverse spaces, adult sea urchins developed a wide variety of radial shapes, sizes, colors, toxicity levels, and age lengths. *Strongylocentrotus purpuratus* (i.e., the purple sea urchin), as its scientific and common namesake implies, is deep purple in color, covered in articulating sharp spines (figure 1A), and lives along the kelp beds in the West Coast of the U.S (Worley, 2001). Moving more than 500 miles West from the U.S. to Hawaii, we encounter the maroon colored sea urchin, *Colobocentrotus atratus*, commonly known as ha'uke'uke or the "shingle urchin" (figure 1B). These sea urchins possess flat, paddle-like spines which allow them to endure the crashing waves of the rocky ocean shoreline (Mah, 2008; Waikiki Aquarium, n.d.). Over in Australia and Japan, we dive into the salt water to encounter the elegant and dangerous *Toxopneustes pileolus*, or the "flower urchin" (figure 1C). They are covered in pedicellaria, claw-shaped appendages, that are venomous and can cause injuries to human and non-human animals (Miskelly, 2009). Heading back to the U.S. on the East Coast, we encounter *Lytechinus variegatus* (*L. variegatus*), the "green

urchin" which, unlike its common name implies, comes in a wide variety of colors (figure 1D), is well-known for its knack for carrying items, and is the main sea urchin we learn from in the McClay lab and is the focus of this research (Watts et al., 2020).

As adults, most sea urchins graze on the local aquatic vegetation such as sea grass, kelp, and algae, but are known to eat detritus, dead or decaying organic matter in the ocean when they encounter it (Lawrence et al., 2020; Watts et al., 2020). In terms of their predators, adult sea urchins are often eaten by fish (e.g., triggerfish, eels), crustaceans, their fellow echinoderms, and mammals such as sea otters and humans (Boudouresque and Verlaque, 2020; Rogers-Bennett and Okamoto, 2020). Select bodies of sea urchins also serve as their own separate habitat for protection of aquatic organisms such as shrimp, or work in collaboration with crabs to protect one another (Berge, Vader, and Lockhart 2004).

In their outward appearance, mature sea urchins are reminiscent of rocks, swaying flowers, or perhaps a marine sponge. Looking internally, one can see defining characteristics of an animal. Below the spines and a thin epithelium, adult sea urchins possess a calcite endoskeleton with pores throughout its structure that allow tube feet controlled by a water vascular system (figure 1E). Below the skeleton is a large fluid filled cavity called a coelomic cavity, which gives space for internal organs such as their intestines, radial nerve, and gonads(Ebert, 2020; Holland, 2020; Reis, 2011; Walker et al., 2020). The coelomic cavity also houses the urchin's immune system, consisting of an

estimated four immune cell types called coelomocytes (Silva, 2020). To readers unfamiliar with sea urchins, this complexity may seem straightforward, but about 80% of sea urchins go through a planktonic larval stage (figure 1F) before metamorphosizing into a radially symmetric adult (Ebert, 2020; Lyons et al., 2014; Metaxas 2020). The developing larval is the focus on the cellular and molecular work of this dissertation, but the larva and the adult are both relevant for a naturecultural perspective on the sea urchins.

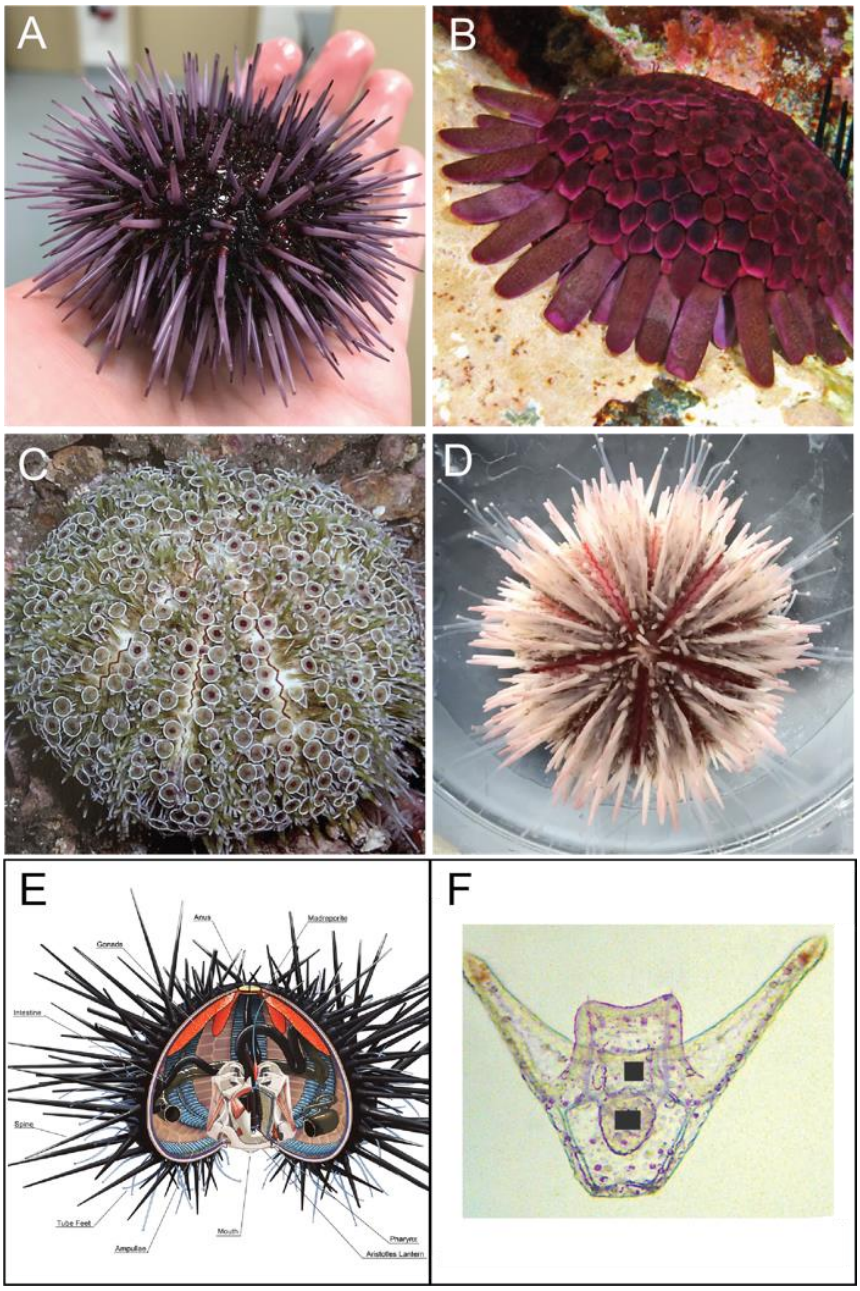


Figure 1: A world tour of sea urchins, their anatomy, and pluteus

(A) An adult of the sea urchin *Strongylocentrotus purpuratus* outside the water. (B) An adult of the sea urchin *Colobocentrotus atratus* in the water (modified from Waikiki Aquarium, n.d.). (C) A close up image of *Toxopneustes pileolus,* showing it's pedicellariae

(modified from Miskelly, 2009). (D) A picture of a color morph [red/pink/white] of *Lytechinus variegatus* caught in Beaufort, NC [Lumbee & Neusiok Lands (Native Land Digital, 2021)] and currently on the Echinobase website. (E) A generalized anatomy of an adult sea urchin based on *Arbacia* (modified from Ries, 2011). Artist's original prints are available on DeviantArt (@Abiogenisis) - https://www.deviantart.com/abiogenisis/art/Sea-Urchin-Anatomy-271355683. (F) A pluteus larva of Lytechinus variegatus (modified from Morill and Marcus, 2005).

1.2.2 Sea urchins and their impact on societies

The sea urchin namesake comes from the Middle English word *urchone*, which is derived from the Latin word, '*ericius*," both meaning hedgehog (The American Heritage dictionary of the English language, 2020). In a Western context, sea urchins are 'sea hedgehogs.' Looking more into Western societies' relationship with the word 'urchin,' it has been used as a name for elves or pixies that can shapeshift into hedgehogs, and as a term to describe children who are mischievous (McNamara, 2007). Societies and cultures outside the West have different relationships with sea urchins, but a survey of current Western popular media portrays sea urchins as these mischievous children, or villains.

The popular animated television series SpongeBob SquarePants, whose main protagonist is an anthropomorphized sea sponge and is best friends with a sea star, is a prime example of sea urchin portrayal in media. Sea urchins throughout a majority of

the series are background accents to this show, appearing as black balls with spikes on rocks (figure 2A) which may scatter when approached or interacted with by the show's characters (SpongeBob SquarePants Official, 2020). Later in the series, in an episode titled "Eek an Urchin!", sea urchins are the antagonist where they have infiltrated SpongeBob's place of work (figure 2B) and are seen as pests (Nickelodeon UK, 2016). In the British children's animated series, The Octonauts, a similar plot takes place in an episode called "Octonauts and the Urchin Invasion" where sea urchins are detaching kelp (figure 2C) and causing chaos to the show's self-declared adventurers (Octonauts, 2021). In both of these episodes, the sea urchins that are interacted with do not possess anthropomorphic eyes or voices, but in another episode of the same series, a specific sea urchin (figure 2D) is given a speaking role and bilateral eyes (Octonauts, 2020). Searching through news and media outlets, you come across articles, videos and podcasts referring to sea urchins "hordes," or "plagues" (BBC Earth, 2012; Sommer, 2021; Sommer et al., 2021). Rummaging through the popular online video platform YouTube, you encounter videos of people stepping on or being stung by sea urchins and their reactions (Brave Wilderness, 2017). Where does this antagonistic portrayal of sea urchins in Western popular culture come from?

Fortunately, it's not all negative reactions to sea urchins in popular media. Many articles and videos exist of the delicacy commonly known as "uni" or sea urchin's roe/egg producing gonads (Ramsay, 2020). Depending on the species eaten, the roe can

be described as “fresh, salty, and creamily opulent” (Jonze, 2018). Sea urchins even made their debut in popular video game franchises such as Animal Crossing New Horizons (figure 2E), and Pokemon Sword & Shield (Game Freak, 2019a; Game Freak, 2019b; Nintendo EPD, 2020). The latter of these mentioned games introduced the “sea urchin Pokemon” pincurchin (figure 2F), an anthropomorphized sea urchin with two large eyes and relatively competitive game stats for the player's use with the creature (The Pokémon Company, n.d.). In the Western Science communication realm, snippets of documentaries and TedTalks talk about interactions with and the development of sea urchins, and even the occasional sea urchin carrying 3-D printed objects, such as a hat (Deep Look, 2016; TED, 2018; TED-Ed, 2012; Whalen, 2020). An overarching theme in these media portrayals of sea urchins stands out - sea urchins are to be seen as odd, weird, or exotic.

Figure 2: Sea urchins in popular culture

(A) Screen Capture from SpongeBob SquarePants Official (2020) of an episode clip of SpongeBob Squarepants showing generic animations of sea urchins circled in a

yellow dashed line. (B) Screen Capture from Nickelodeon UK (2016) of the SpongeBob SquarePants "Eek an Urchin!" episode showing an animated sea urchin, the episode's main antagonist. (C) Screen Capture from Octonauts (2021) of an Octonauts episode where sea urchin are eating kelp. (D) Screen Capture from Octonauts (2020) of an Octonauts episode where main characters help a sea urchin and crab. (E) Screen Capture provided by Valerie Gartner from the video game Animal Crossing: New Horizons (Nintendo EPD, 2020) of playable character catching a "slate pencil urchin." (F) An image of the Pokémon Pincurchin that is based off a sea urchin [modified from The Pokémon Company (n.d.) online Pokédex].

1.2.3 Broader impacts of sea urchins in research

Western researchers and Western Science communicators sell the notion of studying sea urchins in a variety of ways that emphasize the "weirdness" or "uniqueness" of the different species. The structures and mechanics of the sea urchin jaw is one example. The pentaradial jaw consists of five teeth that slowly and rhythmically gnaw at organic and inorganic substrates, which could be a creature straight out of a Western Science fiction movie (figure 3A-B). This mesmerizing structure has been an inspiration for a radial ground sampler in engineering (Frank et al., 2016). Zooming in, the discovery of the self-sharpening teeth of the adult has also been

influential in thinking about how boring apparatuses may be designed in the future (Cell Press, 2019). Sea urchins are even making their way into the economics and aquaculture industry (Urchinomics, n.d.; Sun and Chiang, 2015).

In public facing environmentalism, sea urchins are currently portrayed as pests to be controlled or exterminated. The most infamous instance in the U.S. is for the purple sea urchin (*S. purpuratus*) on the West Coast of the country. Western media and Science outreach initiatives portray the sea urchins as out of control, "voracious," or that they are maliciously destroying kelp beds (Associated Press, 2019; Sommer et al., 2021). In these publications, it is either briefly or never mentioned that it's because of human influence that the ecosystems are disrupted. Climate change is affecting the growth of kelp, and the natural predators of the sea urchin have been over hunted, overfished, or had declines in population due to disease (Sweet, 2020). Parallel to these events, the preservation of coral reefs has taken up public interest, and sea urchins are shown to be vital in locations to the algae that overtakes and kills the coral (Steneck, 2020). Not by accident though, sea urchins population increases are still referred to as "outbreaks" in these beneficial incidences (The Nature Conservancy, n.d.).

Sea urchins are of interest in Western Science for their "unique" ability to regenerate. Their echinoderm relatives, sea stars and sea cucumbers, are the gold star species in their ability to regenerate because they replace entire bodies from a single arm or their entire digestive tracts after evisceration, respectively (García-Arrarás and

Greenberg, 2001; Khadra et al., 2015). Sea urchin adults regenerate to a lesser extent than sea start. They replace spines (figure 3C-D) and tube feet easily and efficiently, and damage to their ambulacrum leads to imperfect wound repair and regeneration (Narvaez et al., 2020; Reinardy et al., 2015). Sea urchins and their impact on science and cultures is pervasive but subtle. If we were to erase the research done on sea urchins these past centuries, discoveries and impacts in Western Science, technology, engineering, art, mathematics, and medicine would not be where they are today.

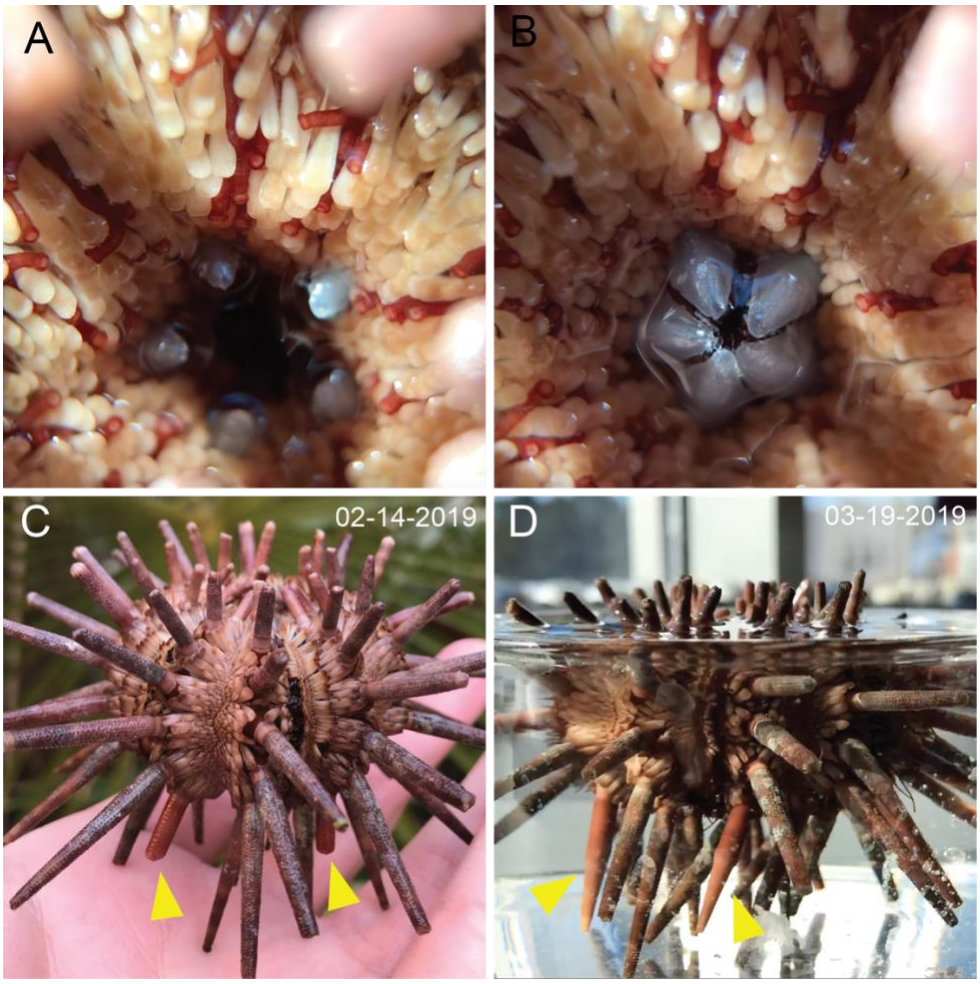


Figure 3: The mouth and spine regeneration of a single *Eucidaris tribuloides*

(A-B) The opening and closing of the pentaradial mouth of *Eucidaris tribuloides*. (C-D) Images of the same individual of Eucidaris tribuloides one month apart. (C) Yellow arrows showing two spines that were regenerating when the individual arrived. (D) One month later, the regenerating spines of the specimen had regenerated their spine points. It is unclear if these are the exact same spines, since there were a few others broken elsewhere.

1.2.4 The "non-traditional" model organisms

Hundreds upon hundreds of peer-reviewed academic articles across disciplines are published on sea urchins, some over a century ago. The genomes of multiple sea urchin species are publicly accessible, and different species exist within all the world's oceans (Cary et al., 2018). Newly characterized species even have generation times about as long as a mouse, making them amenable to transgenic work (Yaguchi, 2019). If all of this history and resources exists on these organisms, why are they still called "non-traditional" model organisms? And why are the same types of organisms funded and researched while close relatives or diverse species "othered" (Sánchez Alvarado, 2016).

A topical and short-term answer and method of addressing these questions involves similar discourse on the field of Diversity, Equity, and Inclusion (DEI) in Western Science, and academia in general. To address both the questions of exclusion of model organisms and diversity in Western Science, we need to take a systematic, multidisciplinary, Indigenous & queer perspective and approach. Chapter 6 of this dissertation explores both of these questions and proposes a future of what inclusive and anticolonial research with sea urchins may look like.

1.3 Development of the feeding planktonic larva

1.3.1 Fertilization and cleavage

Fertilization, the fusing of a spermatocyte (sperm cell) and oocyte (egg cell), occurs externally in echinoderms (Deep Look, 2016). This external fertilization process in

Lytechinus variegatus naturally occurs via colony spawning which is initiated by an unknown chemical cue. When one urchin releases its gametes (a mature germ cell) from its gonopores into the water column, this also signals to nearby sea urchins of the same species to shed their gametes as well. From signaling cues, the spermatocyte migrates to the oocyte and the gametes fuse cell membranes and pronuclei, creating a zygote. Before zygote formation, the unfertilized egg already possesses asymmetric maternal determinants that give rise to the animal-vegetal axis of the embryo which develops into the anterior-posterior axis of the larva (Wikramanayake et al 1998; Sun et al 2021).

The zygote begins its cleavage stages soon after fertilization, dividing the first two times meriodonally into first a 2-cell and then a 4-cell stage embryo (figure 4), both of which have the potential to become completely separate larvae if disassociated (Kearl, 2012; McClay, 2011). The next division occurs equatorially, creating 8-cell stage blastomeres that are now distinguished as the animal and vegetal regions of the embryo, with the vegetal blastomeres containing an enrichment of beta-catenin (Logan et al., 1999; Wikramanayake et al., 1998). To form the 16-cell stage embryo, the animal blastomeres divide meridionally forming the mesomeres. The vegetal blastomeres divide equatorially and unequally to form the larger macromeres, and smaller micromeres. At the 32-cell stage, the macromeres divide into the Veg1 and Veg2 layers, and the micromeres into the large and small micromeres (McClay, 2011; Morill and

Marcus, 2005). The vegetal cells in early cleavage stages are the progenitors of the larval mesenchyme, and will be the main focus of this research.

The large micromeres are the progenitors to the "primary mesenchymal cells" or "skeletal cells" of the larva (figure 4). The skeletal cells are responsible for depositing and shaping the calcite skeleton of the larvae (Lyons et al., 2014). The small micromeres develop into the primordial germ cells, ingress and home to the coelomic pouch of gastrulating embryo. The Veg 1 and 2 cells at this stage in development, are beginning their programming to become the "secondary mesenchymal cells" or the "non-skeletal mesenchyme" (NSM) cells, and the presumptive endoderm. The large micromeres, through the delta-notch signaling pathway, signal to the neighboring Veg2 cells to become the NSM (Croce and McClay, 2010; Sherwood and McClay, 1999). Experiments where the micromeres are removed at the 16 and 32-cell stage, showcase the phenomenon known as "transfating" or cell reprogramming (Cheng et al., 2014). Lacking the micromeres signals, the NSM reprogram and replace the missing skeletal cells.

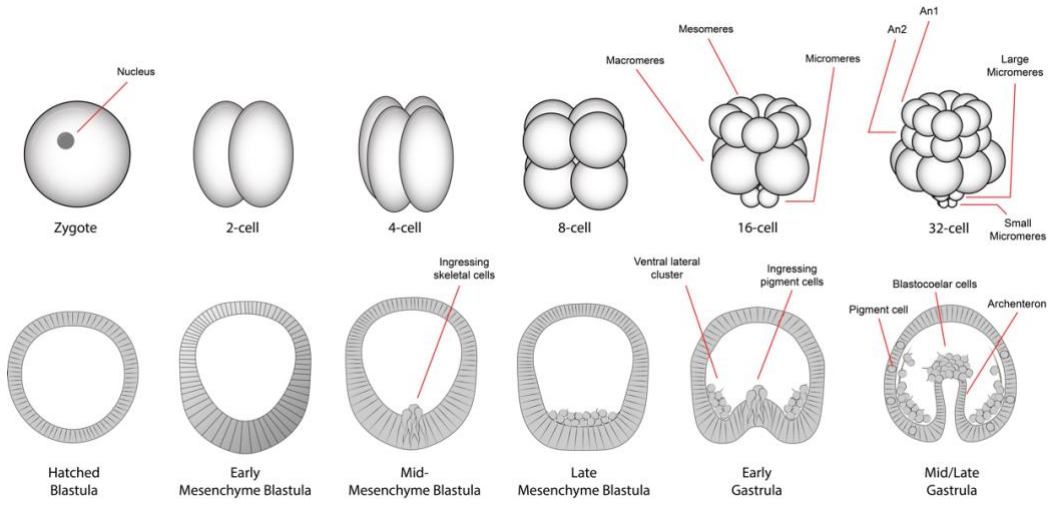


Figure 4: Schematic of the early and later development of the *Lytechinus variegatus* sea urchin larva

(Top) From left to right, the one-cell stage embryo (zygote) divides during its early cleavage stages until the 32- cell stage. (Bottom) Later in development (post-cleavage) the blastula has hatched, goes through multiple EMTs the mesenchyme progenitors, and gastrulates.

1.3.2 Blastula to early pluteus

1.3.2.1 Skeletal mesenchyme development

As the embryo proceeds through its early cleavage stages, it develops a fluid-filled cavity called the blastocoel (figure 4). The previously distinguishable and easily countable cells of the embryo are now an epithelial sheet of about 500 cells that surround the blastocoel, and the embryo, now called a blastula, has started to move inside and "hatch" from its fertilization envelope (Morill and Marcus, 2005). The vegetal side of the blastula proceeds through major developmental and morphological changes, where the region thickens, referred to as the vegetal plate. The skeletal cell progenitors lie directly in the most vegetal region of this area, surrounded by the progenitors to the NSM. At this mesenchyme-blastula stage, the skeletal cell progenitors go through a series of cell states of dissolving the basement membrane, gaining motility, apically constricting, and losing their apical-basal polarity that defines a epithelial cell (Lyons et al., 2014; Saunders and McClay 2014). An average number of 32 skeletal cells enter the blastocoel and have completed their epithelial-to-mesenchymal transition (EMT).

After ingression, the skeletal cells divide once more for an average number of 64 skeletal cells (Lyons et al., 2014). Through a series of intersecting signaling events, the epithelium begins expressing cell signaling molecules (e.g., VEGF) in two lateral regions (left/right axis) called the ventrolateral clusters (McIntyre et al., 2014). The skeletal cells express a VEGF receptor, receive the epithelial signal, migrate to the signaling ring, and form a syncytium amongst themselves. The VEGF signal later localizes into the left-right

sides of the embryo, creating two aggregations of skeletal cells named the ventro-lateral clusters (Duloquin, et al., 2007). These clusters of skeletal cell begin depositing and shaping amorphous calcium carbonate into two triradiant spicules that nucleate the rest of the larval skeleton (Lyons et al., 2014; McIntyre et al., 2014). The overall shape of the larval skeleton (i.e., where skeleton is to be deposited) is based on cues external to the skeletal cells, while the skeletal details (e.g., smooth or variegate rods) is based on a species internal programming (Armstrong and McClay, 1994).

1.3.2.2 Non-skeletal mesenchyme development

In *L. variegatus*, after the skeletal cells ingress, the pigment cells proceed through an EMT process. This is the point where the embryo begins to gastrulate, the blastopore forms, and the animal-vegetal axis becomes the anterior-posterior axis. Once within the blastocoel, the pigment cells stay localized near the posterior region of the embryo. The pigment cells proceed through a mesenchymal-to-epithelial transition (MET) near the posterior side of the embryo, entering the ectoderm (Calestani and Rogers, 2010; Ettensohn, 1992). The pigment cells do not enter the oral region of the ectoderm, and develop their distinguishable reddish-granule pigmentation late in gastrulation (Ho et al., 2016). The EMT timing of the developing mesenchyme varies between planktonic sea urchin species. In *S. purpuratus*, the pigment cells ingress later relative to L. variegatus, and do so after invagination has begun.

As gastrulation proceeds, the tip of the developing gut (i.e., archenteron) carries developing blastocoelar cells near the animal pole, undergoing EMT at mid- and late gastrulation (Solek et al., 2013). Not much is known about the continued differentiation of the heterogeneous blastocoelar cell as they ride the gut and proceed through EMT, and how or if develop into the five categorized immune cells of the larvae (Buckley and Rast, 2019; Ho et al., 2016) There is some evidence that some of these immune cells are sparse (e.g., 2-5 globular cells) or develop under immune challenge (e.g., ovoid cells), and it is unclear, due to a lack of molecular markers, if current categories of immune cells are the same cells but different morphs (Ho et al., 2016). The most numerous and easily observed in the later prism and early pluteus are the filopodial cells which form an intricate network (Tamboline and Burke, 1992).

1.3.2.3 Through-gut development

As Filopodia extend from the mass of cells at the tip of the invaginating gut. A subgroup of these cells will develop two left and right structures known as the coelomic pouches (Martik and McClay, 2015). Concurrently, the primordial germ cells ride atop the rising archenteron (Martik and McClay, 2017). The primordial germ cells will pick one of the coelomic pouches to reside in, and will become the adult rudiment that develops into the adult sea urchin during metamorphosis. In the anterior-oral portion of the late gastrula stage embryo, ectodermal tissue begins to differentiate into the future mouth of the larva. This tissue invaginates as well and will become the mouth and

foregut of the larvae, and will soon meet and fuse with the tip of the archenteron (Christiaen et al., 2007; Suzuki and Yaguchi, 2018).

1.3.3 Larva formation and metamorphosis

Now that the larva can eat and digest food, it has become what is called the "feeding pluteus." If kept in culture and unfed, the larvae will continue to grow the length of their oral and aboral arms and eventually perish. If fed the appropriate combination of algae, the larvae will grow larger, more arms, and develop more cell types and its rudiment. Depending on the species, a planktonic larva will become competent to receive an environmental cue to metamorphosize (Nesbit et al., 2019; Yaguchi, 2019) . During metamorphosis, the rudiment will go through an eversion two where it is now external to the larval body, and begins to resorb the larval tissues. The end result is a small, sexually immature, juvenile sea urchin (Deep Look, 2016).

1.3.4 Developmental gene regulatory networks

To pair with years of classical embryological and cell biology work on the sea urchin embryo, researchers in the field have focused on building extensive developmental gene regulatory networks (GRNs) which incorporate spatial and temporal information (Adonin et al., 2021; Davidson et al., 2002; Ettensohn, 2020; Israel et al., 2016; McClay, 2011). We have diagramed data on signal transduction pathways, transcription factor regulation, and tissue-specific molecules for many tissues and cells in the larvae. Sea urchin GRNs contain homologous factor subcircuits that are applicable

to other systems, one example is in *Drosophila* eye specification (Martik and McClay, 2015).

Principal GRN subcircuits are available and are being worked on for tissues throughout the sea urchin embryo. Researchers are verifying developmental GRNs across planktonic sea urchin species, comparing them evolutionary to lecithotrophic larvae, and building new ones for other echinoderm embryos (Hinman et al., 2003; Israel et al., 2016; McCauley et al., 2010; McCauley et al., 2012; Saunders and McClay, 2014). Even with the myriad of cellular, morphogenic, and molecular data on larval development, the immune system GRN of the sea urchin larva is sparse. This is due in part to immune systems' relatively quick diversification across species, and the sea urchin's non-pigmented mesenchyme (Buckley and Rast, 2015; Hibino et al., 2006). The laboratory based work of this dissertation explores the complex immune system of *L. variegatus* by exploring candidate immune genes across species in the sea urchin, characterizing a short but complex family of cytokines, and exploring a newly characterized wound repair ability of the larva.

2. Materials and Methods

2.1 Embryo culturing

Adult *Lytechinus variegatus* were taken from Lumbee and Neusiok lands (Beaufort, NC, United States) through the Duke University Marine lab; Guarungumbe, Seminole, and Taino lands (Key West, FL, United States) through the Pelagic Corporation; and Cuchiyaga, Seminole, Taino lands (Torch Keys, FL, United States) also by the Pelagic Corporation (Native Land Digital, 2021). The single adult *Eucidaris tribuloides* that was imaged was from Pelagic Corporation. Gametes (sperm and oocytes) were collected from adult *L. variegatus* by injecting 0.5 M KCl into the adults (Adams et al., 2019). Embryo and larva were raised in artificial sea water (ASW) at 23℃ or 18℃.

2.2 Cloning genes & in situ hybridization

Select *L. variegatus* gene coding sequences were cloned via PCR from embryonic cDNA with Phusion High Fidelity DNA Polymerase (NEB) (Table 1). Cloned regions were inserted into the p-GEM T Easy Vector (Promega), or had a T7 promoter added to through PCR (Slota et al., 2018). Other gene loci were synthesized by Twist Bioscience by adding restriction enzyme sites to a gene's 5', coding sequence, and 3' region based on the Lv 3.0 genome and inserted into pTwist Amp High Copy vector (figure 5). A handful of cloned genes have been published and extensively used in sea urchin development research (Table 2). Digoxigenin (DIG) or Fluorescein (FLU) RNA probes were

synthesized *in vitro* with a T7 or Sp6 RNA polymerase (Davidson et al., 2020; Slota et al, 2019).

Single and double whole mount *in situ* hybridization (WMISH) was performed on embryos fixed in 4% paraformaldehyde and stored in -20℃ in methanol. For single WMISH, anti-DIG antibodies (1:1500, Roche) were used to attach to the DIG RNA probes, and they were visualized by an NBT/BCIP color reaction (Slota et al., 2019). For double WMISH, DIG and FLU RNA probes were used and visualized with a Tyramide Signal Amplification Kit (TSA-plus kit, Perkin Elmer) (Slota et al., 2019). Single WMISHs were imaged using a Ziess Axio Imager or a Ziess Axioplane Upright Microscope in the DIC channel. Double WMISHs were imaged with a Ziess LSM 510 Inverted Confocal.

Table 1: Primer cloning sequence

Lv Gene Primer	**Sequence**
Astacin4 Forward	TCACTGATTTTGGGGTGCATGAGCAT
Astacin4 Reverse	ACACTCAATTTGAGGTAGCAACTGCACT
Irf4 Forward	ATCTGAGGCTCTTTCTGAGCTGAAGGA
Irf4 Reverse	CAATGTGGAATTTGTGGCTGGGGGAT
Mif1_2 Forward	AGTGAAAGCAGACGAAAAGTCCAGGAT
Mif1_2 Reverse	ATTCCCGCACAAGTGGTGTTGTTGTA
MifL1_3 Forward	TGCGCCTACAACATTGCGTAACAACAGT
MifL1_3 Reverse	TCATTTTCTGTCGCATGCTAGTCCTGTACTTGT
Mif4 Forward	CATTCAAAGCTGTCAAAGATCATTTCGGAGTTGAC
Mif4 Reverse	CTATGATCGCATGTTATCGAAGAGCTCGATATGTG
Mif5 Forward	GGTGCTTATTCAAGATGATGCGTTGTGTGG
Mif5 Reverse	CTTGCCTGAGGTATCCCTGAATCTTTTTGTAATAGC
Pak1 Forward	ATTGTCAGTGTTGGAGATCCTAAGAGGAAG
Pak1 Reverse	CAATTTCACGGATGCAAGCTGATGATCC
Pak1 Reverse + T7	GCGTAATACGACTCACTATAGGGAGACAATTTCACGGA TGCAAGCTGATGATCC

>Lv-Mif4

TAATACGACTCACTATAGGGCGAATTGGGCCCGACGTCGCATGCTCCCGGCCGCCATGGCG-
GCCGCGGGAATTCGATTTCTTGACCGGTCTAGTCCGCTCTTCAGCTTGAATAATTGACCCACATT
AAACACCGAACTTCCCTTGTTTTTGGCGCCAAGTAAAGATTCCTGAACAACATGCCTCTCTGCAT
CTTCCATACCAACATTTCTTCTGATCGCATCAATGATGAGATTCATTCAAAGCTGTCAAAGATCAT
TTCGGAGTTGACGGGGTCAAACGAAGCGTCCATCACCGTCGAGCTCCGTACGGGCAAGAACAT
GTGCAGGGCGGCTCAACCAGAAGCAGACTGGGGTTGCTTTTTCATGATGCATAACAATGACCCG
AAGCACATTGGACTAGGTGACAACAAGTTGTGTGCTGATAAACCCCACTTCAAGAACTACACAC
ATATCGAGCTCTTCGATAACATGCGATCATAGCTCCTGAATTGACAAGAGGCGGCGCCGCAAGG
AAAGGGAGGGGGGGGCATATCACTAGTGAATTCGCGGCCGCCTGCAGGTCGACCATATGGGAG
AGCTCCCAACGCGTTGGATGCATAGCTTGAGTATTCTATAGTGTCACCTAAAT

>Lv-Mif5

TAATACGACTCACTATAGGGCGAATTGGGCCCGACGTCGCATGCTCCCGGCCGCCATGGCG-
GCCGCGGGAATTCGATAAAACTCGAAAGAAGAACAAGTTTGAAATTCATCAGTCCATATCAATAT
CATCTGGGTTTTTTTGCCTGGTTTCGGTTGCCATATTGGTGCTTATTCAAGATGATGCGTTGTGTG
GTACATGTGAACACGCCAGTCAACAAACTAAGCGATGGTTTCAGGAATCAGCTGACGACTATGC
TTTCAAAACGATACAATCTTGAAAAAGGGAACATCGTCGTTTTACTTCGAGAAGGTCTCCGTCTT
ATGCGAACTGGGACCCTGGATCCATGCGGGTATATCGAGCTCTTTGGTTGTGAAGGTGTGTTTGA
CGACGCGGAGAAGAACAGAGAGGATACAACGGCTCTTATTCAGCTATTACAAAAAGATTCAGG
GATACCTCAGGCAAGACTCTTCGTACTCATGAGACCTCAATGGAGTTCTAACTGGGGACAGATC
GGTGGACAAAGCTTGGTCATGGATGCCTGAAACCATACTCCCAGAAGATTCCTATCCAGACTTCA
AGAAACACTCGTTGAATCACTAGTGAATTCGCGGCCGCCTGCAGGTCGACCATATGGGAGAGCT
CCCAACGCGTTGGATGCATAGCTTGAGTATTCTATAGTGTCACCTAAAT

>Lv-Mif7

TAATACGACTCACTATAGGGCGAATTGGGCCCGACGTCTGGTTGATTTGTGCATGGTTAAA-
TATACATTATAATCGCTTTTTGATATATATATATATATTTTCAATGGTTTATCAGATTGATAACAGTTGA
TAGCACCATGCCTTGCATTGAGATGTACACAACCCTGTCTGCTGATAAAGTACCAGCTAACTTCTT
CGATGTTCTGACAGAGTTCTTCTGTGGGTTGCTTGATAAGGACCCAAGGGGAGTTGTTCTGAAC
TTGTATACAGACCAGCGGATACATACGGGTTCAGATCTCAATGCTACCATGCTTATGATACAGATC
TACAATGCCGAAGCCTGATCTACTGTTCTGTTGATCACCGTTCCATCTCACCAAGTTGGCACGCC
AGGGTCGACCATATGCTATAGTGTCACCTAAAT

Figure 5: MIF gene loci synthesized by TWIST BioScience

Mif4, Mif5, and Mif7 gene loci based on the Lv 3.0 Genome flanked by cloning tools submitted to Twist BioScience for synthesis in the pTwist Amp High Copy vector. Orange: T7 promoter sequence; Blue: Restriction enzyme sites; Black: 5′ and 3′ regulatory regions; Green: coding sequences; Red: Sp6 promoter sequence.

Table 2: Published genes

Gene	Publication(s)
Alx1	Saunder and McClay, 2014
Ese	Rizzo et al., 2006; Slota et al., 2019
GataC	Solek et al., 2013
Gcm	Solek et al., 2013
Hey	Slota et al., 2019
Pks1	Solek et al., 2019

2.3 Immunostaining

After single WMISH with NBT/BCIP for Pak1, color stained embryos were placed in a primary antibody for the skeletal cells (1d5 - undiluted) and incubated overnight. The embryos were washed in TBST and incubated in Cy3-conjugated secondary antibody. After TBST washes, the embryos were briefly stained with Hoechst diluted in TBST (1:2000, Molecular Probes). Embryos were imaged using a Ziess Axio Imager with an Apotome2.0 using the green channel and far red channel to visualize the NBT/BCIP reaction (Trinh et al., 2007).

For only immunostaining, larvae were fixed in 4% paraformaldehyde overnight at 4℃ and stored in freezing methanol at -20℃. The larvae were washed in TBST and blocked in 4% NGS/TBST. The antibody 295, which marks the ciliary band, was diluted

1:200 in 1d5 and incubated overnight at 4℃. The larvae were washed in PBST and incubated in Cy2 and Cy3-conjugated secondary antibody. After washes, larvae were briefly incubated in Hoechst. Fluorescent larvae were imaged using a Ziess Axio Imager.

2.4 Drug treatments and microsurgeries

4-IPP (Sigma) was dissolved in DMSO to a concentration of 10 mg/mL and aliquoted and stored at -20℃ until ready to use. 4-IPP drug treatments were carried out in 6-well plates or spinning in 50 mL screw capped tubes, the latter had to be periodically aerated. For pulse experiments, 4-IPP aliquots were diluted 1:2500 in ASW, control embryos were treated with DMSO 1:2500.

Separate groups of embryos were injected with FITC and TMR, and treated with the 4-IPP drug at the same dilution from fertilization to the 16-cell stage. Microsurgeries were performed where the micromeres of FITC labeled embryos were transplanted on TMR labeled hosts (figure 6). For protocol details on embryo microsurgeries, see George and McClay (2019). In all drug treatments, embryos were rinsed multiple times in fresh ASW. These drug treatments were performed by Michael Wen, and microsurgeries by Dave McClay.

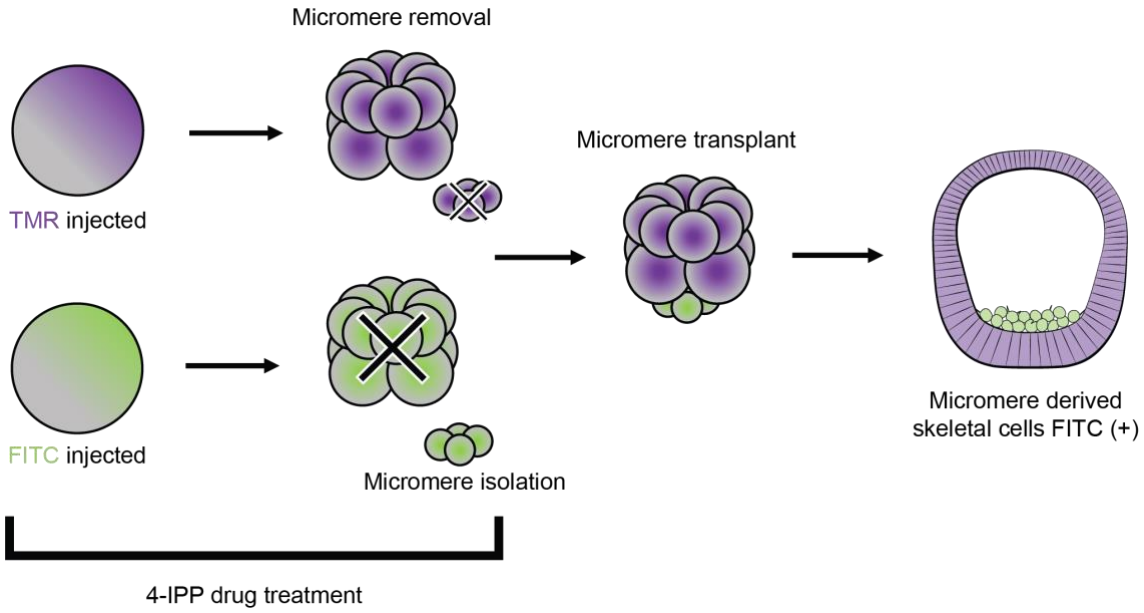


Figure 6: Schematic of 4-IPP treatment and micromere transplant

Two sets of unfertilized eggs were microinjected with TMR (purple) and FITC (green). Injected eggs were treated with 4-IPP drug from fertilization to the 16-cell stage. TMR injected embryos had their micromeres removed. FITC injected embryos had their micromeres isolated. Isolated FITC+ micromeres were placed on TMR+ embryos that had their original micromeres removed. Micromere transplants had 4-IPP washed away and they were allowed to develop with skeletal cells derived from the FITC+ micromeres.

2.5 MASO and mRNA microinjections

Translation blocking morpholino antisense oligonucleotides (MASOs or morpholino) were generated by GeneTools based on submitted sequences. MASOs were dissolved in 100 uL molecular grade water and stored at 4℃. MASOs were vortexed before being used and diluted to working concentrations in FITC and glycerol. Table 3 contains the morpholinos and their concentrations used for knockdown experiments. Membrane-GFP was synthesized and injected into embryos at the 2 and 4-cell stage embryos (Saunders and McClay, 2014; von Dassow et al., 2019).

Table 3: Morpholino sequences and knockdown concentrations

Gene	Morpholino Sequence	Concentration - WMISH	Concentration - Injury Assay
Astacin4	ACCCCAAAATCAGTGAAAACTCCAT	-	750 uM
Atp6v0a1	ACTGCATAAGTATGAAAGTTGAAGC	750 uM	750 uM
Delta	GTGCAGCCGATTCGTTATTCCTTT	428 uM	500 uM
Ese	GGCTTCCTTTCACTGTTGCCATG	-	750 uM
Gcm	GCTTTGGGCTTTTCTTTTGCACCAT	500 uM	500 uM
Irf4	TTTTGTTATGGGACATCATCACG	750 uM	500 uM
Mif4	GTTGTTCAGGAATCTTTACTTGGCG	750 uM	600 uM
Mif5	ATGTACCACACAACGCATCATCTTG	750 uM	750 uM
Mif7	GTACATCTCAATGCAAGGCATGGTG	750 uM	750 uM
Mitf	TCTTACCGTCTCAAGCTCTACCAGC	-	1 mM
Six1/2	AGTAAAACCAAAGGACGGGAGCAT	500 uM	600 uM

2.6 Live imaging protocols

2.6.1 Crush assay

To deciliate embryos, 12ul of larvae in ASW were hand collected in a pipette and placed in 90 ul of 2X ASW. In less than 10 seconds after 2X ASW treatment, 1 mL of fresh ASW was added to bring the larvae back to normal ASW concentration. Larva were placed between a glass slide and cover slip with clay feet, the slide and cover slip were treated with protamine sulfate. While viewed under a dissecting scope, the corners of the cover slip were gently compressed until the larvae were visibly compressed between glass. A visual cue for compression are the larva's legs will splay. Slides were sealed with a hot Vasoline, lanolin, and paraffin wax (VALAP) mixture, and they are immediately ready to be imaged (George and McClay, 2019).

2.6.2 Live imaging crushed larvae

Immobilized larvae were imaged with a Ziess Axio Imager using the DIC color channel. On average, time series were taken every 30 seconds for one hour with 3-4 larvae imaged simultaneously. Depth of Z-stacks varied between imaging sessions. In knockdown experiments where FITC co-injected, fluorescence was checked in larvae with the GFP channel. When membrane-GFP was injected, we imaged in both the DIC black and white channel, and GFP channel. Exposure of the fluorescent channel was adjusted on a case by case basis.

2.6.3 Image processing

Images and videos were processed in the Zen Pro program and FIJI (Schindelin et al., 2012). In FIJI, the Linear Stack Alignment with SIFT was selectively used in time series of injured larvae to stabilize the larva's movement during videos (George and McClay, 2019). Pigment cells were manually tracked using the MTrackJ plugin for FIJI (Meijering et al., 2012). Colored tracks and measurements were done through the MTrackJ program.

3. Uncovering molecules in the mesoderm related to immune and/or wound repair function

3.1 Introduction

Research on the skeletal cells, pigment cells, and the heterogeneous blastocoelar cells have led to discoveries in the area of mesenchyme development, plasticity, and immune-like function. The skeletal cells (i.e., skeletal mesenchyme) are the most intensely studied amongst the larval mesoderm. They are the first to emerge in the cleavage stage embryo, the first cells to undergo an epithelial-mesenchymal transition (EMT) into the blastocoel, and they build a stereotyped calcite skeleton (Lyons et al., 2014). The transcription factor Alx1 is a common marker for skeletal cells, since it's expressed strongly and solely in the skeletal cells pre-EMT and during skeleton formation (figure 7A). Along with their differentiation, skeletal cells have also been studied to investigate their cell-to-cell signaling function, syncytium formation, and ability to deposit and shape specific calcite skeleton forms (Ettensohn and Adomako-Ankomah 2019; Ettensohn and Dey 2017; Schatzberg et al., 2015).

Pigment (i.e., pigmented mesenchyme) and blastocolar mesenchyme development has been more difficult to work with in the sea urchin larva. One reason being is they share key developmental factors with the skeletal cells (figure 8), and temporary factors amongst themselves. Examples include the transcription factors Gcm and GataC (Croce and McClay, 2010; Davidson et al., 2002; Solek et al., 2013). Another reason is that during gastrulation, pigment and blastocoelar cells both are unpigmented

and cannot be morphologically distinguished. Pre-EMT, pigment cells express Pks1, a polyketide synthase (figure 7C). After their EMT and mesenchymal-epithelial transition (MET), pigment cells continue to produce Pks1 and now echinochrome A, the latter appears as easily distinguishable reddish granules. In comparison, the blastocoelar cell populations continue to be optically clear and undergo EMT from the top of the invaginating gut throughout gastrulation (Buckley and Rast, 2019; Calestani et al 2003; Tamboline and Burke, 1992). During the feeding pluteus stage, pigment cells and the different subtypes of blastocoelar cells are morphologically different, and more easily distinguished amongst the mesoderm within the larger larva blastocoel (figure 9).

Skeletal cells and their progenitors were the first to be isolated for RNA-sequencing experiments (Rafiq, 2014), through methods such as micromere removals and "panning" (Ettensohn and McClay, 1987). In recent years, single-cell RNA-sequencing has opened new doors for generating databases of candidate molecules for research topics of interest. Work by Massri et al., (2021) has opened one of these doors for the sea urchin by creating a developmental single-cell RNA-seq database for the *Lytechinus variegatus* embryo. As you will see below, this database has allowed us to track pigment and blastocoelar cell lineages and create an atlas of immune-related molecules that are uncharacterized in sea urchin development and invertebrate innate immunity. A newly published genome for *L. variegatus* has allowed for higher quality access to sea urchin coding sequences, non-coding sequences, and higher resolution

looks at gene duplications (Davidson et al., 2020). This chapter uses these resources to confirm, and investigate genes within the skeletal, pigment, and blastocoelar cell populations. Using a combination of literature on the immune system, *in situ* hybridization (ISH) data (generated by myself, former undergraduate researcher Michael Wen, and research scientist Esther Miranda), and bioinformatics data, I will make an argument for the classification of mesoderm subtypes and key molecules to investigate further.

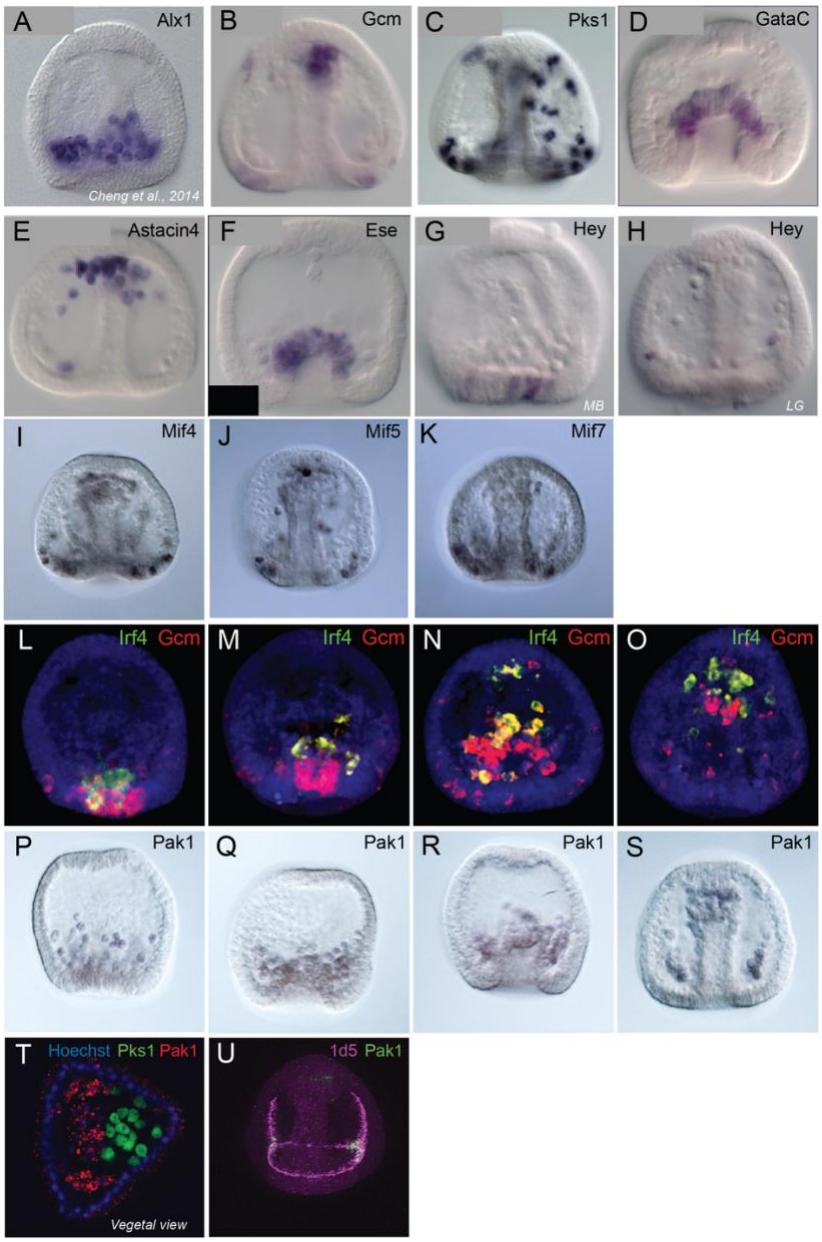


Figure 7: ISH expression data on mesodermal factors

(A) Alx1 ISH expression (modified from Cheng et al., 2014). (B) Gcm ISH expression at late gastrulation. (C) Pks1 ISH in pigment cells. (D). GataC ISH expression mid-gastrulation (cr. E.M.). (E) Astacin4 ISH expression at late gastrulation. (F) Ese ISH

expression in early gastrulation. (G-H) Hey (ISH) expression in early and late gastrulation. (I-K) Mif 4, 5, 7 ISH expression in the pigment cells late gastrulation. (L-O) Irf4 (green) and Gcm (red) double ISH from early to late gastrulation. (P-S) Pak1 ISH expression from early to late gastrulation. (T) ISH of vegetal view of gastrulating embryo showing Pak1 (red) does not co-express with pigment cells (Pks1 - green). Hoechst is blue showing nuclei. (U) ISH and immunostaining of late gastrula showing Pak1 (ISH green) is expressed at the tip of the archenteron and the ventral lateral cluster (skeletal cells immunostained by 1d5). Credit to Esther Miranda for B, D, E-H, & L-O, and Michael Wen for P-U.

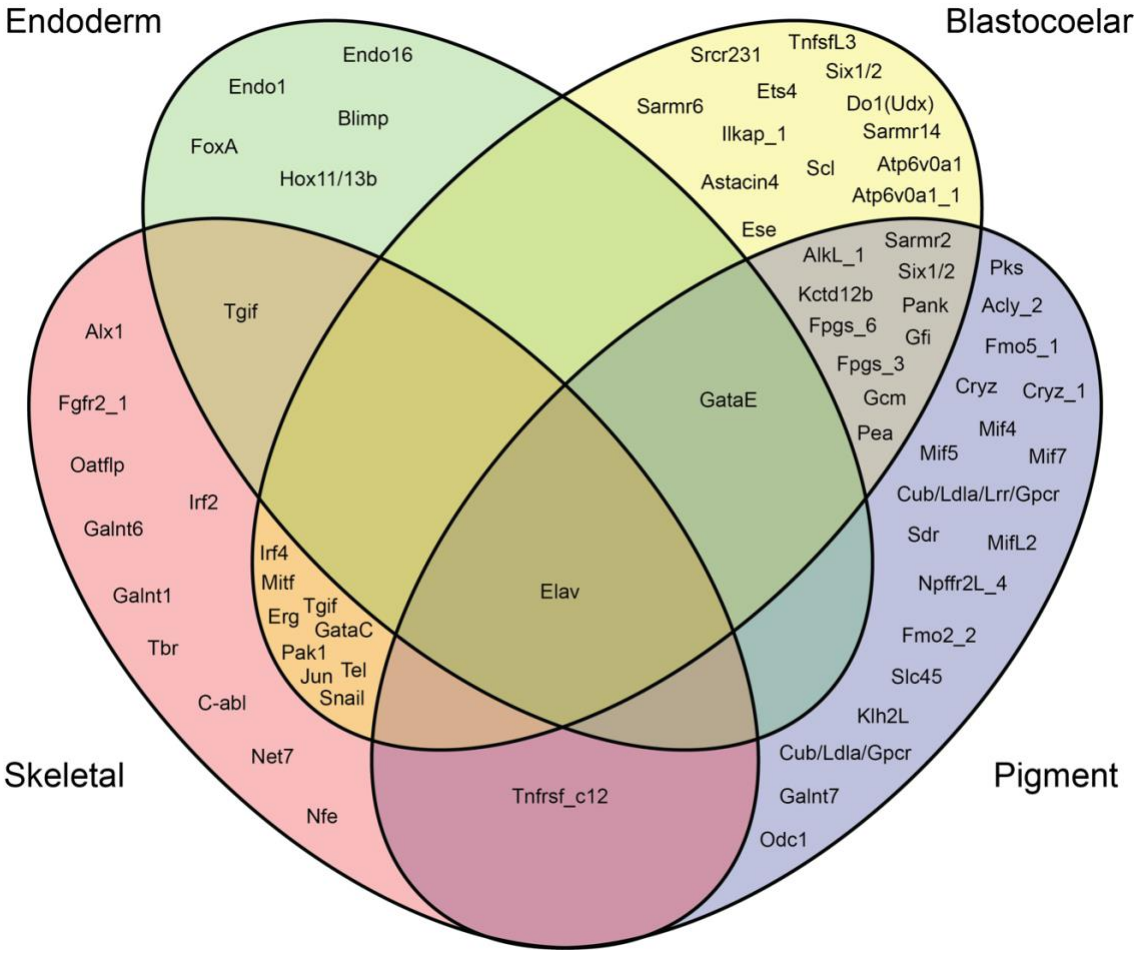


Figure 8: Venn diagram of mesenchymal and endodermal factors

A schematic showcasing published and unpublished factors determined to be in cell types via ISH, immunostaining, or the developmental single-cell RNA-seq *L. variegatus* database (Massri et al., 2021).

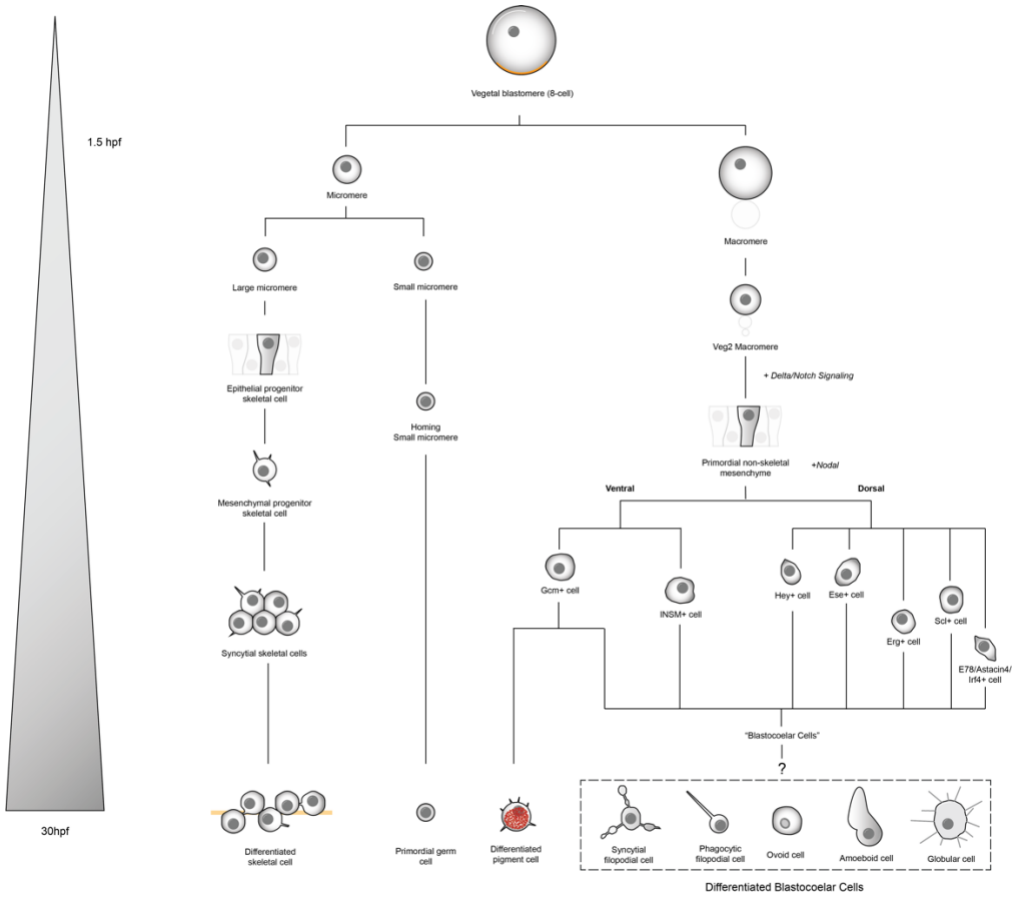


Figure 9: Mesenchymal lineage tree for the *L. variegatus* embryo

A schematic showing the rough timing and derived lineages of the mesenchymal cells and tissues in the *L. variegatus* embryo to larva.

3.2 The larval mesenchyme and its connection to hematopoietic cellular and molecular factors across species

The sequencing of the first echinoderm and sea urchin genome showed that sea urchins possess a large amount of immune gene homologs (Hibino et al., 2006). Many of these immune gene families are expanded in the sea urchin genome compared to other systems. An example of such a gene family is the Toll-like receptors, where *Nematostella vectensis* (i.e., the Starlet Sea Anemone) has one, *Homo sapiens* has 11, and the sea urchin *Strongylocentrotus purpuratus* has 253 (Buckley and Rast, 2012; Hibino et al., 2006; Miller et al., 2007). Micromanipulation experiments, lineage-tracing, antibody generation, *in situ* hybridization, reporter constructs, and knock-down experiments have led to a basic but limited understanding of the larva's immune system (Buckley and Rast, 2019; Ettensohn and McClay, 1988; Ho et al., 2016; McClay and Logan, 1996; Solek et al., 2013; Tamboline and Burke, 1992).

The ontogeny of what we call the skeletal, pigmented, and blastocolar mesenchyme is well understood morphologically until around early-gastrulation. Developmental gene regulatory networks exist for these tissues until early-gastrulation (Davidson et al., 2002; Israel et al., 2016; Saunders and McClay, 2014). After this stage of development, there are gaps in knowledge in what and how the pigmented and blastocoelar mesenchymes develop further. The feeding larva stage offers a future snapshot of immune-cell development, but it's unclear how mesenchyme differentiation occurs and their cellular and molecular origin (figure 9). Simultaneously, the larvae

reared in the lab are in artificial sea water, giving them an immunoquiescent state where it's unclear how the immune-like cells' numbers, morphology, locations, actions, and molecular state differ from the wild. By assessing the literature and current state of sea urchin immunology and hematopoiesis across species, I propose a restructuring in how we classify, name, and approach the skeletal and non-skeletal mesenchyme of the larva. Primarily, I propose that these mesenchyme populations can be classified as macrophages.

Macrophages across vertebrate species have a wide range of broad and specific abilities. Furthermore, classifying and comparing the hematopoietic systems within and across species has been difficult and confusing. To bridge these gaps in interspecies immune knowledge and integrate sea urchin immunology into the macrophage discourse, I will discuss observations in the larval mesenchyme development and connect them to macrophages across species. In mammal development, this will include monocytes, macrophages, and dendritic cells which share a common lineage (Rad, 2006). In invertebrates, this will be their macrophage-like cells called hemocytes (Edholm et al., 2017). The following are reasons why the skeletal and non-skeletal mesenchyme can be categorized as macrophages: (1) phagocytic activity, (2) expression of immune-related genes, and (3) molecular & cellular reprogramming and plasticity.

3.3 Phagocytosis within the pigmented and blastocoelar mesenchyme

An important moment in the field of immunology was the discovery of mesenchyme within the larva sea star that could wrap around and break down non-larval bodies, later categorized as phagocytosis (Tauber, 2003). Macrophages, as their name implies, are known for this phagocytic ability when a foreign body is present in the organism, and during wound healing and tissue homeostasis to eliminate apoptotic or damaged cells. In the mammalian system, macrophages are broadly categorized as tissue-resident and monocyte-derived populations (Italiani and Boraschi, 2017). Macrophages are further sub-categorized depending on the tissue, such as Kupffer cells, microglia, osteoclasts, etc.

Pigmented and blastocoelar mesenchyme subtypes have been documented multiple times for their ability to migrate and engulf foreign particles, including pathogenic bacteria (Buckley and Rast, 2019; Ho et al., 2016). Similar to macrophage cross-talk with cells in the gastrointestinal system, pigmented and amoeboid-like mesenchyme have also been shown to interact with the larval gut through their pseudopodia (Buckley and Rast, 2019; Muller et al., 2014). There are no published cases of apoptosis taking place in developing sea urchin embryos (i.e., across sea urchin species) and early feeding sea urchin larvae, and no published work on non-pathogenic injury to the larva resulting in wound recovery through immune cells. Chapter 5 of this

dissertation investigates non-pathogenic injury and provides further evidence of phagocytosis for blastocoelar mesenchyme.

3.4 Immune-like factors are present amongst the mesoderm lineages

Sia Immune-related gene families and homologues are present throughout sea urchin genomes and scattered across their chromosomes (Davidson et al., 2020; Hibino et al., 2006). A relative handful of these immune genes have been cloned and perturbed to determine function in the immunoquiescent embryos and larvae. We currently use RNA based ISH in the lab to determine gene expression; rarely we use antibodies from other species or generate our own (Slota et al., 2020; Wessel and McClay, 1986). Until recently, these methods used in the sea urchin immune system were labor intensive and involved multiple controls when working with sea urchin species that were not *S. purpuratus*.

Before the recent publication of the *L. variegatus* genome and *L. variegatus* single-cell data set, it was difficult to clone and determine ISH patterns for these immune-related genes across sea urchin species (e.g., *L. variegatus*) solely from the *S. pupuratus* sequence. This is due to the high rate of evolutionary change said to happen in immune systems (Hibino et al., 2006). Another reason for the difficulty is that select immune-related molecules have lower transcript levels compared to non-immune genes, potentially caused by the immunoquiescent state the embryos and larvae are reared in (Hibino et al., 2006; Howard-Ashby et al., 2006). The 2020 *L. variegatus* 3.0 genome,

publicly available on Echinobase, now allows us to clone whole or partial regions of gene loci (Cary et al., 2018; Davidson et al., 2020). The following subsections list immune factors already published on within the three mesenchymes mentioned above, and pairs them with unpublished gene expression patterns and new candidate genes from the developmental single-cell RNA-seq (sc-RNA-seq) dataset to be further investigated (Massri et al., 2021).

3.4.1 Skeletal mesenchyme possess hematopoietic markers

Skeletal mesenchyme cells express the following immune gene homologues: Mitf, a VEGF receptor (Vegfr-10-Ig), GataC (Gata1/2/3), Tbr (Eomes-like), Tel (ETV6), c-Jun, Scl (Scl/Tal-2/Lyl-1) and Erg (Croce et al., 2001; Ettensohn and Adomako-Ankomah, 2019; Rizzo et al., 2006; Russo et al., 2014; Shashikant et al., 2018; Solek et al., 2013). Across species, homologues of these factors or their activation molecules are varyingly present in macrophages, their subsets, and/or cell's in their hematopoietic lineages (e.g., Natural Killer cells or monocytes) (Dong et al., 2013; Fu et al., 2017; Lin et al., 2020; Lu et al., 2014; Rasighaemi et al., 2015; Rodrigues et al., 2008; Vagapova et al 2018; Wheeler et al., 2018). In the framework of categorizing the skeletal mesenchyme as macrophages/immune cells, I mined the sc-RNA-seq data from Massri et al., (2021) and created a list of immune factors for further study (figure 10; Table 4).

Irf4 and Pak1 were cloned, and show matching expression patterns to the sc-RNA-seq data, with partial expression in the skeletal mesenchyme (figure 7L-S; figure

10). Irf4 is a transcription factor with homologues that are present in vertebrate macrophages with roles in their differentiation and their M1/M2 polarizations (Chistiakov et al., 2018). Pak1 is a serine/threonine-protein kinase and is shown to be involved with inflammatory macrophage polarization, and shows expression in the blastocoelar cells as well. (Zhang et al., 2015). As a proof of concept, the sc-RNA-seq database can match gene expression patterns for larval immunology up to the end data point of 24 hour post fertilization.

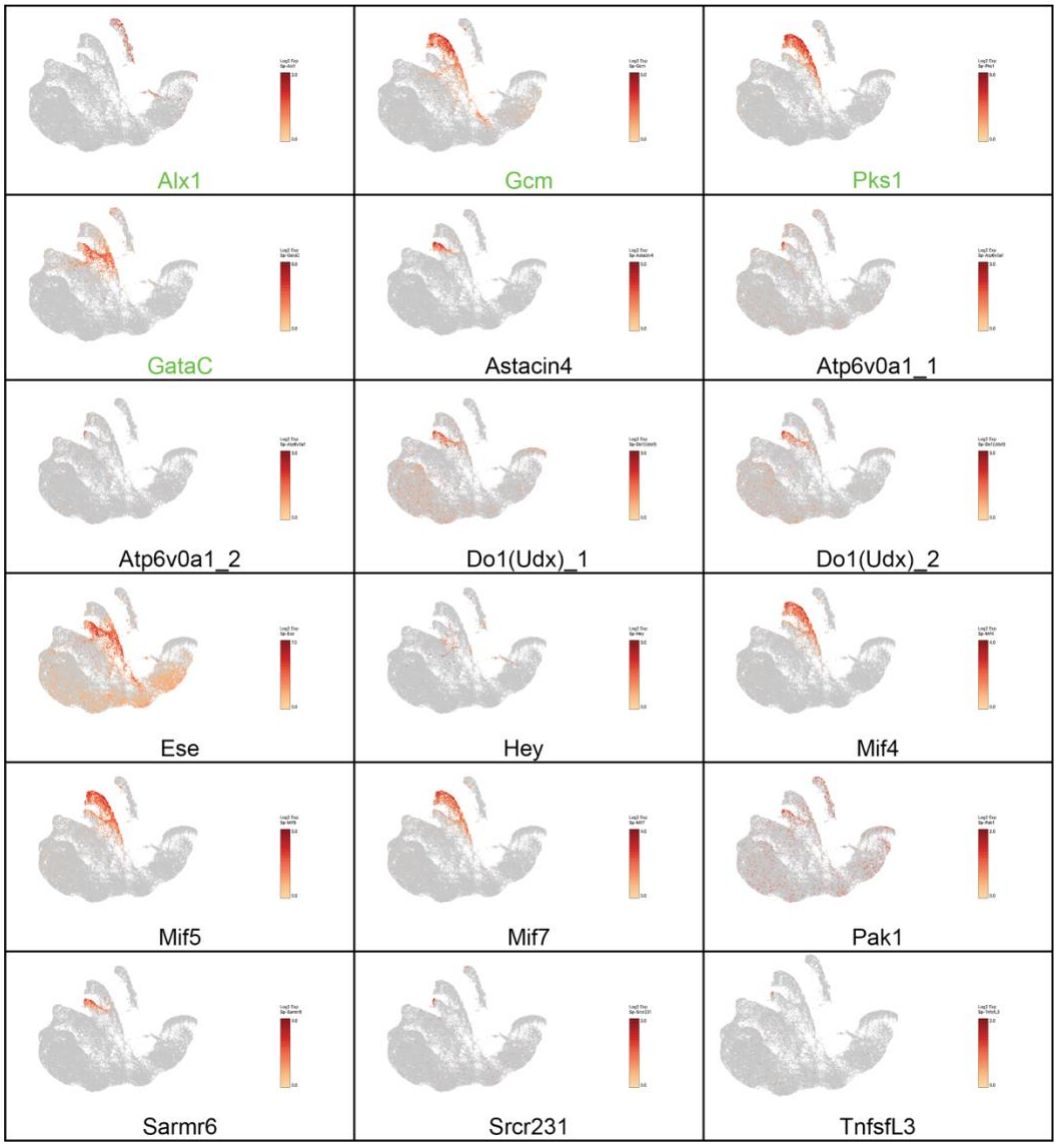


Figure 10: Loupe Browser data from L. variegatus sc-RNA-seq atlas

Images from the *L. variegatus* sc-RNA-seq database showing standardized molecules in green (Alx1; Gcm; Pks1; GataC) and molecules of interest related to immunity. For further molecules details, see Table 4.

Table 4: Candidate immune system genes

Skeletal Mesenchyme	Aliases
B3gt1	beta-1,3-galactosyltransferase 1
Fgfr2_1	fibroblast growth factor receptor 2-like
Galnt1	polypeptide N-acetylgalactosaminyltransferase
Galnt6	polypeptide N-acetylgalactosaminyltransferase
Oatflp	CAS1 domain containing 1; N-acetylneuraminate 9-O-acetyltransferase
Pigmented Mesenchyme	
Acly_2	ATP-citrate synthase; citrate cleavage enzyme / ATP-citrate (pro-S-)-lyase
Cryz	quinone oxidoreductase-like; NADPH:quinone reductase; zeta-crytallin
Cryz_1	quinone oxidoreductase-like; NADPH:quinone reductase; zeta-crytallin
Cub/Ldla/Gpcr	
Cub/Ldla/Lrr/Gpcr	
Fmo2_2	dimethylaniline monooxygenase [N-oxide-forming] 2
Fmo5_1	dimethylaniline monooxygenase [N-oxide-forming] 5 / hepatic flavin-containing monooxygenase
Galnt7	N-acetylgalactosaminyltransferase 7; UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 7
Klhl2L	kelch-like protein 2; Mayven-like
Mif4	Macrophage migration inhibitory factor
Mif5	Macrophage migration inhibitory factor
Mif7	Macrophage migration inhibitory factor
MifL2	Macrophage migration inhibitory factor-like
Npffr2L_4	somatostain-like receptor F_48D10.1; neuropeptide FF receptor 2-like-4
Odc1	ornithine decarboxylase 1
Sdr	3-oxoacyl-acyl-carrier-protein reductase-like; short chain dehydrogenase family; short-chain dehydrogenase/reductase
Slc45	membrane-associated transporter protein; AIM-1 protein; Melanoma antigen AIM1; Solute carrier family 45 member 2
Blastocoelar Mesenchyme	
Astacin4	zinc metalloproteinase nas-13-like
Atp6v0a1	V-type proton ATPase 116 kDa subunit a; Clathrin-coated vesicle/synaptic vesicle proton pump 116 kDa subunit
Atp6v0a1_1	V-type proton ATPase 116 kDa subunit a; Clathrin-coated vesicle/synaptic vesicle proton pump 116 kDa subunit
Do1 (Udx)	dual oxidase 1; urchin dual oxidase
Ilkap_1	Integrin-linked kinase-associated serine/threonine phosphatase 2C
Irf4	interferon regulatory factor 4
Sarmr6	Sterile α and HEAT Armadillo motif-containing protein

3.4.2 Pigmented mesenchyme possess high levels of atypical cytokines

The most well characterized transcription factor that is necessary for pigmented mesenchyme differentiation is Gcm (glial cell missing) (figure 7B). As the name suggests, knockdown of Gcm in *Drosophila* leads to the loss of glial cells and overexpression leads to glia (Jacques et al., 2009; Soustelle and Giangrande, 2007). In the sea urchin larva, Gcm first appears after Delta/Notch signaling from the micromeres, in a ring-like pattern of cells (Croce and McClay, 2010; Solek et al., 2013). This ring of Gcm+ cells later constricts to the aboral side of the embryo. The pigmented mesenchyme later express Eph. When Eph was inhibited, pigment cells stayed in a rounded, non-dendritic-like form (Krupke et al., 2016). Krupke et al. (2016) also show Eph to be an example of a mesenchyme and neural gene used to direct to targeted location since it was shown to be part of the pigmented mesenchyme homing mechanism to the dorsal ectoderm. In vertebrates, bone marrow macrophages are shown to be Ephrin positive and be important for their interactions with erythroblasts (Hampton-O'Neil et al., 2020). One last set of immune related factors shown to be heterogeneously expressed in the pigmented mesenchyme are Tecp2 (thioester-containing protein) and Srcr142 (scavenger receptor) (Ho et al., 2016).

Immune systems are well-known for their production and signaling through cytokines/lymphokines. Homologues of the cytokine family known as Macrophage Migration Inhibitory Factors (MIFs) show up in the sc-RNA-seq data in the pigmented

mesenchyme branch (figure 10). We successfully cloned Mif4, Mif5, and Mif7 which have matching expression patterns (figure 71I-K). Surprisingly, in the literature these genes are shown more to influence macrophage movement via extracellular signals than produce the cytokine but also to inhibit the growth of bacterial infections (Oddo et al., 2005). One of these MIF orthologues appear in the sea urchin literature referred to as "DopT", but has no characterization since, and Mif5 has also been cloned and its expression pattern shown in pigmented mesenchyme (Perillo et al., 2020; Rast et al., 2002). Chapter 4 of this dissertation further investigates and perturbs the Mifs expressed in the pigmented mesenchyme.

3.4.3 Blastoceolar cell subpopulations express markers for biomineral resorption

The heterogeneous blastocoelar mesenchyme cells share expression of the following genes at different time points with either the skeletal or pigmented mesenchyme: Gcm, GataC, Erg, Mitf, c-Jun, Tel, Pea, Scl and Irf4 (Croce et al., 2001; Croce and McClay, 2010; Rizzo et al., 2006; Russo et al., 2014; Shashikant et al., 2018; Solek et al., 2013). MacpfA2, a perforin-like gene, is solely expressed in a subset of the blastocoelar mesenchyme, referred to as "globular cells." (Ho et al., 2016). The homologue of this gene known as MPEG1 was originally discovered in macrophages and the superfamily continues to be investigated in pore formation (Bayly-Jones et al., 2020).

Ese (Elf-3), Hey (Herp), and Astacin4 were cloned, and show tissue expression patterns predicted by the *L. variegatus* sc-RNA-seq database (figure 7E-G; figure 10). Ese and Hey are transcription factors which show roles in monocytes and effects on osteoclasts, respectively (Grall et al., 2005; Salie et al., 2010). Astacin is a large family of metalloendopeptidase genes with members including BMP1 and Tolloids. It is currently unclear which member *L. variegatus* Astacin4 is more closely related to but there are precedents for BMPs participating in osteoclastogenesis (Omi et al., 2019). These molecules in mammalian systems are known to be involved in immunity, and we show their sea urchin homologues are expressed in a subset of immune cells.

Overlapping with Irf4 expression, a blastocoelar mesenchyme subset of the sc-RNA-seq dataset is positive for immune-related, glial factors, and cilia function: Atp6v0a1 (an Atpase subunit), Do1 (Udx), Sarmr6 (sarm-related 6), Srcr231, and TnfsfL3 (figure 10) (Cheon et al., 2020; Furukawa et al., 2012; Idriss and Naismith 2000; Liberati et al., 2004; Matsumoto et al., 2018). Tnfsfl3, a tumor necrosis factor, is a major molecule of interest since the cytokine superfamily is highly involved in immune function and signaling, and it shows subtle expression in immunoquiescent embryos (Vanamee and Faustman, 2018). Atp6v0a1 and Do1(Udx), there are two hypothetical copies of each, are both known to be involved in osteoclast function and genesis, respectively (Cheon et al., 2020; Matsumoto et al., 2018). Apt6v0a1 is further investigated in wound healing in Chapter 5 of this dissertation.

3.5 Macrophages and larval mesenchyme reprogram

Mammalian macrophages are known for their ability to switch between M1 and M2 stages, with a spectrum existing between them. M1 is associated with inflammation and M2 with activation/regeneration (Edholm et al., 2017). Their plasticity and ability to reprogram states is dependent on environmental inputs such as signaling molecules, injuries, and niches (Edholm et al., 2017; Kelly and O'Neill., 2015). When the skeletal mesenchyme or their progenitors are removed in the developing sea urchin embryo, the blastocoelar mesenchyme reprogram to a skeletal mesenchyme-like state and build the skeleton (Cheng et al., 2014; Ettensohn and McClay 1988). This is shown through ectopic skeletal specific markers such as Alx1 in Scl positive blastocoelar cells (Sharma and Ettensohn, 2011). When skeletal, pigmented, and blastoceolar mesenchyme are surgically removed during development, the presumptive endoderm replaces the skeletal mesenchyme (McClay and Logan, 1996). In this mesenchyme removal surgery, pigmented mesenchyme are not replaced and the embryo is an albino.

Current hypotheses for this phenomenon of lost skeletal mesenchyme replacement include (1) the skeletal mesenchyme produces a signal that keeps blastocoelar mesenchyme in their cell state and/or (2) the skeletal mesenchyme sequester signaling molecules and prevent them from reaching neighboring cell types. There is evidence in support of the hypothesis that the skeletal and blastocoelar mesenchyme express a VEGFR at different levels, and the original skeletal mesenchyme normally

sequester the VEGF produced by the ectoderm (Ettensohn and Adomako-Ankomah, 2019). The hypothesis states that removal of skeletal cells allows VEGF to signal to blastocoelar cells thereby causing them to reprogram.

We found an additional phenomenon for the reprogramming process. When mesenchyme blastula stage embryos are meridionally bisected, there is a partial recovery of the larvae. In this partial recovery, a swimming larva is developed but is missing portions of tissue such as an arm or portion of the oral hood. This is consistent where portions of the surgically removed ectoderm maintain a region-specific state (e.g., oral hood) that directs inserted skeletal mesenchyme to build a region-specific section of the skeleton or invaginate a mouth (Hardin and Armstrong, 1997). Removing the skeletal mesenchyme first, and then bisecting the embryos at mesenchyme blastula stage resulted in the recovery of larval bilateral symmetry (figure 11). This provides evidence in support of the hypothesis that the skeletal mesenchyme provides important signaling cues for surrounding tissues. Unfortunately, due to the technical, cellular, and molecular complexity of these experiments, the phenomenon was not studied further.

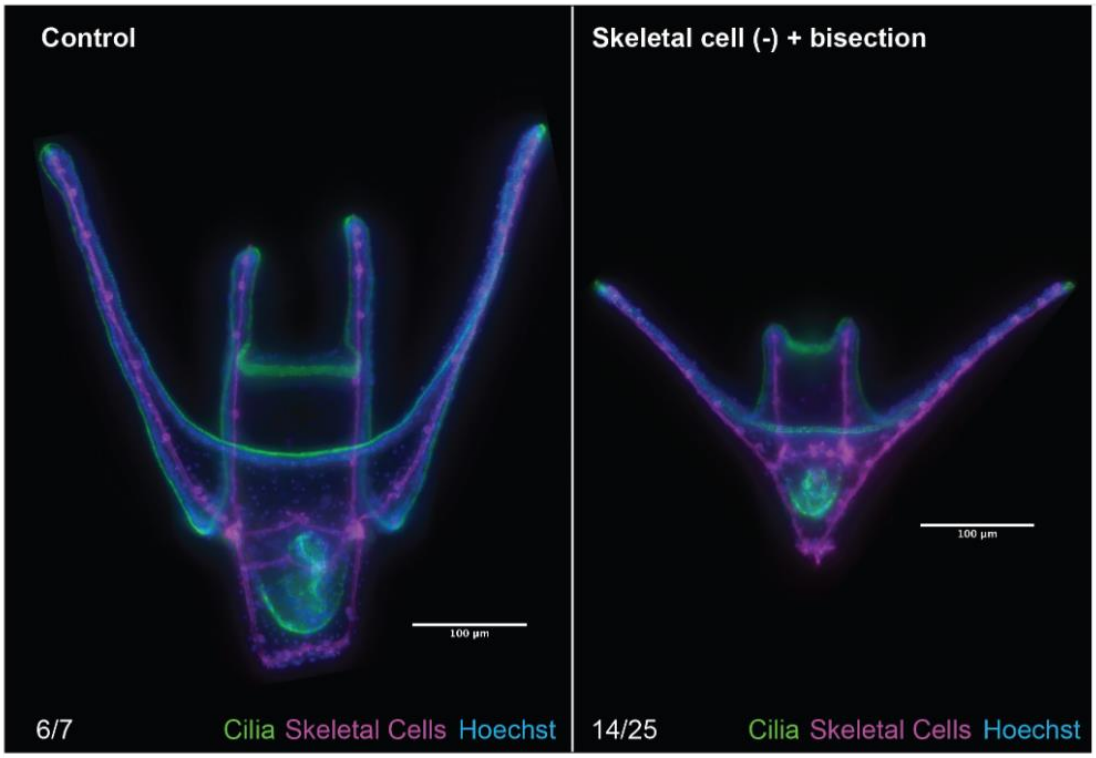


Figure 11: Removing skeletal cells recovers bisected embryos

(Left) A control pluteus larva immunostained to show structures. (Right) An experimental, recovered larva that had its skeletal cells removed at mesenchyme blastula stage and bisected meridionally soon after at the mesenchyme blastula/early gastrula stage. Cilia (green) stained with the 295 antibody, skeletal cells (purple) stained with the 1d5 antibody, and nuclei (blue) stained with Hoechst. Scale bar is 100 microns.

3.6 Conclusions

This chapter covers years of work in sea urchin immune system development, and touches upon the overwhelming complexity of immunology across species. With access to new bioinformatics information, we've been able to dive deeper into our understanding of the larval immune system by connecting immune factors and cell behaviors to the larva's mesodermal populations. Specifically, we looked into the phagocytic activities of the different mesenchymes, compiled and confirmed immune-related expression data, and recapped the history of cell reprogramming in the embryo and its parallels to immune system plasticity.

For phagocytosis, a cell function pervasive in immunology, is found in the pigmented and blastocoelar mesenchyme. Paired with chapter 5, we see this phagocytosis within these cell populations applies to skeletal remodeling and resorption. Our literature review of the inter-species immune molecules allowed us to conclude the reliability and power of the *L. variegatus* sc-RNA-seq database through efficient *in silico* and *in situ* gene expression patterns. Finally, connecting the parallels in sea urchin transfating/cell reprogramming in the mesenchyme to the replacement and plasticity in the mammalian immune system, we are able propose an applicable model for studying reprogramming in the sea urchin embryo and larva.

Based on information in this chapter, paired with select information in chapters 4 and 5, I propose the sub-field of immunology in sea urchin development moves away

from historical thinking and classifications of the “immune system” within the community. This work shows multiple novel discoveries of the echinoid mesoderm by looking into single-cell expression data and matching it to “unexpected” immune cells factors across species and tissues. One example is, if we did not connect osteoclasts to the larval mesenchyme, before this dissertation there were no examples of the mesenchyme resorbing skeleton (chapter 5), we would not have made these discoveries. By reclassifying, either officially or personally, the mesodermal populations generally to mesenchyme (e.g., skeletal cells to skeletoblastic mesenchyme) or specifically macrophages, we may be able to continue making more novel discoveries in the area of sea urchin immunology.

4. The MIF cytokine superfamily in the sea urchin

Data from this chapter will be included as part of a paper on separation of pigment cell and blastocoelar cell fates in response to Nodal signaling, in both cases setting up an ability to undergo EMTs, though those EMTs are surprisingly different from each other.

4.1 Introduction

Macrophage Migratory Inhibitory Factors (MIFs) are a conserved family of atypical chemokines involved in a myriad of cell processes in vertebrate and invertebrate animals and plants, but how they operate throughout development across systems is not well known (Ito et al., 2008). The first MIF was discovered in the 1960's and was fully cloned in mammals in 1993. These molecules have been shown to be important in vertebrate specific CD74 and chemokine receptor binding, as an upstream activator of ERK1/2, AMPK, and AKT signaling pathways, as an *in vitro* enzymatic role acting as a phenylpyruvate tautomerase, and as a growth inhibitor to bacteria (Jankauskas et al., 2019). In invertebrate systems, MIF-like genes were cloned and perturbed in the larval starfish *Patiria pectinifera* to examine the larval immune system but their tissue expression patterns and mechanistic action in embryo development was not addressed (Furukawa et al., 2016). A Mif-like gene was cloned in 2002 in *S. purpuratus* with limited expression data, named "DopT," and a "Mif5" gene as well in 2020 (Perillo et al., 2020; Rast et al., 2002). To date, however, very little is known about how these chemokines function in development and invertebrates.

In *homo sapiens* (Hs), there are two members of the MIF family, MIF1 (Hs-Mif1)and MIF2 (Hs-Mif2). MIF2 is commonly referred to as D-DT (D-dopachrome tautomerase). The coding sequences of Hs-Mif1 and Hs-Mif2 are 348 base pairs (bp) and 357 bp respectively . Translation of the mRNA sequences results in Hs-Mif1 having a protein length of 115 amino acids (aa), and Hs-Mif2 with 118 aa (figure 12A-B). These relatively short molecules become more enigmatic when processed in the online InterPro program that classifies protein domains, with the entire protein categorized as "MIF" or "D-dopachrome decarboxylase" (figure 12C) (Blum et al., 2020). In non-mammalian species, the number of MIF homologues varies - examples include bacteria (1 MIF), plants (3 MIFs), and birds (1 MIF) (Sparkes et al., 2017).

This chapter examines the MIF homologues present in the sea urchin *L. variegatus* in early to late larval development. Using a combination of sequencing, bioinformatic, and *in situ* hybridization (ISH) data, we show expression patterns of MIFs in the cleavage stage embryo and mesenchymal cells of gastrulae. By perturbing the MIFs and candidate interacting partners through drug treatments and morpholino antisense oligonucleotides (MASOs), we have defined their position in a pigment cell developmental gene regulatory network, and identified a possible functional role given that perturbation of MIFs causes deadhesion defects, atypical cell localization, and an apparent cellular reprogramming event. This work was done in collaboration with Esther Miranda and Michael Wen.

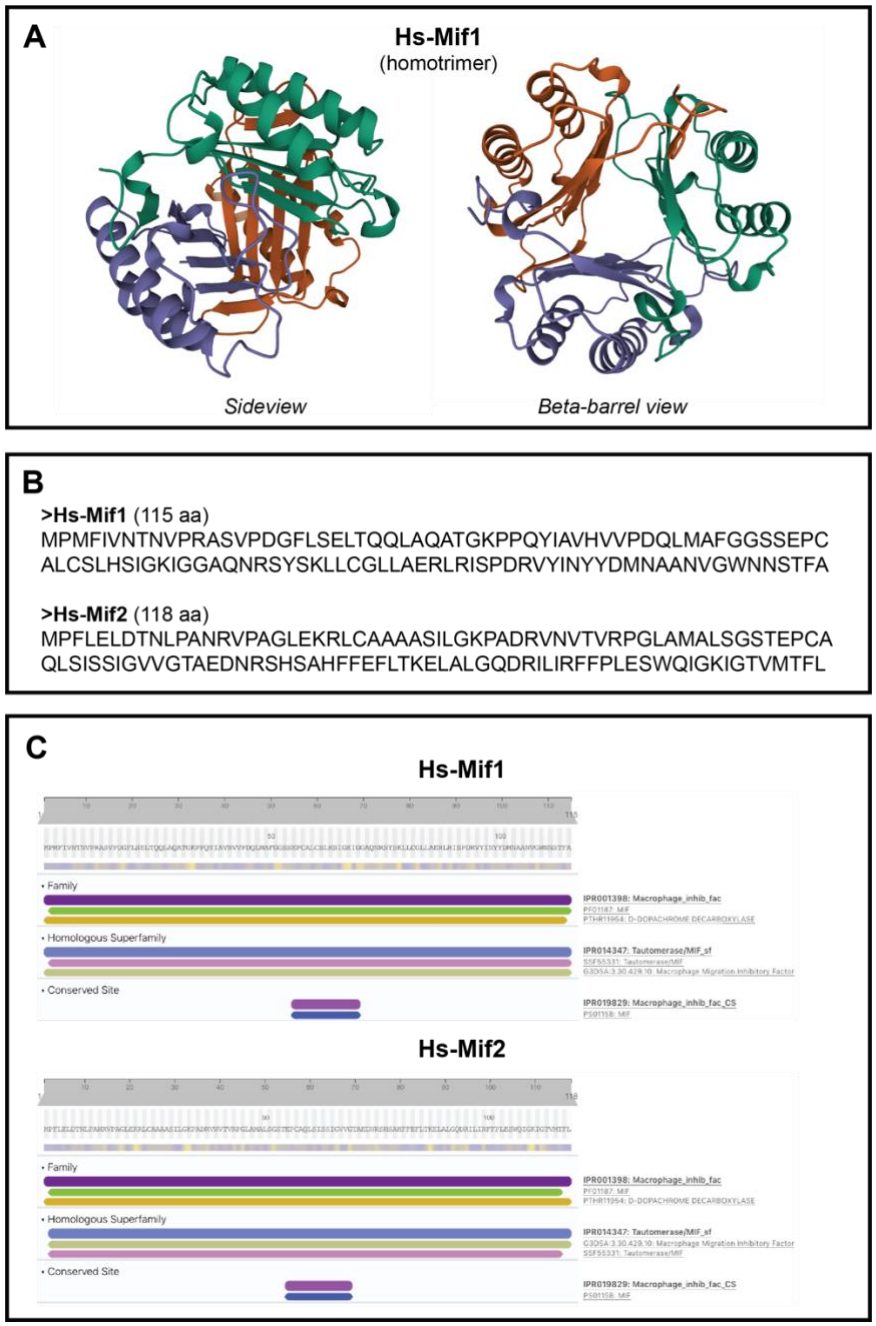


Figure 12: Hs-Mif1 and Hs-Mif2 (D-DT)

(A) Hs-Mif1 homotrimer from the protein data bank. (B) Hs-Mif1 and Hs-Mif2 coding sequences. (C) InterPro analysis of Hs-Mif1 and Hs-Mif2.

4.2 MIFs are expanded in sea urchin genomes

The sequencing of the *S. purpuratus* genome showed an expansion of MIF genes, 9 homologues in total (Hibino et al., 2006). The *L. variegatus* (Lv) genome has shown a greater expansion of MIF homologues with 16 MIF-like genes and one fragment gene (table 5) (Davidson et al., 2020). We discovered a greater number of Lv MIFs through BLAST of Hs and Sp MIF homologues in Echinobase, and running genes adjacent to the MIFs through InterPro (figure 13). We decided to do the latter when the neighboring gene was relatively small and had three exons. An example of this is that D-dopachrome decarboxylase-like (Chrom 15 - XM_041626269.1) is adjacent to an uncharacterized locus (LOC121428845). When the amino acid sequence of this uncharacterized locus is put through InterPro, it is categorized as having a MIF/D-DT domain. With the increased number of MIFs in Lv, and many of them not having names, we propose a temporary renaming of them to reflect their relation to MIF genes and their relation to one another (table 5). Using the example loci above, D-dopachrome decarboxylase-like (XM_041626269.1) is renamed to Mif2-like (Mif2L) since Hs-Mif2 is also called D-DT. We also add an "_2" to it since there is a loci duplication earlier in Chromosome 15 (figure 13).

Sc-RNA-seq data shows tissue specific expression during embryo development for 10 out of the 16 MIF genes (figure 14). As originally shown in chapter 3, Mif4, Mif5, and Mif7 show expression patterns in the pigment cell lineage (figure 7I-K). MifL1_3,

MifL2, MifL_1, and MifL_2 are also predicted to be in the pigment cell lineages. Mif1_2 indicates potential expression in the coelomic pouch and neural region of the embryo. MifL1_1 indicates maternal expression at early cleavage stages (Massri et al., 2021).

We cloned selected Lv MIF genes with candidate tissue specific expression. There were molecular biology barriers for some, and others were only recently discovered through BLAST. We successfully cloned and created RNA probes for Mif4, Mif5, and Mif7 which showed expression in early and late gastrulation within the embryo's pigment cells through ISH (figure 15). We also cloned Mif1_2 and MifL1_3. Mif1_2 shows a unique expression amongst the Mifs where it's expressed in the coelomic pouch and potentially neural cells, based on physical location and the sc-RNA-seq data (figure 14A; 16). MifL1_3 is expressed later than Mif4, 5, & 7, at early prism and it appears in a non-skeletal mesenchyme cell population, which is believed to be the pigment cells due to locations and sc-RNA-seq data (figure 14B; 16). We did not pursue Mif1_2 and MifL1_3 further, and Mif4, Mif5, and Mif7 will be the focus of later parts of this chapter.

Next we compare the sequences of the predicted Mifs to each other and Hs homologues. The coding sequences of the Lv homologues vary in size (table 5). The shortest is the "Mif (fragment)" which is 69 aa long and does not have a labeled locus in the Lv 3.0 Genome browser. The two longest Lv Mif genes are MifL_1 and MifL_2 both at 141 aa long, 23 residues longer than Hs-Mif2. Predicted protein translation with NCBI

indicates that Mif1_1, Mif5, and the Mif (fragment) are missing the highly conserved proline of the MIF family at the start of the protein. From the sequence information alone, and without defined motifs, it's impossible to posit how these shorter and longer Lv MIF proteins may or may not function.

At the genomic level, Lv MIF genes are present on five different chromosomes (figure 13). Chromosome 15 has a total of seven. Mif4 & Mif5, MifL_1 & Mif2L_1, and Mif2_2 & MifL_2 are next to each other respectively on chromosome 15, and share 5' regulatory regions. Setting aside the Mif fragment which doesn't have defined features, all the other Lv Mif genes have three exons (table 13). Via ATAC-seq data at the gastrula stage, Mif4 and Mif5 share an open 5' upstream regulatory region, and they are transcribed in opposite directions (figure 17). Lv-Mif7 is located on chromosome 3. These bioinformatics data and loci observations on the MIF family show that these genes are even more complex than originally thought. While not the focus of this study, the genomic evolution of MIFs across echinoderms, such as the duplications, and range of expression patterns, may well provide new insights into the evolution, and perhaps function, of this family of genes.

Table 5: *Lv* MIF family bioinformatic data

Gene Rename	Chrom	Location	NCBI #	Exons	bp	AA	2Pro?
Mif1_1	2	69371033-69371205	XM_041600471.1	3	351	116	No (S)
Mif1_2	2	69379684-69379857	XM_041600470.1	3	354	117	Yes
Mif1_3	2	69387278-69387450	XM_041600472.1	3	348	115	Yes
Mif7	3	59274016-59274200	XM_041604275.1	3	423	140	Yes
MifL1_1	3	72465964-72466146	XM_041604766.1	3	417	138	Yes
MifL1_2	3	72473681-72473864	XM_041605186.1	3	330	109	Yes
MifL1_3	3	72484734-72484917	XM_041604767.1	3	366	121	Yes
MifL1_4	3	72493529-72493707	XM_041605187.1	3	363	120	Yes
Mif (fragment)	9	29485455-29485597			210	69	No (V)
MifL2	11	20935465-20935639	XM_041619779.1	3	372	123	Yes
Mif6	15	1144122-1144302	XM_041626100.1	3	321	106	Yes
MifL_1	15	21231428-21231606	XM_041626292.1	3	426	141	Yes
Mif2L_1	15	21254055-21254232	XM_041625513.1	3	372	123	Yes
Mif2L_2	15	22315545-22315722	XM_041626269.1	3	372	123	Yes
MifL_2	15	22324901-22325079	XM_041625710.1	3	426	141	Yes
Mif4	15	26039014-26039196	XM_041625735.1	3	414	137	Yes
Mif5	15	26042757-26043191	XM_041626163.1	3	366	121	No (M)

Chrom = chromosome; bp = base pairs; AA = amino acids; 2Pro - 2Proline

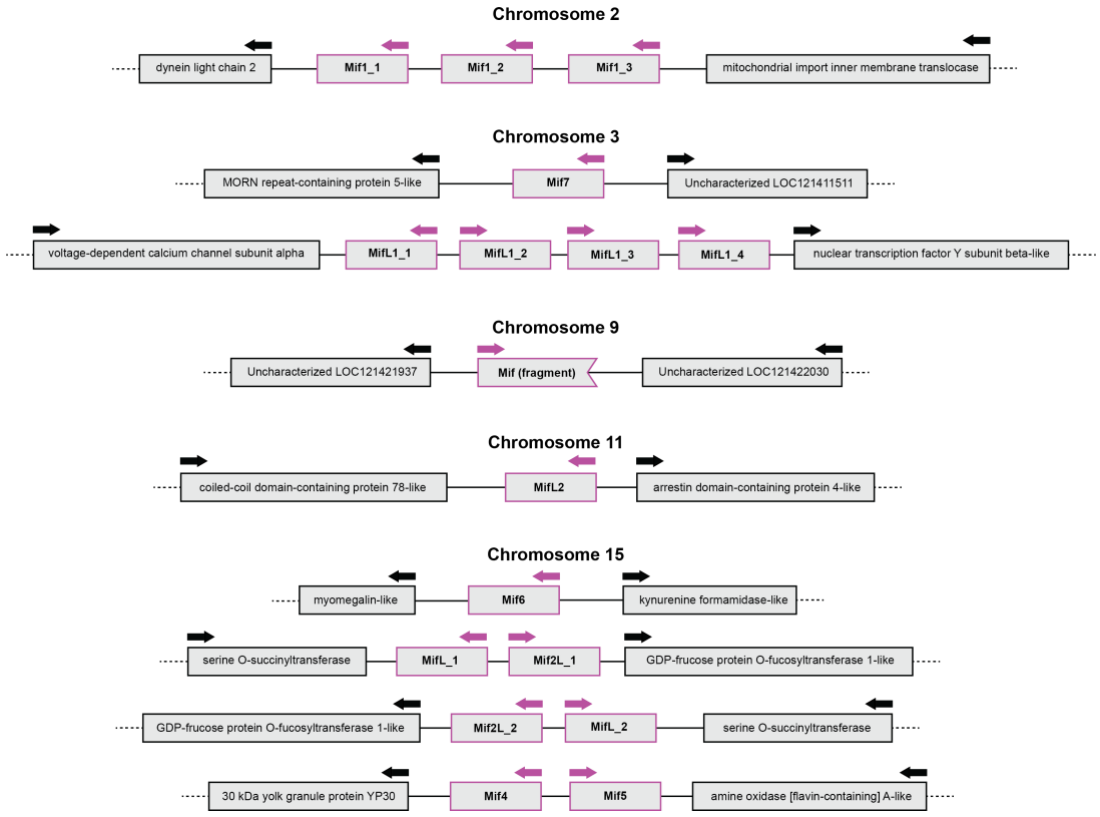


Figure 13: Schematic of MIF loci in the *Lv* Genome

Purple boxed genes showcase MIF family members. Arrows indicate gene direction.

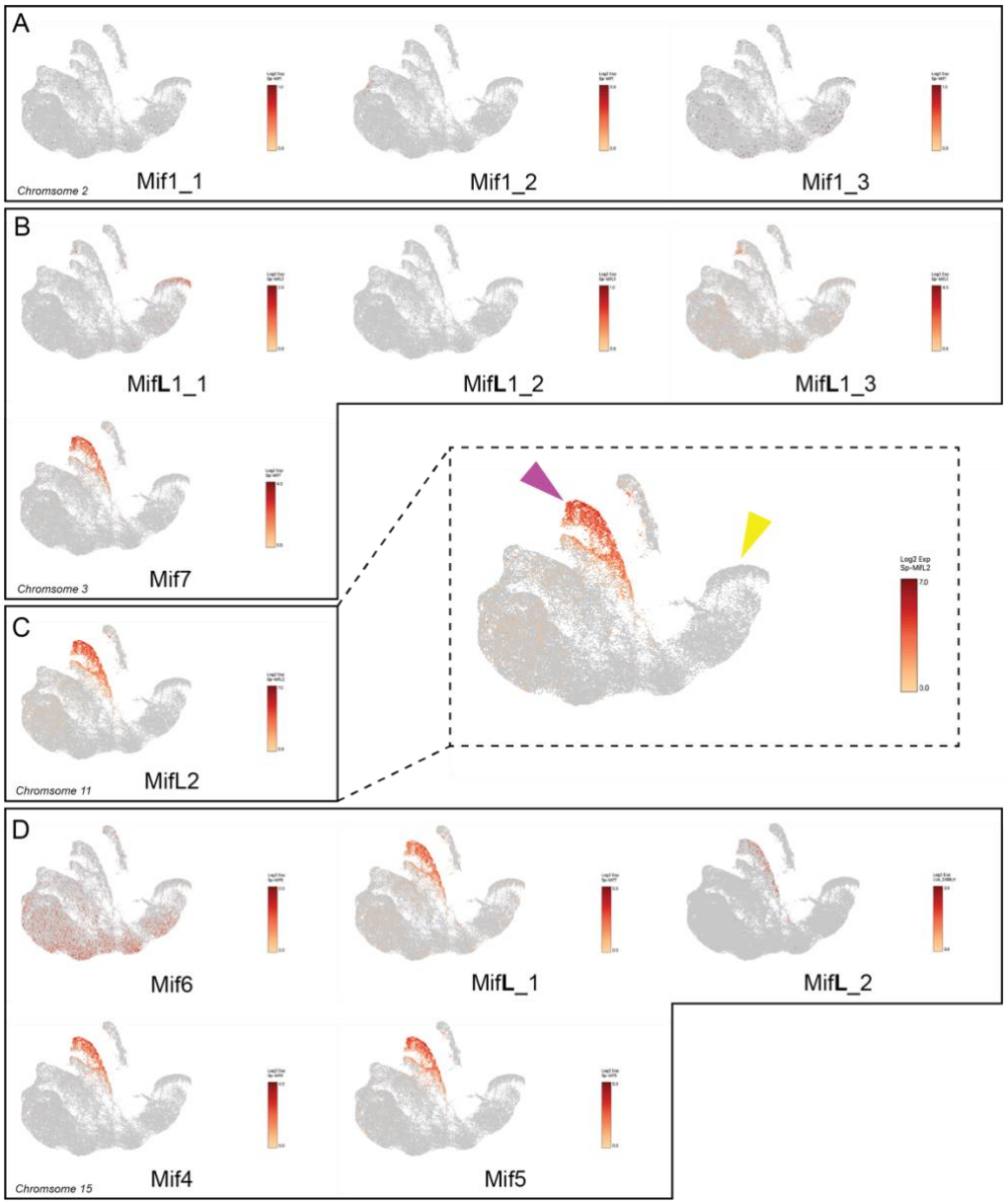


Figure 14: Loupe browser data for Lv MIF family in development

UMAP images from the *L. variegatus* sc-RNA-seq database that show tissue specific expression patterns. MIF genes separated by chromosome location. (A) Chromosome 2 MIF genes. (B) Chromosome 3 MIF genes. (C) A chromosome 11 MIF gene, enlarged region in dashed box with yellow arrowhead (right) showing the earliest

developmental time points and the purple arrowhead (left) showing the pigment cell lineage branch. (D) Chromosome 15 MIF genes. The red and orange dots represent cells expressing that particular gene.

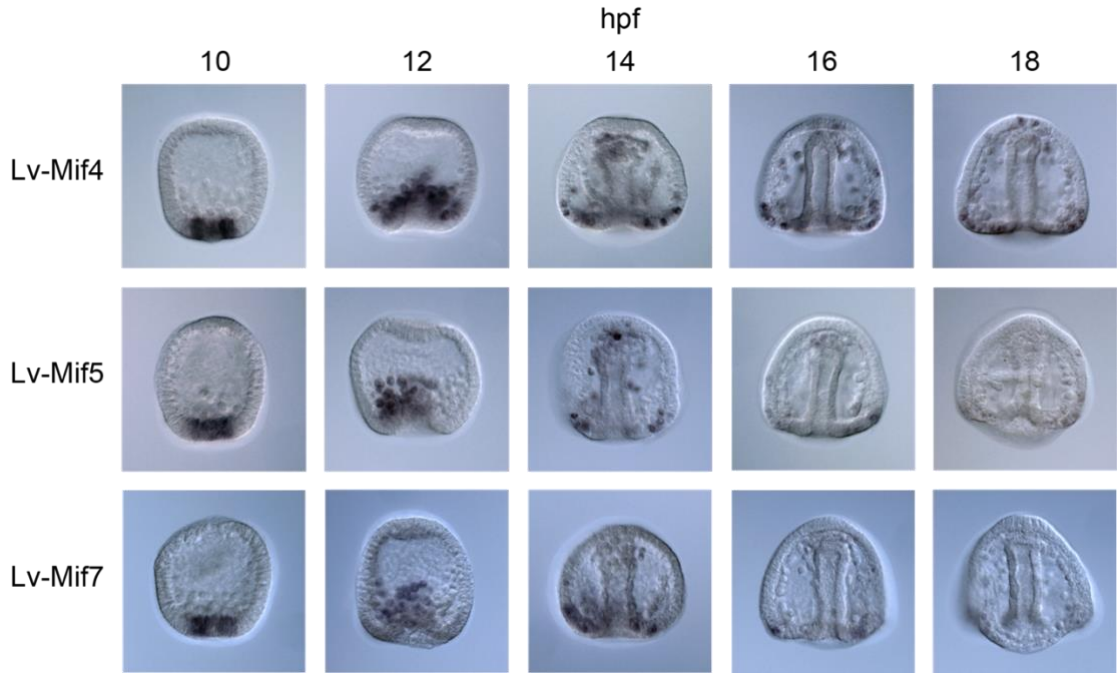


Figure 15: Developmental expression of Mif4, Mif5, and Mif7

Mif4, Mif5, and Mif7 in situ hybridization throughout development in the pigmented mesenchyme. 14 hpf timepoint frames are seen in figure 7I-K in chapter 3. Hpf = hour post fertilization.

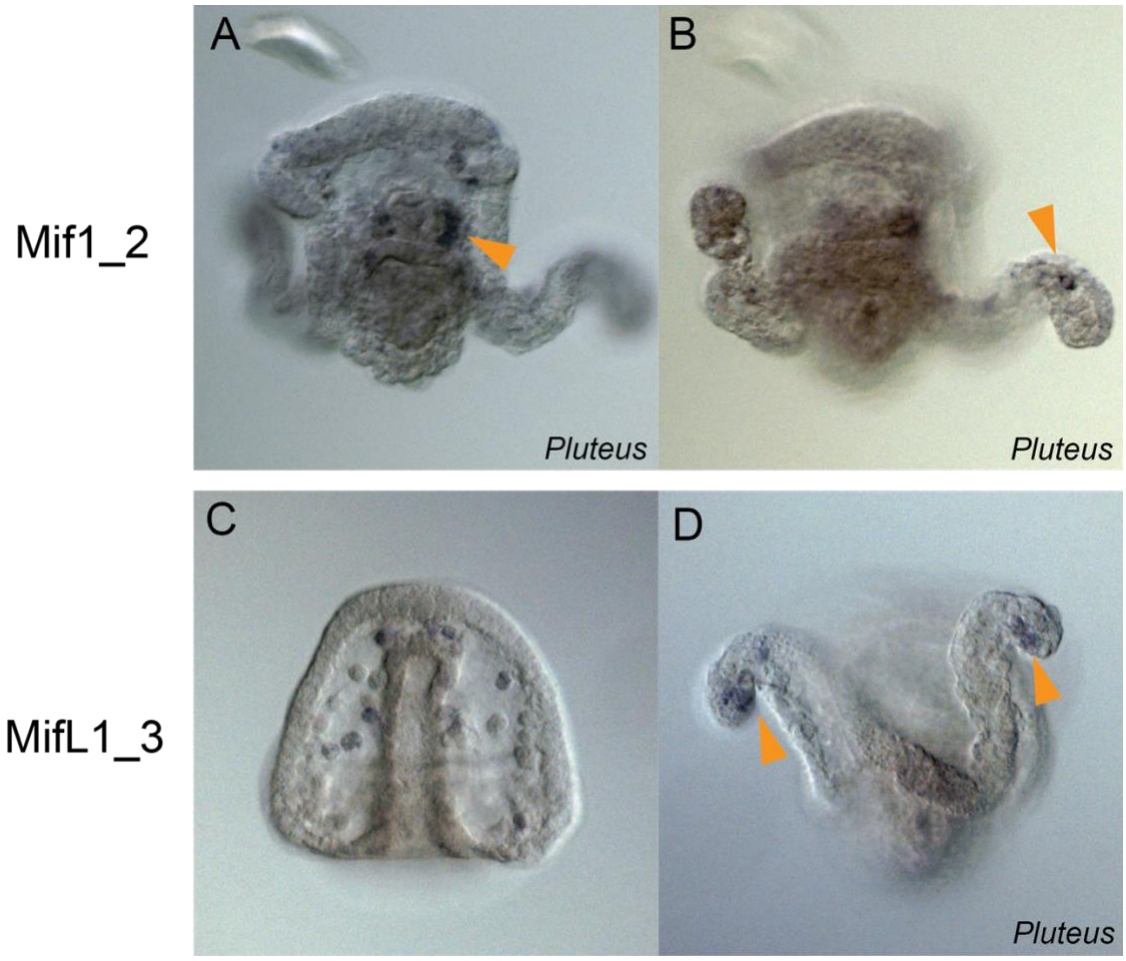


Figure 16: Mif1_2 and MifL1_3 show expression later in development

(A-B) Mif1_2 in situ hybridization in a pluteus larva. (A) Mif1_2 expression in the coelomic pouch marked by orange arrowhead. (B) Mif1_2 expression near the tips of the post oral arms, potentially neural cells, marked by orange arrowhead. (C-D) MifL1_3 in situ hybridization in a late gastrula and pluteus larva. (C) MifL1_3 expression in mesenchymal cell populations. (D) MifL1_3 expression in the tips of the post oral arms marked by two orange arrowheads.

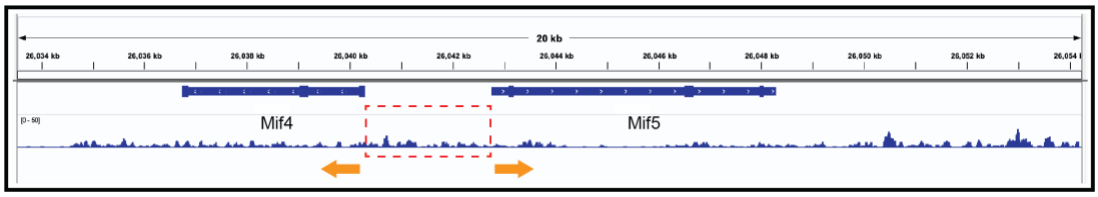


Figure 17: Mif4 and Mif5 5' regulatory region

ATAC-seq data from Philip Davidson showing the Mif4 and Mif5 loci. Peaks show open regions of the genome. Red square showing the shared Mif4 and Mif5 5′ regulatory region. Orange arrows indicate direction of transcription.

4.3 MIF function in the developing pigmented mesenchyme

To begin determining the roles of MIFs expressed in the pigment cells, we perturbed the upstream signals and transcription factors and the MIFs themselves. Delta initiates a signal through the Notch receptor and is necessary for activation of the cell lineage that leads to pigment cells. Gcm is the primary transcription factor activated by Delta-Notch at the top of the GRN leading to pigment cells (Croce and McClay, 2010; Davidson et al., 2002; Sherwood and McClay, 1999; Sweet et al., 2002). In Delta and Gcm MASO knockdowns, Mif4, Mif5, and Mif7 expression is eliminated, as previously shown for all pigment cell molecular components (figure 18). Thus, Mif4, 5, and 7 are an effector molecule in pigment cells. We next generated morpholinos (MOs) to Mif4, Mif5, and Mif7 (Table 3). The embryos injected with Mif5 MASO exhibited no phenotypic changes. This could be an indicator that Mif5 is a relatively new duplication amongst sea urchins that is non-functional. It is one of the Mifs that is missing a conserved amino acid, but without a demonstrable function we decided to not pursue this gene further.

Normally pigment cells are specified in the epithelium of the blastula. Shortly after the skeletogenic cells undergo an epithelial-mesenchymal transition (EMT), the pigment cells also leave the epithelium via an EMT, and they move to the posterior end of the embryo where they re-enter the epithelium through a mesenchymal-epithelial transition (MET) (Gibson and Burke, 1987; Wessel et al., 2020). They then spread out in the dorsal ectoderm where they conduct immunosurveillance throughout the larval life

(Krupke et al., 2016). Injection of the Mif4 MASO and Mif7 MASO resulted in a pigment cell deadhesion defect, or an inability of the cells to complete their EMT. This is similar to a knockdown previously seen when the transcription factor Twist was knocked down (figure 19) (Wu et al., 2008). The deadhesion defect appears in an embryo that is delayed in gastrulation, but after a short interval gastrulation occurs but the pigment cells fail to undergo EMT and instead remain at the tip of the archenteron, and appear stuck there. The marker for pigment cells, Pks1, is still expressed (figure 19). Other cell types appear to undergo their normal EMT (non-Pks1 mesenchyme which includes skeletogenic cells and blastocoelar cells), so it appears that the Mif4 and Mif7 knockdown of the EMT deadhesion is specific (neither Mif4 nor Mif7 is expressed in either skeletogenic cells or blastocoelar cells, and both undergo their EMTs in the Mif4 and Mif7 knockdowns).

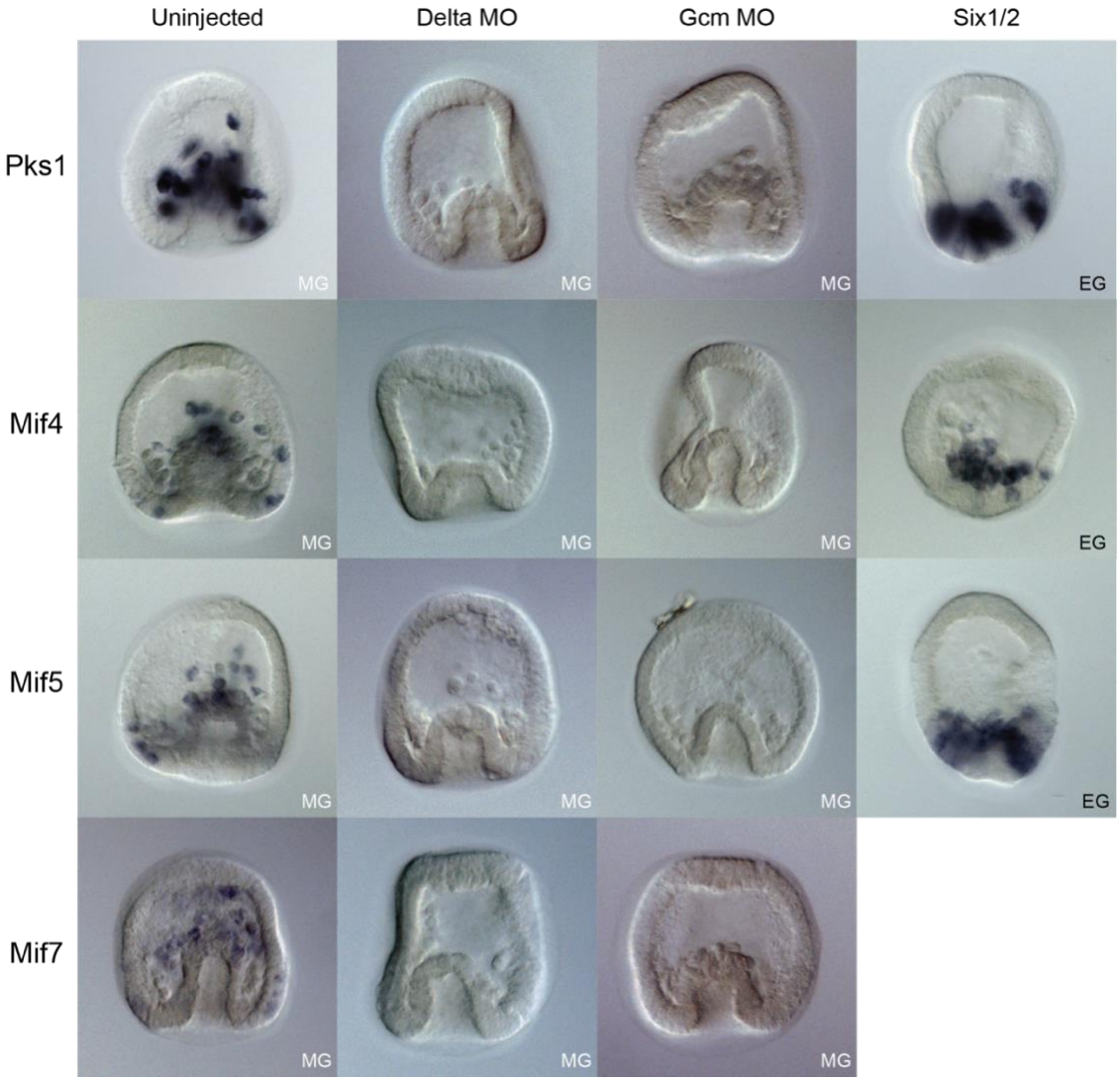


Figure 18: Delta and Gcm knockdowns eliminate pigment cell MIF expression

Morpholino injected embryos raised until mid-gastrulation (MG) and early gastrulation (EG). Delta and Gcm morpholino injected embryos gastrulate and do not express pigment cell marker Pks1, and Mif4, Mif5, and Mif7, but Six1/2 morpholino injected do.

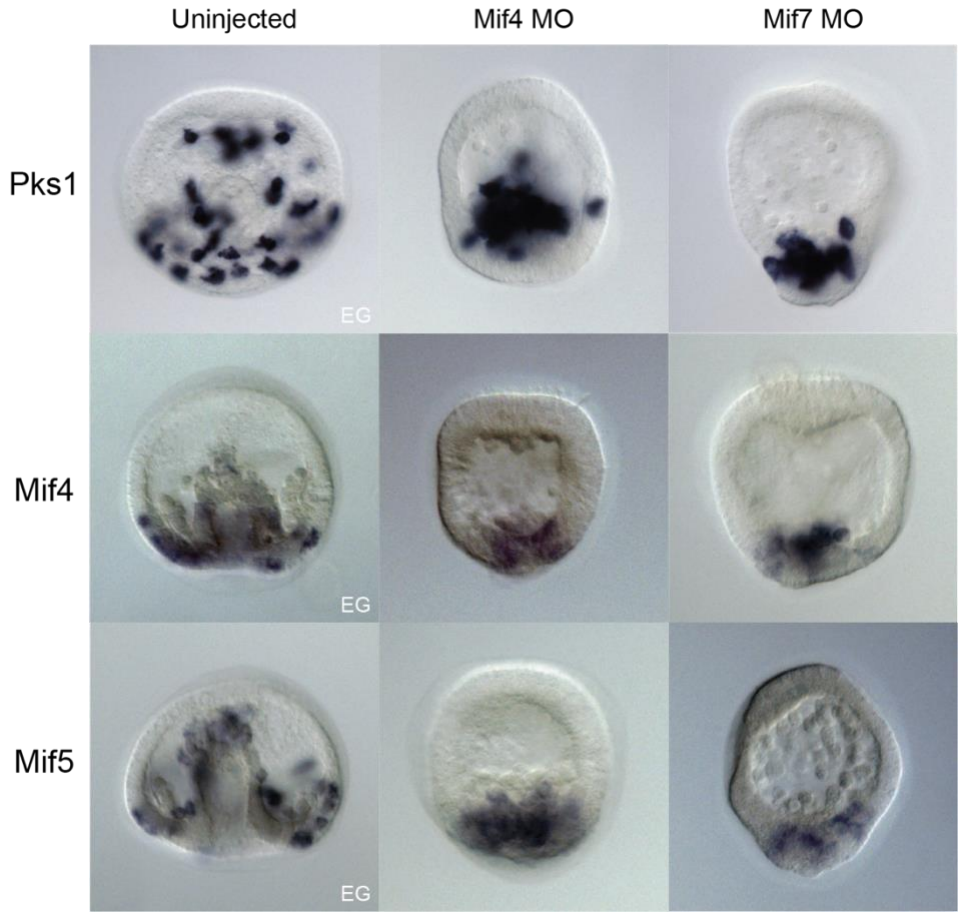


Figure 19: MIF morpholino knockdowns show EMT defects

Morpholino injected embryos raised until early gastrulation (EG). Mif4 and Mif7 morpholino injected embryos have delayed gastrulation and pigmented mesenchyme stay within the epithelium.

4.4 Broad inhibition of the MIF protein in the embryo results in cell reprogramming

At the beginning of this research project we attempted to understanding the cell reprogramming process that occurs when skeletal cells or their progenitors are removed from the embryo (Cheng et al., 2014; Sharma and Ettensohn, 2011). Within 24 hrs those skeletogenic cells are replaced through transfating of other mesoderm cells, including it was thought, the pigment cells and more likely the blastocoelar cells, both included as part of the non-skeletal mesenchymal progenitors (NSM). The prevalent hypothesis was that the skeletal cells produced an unknown signal that was received by NSM (Sharma and Ettensohn, 2011). Data from an RNA-seq study Barsi et al., (2015) suggested that MIF genes were differentially expressed by skeletal cells in *S. purpuratus*. At the time, based on the signaling hypothesis, we were looking for a signal produced by skeletogenic cells, so the Mif cytokine expression drew our attention. In order to perturb the system and assay for reprogramming, we decided to use a drug inhibitor.

4-IPP is a specific, irreversible MIF protein inhibitor that forms a covalent bond with the Pro-1 proteins (Rajasekaran et al., 2014). We tested the inhibitor for a working concentration. We found that when early staged embryos are treated with 4-IPP from 0-2.5hpf, the marker for skeletal cells is ectopically expressed at the tip of the archenteron, the location we expected to see transfating first occurring (figure 20). Past work had already shown that when skeletogenic cells are removed, that same marker is first expressed by NSM near the tip of the archenteron (Sharma and Ettensohn, 2011). This

suggested to us that perhaps MIFs were part of the signaling pathway of the transfating. To make sure the marker expression came from transfated cells we took FITC injected eggs and treated them in 4-IPP for 2.5hrs. Using microsurgeries at the 16-cell stage, we transplanted FITC injected and drug treated micromeres onto TMR injected 16-cell untreated embryos. These chimeric embryos developed skeletal cells that were FITC+. We imaged the larvae and saw co-localization of FITC and TMR in the skeletal cells, indicating that TMR+ NSM cells reprogrammed and joined the syncytium of skeletal cells, mixing their cytoplasms (figure 21). We performed pilot assays of 4-IPP on gastrulating embryos to test for pigment cell MET, and on swimming larva to test for pigment cell migration after injury. Both experiments were negative.

This experiment was repeated and we were initially excited, thinking that MIFs might be the hypothesized signal. However, complications began to appear. First, with the exception of one MIF that is expressed very early in most cells, all other MIFs appear to be expressed by pigment cells and not by skeletogenic cells. Then, knockdown of those other MIFs with morpholinos did not lead to the skeletogenic marker expression and thus were different from the drug response. Ultimately, without a specific skeletogenic marker, we abandoned the idea of the skeletogenic MIF signal.

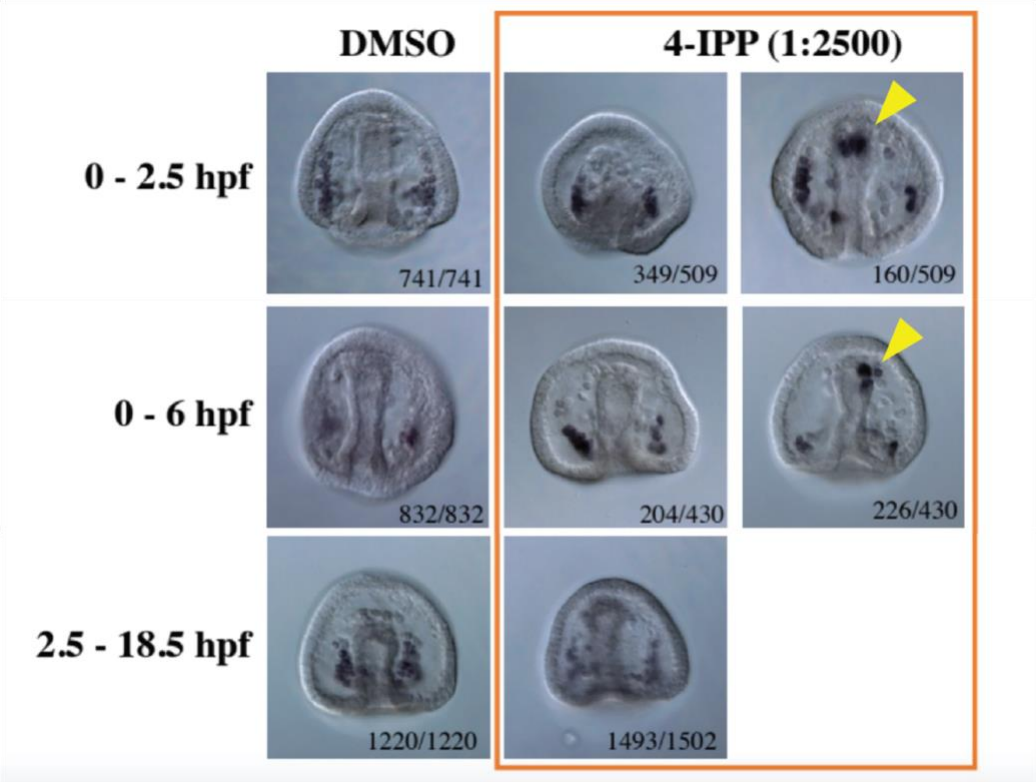


Figure 20: 4-IPP treated embryos express ectopic Alx1

DMSO control treated embryo compared to 4-IPP (1:2500) drug treated embryos. Right panels show ectopic Alx1 at the tip of gastrulating embryos, emphasized by yellow arrowheads. Hpf: hours post fertilization. Work done by Michael Wen.

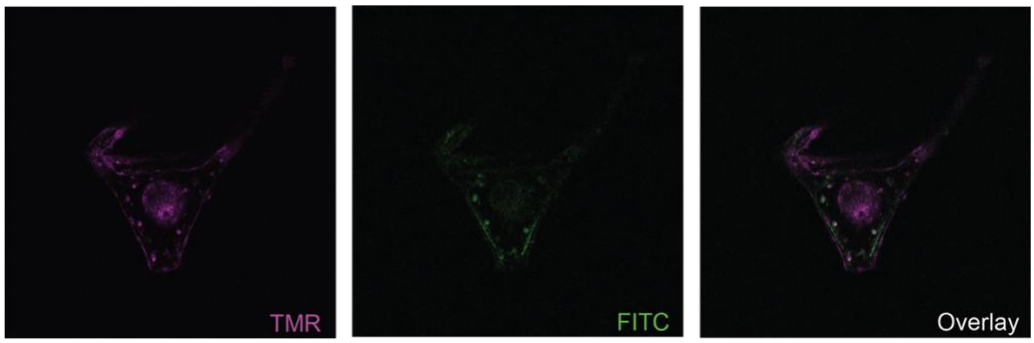


Figure 21: Micromere transplants and 4-IPP drug treatment larva

The developed larva of a chimeric embryo with transplanted micromeres (FITC+) and host being TMR+. The donor embryo was treated with 4-IPP until 16-cell stage at which time the FITC-micromeres were transplanted to the TMR control hosts. Left panel is the red fluorescent channel (pseudo colored purple). Middle panel is the green fluorescent channel (pseudo colored green). Right channel is the overlay of the two channels with white showing TMR and FITC overlap indicating that the transplanted skeletal cells (FITC+) formed a syncytium with 4-IPP induced skeletal cells from the host (TMR+). Work done by Michael Wen.

4.5 Conclusions and future directions

High quality genome sequencing and single-cell data to tease apart subtle differences amongst the sea urchin MIFs provided us with very useful molecular information on the MIFs. The sea urchin genome has many more MIFs than in mammals. The presence of many MIFs became problematic when we focused on the results of the IPP inhibition since the drug is a pan-MIF inhibitor. As we learned more about the MIFs our initial hypothesis of MIF function was not well supported and we could not find a MIF that fit the hypothetical function initially proposed. This sent us in several directions to explore other possible MIF functions.

Based on MIF expression patterns and gene knockdowns, we discovered a role for MIFs in pigment cell development. Based on the phenotypes on the Mif MASO knockdowns, Mif4, Mif5, and Mif7 do not have redundant functions. Mif5 appears to not be translated from mRNA, which could be confirmed by creating a reporter construct with a hybrid Mif5 and fluorescent reporter. MIFs are known to be involved in intracellular signaling cascades. Mif4 and Mif7 offer evidence for this in the sea urchin by their effects on deadhesion. It remains unclear, however, whether the observed function is due to intra- or inter-cellular signaling.

Future directions for the pigment cell MIFs is to create ISH probes or reporters for the Mifs expressed in pigment cells. This will be important because MIFs in other species create homotrimers with one another, and it's unclear how the MIFs present in

the pigment cells interact with one another. Also, in chapter 5, cytokines are known to be secreted during skin wounding. Thus, one hypothesis is that the pigment cell MIFs are used to signal to neighboring mesenchymal cells for wound healing. Thus, while there are many unanswered questions about the MIF genes in sea urchin development, the results on their role in pigment cell EMT will be incorporated into a paper that compares EMTs of three different mesenchymal cells. The MIF results recorded here are among a number of observations indicating that the three EMTs are each unique in that they are triggered by three different signals, controlled by overlapping but different sets of transcription factors, and conducted by somewhat different sets of effector proteins.

5. Non-skeletal mesenchyme responds to larval wounding

Introduction

5.1 Injury in the sea urchin embryo

5.1.1 The larval immune system and injuries

La The focus of immune system research in the sea urchin larvae has and is primarily focused on pathogenic bacterial infections and, more recently, the microbiome (Carrier et al., 2021; Fleming et al., 2021). Besides combating pathogenic infections, immune systems across species play an extraordinarily important role in wound repair and tissue homeostasis. Beyond pathogenic infections, there is a major deficit in knowledge in how the larval immune system responds to other forms of injury throughout all stages of development. These types of injuries include cell and tissue loss (e.g., scrapes or encounters with predators), crushing and twisting, sudden osmotic changes, UV damage, punctures (internally and externally caused), stretching, and chemical injury amongst many other possibilities.

In a simple definition of injury, where it involves a non-purposeful loss of tissue, classical microsurgeries may be classified as subtype of injury. Published microsurgeries (i.e., surgeries performed at the microscopic level) in the sea urchin have involved single cells, clusters of cells, tissue or sections of gastrulae being completed removed or replaced (Cheng et al., 2014; Hardin and Armstrong, 1997; Hörstadius, 1973). Microsurgeries in early sea urchin embryo development have shown the developmental

plasticity within the 2-cell and up until the early gastrula. Separation at 2-cell stage results in identical twin larvae, and complete removal of the skeletal mesenchyme results in morphologically wild-type larva with a skeleton. Though there are no explicit "immune cells" at these very early cleavage stages of development, the embryo still physiologically reacts and cells change state.

As we've talked about in prior chapters of this research, what dominant Science categorizes as the immune system within and across species is overwhelming complex, second in complexity to the nervous system (Kurzgesagt – In a Nutshell, 2021). We know a decent amount about the development of the sea urchin quiescent immune system (immunoquiescent - IQ), but we wanted to investigate other types of mechanical injury that were easy to reproduce, obvious, and quantifiable (Buckley et al., 2019). To do so, we turned to the most obvious immune cell type in the larval, the pigment cells, since changes in shape, size, locations, and appearance are easily observable under a microscope. The following sections show our work in characterizing a "crush assay" that consistently yields a visible injury in the larval epithelium, and blatant movement of pigmented cells to the site of injury. In later subsections we describe novel findings about the heterogenous blastocoelar cells, including their ability degrade skeletal matrix and extending filopodia outside of the epithelium.

5.1.2 Sites of epithelial injury in the larvae

Certain areas in humans are more prone to injury than others due to their use and flexibility. Take a human knee for instance, because we are able to extend and rotate it in a wide variety of angles means that it's highly susceptible to injury when overextended or rotated in the wrong way. The pluteus larva is no different in that there are areas in its anatomy that grant it beneficial abilities but also leave it open to injuries in those regions. We explored what those weaknesses in the anatomy could be.

The swimming pluteus larvae of *L. variegatus* is shaped somewhat like an artist's easel (figure 22A). The mouth of the larva sits within the easel's legs, creating a basket-like structure that captures algae as the larvae swim. Beneath the larva's epithelium, the rigid calcite skeleton of the larva grows into this easel and creates long extending structures termed arms (figure 22B). The "oral hood" is the rounded structure that is lateral to the mouth, and contains two skeletal rods called the "anterolateral (AL) rods" which develop into oral arms. On the polar opposite, or posterior side of the larva are the aboral arms which contain much longer skeletal rods that are called the "post-oral (PO) rods". The tip of the larva contains regions of the skeleton called the "posterior crests" (Morrill and Marcus, 2005). As the larvae continue to swim unfed, the rods in the oral hood will continue to grow and give the oral hood a "U" shape, and their aboral arms will continue growing. These elongating skeletal tips become like needles and

consistently grow into the epithelium and become easy sites of skeletal rod penetration. How has the larva adapted to respond to these wounds?

While the larval skeleton grows and becomes more complex, the pigmented mesenchyme in the immunoquiescent larvae undergo EMT then MET into the aboral ectoderm (e.g., oral hood) where they become evenly spaced. Some pigment cells congregate in the tips of the aboral arms, and others are known to interact with the gut when in the blastocoel (Buckley et al., 2017). Another immune cell type, the blastocoelar cells, also undergo mesenchymal dynamics and while their function is not well understood in these IQ larvae, these cells reside throughout the blastocoel on the surface of other structures (the gut and the skeletal extracellular matrix). The filopodial population of blastocoelar mesenchyme creates an extensive and elegant network within the blastocoel as part of the behavior of these cells in attaching to the matrix surrounding the skeleton (Buckley and Rast, 2017; Tamboline and Burke, 1992). Some subtypes of blastocoelar cells migrate around and are interpreted as immunosurvielling, and others are thought to appear only during immune response (Ho et al., 2016).

This chapter focuses on the switch of from IQ to a wound-healing response. Injured swimming *L. variegatus* larvae undergo a massive level of pigment cell migration. Protruding skeletal rods are resorbed to allow the epithelium to cover the wound. Blastocoelar cells are recruited to the wound site to participate in the repair. Live-imaging of these cellular events *in vivo* showcases a resilient larval immune and

wound repair system. Specific conclusions are that a blastocoelar cell subpopulation provides an osteoclast-like, or "skeletoclastic" (Greek: *clast* = to break) property in remodeling the skeleton, and non-skeletal mesenchyme are able to breach and return to the larva's epithelium. Broad takeaways from this research are the tips of the pluteus arms are a new model for epithelial wound healing, and the larval immune system contributes to the resilience of the embryo to microbial invasion.

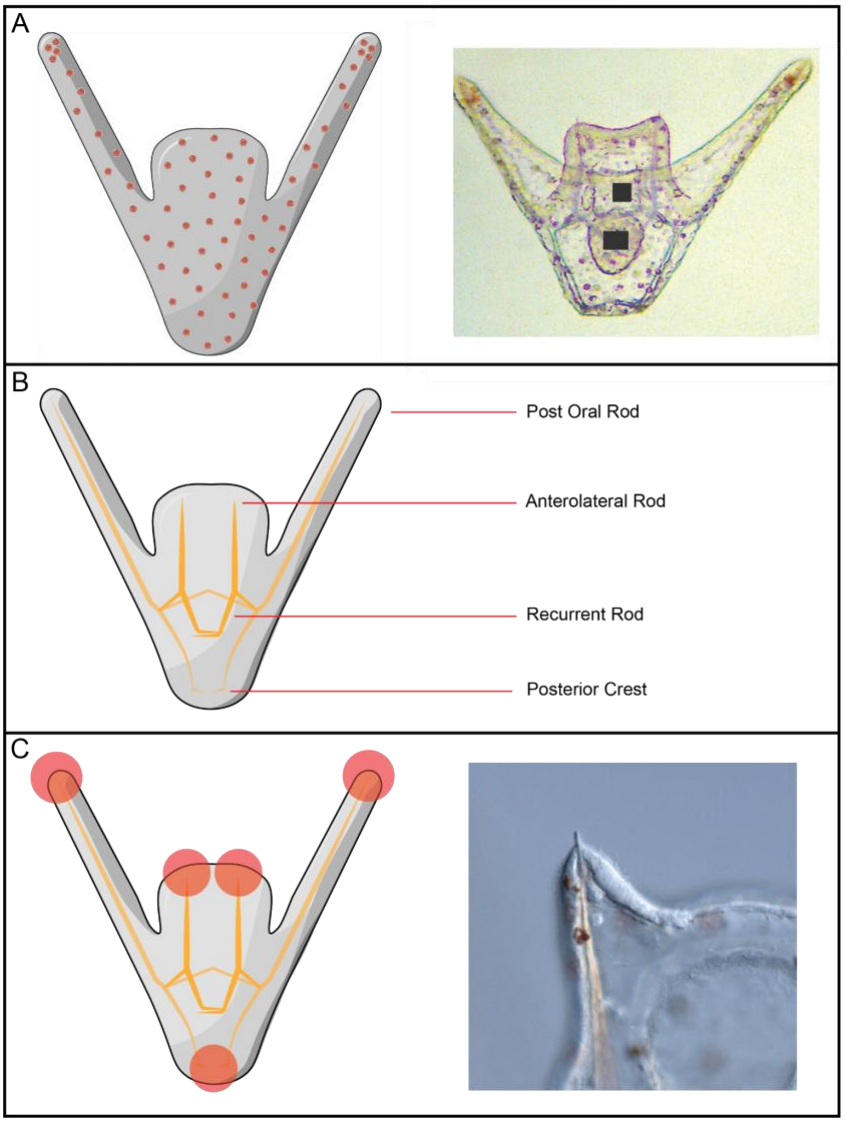


Figure 22: Pigment cells & skeletal rods in the larva

(A) Left - a schematic of the pluteus larva showcasing the distribution of pigment cells in the oral hood and arms. Right - an image of a *Lytechinus variegatus* pluteus larva modified from Morrill and Marcus (2005). (B) A schematic of the pluteus larva skeleton. (C) Left - Injury points of the larval skeleton. Right - an example of an anterolateral rod puncturing the epithelium of the oral hood.

Results

5.2 A crush assay to assess larval wound response and repair

In pilot experiments the tips of the larval arms of swimming pluteus larva were found to be continuously prone to injury,. The skeletal rods (AL and PO rods) grow up against and are covered by epithelial tissue. In handling embryo or often seen in embryos collected from sea water, the skeleton frequently penetrates the epithelium. We decided to use this penetration injury as an assay to understand how the embryos responds and repairs these wounds.

To develop a reliable and repeatable assay, we first removed the cilia from swimming larvae to temporarily immobilize them (a short exposure to 2X seawater). The larvae are then mounted on a protamine sulfate coated slide and coverslip. The posterior region of the pluteus and the fully developed oral hood are flat. Because of the flat surfaces, the mounted larva tends to orient with these sides either at the top or the bottom. Pressure is applied until the larvae are visibly compressed (i.e., the arms are splayed) and uncompressed. This approach causes the skeletal rods in the arms to puncture the epithelium, and allows for an easy and highly repeatable visualization of the larva's z-planes and punctured arms.

What then follows immediately after crushing is a dramatic multi-tissue response to repair the region. In an average "crush assay" where the larvae are wounded and observed from late gastrulation (~18 hours post fertilization [hfp] at 23℃)

to swimming unfed larva > 2 days post fertilization (dpf); AL rods extend through the hood and the PO rods penetrate the covering epithelium (figure 23). Within one hour: (1) pigmented mesenchyme rapidly and directionally migrate from nearby regions to the tips of the arms, (2) filopodia from an unknown blastocoelar cell-type to wrap around the extended skeletal tip, (3) the tips of puncturing skeletal rods are degraded, (4) and the epithelium is pulled over the skeletal rods.

While repair occurs at all these stages, the most dramatic and easiest time frame to visualize and score is at 2 dpf or greater. Thus, most of the data for this set of experiments were gathered with >48hpf pluteus larva. We found that the oral hood is originally rounded in structure during early pluteus stages and the AL rods do not extend as deeply into the epithelial tissue. Later the oral hood flattens and AL rods and the epithelia develop more prominent arm protrusions that appear to stretch the epithelium (figure 24). At this stage the arms easily puncture the epithelium in the crush assay. Sometimes even the initial deciliation is enough to promote the epithelial puncture. Crushing the larva increases the likelihood of skeletal rods puncturing the epithelium, and as a practical matter for imaging, it helps secure the larva in place longer since the cilia regenerate within the hour causing the larva to swim away.

We posited that other regions of the skeleton would be able to puncture the epithelium and cause a wound response similar to the AL and PO rod tips. Candidates included the “recurrent rods” which are dorsal to the AL rods, and the posterior crests

(figure 22C). In our experiments, we observed these regions and found that the recurrent rods and posterior crest puncture the epithelium less frequently, and are more difficult to image relative to the AL and PO rods given the geometry of the larva on the slide. However, we documented one instance of a puncture by the posterior crests (by crushing the larvae dorsal-ventrally, as opposed to the usual anterior-posterior method). In the posterior crest puncture we observed the pigmented mesenchyme rapidly migrate to the site of injury, the epithelium opened to the seawater, and unpigmented mesenchyme arrived and surrounded the posterior crests and extended filopodia. Then, in less than two hours, the epithelium closed, and the posterior crest epithelium again covered the larva (figure 25). Prior research in pigment cell function in *S. purpuratus* and *L. variegatus* larva did not document these massive (pigment cells throughout the oral hood) and rapid (<30 minute) migration to sites of injury in the larva. These injury responses became the focus of our follow up work.

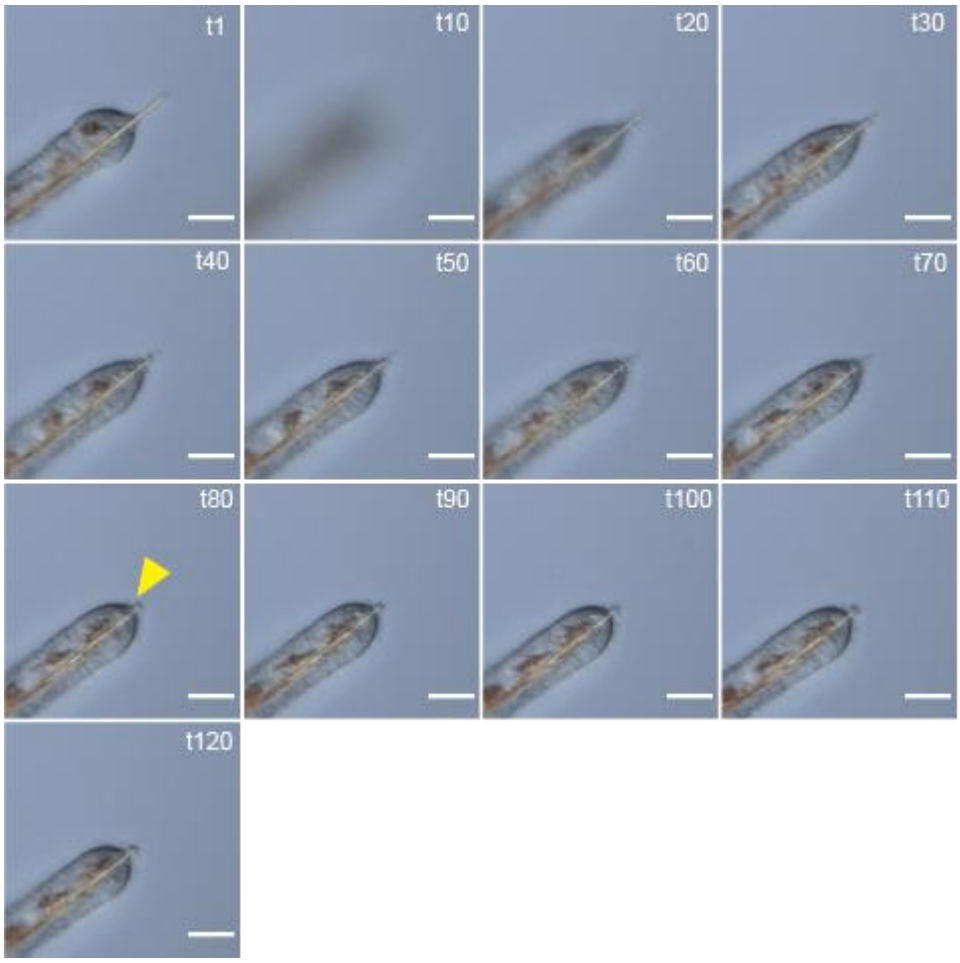


Figure 23: Postoral arms recover and degrade skeleton during injury

60 minute time course of injured larva's postoral (long) arm tip. Images taken every 30 seconds and time (t) is out of 121. t1 to t20 show epithelium extending further up the skeletal tip. At t80 the tip of the skeletal rod is broken and brought into the larva (yellow arrow). A faint cellular structure (filopodia) is present throughout the time course. Scale bar is 20 microns.

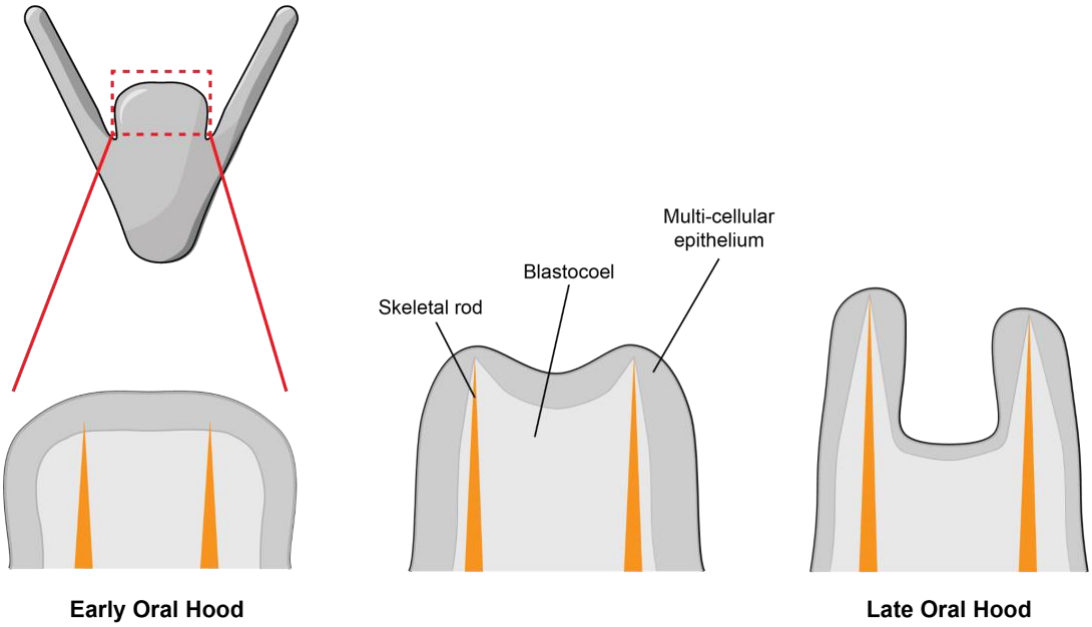


Figure 24: Development of the oral hood

Schematic of the developing oral hood of the pluteus larva. The two antero-lateral skeletal rods are orange. The fluid-filled cavity (blastocoel) is light grey. The multi-cellular epithelium, formerly referred to as the ectoderm, is dark grey. The transition from the early to later staged oral hood consists of the skeletal rods growing and extending sharply into the epithelium. As this occurs, the rounded shape of the epithelium becomes two points and usually develop slight asymmetries.

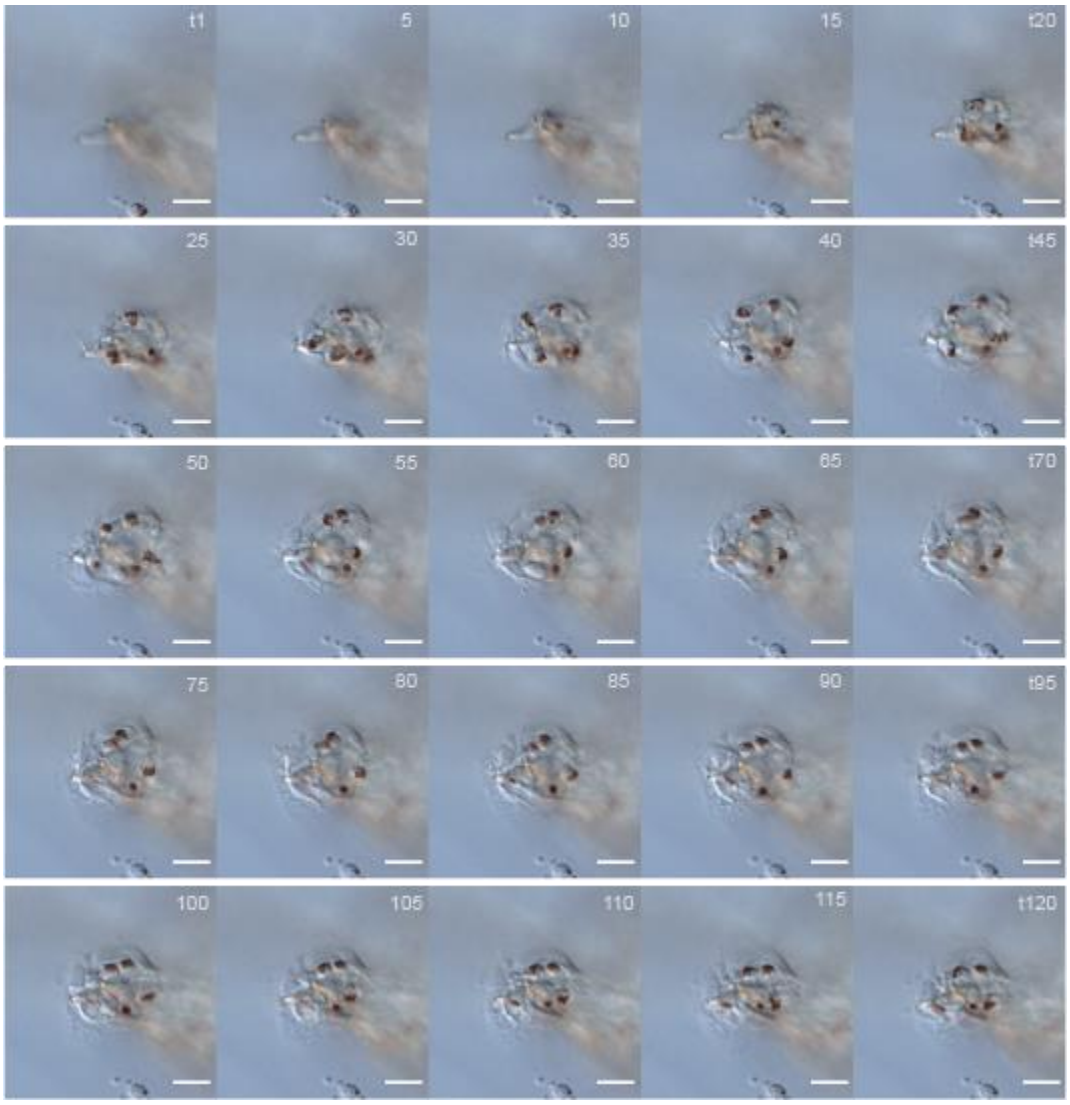


Figure 25: Non-skeletal mesenchyme remodel posterior crest during injury

Time course of crushed pluteus larva for one hour post injury. Larva mounted with the posterior crest pushed against the coverslip, causing the skeleton to puncture the epithelium. The pigment and blastocoelar cells open the epithelium and move around the injury. Near the end of one hour post injury, the non-skeletal mesenchyme

have started to close the opening they created and bring the skeleton back within the larva. Time (t) is out of 121 time series taken every 30 seconds. Scale bar is 20 microns.

5.3 Pigmented mesenchyme respond to skeletal-epidermal injury with directed migration to the site of the injury

5.3.1 Pigment cells mass migration

The echinochrome-filled pigment cells have the most striking and consistent response in the crush assay. Almost immediately after the slide is prepared and the wound initiated, pigment cells begin migrating in mass to the nearest wound site at the tip of the larval arm (figure 26). In the oral arms, pigment cells within the oral hood move towards the left and right arms, whichever is closest. When pigment cells arrive at the wound site they display one or more of the following behaviors: MET into the epithelium near the skeletal tip, move into the blastocoelar cavity by the tip, and/or change from their rounded to stellate shape (Ho et al., 2016). A similar process happens within the aboral arms, but because the long arm is closely surrounded by the epithelium, there is no visible angular convergence on the wound since the arriving pigment cells are forced to move parallel to the skeletal rod (figure 27). Furthermore, the process differs since these arms already have a higher number of localized pigment cells near the tip.

Our quantifications on pigment cells using MTrackJ show drastic differences in how pigment cells respond to the skeletal rod punctures. In one instance within the oral hood, one pigment cell traveled (Track 1 – purple) a total distance of 228 microns and moved 65 microns away from its original position (figure 28). Meanwhile, the other pigment cell (Track 2 – green) stayed relatively in the same location, traveling a total

distance of 93 microns and being only 18 microns away from its start point (figure 28). A similar situation occurred in the PO arm. One pigment cell migrated from the lateral side of the oral hood to more than half way up the PO arm, changed direction and went back to the start of the PO arm, and changed direction again went farther up the PO arm towards the skeletal rod tip (figure 27 – Track 1). A different pigment cell, closer to the site of PO arm injury, in the same larva stayed in the same location the entire time (figure 27 – Track 2).

When multiple pigment cells are tracked, we see the distance traveled and directionality remains heterogenous. In figure 26, more proximal pigment cells stay in the same area over the course of 30 minutes (purple and orange track). Distal pigment cells closer to the oral hood skeletal rod tips meanwhile both migrate closer to the site of injury, and did so toward the left (green and red track) and right (yellow and blue track) sides whichever is closer (figure 26). Observing the red and green tracked pigment cells, the pigment cells also change from their round to stellate shapes during the injury response (figure 26).

In rare instances, we observed pigment cells extend parts of themselves outside the epithelium into the seawater or completely remove themselves from larva (i.e., cell shed) (figure 29). This happens near the exposed skeletal tips, and other areas in the epithelium. In one example, a pigment cell was released from the larvae into the seawater, then extended a long filodpodium back to the larva. These extra-larval

movements have not been documented previously in this system. A possible explanation is that the tissue damage is so extensive to the epithelium, the normal regulatory process that keeps pigment cells localized is not enough to keep them from shedding in a self-destructive manner.

To assay for pigment cell migration function we performed a series of gene knockdowns and inhibition experiments (Table 3 - excluding Delta and Gcm morpholino knockdowns) and assayed for pigment cell migration to the larval arms. In all of the perturbations, pigment cell migration to the area of injury was never abolished.

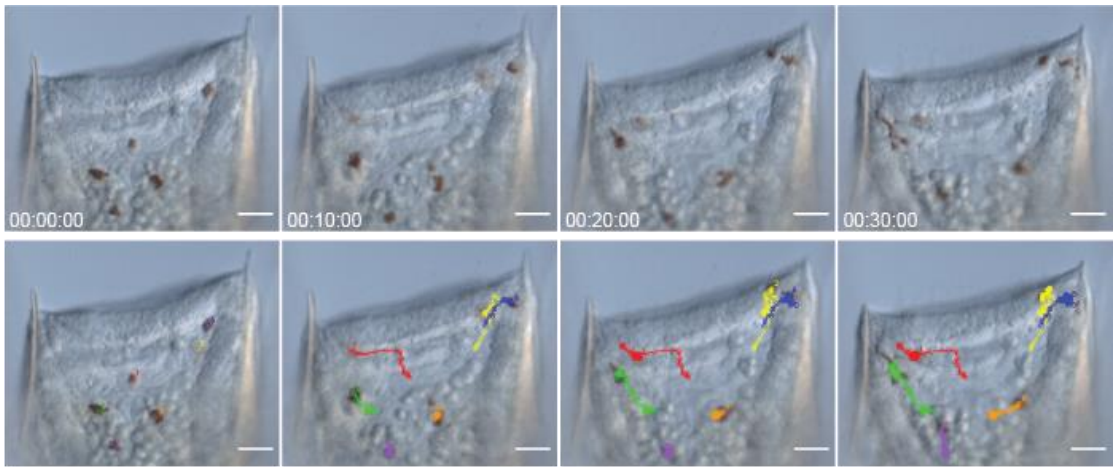


Figure 26: Pigment cells migrate quickly to epithelial injury

Larval pigment cells migrate early and quickly to the oral hood epithelium when injured. From 0 to 10 minutes post-injury (00:00:00 - 00:10:00), select pigment cells have migrated to the edge of the oral hood when the antero-lateral rods puncture the epithelium. Pigment cells continue to move within the oral hood, but they do not travel the same distance within the hour.

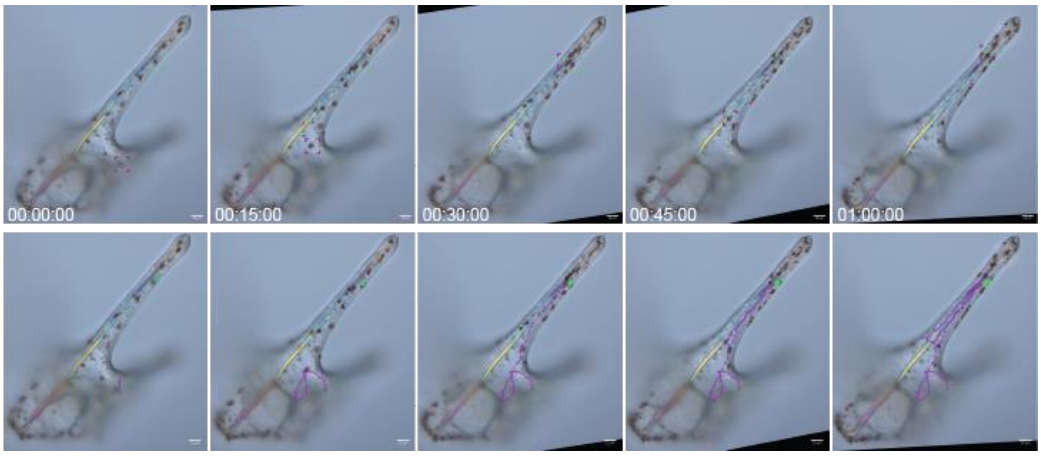
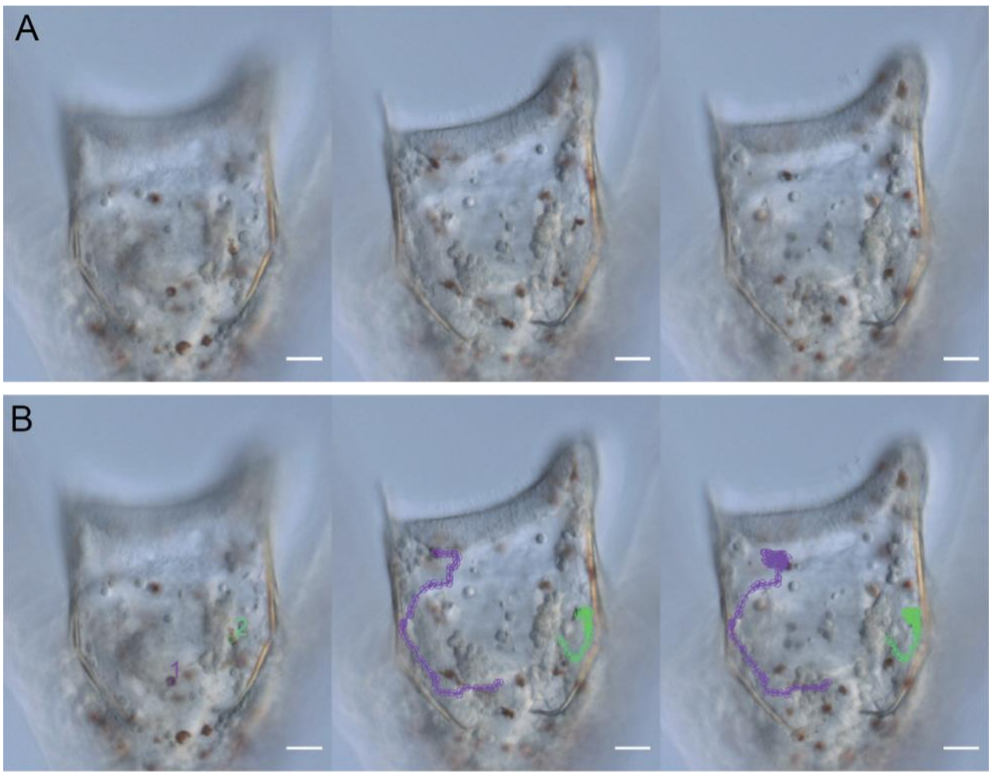


Figure 27: Heterogeneity in pigment cell movement during injury

Top row - time course of crush assayed pluteus larva injected with a Irf4 morpholino until one hour post injury. Purple and green dashed boxes show two pigment cells of interest. Bottom row - two manually tracked pigment cells with MTrackJ in the injured pluteus larva show differences in migration pathways. Track 1 (purple) shows a pigment cell travel distally in the post-oral (long) arm, reverse direction, and travel again distally toward the tip of the arm. Track 2 (green) shows a pigment cell in the epithelium, closer to the tip of the arm that travels minimally within the arm. Scale bar is 20 microns.



C

MTrackJ Quantifications

	Length (μm)	Distance from Start [D2S] (μm)
Track 1	228	65
Track 2	93	18

Figure 28: MTrackJ quantification of oral hood injury

(A-B) Time course of 60 minutes of crush injured pluteus larva. (B) Two manually tracked pigment cells with MTrackJ in the injured pluteus larva show differences in migration pathways. Track 1 (purple) shows a pigment cell migrating proximally to distally in the oral hood to the postoral rod. Track 2 (green) shows a pigment cell that stays in the same relative position over time. (C) MTrackJ

quantifications of length and distance from the start of the two pigment cells over the course of the hour.

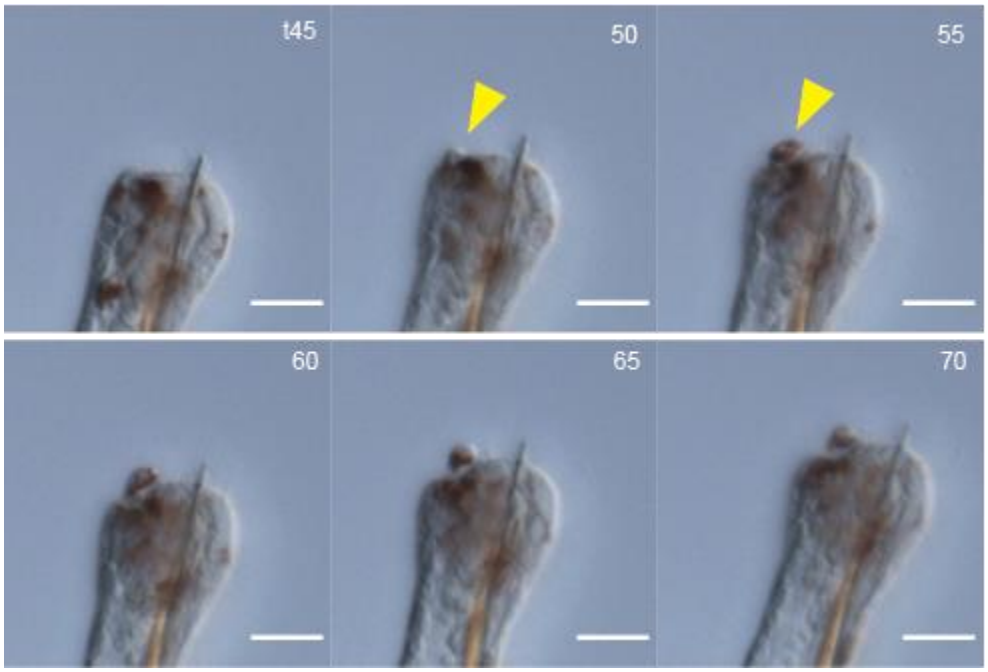


Figure 29: Pigment cells can shed from the larva

Larva injected with Irf4 morpholino and subjected to a crush assay. Area near skeletal rod puncture possess a large number of pigment cells. During recovery a pigment cell exits the epithelium (yellow arrow). Time (t) is out of 121 time series taken every 30 seconds. Scale bar is 20 microns.

5.3.2 The role of pigment cells in the wound response

In section 5.2, we summarized tissue responses observed beyond pigment cell migration. Since pigmented mesenchyme showed the most drastic visual response to injury, and have been known to surround and phagocytose foreign materials, we hypothesized pigment cells were also responsible for the cell-wrapping around the skeletal tips, degradation of the skeletal tips, and/or the recovering of the skeletal rods with epithelium. The rationale for this hypothesis originated from the general understanding that blastocoelar cell populations rarely had been observed to engulf foreign bodies compared to pigment cells. One way to test if and how pigment cells were required for these other phenotypes of wound repair was to eliminate pigment cells and observe the wound response.

We eliminated or significantly decreased the number of pigment cells through morpholino knockdowns of Delta and Gcm. We also periodically imaged uninjected larvae that produced only a few pigment cells. In larval arms lacking pigment cells, skeletal rods tips continued to be degraded and the cell-wrapping of the tips still took place (figure 30). The ability to cover the skeletal rods with epithelium appears to be slowed or inhibited when the pigmented mesenchyme is missing but not abolished. These results indicate that mechanisms other than pigment cells, are responsible for cell-wrapping and skeletal rod degradation. Pigment cells may aid in the epithelial remodeling during injury but those processes can occur in the absence of pigment cells.

And since the wound repair can occur in the absence of pigment cells, yet the pigment cells normally move to the injury site rapidly, it is likely that they function exclusively in an immune responsive role as shown elsewhere (George, Thesis, 2019).

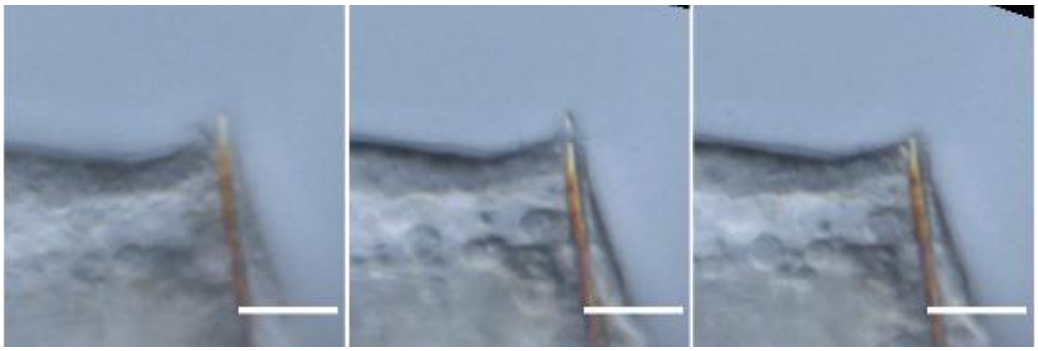


Figure 30: Pigment cell lacking oral hood shows skeleton resorption

An uninjected larva that had a naturally occurring low number of pigment cells, showcased by no pigment cells present in the oral hood region imaged. Time points (t62, t92, t94) showing an exposed skeletal rod degraded and resorbed. Time (t) is out of 121 time series taken every 30 seconds. Scale bar is 20 microns.

5.4 Blastocoelar mesenchyme populations response to skeletal-epidermal injury

We turned to the function of the blastoceolar cells in the wound response. If pigment cells are not involved in the repair we thought that function might instead involve blastocoelar cells. In the absence of pigment cells we observed excision and resorption of the skeletal rod (figure 30). Imaging showed instances of the transparent blastocoelar cells exiting the epithelium and wrapping themselves around the skeletal rod. Shortly thereafter the skeletal rod tip that protrudes beyond the wounded epithelium is broken in the vicinity of the blastocoelar cells. The tip is then either lost, or if retained by the embryo, resorbed. These wrapping blastoceolar cells match earlier descriptions of a subset of blastoceolar cells called "globular cells," based on their morphology in terms of overall shape and filopodial projections (Ho et al., 2016).

To image these transparent cells more clearly, we injected single cells at the 2 and 4-cell stage embryos with mRNA expressing a transmembrane segment attached to GFP. This resulted in a half or a fourth of the larva to fluoresce. Some of the fluorescent blastocoelar cells move into the unlabeled portion of the embryo to enable a detailed view of the blastocoelar cell behavior. A time series example shows a membrane-GFP+ globular cell extending the filopodial projections we usually observe, and interacting with what appears to be a network of blastocoelar cells within the unlabeled oral hood tip (figure 31). Observing the epithelium in other areas of the larva, it is not uncommon to observe these globular cells extend filopodia into the seawater. Similar to the pigment

cell shedding observation in the injured larva, it's unclear if this happens in uninjured larva or in response to larval pathogens.

In injuries where the skeletal rods are not quickly (<1 hour) covered by the epithelium, the tips of the exposed rods are broken off and engulfed by the neighboring tissue. Less often, the skeleton will be degraded within the larva, leaving a large portion of a skeletal rod to be resorbed. A small region in the rod is resorbed, and within 2 hours that portion of broken skeleton is resorbed by a blastocoelar cell subtype (figure 32). These resorption mechanisms are reminiscent of osteoclasts in vertebrate systems. The sc-RNA-seq data shows one of these candidates is an H-ATPase subunit (Atp6v0a1) expressed in osteoclasts (Matsumoto et al., 2018). We designed and injected a morpholino for this factor, and the skeletal tips were still resorbed.

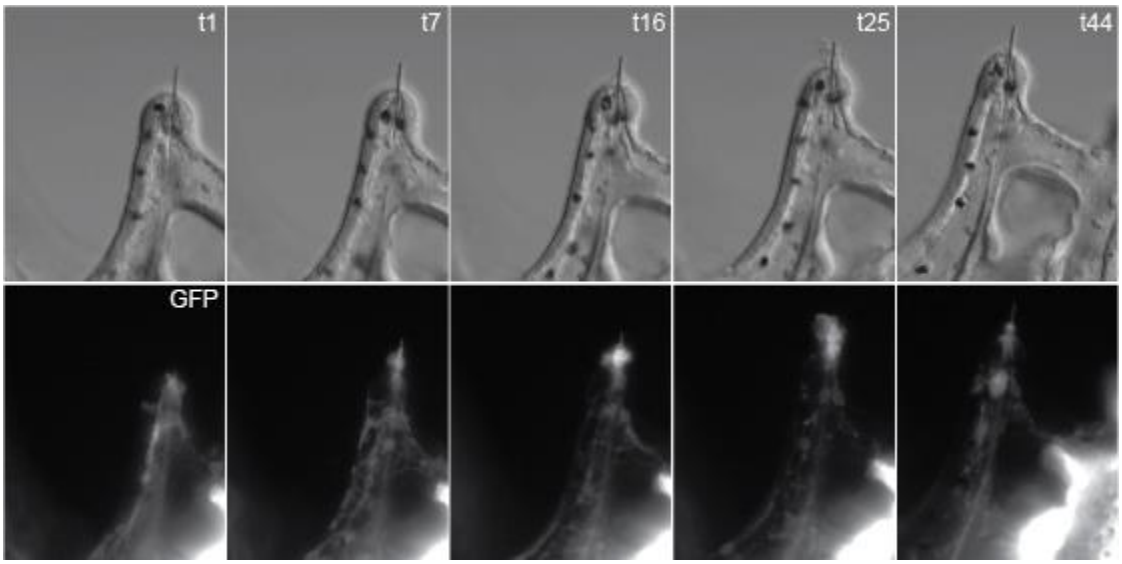


Figure 31: Blastocoelar cells use elaborate filopodial extensions during injury

Larva that was injected with membrane-GFP at the 2 or 4-cell stage that was cultured until two days post fertilization. The crush assay was performed and the oral hood imaged. Top row shows DIC images taken and show a blastocoelar cell protruding filopodia. Bottom row show the GFP channel and the elaborate interconnected network of the GFP+ blastoceolar cells during injury.

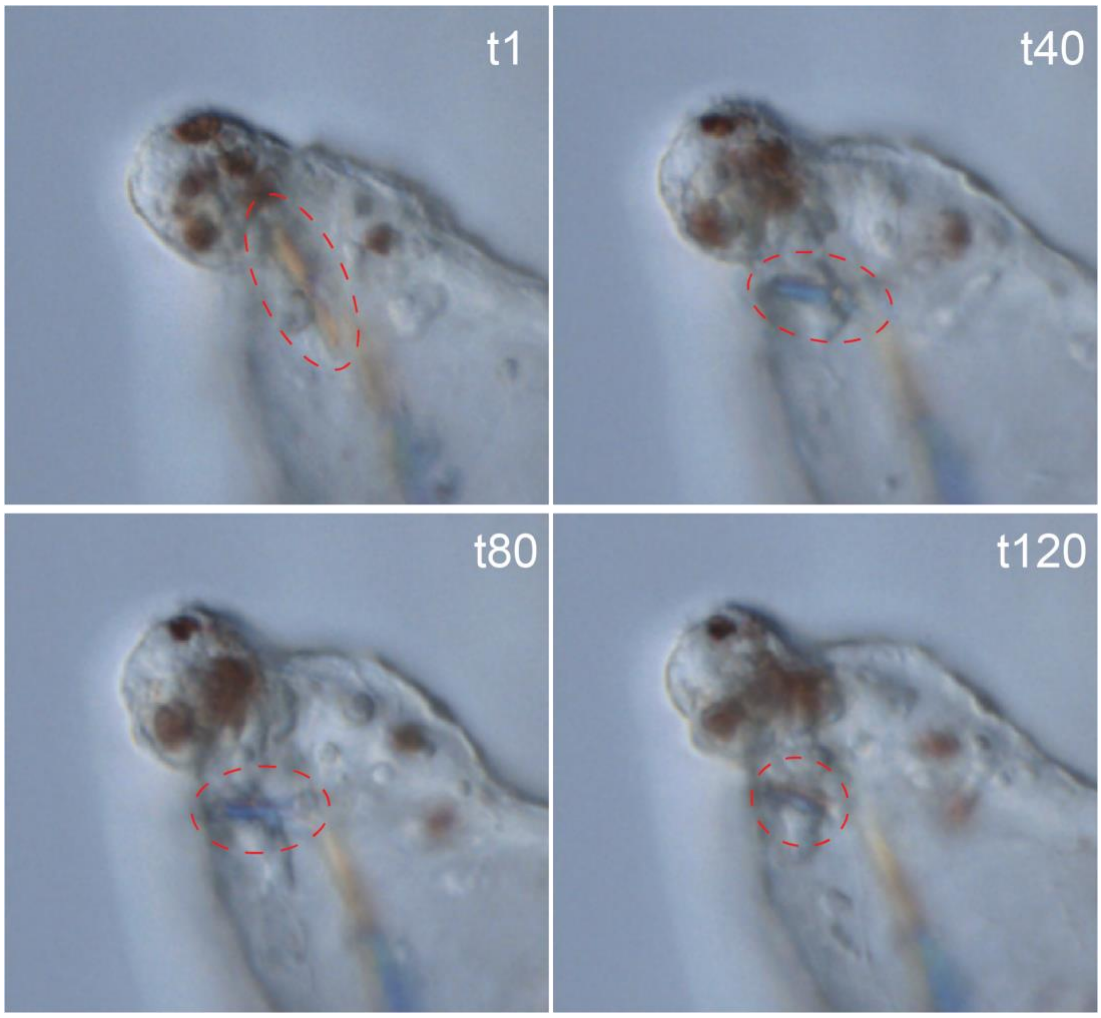


Figure 32: Mesenchyme can resorb skeletal rods during injury

An uninjected larva that had an injured post oral arm. Red dashed circle highlights where the skeletal rod is broken within the larva and shrinks (i.e., degraded) over time. Time (t) is out of 121 time series taken every 30 seconds.

5.5 Conclusions and future directions

This chapter describes an emerging model for wound repair and immune function in the sea urchin larva. The sites of skeletal rod punctures are an intersection of epithelium, resident and migratory mesenchyme, and extracellular matrices (i.e., skeleton, blastocoelar fluid) similar to skin wound healing (Piipponen et al., 2020). We've provided incipient observations and molecular data on what may be happening in the system. We know that pigment cells consistently and in groups migrate to sites of injury, and may be responsible for remodeling the epithelium to preserve the skeleton. We also show that the blastocoelar cell subpopulations are highly dynamic during injury and are able to resorb skeletal structures when needed, and may possess factors associated with bone resorption in vertebrates. These skeletoclastic cells demonstrate cell behaviors that include surveying the wound site, an ability to undergo an EMT, an apparent directional movement toward the site of the wound, in addition to their ability to resorb the skeleton.

Future directions for the project include cloning and inhibiting sc-RNA-seq candidate molecules found in osteoclasts, and creating a RNA-seq or sc-RNA-seq database on injured larvae. The latter will be the most important for investigating candidate molecules across species. Many genes found to be important in immune signaling in vertebrates are not expressed in the sc-RNA-seq dataset we have. We hypothesize that this is due to the IQ larva used in generating the dataset. By using the

crush assay on whole slides of embryos, it's possible to generate a wound-healing RNA-seq database. From there, follow-up would include cloning candidate genes and perturbing their function.

6. Anti- & Decolonial Sea Urchin Science

6.1 Introduction

My scientific career in Western institutions has and is accompanied by work "outside" of the discipline of Biology. These interests include language, art, sociology, psychology, Indigenous studies, queer and gender studies, government, and policy. Concurrently, as a queer and racialized person, I have been asked and expected by Western institutions, organizations, and individuals within them to participate in, be trained in, contribute to, and teach peoples about Diversity, Equity, and Inclusion (DEI). As of 2021, this constantly changing field of DEI work focuses on: bringing in peoples from diverse (n.b. depending on the individual asked, the definition of diversity varies) backgrounds, making diverse individuals feel included in the work spaces, and offering opportunities, funding, or support so they may have the same experience as everyone else within the space. You may read this definition of DEI and think, "Wait a minute, this seems vague!" You would be correct, and that's because DEI work is vague, and can be and is used in a context dependent manner. During the writing of this thesis, the current discourse on DEI in science, technology, engineering, and mathematics (STEM) is constantly changing and being co-opted by different groups.

To be clear early on, this chapter is not about DEI in STEM, though my experiences in DEI have shaped and pushed my thinking and work in this chapter. This chapter is also not written as a "resource" understandable or relatable to every person,

and is not to be appropriated and used as capital. My consistent experiences in interdisciplinary courses and seminars, readings, workshops, conferences, student organizations, and a minor in Science & Society, have solidified into a dissertation topic applicable to my research, peoples, and communities. If you are interested in DEI in STEM, please see published work on a course I and other Duke graduate students created (Simha et al., 2021). The culmination of this work, which I now understand as being in the realm of Science and Technology Studies (STS), leads to this chapter and its analysis of settler-colonialism in sea urchin science and paths that can be taken to do anticolonial and potentially decolonial research practices.

If you have read the Introduction chapter of this thesis, you will notice that I am specific in my wording and phrasing. The prime example is my use of "Western Science" instead of "science," the latter commonly used by academics or the general public. My specific phrasing will come up throughout the remainder of this chapter, along with my use of "I" as opposed to the royal "we" or an unnamed perspective common in Western Science writing. In performing these actions of specificity, I aim to (1) state the hierarchies that exist in the areas I talk about, (2) note the scale to which I'm referring to (e.g., individual versus institutional), (3) decenter monolithic exaggerations or binaries, (4) cite "non-academic" and "atypical" sources in a Biology thesis, and (5) be transparent of who I have obligations to in writing about this work.

Circling back to my use of "little-*s*" science in the chapter title, I work and build from the definition stated by Indigenous STS scholar Dr. Kim TallBear:

> What I have tended to do is to think about knowledges as knowledges and I don't like to use the word traditional [ref: traditional knowledge]. . . because that word can tend to imply stasis, it can tend to imply that that knowledge wasn't gotten also through observation and experimentation. . . I am often distinguishing between big-*S* Science and little-*s* science. Little-*s* science being the scientific method, big-*S* science being the kind of science tied up with capitalism and militarism. I think those two things should not be conflated. Many peoples around the world have done little-*s* science, while that is entangled with big-*S* Science we should be clear they are not synonymous. (Estes, 2020, 47:18)

Not completely synonymous names for Western Science include Settler Science and dominant science. Dr. Max Liboiron provides further context in their book *Pollution is Colonialism*:

> I use the term *dominant science* instead of *Western science* for two reasons. First, *dominant* keeps the power relations front and centre, and it's these power relations I am usually discussing. Western science is a cultural tradition where ways of knowing start with Ancient Greeks, get influenced by various forms of Christianity and Judaism, and move through the Enlightenment. . . not all

> Western science is dominant. Midwifery, alchemy, and preventative medicine are part of Western science that suffer at the hands of dominant science. (Liboiron, 2021, p20f77)

For consistency with the contemporary discourse on what is colloquially called "Western science," and to continually acknowledge the underlying roots of European cultures on academic Science, I will continue refer to big-S Science as Western Science.

On a spectrum of uses, I write "science" as an umbrella term to include all observations and experiments done throughout the world, especially on sea urchins. This includes experiments on sea urchin embryos by Sven Hörstadius in the early 1900s, the generated knowledges of different sea urchin species by Indigenous peoples throughout the world in the past and present, the current research done at Western academic institutions funded by private and public funds, and casual and consistent observations and experiments by a person in a local context. With this definition of science, I can begin to identify and be critical of sea urchin science that is colonial, anti-colonial, and decolonial in order to acknowledge my obligations to my aquatic, spiney relative.

6.2 Relationality or kinship in little-s science

Definitions and priorities of what relations, relationships, and kinship mean depend on cultural contexts and are always changing. An example is that the U.S. prioritizes the nuclear family, as opposed to entire communities. I am working from an

Indigenous framework, a blend of personal, cultural, and Indigenous STS, that knows and defines relations away from individualistic, human-centric, and hierarchical. My use of kinship through a queer Indigenous lens means that I am in relations to: my family (including blood and chosen), my tribe (Lac du Flambeau Band of Lake Superior Chippewa Indians), friends, co-workers, past and present academic advisors, fellow Duke University students, racialized communities, research collaborators, the sea urchins we use in lab, the land I walk and live on, the land the lab receives sea urchins from, people whose work I cite in my research, and peoples I interact with day-to-day or once a year. I do not live in a vacuum as a researcher; my ideas, questions, thoughts, and place within my communities and within the world are dependent on past and present relationships. This is especially important for the academic work I do. Ed Yong phrases it well in an introduction to a piece on lichens in The Atlantic (2019):

> Science is sometimes caricatured as a wholly objective pursuit that allows us to understand the world through the lens of neutral empiricism. But the conclusions that scientists draw from their data, and the very questions they choose to ask, depend on their assumptions about the world, the culture in which they work, and the vocabulary they use.

The non-exhaustive list in the prior paragraph emphasizes three important points. The first point is that I, and my academic peers (especially those who are living by themselves, are not in a romantic relationship or work on a unique project within a

lab) who are categorized as being alone in research, are in an intimate and complex web of relationships. Dr. TallBear dispels this myth of being "alone" in a podcast episode of *All My Relations*, "I don't live alone in this world, I have all kinds of relations, and meaningful relationships. . . We are all in relation. All the time. Whether those relations are good or not, we're in relation." (Wilbur and Keene, 2019, 40:30). Dr. TallBear touches on another important point: relationships include individuals, groups, or entities with whom you are in "bad," harmful, or negative relationships with.

The second point involves extending relationships beyond human animals. I am in relation to the non-human animals in my research, and I am a relative to these non-human animals. Western Science communication emphasizes an analogous but not synonymous point when describing how DNA is shared between all of the organisms on the planet. Confusingly, Western researchers and the general Western public selectively emphasize similarities and the differences that exist between human animals and non-human animals. The latter way of thinking results in the current discourse on "model" and "non-model" organisms in Western biomedical research, and results in a subjective view on why Western researchers and funding agencies should invest into studying non-human animals to benefit human animals. For further information on subjectivity in science, science writer Angela Saini talks deeper on the subject in a *Nature* article (Saini, 2020).

The final point is about the hierarchies of relationships that exist within Western science. Academia is commonly called an "ivory tower," and talking to individuals outside of secondary education, it becomes clear the way academia works is mysterious and confusing. What do you mean you're still a student? What does getting a Masters or Ph.D mean? In the context of Western society, a hierarchy exists between academic science researchers and Indigenous groups who have lived in the area for millennia, and since time began. Western produced knowledge is prioritized, and Indigenous produced knowledge is not, unless it's rediscovered or appropriated by Western researchers.

I see the topic of this S/science & Society chapter being a well-synchronized dance of my obligations to my kin and relationships that are not normally prioritized in biology/biomed research. These include the following kin: Indigenous folks, sea urchins, and my graduate school academic mentors. My obligation to my Indigenous relations manifests itself with an emphasis on demystifying and making known the coloniality present in biological research, and standing in solidarity with science produced by Indigenous folks. My understanding of this comes from teachings and works by my Indigenous family, community, and tribe, and Indigenous academics, media producers, teachers, writers, artists, water and land protectors whom I cite and make reference to throughout this work. With these teachings comes the understanding that I may not, should not, and will not be writing about knowledge not produced by Western Science. In my limited time and resources to build relationships with Indigenous folks that live

with sea urchins in their native regions and my position as a scientist produced in a colonial system, I would only cause harm through appropriation. My goal is to continue this STS work after graduate school by writing a book in close relations to Indigenous groups and my/their sea urchin relatives.

Another major obligation I have is from my relatively new relationship to sea urchins. I did not take courses in biology with the goal to learn from sea urchins, and to learn about the work that has been done on them in Western Science. As a person whose Indigenous homelands are located in what is currently called Wisconsin, a place with no direct connection to oceans, my original infatuation with the sea urchins began with the "alien" and "exoticness" they had to me and Western society. As I have become more familiar with the species *Lytechinus variegatus*, and sea urchin species used in developmental biology, I never fully assimilated into the Western Science perspective on non-human animals, especially invertebrates. This perspective is complex and context dependent, but key takeaways are that non-human animals can and should be used to benefit human animals, and invertebrate animals are not afforded the same care and rights as vertebrate animals. Sea urchins, and other non-human animals used for research, are more than just tools. They have been my teachers during my time in graduate school, there are homes for other non-human animals, and they are a part of the **L**and. I understand my obligation to sea urchins during this stage of my life as

writing about the history of why they were and still are used in developmental biology and critically assessing it under an anti-colonial perspective.

My final and academic obligation is to my academic mentors in graduate school. This obligation is stereotyped in the academic system and results is this written doctoral dissertation that must fit within expectations and formatting defined by Duke University's Graduate School. This minor chapter fits within these rules, and is unique to the Biology Department with special obligations to a minor advisor. Unfortunately this dissertation will only provide a snapshot on the ways I show my relationality to others. Other examples will include the public seminar and published media I do beyond this writing. With background explained, my aim is that you, the reader, will be able to continue or build new relationality to me, sea urchins, and a broader body of scholarly and grass root work and peoples in doing science.

6.3 Colonialism in the field of sea urchin research

6.3.1 Stone mask - Seeing colonialism

Colonialism comes in many forms and names, including external colonialism, internal colonialism, settler-colonialism, etc. Tuck and Yang (2012) define these terms:

> *External colonialism* (also called exogenous or exploitation colonization) denotes the expropriation of fragments of Indigenous worlds, animals, plants and human beings, extracting them in order to transport them to - and build the wealth, the privilege, or feed the appetites of - the colonizers, who get marked as the first. . .

> *internal colonialism*, the biopolitical and geopolitical management of people, land, flora and fauna within the "domestic" borders of the imperial nation. . . *Settler colonialism* operates through internal/external colonial modes simultaneously because there is no spatial separation between metropole and colony. (p. 4-5)

The United States, which currently occupies Indigenous lands on which my research was performed on and is a former colony of the British Empire, is a prime example of a settler-colonial state historically and contemporarily enacting colonialism. These colonial actions include the theft of lands, peoples, and Indigenous produced knowledge inside and outside North America to grow and develop the US state.

> . . . imperialism and colonialism brought complete disorder to colonized peoples, disconnecting them from their histories, their landscapes, their languages, their social relations and their own ways of thinking, feeling and interacting with the world. It was a process of systematic fragmentation which can still be seen in the disciplinary carve up the indigenous world: Bones, mummies and skull to the museums, artwork to private collectors, languages to linguistics, 'customs' to anthropologists, beliefs and behaviors to psychologist. (Smith, 2012, p. 29)

Fortunately, Indigenous peoples, communities, tribes and nations still live on these occupied lands and do a wide variety of science (Kimmerer 2013, TallBear 2013, Treuer 2019).

Colonial science, which includes practices, methods, questions, etc., is broad in scale and impact. They may be identified by connecting general colonial practices directly to one's research methods, and/or by listening to Indigenous peoples and groups. An example of colonial science is a non-Indigenous (e.g., settler) person researching a plant on occupied lands that are not their Indigenous homelands. The scale of impact ranges depending on the details. For example, if a non-Indigenous researcher did or did not have permission from the local Indigenous population to perform the research, the harm to the Indigenous group differs.

6.3.2 Scary monsters - A history of colonialism in sea urchin research

If you are a student or researcher in Western Science, instead of asking yourself, "are" you doing colonial science, you'll find it more productive to ask "how" you are doing colonial science. This is not to imply that *all* Western Science practices are colonial, but as an operating principle to address colonial practices that are subtle or normalized into research methods. This is especially important because the current academic research system has historically and currently excludes Indigenous folks or multidisciplinary thinkers who are trained to identify colonialism and its many forms and monikers. As a developmental biologist working with sea urchins, I will answer the latter question, how is sea urchin developmental biology research colonial? I will provide historical and contemporary examples for readers. May this paragraph serve as a content warning for the rest of this chapter since the histories and actions of settler-

colonialism in Western Science is one fraught with harm to Indigenous peoples, and colonization is an ongoing event. The process of decolonization is one that "unsettles" the settler, meaning this process is not associated with positive emotions.

To begin, we'll look into the history of sea urchin developmental biology or why Western Scientists started using sea urchins for research. Digging through research articles in the past century on sea urchin developmental biology, you'll come across citations from "pioneering" works from individuals in the field including: Johannes Müller, Oscar Hertwig, Hans Driesch, Sven Hörstadius, and others (Figure 33). German scientist Johannes Müller is credited in Western Science with the "discovery" of the pluteus larvae during his first visit to Helgoland, an archipelago currently under the control of Germany (Florey, 1995; Müller, 1846). Müller's career in Western Science ranged from his early work on insects to being credited with the discovery of the "Müllerian" duct. Earlier in his academic career, he studied and published his first known work on marine invertebrates, crustaceans (Steudel, 2021). It is at this point I can trace the beginning of Western work on marine invertebrates that will lead to the study of sea urchins in developmental biology. What is beyond this dissertation, and perhaps unknowable at this point in time, is why Müller decided to study and become familiar with marine invertebrates in his life. Was it his mentor(s)'s influence? Did he grow up along the shore and develop general marine knowledge before his academic work? Or,

did he have a relative who introduced him to the wonders of aquatic, non-human animals?

After an economic decline due to it losing military interest, the Helgoland economy was forced to become a tourist location:

> Helgoland became an attraction not only for those seeking relaxation or holiday fun, but also for naturalists. In the beginning, it was mainly the algae that caught the attention of amateur and professional botanists alike. Helgoland, after all, represents the only rocky coast in all Germany. . . (Florey, 1995, p. 79)

During his time in Helgoland, Müller mentored a man named Ernst Haeckel, who later became a German scientist, proponent of Darwinism, and known eugenicist (Florey, 1995; Haeckel, 1904; Robinson, 2021). Influenced by Darwin's theory of evolution, Haeckel preached his theory to individuals such as Anton Dohrn (Colonna, 2014). Dohrn is recognized as the founder of the Anton Dohrn Zoological Station in Naples, Italy in 1872 (Colonna, 2014; Groeben, 1985). A researcher himself, Dohrn was "in pursuit of research material, particularly marine organisms," and:

> . . . Dorhn and colleague Nicolai Miclaoucho-Maclay proposed to create a network of zoological stations. Dohrn proposed that those stations should be equipped with laboratory rooms and experimental instruments and supplies for scientists to collect research materials, make observations, and perform experiments before potentially moving to the next station. (Colonna, 2014).

What is particularly striking and colonial about this goal of creating this and other zoological stations is that Dohrn assumes entitlement to marine organisms (i.e., the land) outside of his home country, including Italy. It becomes clear that the colonial aspects of Western academia, which have their roots in European ideals, come through in Dohrn's and other's European scientific goal of producing and appropriating knowledge from the land that they have no kinship or relations to in order to further their careers. The relationships to marine, non-human animals is also made clear by Dohrn that they are just research materials, no matter what ocean they may come from.

Dohrn's colonial wishes came true as his zoological station became established. European researchers building off knowledge produced on prior work such as Müller's made sea urchins a model organism (Colonna, 2014). These researchers and their work at the station included Oscar Hertwig [fertilization], Jacques Loeb [artificial parthenogenesis], Theodor Boveri [cytoplasmic specification], and Han Driesch [egg micromanipulation] (Britannica, 2021; Colonna, 2014; Maayan, 2011). What influenced their respective research and their questions in developmental biology/embryology is important to understand for a more in-depth look at settler-colonialism today but it will not be covered in this work.

Influenced by the work of sea urchin researchers at the Anton Dohrn Zoological Station, and the work of other embryologists/developmental biologists, Sven Hörstadius published multiple works on echinoderms (Hörstadius, 1928; Hörstadius, 1939;

Hörstadius, 1973; Jacobson, 2000). These works have been instrumental for influencing mid- and late 20th century developmental biologists, and providing foundation for a resurgence in sea urchin developmental biology. We can see direct connections interpersonally and institutionally that influenced the colonial history of sea urchin science in the field of development. When compared to the most popular model organisms in biomedical/basic bio Western Science today, many of which have direct colonial ties in research, sea urchins take-up a smaller piece of the research grants and focus at conferences. Though marginalized in Western Science, sea urchin research continues to be colonial.

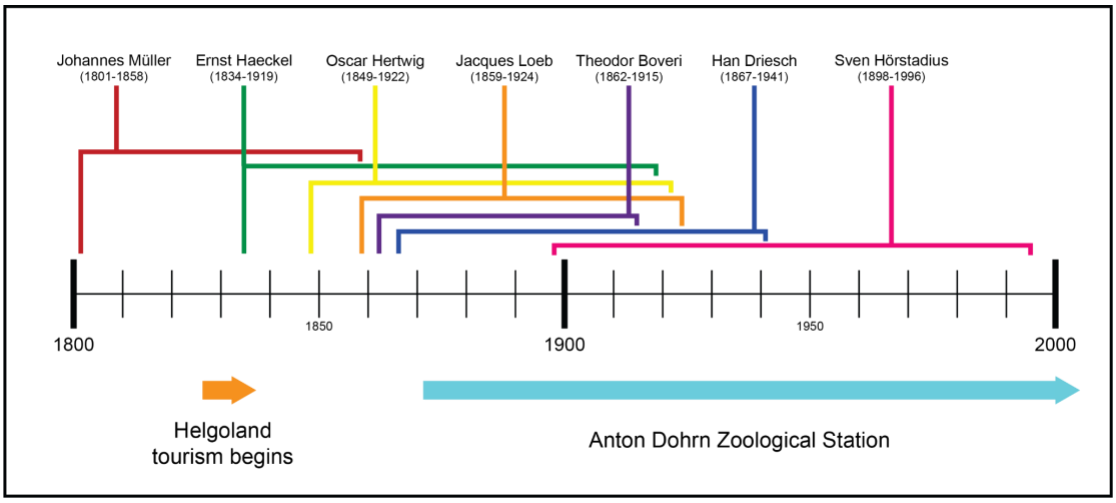


Figure 33: European researchers and research locations in the history of sea urchin developmental biology

From 1800 to 2000 A.D., a timeline showing the life of researchers who started and influenced different areas of sea urchin developmental biology (Top). The bottom portion of the timeline indicates the rough time period Helgoland became a tourist destination and the founding of the Anton Dohrn Zoological Station in Naples, Italy. The former location is where Johannes Müller described an echinoderm pluteus larva. The latter Zoological Station is where many of the above scientists did work on sea urchins.

6.3.3 The World - Contemporary colonialisms amongst sea urchin science

Everywhere you look today, you'll be able to locate harm that is being caused by Western Science. Colonialism continues today, but it's not often talked about, or it's recast as being benign or in the past. Evening writing and talking about it causes subtle and sometimes blatant instances of harm to me by individuals and institutions in the settler-colonial system. To mitigate harm, I will generally (not specifically) talk about observations of settler-colonialism in today's research environment with sea urchins. This is not an exhaustive list, and reading it as an Indigenous versus non-Indigenous person will be a different experience.

Let's talk about the socio-politics of sea urchins. As mentioned in the Introduction chapter, echinoderms are present throughout all the oceans of the world, and there are hundreds of sea urchin species living solely or alongside other sea urchins. In a binary context, research labs can locally *take* (or *harvest* if you're an Indigenous lab) sea urchins or ship them from one location to another. If a group's only relationship to a sea urchin species is for Western research, this is colonial science. An Indigenous group living alongside sea urchins will/could know the sea urchins' stories and history, the quirks they have (e.g., none live in this specific bay or depth), if you should avoid them, who eats them, and what they do.

A colonial research group will not know the history a sea urchin species has, will generate knowledge that is already known, will appropriate the knowledge from an

Indigenous group, and/or generate knowledge relevant to their own institutions. This can be an indicator for how an individual or research group is benefiting a researcher, a research group, a research institution, and/or "first" world countries and governments, at the expense of Indigenous lands, which include the peoples. The goal of a colonial system is to displace, erase, and replace Indigenous peoples, and Western scientific practices do that explicitly or implicitly on a daily basis.

Colonial scientists will participate and be complicit in the regulation of sea urchins. This can be doing the minimum protocol requirements when working with sea urchins in the lab (e.g., killing sea urchins that aren't used as opposed to returning them to their respective home), or being silent when settler-colonial governments and/or institutions regulate (or don't regulate) a sea urchin species on how an Indigenous government or group decides. Seeing and deciding an Indigenous government/group's way of being in relation to sea urchins is wrong or "outdated" is an extremely harmful and institutional way of harming sea urchins and Indigenous peoples.

Finally, colonial science will establish a culture and hierarchy that prioritizes human animals. This is an instance where "othering" comes into play. In a local context, respect and relationships to non-human animals, plants, and lands is encultured. In the current climate of globalization the colonial practice of tourism, which prioritizes "first" world citizens, "second" and "third" world places are viewed as developing, exotic, and/or strange. It's easier to subjugate entities that are strange (i.e., you don't have

relations to) for one's own benefit. It's taken me awhile to pick-up on the behaviors *Lytechinus variegatus* has (e.g., I didn't realize they would wave their tube feet when I turn off the light), as opposed to seeing them as boring, aimless, or odd creatures that don't do much.

A fictitious example of colonial sea urchin science is JoJo Doe (he/him). JoJo is a researcher at an R1 institution in what is currently called California and JoJo studies the made-up Stone Water sea urchin living in the Great Lakes of the U.S. (this is very fanciful because sea urchins only live in saltwater). JoJo is interested in studying how the immune cells of the sea urchin develop and live with a freshwater microbiome, as opposed to their saltwater relatives. There isn't much info published about the species in scientific journals, so JoJo acquires government and internal funding to make the Stone Water urchin a new model organism. JoJo received permission for his proposed IACUC (The Institutional Animal Care and Use Committee) protocol, and found out the states that are connected to the Great Lakes don't regulate invertebrate harvesting. JoJo is overjoyed and spends the next few years publishing scientific papers, presenting at conferences, and doing outreach in his city on the Stone Water urchin.

JoJo's research shows many key cornerstones of colonialism. He has no obvious relations to the Great Lakes region, the fictitious sea urchin, and the Indigenous nations living in the area. JoJo assumes access to these regions, and access to the sea urchins. Besides knowing what state and local regulations are, he shows no interest in building

long-term relationships with the areas and has no idea what local, Indigenous practices are. He sees these sea urchins as an "untapped" resource to further his career, which is what he does, quite successfully. Never does he ask if the knowledge he is generating has already been known by local groups. The cherry on top of this colonial sundae is that JoJo does outreach that only benefits his own scientific community in California. The Indigenous peoples and the Land have had more items and knowledge taken from them.

6.4 Anti- and decolonial possibilities

6.4.1 Ripple - Anti- and decolonial science

Naming colonialism and/or colonial activities is becoming more commonplace in the US, even in science, but identifying and implementing anticolonial or decolonial activities is still difficult. Dr. Liboiron makes a clear point on the former, "Anticolonial sciences are characterized by how they do not reproduce settler and colonial entitlement to Land and Indigenous cultures, concepts, knowledge, and life." (Liboiron, 2021). Returning to the JoJo Doe example in the prior subsection, anticolonial research practices would mean the non-Indigenous researcher (JoJo), and his research group, do not assume they are entitled to research the Stone Water urchin, even if they have permission to do so from their institution, government, and/or settler state's government. A key factor is the involvement, permission, consent, and promoted

sovereignty of the Indigenous communities, this may be seen as the group's (not a single Indigenous person's) consent.

Decolonial science is similar to but not the same as anticolonial science. The word "decolonize" or to describe someone or something as "decolonized" is turning up more frequently in public discourse. The use of the verb decolonize in these situations refers to it as an umbrella word for DEI, anti-racism, social justice work, etc. Unfortunately, this usage is incorrect and turns decolonize into a metaphor. Tuck and Yang (2012) talk about this metaphorical use of decolonize, and define what the process is:

> Decolonization in the settler colonial context must involve the repatriation of land [to Indigenous peoples] simultaneous to the recognition of how land and relations to land have always already differently understood and enacted [by them/us]; that is, *all* of the land, and not just symbolically. (p. 7)

I use Tuck and Yang's definition of decolonize in this work. To be clear, this means if the recommendations and actions talked about within this chapter are not used by readers with the intent goal of repatriating or stopping the theft of Land (i.e., land, earth, water, air, non-human relatives, spirits, humans, other relative entities defined by an Indigenous peoples), it is <u>not</u> decolonial or decolonizing (Liboiron, 2021). In terms of the fictitious JoJo Doe scenario, decolonial science would involve ways to return full sovereignty back to the Indigenous peoples who are and have been in relations to the

Stone Water urchin, including the water it lives in, the air and plants that surround it, the Indigenous names they originally had/have, and to the sea urchin itself.

6.4.2 Golden experience - Supporting generations of anti- and decolonial sciences

Similar to my discussion on colonialism, I will not provide specific possibilities or examples of anti- and decolonial research practices. *Decolonizing Methodologies* by Dr. Smith (2012) and a reading list by Dr. Prescod-Weinstein titled "Decolonising Science Reading List" are great starting points (2015). There are other books present in the Inclusion, Diversity, Equity, and Anti-racism (IDEA) course I and other Duke graduate students created (Simha et el., 2021). Instead, I will provide brief, general practices to start with in sea urchin research.

A major focus is to hire and provide financial and social support for Indigenous peoples in your respective institution, especially individuals who are Indigenous to the lands a research institution stands on. A part of this includes building an equitable, and long-term relationship with local Indigenous groups. Doing research abroad and seasonally is not an exception.

Another important practice will be asking for consent from people living where sea urchins are taken. This includes a settler-colonial government and Indigenous governments. For the latter, the protocols Indigenous groups decide on for non-human animals, plants, etc. must be mandatory for researchers. If research groups have been taking information in the past, it's vital to return what has been taken in a way the

Indigenous group wants. This includes non-human animals (e.g., sea urchins), whether they are alive, deceased, or present in a museum.

A final proposal, and the most difficult, is Land back. How and what ways can you, your group, and/or institutions return the Land? Instead of providing an answer, I request that individuals keep this question on the back of their minds while doing science. The methods to address this colonial conundrum will be complex, take a large amount of resources, and will be unsettling to the settler. To do decolonial scientific work, Land back is a must.

6.5 Conclusions

Model organism research is colonial at its core. Western Scientists harm and use non-human animals primarily to benefit themselves and the settler institutions of which they are a part of. Model organism research takes non-human relatives from their homelands, severs their connection to peoples and the land, keeps and/or breeds their kin in perpetuity or until they are no longer useful to Western researchers, and harms them (e.g., drug trials) with minimal expectation of reciprocity. This chapter is an obligation to my relations in life as an Indigenous person produced and working in a colonial academic system. Readers from academic, especially non-Indigenous, may have expected a panacea or a "how to" guide for finding and addressing colonialism in science. If this were the case, I would have created something that would have perpetuated colonialism through consumption and appropriation. For my fellow

Indigenous readers, my aim was to stand in solidarity with you and my sea urchin relatives, and to mitigate the harm of writing down what I've learned for consumption by non-Indigenous, academic peers.

Though the title of this section is called "conclusions" which is often associated with content feelings, you may find yourself more distraught, confused, and/or unsettled. This is great! As I mentioned earlier, anti- and decolonial work should be unsettling to those who are non-Indigenous or Indigenous peoples who are deep into colonial systems. What you do next will be vastly important if you're dedicated to anti- and decolonial practices in science. Think about your obligations in your work, how you want to mitigate harm, and how you want to yield your power and influence to those it was stolen from and/or built off of.

A great starting point is to read, listen to, and watch the cited works in this chapter, and support and build relationships with those who have done work. If you're in academia in the natural sciences, look into STS studies. If you're Indigenous, please follow my work elsewhere and reach out (Twitter: @Ray_L_Allen). If you're a reader who wanted to learn more about sea urchins, do the best you can to support established relations to our non-human relatives locally.

7. Conclusions

7.1 Summary

The complexity of sea urchin immunity, influence on cultures, and history of colonial science is a wonder to marvel at. Current dominant cultures frequently neglect and vilify sea urchins, even though they have and still do influence areas of science, engineering, art, and digital media. In Chapter 1 we do a deep dive (this is funny because urchins live in the ocean) into the myriad of ways sea urchins are a part of our lives, and some evidence as to why they are treated like pests in a Western context. In the following chapter we transition to talking about standard techniques used in developmental biology research. At the end, we describe an easy to reproduce injury assay that causes skeletal rod punctures in the early prism and pluteus larva. Chapter 3 looks broadly and critically thinks about the mesenchyme in the sea urchin embryo and its relationship to immune systems across different species. I make a case for recategorizing the skeletal and non-skeletal mesenchyme as macrophage-like cells, based on gene expression and functional properties, in order to break out of limited historical thinking. The following chapter of this dissertation characterizes *Lytechinus variagatus*'s expanded MIF family of atypical cytokines. We found hidden members of the family, tease apart the subtle differences between these short genes, and made headway in functional analyses of these them. Chapter 5 details the injury response when skeletal rods puncture the epithelium. We document and quantify how the

pigment cells migrate to areas of injury, and discover the blastocoelar cell ability to degrade skeletal matrix. In the final chapter, I explore the history and contemporary colonialism in sea urchin research. I trace the origins of sea urchins in developmental biology and find marine research stations as a starting point. And I further point out that these research stations were created as a means of Western scientists to gain access to and exploit lands and non-human animals.

7.2 Future Directions

At the end of a majority of chapters, there are aims for future directions. This section lays out some general ideas that are of interest to future researchers working i*n vivo*. I will also propose what my future goal is for my Science & Society work in sea urchins.

MIFs in *L. variegatus* and across echinoderms

Along with the full characterization of the already cloned MIF genes in this dissertation, there are still 8 more MIF genes to be cloned that have candidate expression via sc-RNA-seq (figure 14). As I emphasized in Chapter 4, the MIF family is involved in a multitude of different processes, with little emphasis on development. I propose future researchers prioritize this signaling molecule family in sea urchin research since we can now distinguish the MIF loci from another, clone and generate reporter constructs, and inhibit their function using methods such as CRISPR. And with the beauty of new

echinoderm genomes, an evolution-development analysis will offer novel insights into how this gene family evolved.

Inhibiting non-skeletal mesenchyme response in injury

Our work on perturbing through gene knockdowns and injuring the larva was done efficiently using the well-defined gene regulatory network and tools available to us. Quite surprisingly, we were unable to stop pigment cells from migrating, and blastocoelar cells from migrating and degrading the skeleton. I believe an important focus should be uncovering molecules that when inhibited or knocked down, perturb the injury phenotypes we characterized in these chapters. Fortunately, the Lv 3.0 Genome will allow candidate molecules to be inhibited much quicker than in previous years.

Science, Society, and Sea Urchins

A major section of my Science and Society chapter focused on Western ideas of sea urchins in culture and developmental biology research. An area I did not yet feel comfortable learning and writing about is an Indigenous centered perspective of sea urchins. This comes from the anti-colonial method of building relationships with Indigenous people and peoples before consuming and producing content. My future direction is to produce a non-fiction book that also centers the Indigenous perspective on sea urchins, and will require me to build long-term relationships with multiple

Indigenous groups, and also ask what they may want and need from such a piece. The future is anticolonial and decolonial science, science art, and science art.

Works Cited

Adams, N. L., Heyland, A., Rice, L. L., & Foltz. (2019). *Procuring animals and culturing of eggs and embryos.* In L. Wilson & P. Tran (Eds.), *Methods in Cell Biology* (Volume 150, 3-26). Elsevier.

Adonin, L., Drozdov, A., & Barlev, N. A. (2021). Sea Urchin as a Universal Model for Studies of Gene Networks. *Front. Genet*, https://doi.org/10.3389/fgene.2020.627259

Associated Press. (2019, October 24). *Sea urchin population soars 10,000% in five years, devastating US coastline*. The Guardian. https://www.theguardian.com/environment/2019/oct/24/sea-urchins-california-oregon-population

Armstrong, N., & McClay, D. R. (1994). Skeletal pattern is specified autonomously by the primary mesenchyme cells in sea urchin embryos. *Dev Biol*, 162, 329-38.

Barsi, J. C., Tu, Q., Calestani, C., & Davidson, E. H. (2015). Genome-wide assessment of differential effector gene use in embryogenesis. *Development*, 142, 3892-3901.

Bayly-Jones, C., Pang, S. S., Spicer, B. A., Whisstock, J. C., & Dunstone, M. A. (2020). Ancient but Not Forgotten: New Insights Into MPEG1, a Macrophage Perforin-Like Immune Effector. *Frontiers in Immunology*, 11, doi: 10.3389/fimmu.2020.581906

BBC Earth. (2012, May 2). *Army of Sea Urchins? | Planet Earth | BBC Earth* [Video]. YouTube. https://www.youtube.com/watch?v=ihvKwVDw8Pc

Berge, J., Vader, W., & Lockhart, S. (2004). A survey of amphipod associates of sea urchins, with description of new species in the genera *Lepidepecreella* (Lysianassoidea: lepidepecreellid group) and *Notopoma* (Photoidea: Ischyroceridae) from Antarctic cidarids. *Deep-Sea Research II*, 51, 1717-1731.

Blum, M., Chang, H. Y., Chuguransky, S., Grego, T., Kandasaamy, S., Mitchell, A., Nuka, G., Paysan-Lafosse, T., Qureshi, M., Raj, S., Richardson, L., Salazar, G. A., Williams, L., Bork, P., Bridge, A., Gough, J., Haft, D. H., Letunic, I., Marchler-Bauer, A., Mi, H., Natale, D. A., Necci, M., Orengo, C. A., Pandurangan, A. P., Rivoire, C., Sigrist, C. J. A., Sillitoe, I., Thanki, N., Thomas, P. D., Tosatto, S. C. E., Wu, C. H., Bateman, A., & Finn, R. D. (2021). The InterPro protein families and domains database: 20 years on. Nucleic Acids Research, 49, D344-D354, DOI: 10.1093/nar/gkaa977

Boudouresque, C. F., & Verlaque, M. (2020). *Paracentrotus lividus*. In J. M. Lawrence (Ed), *Sea Urchins: Biology and Ecology* (4th ed., 447-485). Elsevier.

Brave Wilderness. (2017, September 13). *SPIKED by a Sea Urchin?* [Video]. YouTube. https://www.youtube.com/watch?v=xHvVhhFvLtY

Britannica, The Editors of Encyclopedia. (2021, April 3). *Jacques Loeb*. Encyclopedia Britannica. https://www.britannica.com/biography/Jacques-Loeb

Buckley, K. M., & Rast, J. P. (2012). Dynamic evolution of toll-like receptor multigene families in echinoderms. *Front. Immunol.*, https://doi.org/10.3389/fimmu.2012.00136

Buckley, K. M., & Rast, J. P. (2015). Diversity of animal immune receptors and the origins of recognition complexity in the deuterostomes. *Developmental and Comparative Immunology*, 49, 179-189.

Buckley, K. M., & Rast, J. P. (2017). An Organismal Model for Gene Regulatory Networks in the Gut-Associated Immune Response. Frontiers in Immunology, 8, doi: 10.3389/fimmu.2017.01297

Buckley, K. M., & Rast, J. P. (2019). Immune activity at the gut epithelium in the larval sea urchin. *Cell and Tissue Research*, 377, 469-474.

Buckley, K. M., Schuh, N. W., Heyland, A., & Rast, J. P. (2019). Analysis of immune response in the sea urchin larva. In L. Wilson & P. Tran (Eds.), *Methods in Cell Biology* (Volume 150, 333-355). Elsevier.

Calestani, C., & Rogers, D. J. (2010). *Cis*-regulatory analysis of the sea urchin pigment cell gene *polyketide synthase*. *Developmental Biology*, 340, 249-255.

Calestani, C., Rast, J. P., & Davidson, E. H. (2003). Isolation of pigment cell specific genes in the sea urchin embryo by differential macroarray screening. *Development*, 130(19), 4587-4596.

Carrier, T. J., Leigh, B. A., Dealer, D. J., Devens, H. R., Wray, G. A., Bordenstein, S. R., Byrne, M., & Reitzel, A. M. (2021). Microbiome reduction and endosymbiont gain from a switch in sea urchin life history. *PNAS*, 118(16), https://doi.org/10.1073/pnas.2022023118

Cary, G. A., Cameron, R. A., & Hinman, V. F. (2018). Echinobase: Tools for Echinoderm Genome Analyses. *Methods Mol Biol*. doi:10.1007/978-1-4939-7737-6_12, 2018

Cell Press. (2019, September 18). *These pink sea urchins have teeth that sharpen themselves*. ScienceDaily. https://www.sciencedaily.com/releases/2019/09/190918112430.htm

Cheng, X., Lyons, D. C., Socolar, J. E. S., & McClay D. R. (2014). Delayed transition to new cell fates during cellular reprogramming. *Dev Biol*, 391(2), 147-57.

Cheon, Y., Lee, C. H., Jeong, D. H., Kwak, S. C., Kim, S., Lee, M. S., & Kim, J. (2020). Dual Oxidase Maturation Factor 1 Positively Regulates RANKL-Induced Osteoclastogenesis via Activating Reactive Oxygen Species and TRAF6-Mediate Signaling. *Int. J. Mol. Sci*, 21, Int. J. Mol. Sci. 2020, 21, 6416; doi:10.3390/ijms21176416

Christiaen, L., Jaszczyszyn, Y., Kerfant, M., Kano, S., Thermes, V., and Joly, J. (2007). Evolutionary modification of mouth position in deuterostomes. *Seminars in Cell & Developmental Biology*, 18, 502-511.

Christiakov, D. A., Myasoedova, V. A., Revin, V. V., Orekhov, A. N., & Bobryshev, Y. V. (2018). The impact of interferon-regulatory factors to macrophage differentiation and polarization into M1 and M2. *Immunobiology*, 223, 101-111.

Colonna, F. T. (2014, December 22). *Stazione Zoologica Anton Dohrn, Naples, Italy*. Embryo Project Encyclopedia. https://embryo.asu.edu/pages/stazione-zoologica-anton-dohrn-naples-italy

Croce, J., Lhomond, G., & Gache, C. (2001). Expression pattern of *Brachyury* in the embryo of the sea urchin *Paracentrotus lividus*. *Dev Genes Evol*, 211, 617-619.

Croce, J. C., & McClay, D. R. (2010). Dynamics of Delta/Notch signaling on endomesoderm segregation in the sea urchin embryo. *Development*, 137(1), 83-91.

Davidson, E. H., Rast, J. P., Oliveri, P., Ransick, A., Calestani, C., Yuh, C., Minokawa, T., Amore, G., Hinman, V., Arenas-Mena, C., Otim, C., Brown, C. T., Livi, C. B., Lee, P. Y., Revilla, R., Schilstra, M., Clarke, P. J. C., Rust, A. G., Pan, Z., Arnone, M. I., Rowen, L., Cameron, R. A., McClay, D. R., Hood, L., & Bolouri, H. (2002). A Provisional Regulatory Gene Network for Specification of Endomesoderm in the Sea Urchin Embryo. *Developmental Biology*, 246, 162-190.

Davidson, P. L., Guo, H., Wang, L., Berrio, A., Zhang, H., Chang, Y., Soborowski, A. L., McClay, D. R., Fan, G., & Wray, G. A. (2020). Chromosomal-Level Genome Assembly of the Sea Urchin Lytechinus variegatus Substantially Improves Functional Genomic Analyses. *Genome Biology and Evolution*, 12(7), 1080-1086.

Deep Look. (2016, August 23). *Sea Urchins Pull Themselves Inside Out to be Reborn | Deep Look* [Video]. YouTube. https://www.youtube.com/watch?v=ak2xqH5h0YY&t=5s

Dong, H., Ji, Z., Liu, M., Wang, Y., Bai, X., Wang, T., Liu, Z., Wu, Y., Zhang, B., Luo, Y., Li, Z., & Dong, M. (2013). Functional expression of ERG1 potassium channels in rat alveolar macrophages. *J Mol Hist*, 44, 117-124.

Duloquin, L., Lhomond, G., & Gache, C. (2007). Localized VEGF signaling from ectoderm to mesenchyme cells controls morphogenesis of the sea urchin embryo skeleton. *Development*, 134, 2293-2302.

Ebert, T. A. (2020). Growth and survival of postsettlement sea urchins. In J. M. Lawrence (Ed), *Sea Urchins: Biology and Ecology* (4th ed., 95-145). Elsevier.

Edholm, E., Rhoo, K. H., & Robert, J. (2017). Evolutionary Aspects of Macrophages Polarization. In M. Kloc (Ed), *Macrophages: Origin, Functions and Biointervention* (3-22). Springer Nature.

Estes, N. (Host). (2020, August 31). The end of US empire? w/ Kim TallBear [Audio Podcast Episode]. In *The Red Nation Podcast*. The Red Nation. https://directory.libsyn.com/episode/index/show/therednation/id/15815267

Ettensohn, C. A. (1992). Cell interactions and mesodermal cell fates in the sea urchin embryo. *Development*, supplement, 43-51.

Ettensohn, C. A. (2020). The gene regulatory control of sea urchin gastrulation. *Mech Dev*, 162, 103599.

Ettensohn, C. A., & Adomako-Ankomah, A. (2019). The evolution of a new cell type was associated with competition for a signaling ligand. *PLoS Biol*, 17(9), e3000460. https://doi.org/ 10.1371/journal.pbio.3000460

Ettensohn, C. A., & Dey, D. (2017). KirrelL, a member of the Ig-domain superfamily of adhesion proteins, is essential for fusion of primary mesenchyme cells in the sea urchin embryo. *Developmental Biology*, 421, 258-270.

Ettensohn, C. A., & McClay, D. R. (1987). A new method for isolating primary mesenchyme cells of the sea urchin embryo. Panning on wheat germ agglutinin-coated dishes. *Exp Cell Res*., 168(2), 431-8.

Ettensohn, C. A., & McClay, D. R. (1988). Cell Lineage Conversion in the Sea Urchin Embryo. *Developmental Biology*, 125, 396-409.

Fleming, T. J., Schrankel, C. S., Vyas, H., Rosenblatt, H. D., & Hamdoun, A. (2021). CRISPR/Cas9 mutagenesis reveals a role for ABCB1 in gut immune responses to *Vibrio diazotrophicus* in sea urchin larvae. *Journal of Experimental Biology*, 224, jeb232272. doi:10.1242/jeb.232272

Florey, E. (1995) Highlights and sidelights of early Biology of Helgoland. *Helgoländer Meeresunters*, 49, 77-101.

Frank, M. B., Naleway, S. E., Wirth, T. S., Jung, J., Cheung, C. L., Loera, F. B., Medina, S., Sato, K. N., Taylor, J. R. A., & McKittrick, J. (2016). A Protocol for Bioinspired Design: A Ground Sampler Based on Sea Urchin Jaws. *J. Vis. Exp*, 110, e53554, doi:10.3791/53554

Fu, B., Zhou, Y., Ni, X., Tong, X., Xu, X., Dong, Z., Sun, R., Tian, Z., & Wei, H. (2017). Natural Killer Cells Promote Fetal Development through the Secretion of Growth-Promoting Factors. *Immunity*, 47, 1100-1113.

Furukawa, R., Tamaki, K., & Kaneko, H. (2016). Two macrophage migration inhibitory factors regulate starfish larval immune cell chemotaxis. *Immunology and Cell Biology*, 94, 315-321.

Game Freak. (2019a). Pokémon Shield (Switch Version) [Video game]. Nintendo The Pokémon Company.

Game Freak. (2019b). Pokémon Sword (Switch Version) [Video game]. Nintendo The Pokémon Company.

García-Arrarás, J. E., and Greenberg, M. J. (2001). Visceral regeneration in holothurians. *Microsc Res Tech*, 55(6), 438-51.

George, A. N. (2018). *Molecular, Morphological, and Functional Characterization of Pigmented Cells in the Sea Urchin Embryo* (10837623) [Doctoral dissertation, Duke University]. ProQuest Dissertations Publishing.

George, A. N., & McClay, D. R. (2019). Methods for transplantation of sea urchin blastomeres. In L. Wilson & P. Tran (Eds.), *Methods in Cell Biology* (Volume 150, 223-233). Elsevier.

Gibbens, S. (2017, March 23). *Watch a Colorful Sea Urchin Hitch a Ride on a Crab*. National Geographic. https://www.nationalgeographic.com/animals/article/sea-urchin-rides-carrier-crab

Gibson, A. W., & Burke, R. D. (1987). Migratory and Invasive Behavior of Pigment Cells in Normal and Animalized Sea Urchin Embryos. *Experimental Cell Research*, 173, 546-557.

Grall, F. T., Prall, W. C., Wei, W., Gu, X., Cho, J., Choy, B. K., Zerbini, L. F., Inan, M. S., Goldring, S. R., Gravallese, E. M., Goldring, M. B., Oettgen, P., & Libermann, T. A. (2005). The Ets transcription factor ESE-1 mediates induction of the *COX-2* gene by LPS in monocytes. *FEBS Journal*, 272, 1676-1687.

Groeben, C. (1985). Anton Dohrn—The Statesman of Darwinism. *Biol. Bull.,* 168 (suppl.), 4-25.

Haeckel, E. (1904). *The Wonder of Life*. Watts & Co.

Hampton-O'Neil, L. A., Severn, C. E., Cross, S. J., Gurung, S., Nobes, C. D., & Toye, A. M. (2020). Ephrin/Eph receptor interaction facilitates macrophage recognition of differentiating human erythroblasts. *Hematopoiesis*, 105(4), doi:10.3324/haematol.2018.215160

Hardin, J., & Armstrong, N. (1997). Short-Range Cell-Cell Signals Control Ectodermal Patterning in the Oral Region of the Sea Urchin Embryo. *Developmental Biology*, 182, 134-149.

Hibino, T., Loza-Coll, M., Messier, C., Majeske, A. J., Cohen, A. H., Terwilliger, D. P., Buckley, K. M., Brockton, V., Nair, S. V., Berney, K., Fugmann, S. D., Anderson, M. K., Pancer, Z., Cameron, R. A., Smith, L. C., & Rast, J. P. (2006). The immune gene repertoire encoded in the purple sea urchin genome. *Developmental Biology*, 300, 349-365.

Hinman, V. F., Nguyen, A. T., Cameron, R. A., & Davidson, E. H. (2003). Developmental gene regulatory network architecture across 500 million years of echinoderm evolution. *PNAS*, 100(23), 13356-13361.

Ho, E. C. H., Buckley, K. M., Schrankel, C. S., Schuh, N. W., Hibino, T., Solek, C. M., Bae, K., Wang, G., & Rast, J. P. (2016). Perturbation of gut bacteria induces a coordinated cellular immune response in the purple sea urchin larva. *Immunology and Cell Biology*, 94, 861-874.

Holland, N. D. (2020). Digestive system in regular sea urchins. In J. M. Lawrence (Ed), *Sea Urchins: Biology and Ecology* (4th ed., 147-163). Elsevier.

Hörstadius, S. (1928). Über die Determination des Keimes bei Echinodermen. *Acta zool. Stockh*. 9, 1-191.

Hörstadius, S. (1939). The mechanics of sea urchin development, studied by operative methods. *Biol. Rev*. 14, 132-79.

Hörstadius, S. (1973). *Experimental Embryology of Echinoderms*. Oxford University Press.

Howard-Ashby, M., Materna, S. C., Brown, C. T., Chen, L., Cameron, R. A., & Davidson, E. H. (2006). Gene families encoding transcription factors expressed in early development of *Strongylocentrotus purpuratus*. *Developmental Biology*, 300, 90-107.

Idriss, H. T., & Naismith, J. H. (2000). TNFα and the TNF Receptor Superfamily: Structure-Function Relationship(s). *Microscopy Research and Technique*, 50, 184-195.

iHeartRadio. (2018, March 11). *Cardi B Acceptance Speech - Best New Artist | 2018 iHeartRadio Music Awards* [Video]. YouTube. https://www.youtube.com/watch?v=7WbdxpZ6Khc

Israel, J. W., Martik, M. L., Byrne, M., Raff, E. C., Raff, R. A., McClay, D. R., & Wray, G. A. (2016). Comparative Developmental Transcriptomics Reveals Rewiring of a Highly Conserved Gene Regulatory Network during a Major Life History Switch in the Sea Urchin Genus Heliocidaris. *Comparative Study*, 14(3), e1002391. doi: 10.1371/journal.pbio.1002391

Italiani, P., & Boraschi, D. (2017). Development and Functional Differentiation of Tissue-Resident Versus Monoctye-Derived Macrophages in Inflammatory Reactions. In M. Kloc (Ed), *Macrophages: Origin, Functions and Biointervention* (23-44). Springer Nature.

Ito, K., Yoshiura, Y., Ototake, M., & Nakanishi, T. (2008). Macrophage migration inhibitory factor (MIF) is essential for development of zebrafish, *Danio rerio*. *Developmental and Comparative Immunology*, 32, 664-672.

Jacobson, C. O. (2000). Sven Otto Hörstadius. *Biog. Mems Fell. R. Soc. Lond*. 46, 243-256.

Jacques, C., Soustelle, L., Nagy, I., Diebold, C., & Giangrande, A. (2009). A novel role of the glial fate determinant *glial cells missing* in hematopoiesis. *Int. J. Dev. Biol*, 53, 1013-1022.

Jankauskas, S. S., Wong, D. W. L., Bucala, R., Djudjaj, S., & Boor, P. (2019). Evolving complexity of MIF signaling. *Cellular Signaling*, 57, 76-88.

Jonze, T. (2018, October 17). *Sea urchin is in demand. It's the ocean's foie gras: delicious, but not entirely ethical*. The Guardian. https://www.theguardian.com/food/2018/oct/17/taste-of-uni-fresh-salty-opulent-edible-sea-urchin-demand-rising-uk-restaurants

Kearl, M. (2012, January 1). *"The Potency of the First Two Cleavage Cells in Echinoderm Development. Experimental Production of Partial and Double Formations"* (1891-1892), by Hans Driesch. Embryo Project Encyclopedia. https://embryo.asu.edu/pages/potency-first-two-cleavage-cells-echinoderm-development-experimental-production-partial-and

Keesing, J. K. (2020). *Peronella*. In J. M. Lawrence (Ed), *Sea Urchins: Biology and Ecology* (4th ed., 333-336). Elsevier.

Kelly, B., & O'Neill, L. A. J. (2015). Metabolic reprogramming in macrophages and dendritic cells in innate immunity. *Cell Research*, 25, 771-784.

Khadra, Y. B., Ferrario, C., Benedetto, C. D., Said, K., Bonasoro, F., Carnevali, M. D. C., & Sugni, M. (2015). Re-growth, morphogenesis, and differentiation during starfish arm regeneration. *Wound Repair Regen*, 23(4), 623-34.

Kimmerer, R. W. (2013). *Braiding Sweetgrass: Indigenous Wisdom, Scientific Knowledge, and the Teachings of Plants*. Milkweed Editions.

Krupke, O. A., Zysk, I., Mellott, D. O., & Burke, R. D. (2016). Eph and Ephrin function in dispersal and epithelial insertion of pigmented immunocytes in sea urchin embryos. *eLIFE*, 5, e16000. DOI: 10.7554/eLife.16000

Kurzgesagt – In a Nutshell. (2021, August 10). *How The Immune System ACTUALLY Works – IMMUNE* [Video]. YouTube. https://www.youtube.com/watch?v=lXfEK8G8CUI

Lawrence, J. M. (2020). Sea urchin life-history strategies. In J. M. Lawrence (Ed), *Sea Urchins: Biology and Ecology* (4th ed., 19-28). Elsevier.

Lawrence, J. M., Lawrence, A. L., & Watts, S. A. (2020). Ingestion, digestion, and digestibility of regular sea urchins. In J. M. Lawrence (Ed), *Sea Urchins: Biology and Ecology* (4th ed., 165-190). Elsevier.

Liberati, N. T., Fitzgerald, K. A., Kim, D. H., Feinbaum, R., Golenbock, D. T., & Ausubel, F. M. (2004). Requirement for a conserved Toll/interleukin-1 resistance domain protein in the *Caenorhabditis elegans* immune response. *PNAS*, 101(17), 6593-6598.

Liboiron, M. (2021). *Pollution Is Colonialism*. Duke University Press.

Lin, Y., Wang, Y., Peng, Y., Liu, F., Lin, G., Huang, S., Sytwu, H., & Cheng, C. (2020). Cells, 938(9),doi:10.3390/cells9040938

Logan, C. Y., Miller, J. R., Ferkowicz, M. J., & McClay, D. R. (1999). Nuclear beta-catenin is required to specify vegetal cell fates in the sea urchin embryo. *Development*, 126(2), 345-57.

Lu, S., Li, M., & Lin, Y. (2014). Mitf regulates osteoclastogensis by modulating NFATc1 activity. *Experimental Cell Research*, 328, 32-43.

Lyons, D. C., Martik, M. L., Saunders, L. R., & McClay, D. R. (2014). Specification to Biomineralization: Following a Single Cell Type as It Constructs a Skeleton. *Integrative and Comparative Biology*, 54(4), 723-733.

Maayan, I. (2011, March 3). *Theodor Heinrich Boveri (1862-1915)*. Embryo Project Encyclopedia. https://embryo.asu.edu/handle/10776/1690

Mah, C. (2008, April 21). Holding on in a Rough World: *Colobocentrotus* atratus-the Shingle Urchin!! *The Echinoblog*. http://echinoblog.blogspot.com/2008/04/holding-on-in-rough-world.html

Martik, M. L., & McClay, D. R. (2015). Deployment of a retinal determination gene network drives directed cell migration in the sea urchin embryo. *eLife* 2015;4:e08827 DOI: 10.7554/eLife.08827

Martik, M. L., & McClay, D. R. (2017). New insights from a high-resolution look at gastrulation in the sea urchin, Lytechinus variegatus. *Mech Dev*, 148, 3-10.

Massri, A. J., Schiebinger, G. R., Berrio, A., Wang, L., Wray, G. A., & McClay, D. R. (2021). Methodologies for Following EMT *In Vivo* at Single Cell Resolution. *Methods Mol Biol*, 2179, 303-314.

Matsumoto, N., Sekiya, M., Tohyama, K., Ishiyama-Matsuura, E., Sun-Wada, G., Wada, Y., Futai, M., & Nakanishi-Matsui, M. (2018). Essential Role of the α3 Isoform of V-ATPase in Secretory Lysosome Trafficking via Rab7 Recruitment. *Scientific Reports*, 8:6701, DOI:10.1038/s41598-018-24918-7

McCauley, B. S., Weideman, E. P., & Hinman, V. F. (2010). A conserved gene regulatory network subcircuit drives different developmental fates in the vegetal pole of highly divergent echinoderm embryos. *Developmental Biology*, 340, 200-208.

McCauley, B. S., Wright, E. P., Exner, C., Kitazawa, C., & Hinman, V. F. (2012). Development of an embryonic skeletogenic mesenchyme lineage in a sea cucumber reveals the trajectory of change for the evolution of novel structures in echinoderms. *EvoDevo*, 3(17), http://www.evodevojournal.com/content/3/1/17

McClay, D. R. (2011). Evolutionary crossroads in developmental biology: sea urchins. *Development*, 138, doi:10.1242/dev.048967

McClay, D. R, & Logan, C. Y. (1996). Regulative capacity of the archenteron during gastrulation in the sea urchin. *Development*, 122, 607-616.

McIntyre, D., Lyons, D. C., Martik, M., & McClay, D. R. (2014). Branching Out; Origins of the Sea Urchin Larval Skeleton in Development and Evolution. *Genesis*, 52, 173-185.

McNamara, K. J. (2007). Shepherds' crowns, fairy loaves and thunderstones: the mythology of fossil echinoids in England. *Geological Society, London, Special Publications*, 273, 279-294.

Meijering, E., Dzyubachyk, O., & Smal, I. (2012). *Chapter nine - Methods for Cell and Particle Tracking*. In P. M. Conn (Ed), *Methods in Enzymology* (183-200). Elsevier.

Metaxas, A. (2020). Larval ecology of echinoids. In J. M. Lawrence (Ed), *Sea Urchins: Biology and Ecology* (4th ed., 77-87). Elsevier.

Miller, D. J., Hemmrich, G., Ball, E. E., Hayward, D. C., Khalturin, K., Funayama, N., Agata, K., & Bosch, T. C. G. (2007). The innate immune repertoire in cnidaria--ancestral complexity and stochastic gene loss. *Genome Biol*, 8(4), R59. doi: 10.1186/gb-2007-8-4-r59.

Miskelly, A. (2009). *Sea Urchins of the World: Diversity, Symmetry & Design*. Ashley Miskelly.

Morrill, J. B., & Marcus, L. (2005). An Atlas of the Development of the Sea Urchin *Lytechinus variegatus*. Echinobase.org. http://ftp.echinobase.org/pub/WikiDownloads/Atlas_of_Lytechinus_variegatus_development.pdf

Müller, J. (1846). Bericht über einige neue Thierformen der Nordsee. *Archiv. Anat. Physiol*. 101-110.

Muller, P. A., Koscsó, B., Rajani, G. M., Stevanovic, K., Berres, M., Hashimoto, D., Mortha, A., Leboeuf, M., Li, X., Mucida, D., Stanley, E. R., Dahan, S., Margolis, K. G., Gershon, M. D., Merad, M., & Bogunovic, M. (2014). Crosstalk between Muscularis Macrophages and Enteric Neurons Regulates Gastrointestinal Motility. *Cell*, 158, 300-313.

Narvaez, C. A., Padovani, A. M., Stark, A. Y., & Russell, M. P. (2020). Plasticity in the purple sea urchin (Strongylocentrotus purpuratus): Tube feet regeneration and adhesive performance. *Journal of Experimental Marine Biology and Ecology*, 528, 151381.

Native Land Digital. (2021). *Native Land* [Online-resource]. https://native-land.ca/

Nesbit, K. T., Fleming, T., Batzel, G., Pouv, A., Rosenblatt, H. D., Pace, D. A., Hamdoun, A., & Lyons, D. C. (2019). The painted sea urchin, *Lytechinus pictus*, as a genetically-enabled developmental model. *Methods Cell Biol*, 150, 105-123.

Nickelodeon UK. (2016, April 29). *SpongeBob SquarePants | Eek an Urchin! | Nickelodeon UK* [Video]. YouTube. https://www.youtube.com/watch?v=eUctdVLABgM&t=36s

Nintendo EPD. (2020). Animal Crossing: New Horizons (Switch Version) [Video game]. Nintendo.

Octonauts. (2020, January 21). *Octonauts - A Spikey Friendship | Full Episodes | Cartoons for Kids* [Video]. YouTube. https://www.youtube.com/watch?v=hT_aNzQcadY&t=974s

Octonauts. (2021, April 16). *Octonauts - The Urchin Invasion | Cartoons for Kids | Underwater Sea Education* [Video]. YouTube. https://www.youtube.com/watch?v=9g0Nj8RIpfg&t=83s

Oddo, M., Calandra, T., Bucala, R., & Meylan, P. R. A. (2005). Macrophage Migration Inhibitory Factor Reduces the Growth of Virulent Mycobacterium tuberculosis in Human Macrophages. *Infection and Immunity*, 73(6), 3783-3786.

Omi, M., Kaartinen, V., & Mishina, Y. (2019). Activin A receptor type 1-mediated BMP signaling regulates RANKL-induced osteoclastogenesis via canonical SMAD-signaling pathway. *J. Biol. Chem*., 294(47), 17818-17836.

Perillo, M., Oulhen, N., Foster, S., Spurrell, M., Calestani, C., & Wessel, G. (2020). Regulation of dynamic pigment cell states at single-cell resolution. *eLife*, 9:e60388. DOI: https://doi.org/10.7554/eLife.60388

Pierce, M, & Stanley, P. (2017). Deuterostomes. In A. Varki, R. D. Cummings, J. D. Esko., P. Stanley, G. W. Hart, M. Aebi, A. G. Darvill, T. Kinoshita, N. H. Packer, J. H. Prestegard, R. L. Schnaar, & P. H. Seeberger (Eds), *Essentials of Glycobiology* [Internet] (3rd ed., epp. Chapter 27). Cold Spring Harbor Laboratory Press.

Prescod-Weinstein, C. (2015, April 25). *Decolonising Science Reading List*. Medium. https://medium.com/@chanda/decolonising-science-reading-list-339fb773d51f

Rad, A. (2006, August 11). *File:Hematopoiesis (human) diagram.png* [Online-resource]. Wikipedia Commons. https://commons.wikimedia.org/wiki/File:Hematopoiesis_(human)_diagram.png

Raff, R. A. (1987). Constraint, flexibility, and phylogenetic history in the evolution of direct development in sea urchins. *Developmental Biology*, 119(1), 6-19.

Rafiq, K., Shashikant, T., McManus, C. J., & Ettensohn, C. A. (2014). Genome-wide analysis of the skeletogenic gene regulatory network of sea urchins. *Development*, 141, 950-961.

Rajasekaran, D., Zierow, S., Syed, M., Bucala, R., Bhandari, V., & Lolis, E. J. (2014). Targeting distinct tautomerase sites of D-DT and MIF with a single molecule for inhibition of neutrophil lung recruitment. *The FASEB Journal*, 28, 4961-4971.

Ramsay, G. (2020, June 7). *Gordon Ramsay Makes Sea Urchin Scrambled Eggs in Australia | Scrambled* [Video]. YouTube. https://www.youtube.com/watch?v=IqkQx7dF-BE

Rasighaemi, P., Onnebo, S. M. N., Liongue, C., & Ward, A. C. (2015). ETV6 (TEL1) regulates embryonic hematopoiesis in zebrafish. *Hematopoiesis*, 100(1), doi:10.3324/haematol.2014.104091

Rast, J. P., Cameron, R. A., Poustka, A. J., & Davidson, E. H. (2002). *brachyury* Target Genes in the Early Sea Urchin Embryo Isolated by Differential Macroarray Screening. *Developmental Biology*, 246, 191-208.

Reinardy, H. C., Emerson, C. E., Manley, J. M., & Bodnar, A. G. (2015). Tissue regeneration and biomineralization in sea urchins: role of Notch signaling and presence of stem cell markers. *PLoS One*, 10(8): e0133860. https://doi.org/10.1371/journal.pone.0133860

Ries, A. (2011). Sea Urchin Anatomy: Generalised Anatomy based on *Arbacia* [Digital Illustration]. Alexries.com. https://www.alexries.com/non-fiction/i7lv5f79lzdoaixxohh9ynh55zw0m3

Rizzo, F., Fernandez-Serra, M., Sqarzoni, P., Archimandritis, A., & Arnone, M. I. (2006). Identification and developmental expression of the *ets* gene family in the sea urchin (*Strongylocentrotus purpuratus*). *Developmental Biology*, 300, 35-48.

Robinson, G. (2021, August 5). *Ernst Haeckel*. Encyclopedia Britannica. https://www.britannica.com/biography/Ernst-Haeckel

Rodrigues, N. P., Boyd, A. S., Fugazza, C., May, G. E., Guo, Y., Tipping, A. J., Scadden, D. T., Vyas, P., & Enver, T. (2008) GATA-2 regulates granulocyte-macrophage progenitor cell function. *Hematopoiesis and Stem Cells: Blood*, 112(13), 4862-4873.

Rogers-Bennett, L., & Okamoto, D. (2020). *Mesocentrotus franciscanus* and *Strongylocentrotus purpuratus*. In J. M. Lawrence (Ed), *Sea Urchins: Biology and Ecology* (4th ed., 593-604). Elsevier.

Russell, M. P., Gibbs, V. K., and Duwan, E. (2018). Bioerosion by pit-forming, temperate-reef sea urchins: History, rates and broader implications. *PLoS ONE*, 13(2), e0191278. https://doi.org/ 10.1371/journal.pone.0191278

Russo, R., Pinsino, A., Costa, C., Bonaventura, R., Matranga, V., & Zito, R. (2014). The newly characterized *Pl*-jun is specifically expressed in skeletogenic cells of the *Paracentrotus lividus* sea urchin embryo. *FEBS Journal*, 281, 3828-3843.

Saini, A. (2020). Want to do better science? Admit you're not objective. *Nature*, 579, 175. https://doi.org/10.1038/d41586-020-00669-2

Salie, R., Kneissel, M., Vukevic, M., Zamurovic, N., Kramer, I., Evans, G., Gerwin, N., Mueller, M., Kinzel, B., & Susa, M. (2010). Ubiquitous overexpression of Hey1 transcription factor leads to osteopenia and chondrocyte hypertrophy in bone. *Bone*, 46, 680-694.

Sánchez Alvarado, A. (2016, August). *To solve old problems, study new species* [Video]. TED Conferences. https://www.ted.com/talks/alejandro_sanchez_alvarado_to_solve_old_problems_study_new_species?language=en

Saunders, L. R., & McClay, D. R. (2014). Sub-circuits of a gene regulatory network control a developmental epithelial-mesenchymal transition. *Development*, 141, 1503-1513.

Sharma, T., & Ettensohn, C. A. (2011). Regulative deployment of the skeletogenic gene regulatory network during sea urchin development. *Development*, 138(12), 2581-90.

Shashikant, T., Khor, J. M., & Ettensohn, C. A. (2018). Global analysis of primary mesenchyme cell cis-regulatory modules by chromatin accessibility profiling. *BMC Genomics*, 19(206), https://doi.org/10.1186/s12864-018-4542-z

Schatzber, D., Lawton, M., Hadyniak, S. E., Ross, E. J., Carney, T., Beane, W. S., Levin, M., & Bradham, C. A. (2015). H+/K+ ATPase activity is required for biomineralization in sea urchin embryos. *Developmental Biology*, 406, 259-270.

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saafeld, S., Schmid, B., Tinevez, J., White, D. J., Hartenstein, V., Eliceri, K., Tomancak, P., & Cardona, A. (2012). Fiji: an open-source platform for biological-image analysis. *Nature Methods*, 9, 676-682.

Sharma, T., & Ettensohn, C. A. (2011). Regulative deployment of the skeletogenic gene regulatory network during sea urchin development. *Development*, 138(12), 2581-90.

Sherwood, D. R., & McClay, D. R. (1999). LvNotch signaling mediates secondary mesenchyme specification in the sea urchin embryo. *Development*, 126(8), 1703-13.

Silva, J. R. M. C. (2020). Immunology in sea urchins. In J. M. Lawrence (Ed), *Sea Urchins: Biology and Ecology* (4th ed., 227-236). Elsevier.

Simha, A., Allen, R., & Carley, L. N. (2021). Introduction to Inclusion, Diversity, Equity, and Anti-racism in Biology Syllabus. *figshare*. https://doi.org/10.6084/m9.figshare.13611287

Smith, L. T. (2012). *Decolonizing Methodologies: Research and Indigenous Peoples*. Zed Books.

Slota, L. A., & McClay, D. R. (2018). Identification of neural transcription factors required for the differentiation of three neuronal subtypes in the sea urchin embryo. *Developmental Biology*, 435, 138-149.

Slota, L. A., Miranda, E. M., & McClay, D. R. (2019). Spatial and temporal patterns of gene expression during neurogenesis in the sea urchin *Lytechinus variegatus*. *EvoDevo*, 10:2 https://doi.org/10.1186/s13227-019-0115-8

Slota, L. A., Miranda, E., Peskin, B., & McClay, D. R. (2020). Developmental origin of peripheral ciliary band neurons in the sea urchin embryo. *Developmental Biology*, 459(2), 72-78.

Solek, C. M., Oliveri, P., Loza-Coll, M., Schrankel, C. S., Ho, E. C. H., Wang, G., & Rast, J. P. (2013). An ancient role for Gata-1/2/3 and Scl transcription factor homologs in the development of immunocytes. *Developmental Biology*, 382, 280-292.

Sommer, L. (2021, March 31). *In a Hotter Climate, 'Zombie' Urchins Are Winning And Kelp Forests Are Losing*. NPR. https://www.npr.org/2021/03/31/975800880/in-hotter-climate-zombie-urchins-are-winning-and-kelp-forests-are-losing

Sommer, L., Sofia, M., & Ramirez, R. (2021, March 26). *The Purple Urchins Don't Die*. NPR. https://www.npr.org/2021/03/25/981151345/the-purple-urchins-dont-die

Soustelle, L., & Giangrande, A. (2007). Glial differentiation and the Gcm pathway. *Neuron Glia Biology*, 3, 5-16.

Sparkes, A., De Baetselier, P., Roelants, K., De Trez, C., Magez, S., Van Grinderachtr, J. A. V., Raes, G., Bucala, R., & Stijlemans, B. (2017). Reprint of: The non-mammalian MIF superfamily. Immunobiology, 222, 858, 867.

SpongeBob SquarePants Official. (2020, September 4). *SpongeBob Needs Water!* 💧 *"I Don't Need It...I Need It" Full Scene* [Video]. YouTube. https://www.youtube.com/watch?v=m5uayeTCYJQ

Steneck, R. S. (2020). Regular sea urchins as drivers of shallow benthic marine community structure. In J. M. Lawrence (Ed), *Sea Urchins: Biology and Ecology* (4th ed., 255-273). Elsevier.

Steudel, J. (2021, July 10). *Johannes Müller*. Encyclopedia Britannica. https://www.britannica.com/biography/Johannes-Muller

Stevenson, A., & Kroh, A. (2020). Deep-sea sea urchins. In J. M. Lawrence (Ed), *Sea Urchins: Biology and Ecology* (4th ed., 237-249). Elsevier.

Sun, J., & Chiang, F. (2015). Use and Exploitation of Sea Urchins. In N. Brown, & S. Eddy (Eds), *Echinoderm Aquaculture* (1st ed., 25-46). John Wiley & Sons.

Sun, H., Peng, C. J., Wang, L., Feng, H., & Wikramanayake, A. H. (2021). An early global role for Axin is required for correct patterning of the anterior-posterior axis in the sea urchin embryo. *Development*, 148, dev191197. doi:10.1242/dev.191197

Sun, H. W., & Lolis, E. (1996). Image from the RCSB PDB (rcsb.org) or PDB ID 1MIF. DOI: 10.2210/pdb1MIF/pdb

Sun, H. W., Bernhagen, J., Bucala, R., & Lolis, E. (1996). Crystal structure at 2.6-A resolution of human macrophage migration inhibitory factor. *PNAS*, 93, 5191-5196.

Suzuki, H., & Yaguchi, S. (2018). Transforming growth factor-β signal regulates gut bending in the sea urchin embryo. *Development, Growth, & Differentiation*, doiI: 10.1111/dgd.12434

Sweet, H. C., Gehring, M., & Ettensohn, C. A. (2002). LvDelta is a mesoderm-inducing signal in the sea urchin embryo and can endow blastomeres with organizer-like properties. *Development*, 129, 1945-1955.

Sweet, M. (2020). Sea urchin diseases: Effects from individuals to ecosystems. In J. M. Lawrence (Ed), *Sea Urchins: Biology and Ecology* (4th ed., 219-225). Elsevier.

TallBear, K. (2013). *Native American DNA: Tribal Belonging and the False Promise of Genetic Science*. University of Minnesota Press.

Tamboline, C. R., & Burke, R. D. (1992). Secondary Mesenchyme of the Sea Urchin Embryo: Ontogeny of Blastocoelar Cells. *The Journal of Experimental Zoology*, 262, 51-60.

Tauber, A. I. (2003). Metchnikoff and the phagocytosis theory. *Nature Reviews: Molecular Cell Biology*, 4, 897-901.

TED. (2018, October 12). How I became part sea urchin | Catherine Mohr [Video]. YouTube. https://www.youtube.com/watch?v=Uq3MCf_6HtI

TED-Ed. (2012, May 14). *How Life Begins in the Deep Ocean* [Video]. YouTube. https://www.youtube.com/watch?v=i_R7ouD8-Eo

The American Heritage dictionary of the English language. (2020). Boston :Houghton Mifflin Harcourt Publishing Company.

The Nature Conservancy. (n.d.). *Urchins*. https://reefresilience.org/stressors/predator-outbreaks/urchins/

The Pokémon Company. (n.d.). *Pincurchin | Pokédex*. https://www.pokemon.com/us/pokedex/pincurchin

Treuer, D. (2019). *The Heartbeat of Wounded Knee: Native America from 1890 to the Present*. Riverhead Books.

Trinh, L. A., McCutchen, M. D., Bonner-Fraser, M., Fraser, S. E., Bumm, L. A., & McCauley, D. W. (2007). Fluorescent in situ hybridization employing the conventional NBT/BCIP chromogenic stain. *BioTechniques*, 42(6), 756-759.

Tuck, E., & Yang, K. W. (2012). Decolonization is not a metaphor. *Decolonization: Indigeneity, Education & Society*. 1(1), 1-40.

Urchinomics. (n.d.). *Home*. https://www.urchinomics.com/

Vagapova, E. R., Spirin, P. V., Lebedev, T. D., & Prassolov, V. S. (2018). The Role of TAL1 in Hematopoiesis and Leukemogenesis. *Acta Naturae*, 10(1), 15-23.

Vanamee, É. S., & Faustman, D. L. (2018). Structural principles of tumor necrosis factor superfamily signaling. Sci Signal. 511(11), eaao4910 DOI: 10.1126/scisignal.aao4910

von Dassow, G., Valley, J., & Robbins, K. (2019). *Microinjection of oocytes and embryos with synthetic mRNA encoding molecular probes*. In L. Wilson & P. Tran (Eds.), *Methods in Cell Biology* (Volume 150, 189-221). Elsevier.

Waikiki Aquarium. (n.d.). *Shingle Urchin*. Waikikiaquarium.org. https://www.waikikiaquarium.org/experience/animal-guide/invertebrates/echinoderms/shingle-urchin/

Walker, C. W., Lesser, M. P., & Unuma T. (2020). Gametogenesis in regular sea urchins: Structural, functional, and molecular/genomic biology. In J. M. Lawrence (Ed), *Sea Urchins: Biology and Ecology* (4th ed., 29-50). Elsevier.

Watts, S. A., McClintock, J. B., & Lawrence, J. M. (2020). *Lytechinus*. In J. M. Lawrence (Ed), *Sea Urchins: Biology and Ecology* (4th ed., 661-680). Elsevier.

Wessel, G. M., & McClay, D. R. (1986). Two embryonic, tissue-specific molecules identified by a double-label immunofluorescence technique for monoclonal antibodies. *Journal of Histochemistry and Cytochemistry*, 34(6), 703-706.

Wessel, G. M., Kiyomoto, M., Shen, T., & Yajima, M. (2020). Genetic manipulation of the pigment pathway in a sea urchin reveals distinct lineage commitment prior to metamorphosis in the bilateral to radial body plan transition. *Scientific Reports*, 10, 1973, https://doi.org/10.1038/s41598-020-58584-5

Whalen, A. (2020, April 27). *Why Are These Sea Urchins Sporting Cowboy and Viking Hats? There's Science to Their Hot Looks*. Newsweek. https://www.newsweek.com/sea-urchin-hats-saltwater-aquarium-cowboy-viking-top-hat-3d-printing-1500500

Wheeler, K. C., Jena, M, K., Pradhan, B. S., Nayak, N., Das, S., Hsu, C., Wheeler, D. S., Chen, K., & Nayak, N. R. (2018). VEGF may contribute to macrophage recruitment and M2 polarization in the decidua. *PLoS ONE*, 13(1), e0191040. https://doi.org/10.1371/journal. Pone.0191040

Wikramanayake, A. H., Huang, L., & Klein, W. H. (1998). beta-Catenin is essential for patterning the maternally specified animal-vegetal axis in the sea urchin embryo. *Proc Natl Acad Sci U S A*, 95(16), 9343-8.

Wilbur, M., & Keene, A. (Hosts). (2019, March 19). Decolonizing Sex [Audio Podcast Episode]. In *All My Relations Podcast*. All My Relations. https://www.allmyrelationspodcast.com/podcast/episode/468a0a6b/decolonizing-sex

Worley, A. (2001). *Strongylocentrotus purpuratus* [On-line]. Animal Diversity Web. https://animaldiversity.org/accounts/Strongylocentrotus_purpuratus/

Wu, S., Yang, Y., & McClay, D. R. (2008). Twist is an essential regulator of the skeletogenic gene regulatory network in the sea urchin embryo. *Developmental Biology*, 319, 406-415.

Yaguchi, S. (2019). Temnopleurus as an emerging echinoderm model. *Methods Cell Biol*, 150, 71-79.

Yong, E. (2019, January 17). *The Overlooked Organisms That Keep Challenging Our Assumptions About Life*. The Atlantic. https://www.theatlantic.com/science/archive/2019/01/how-lichens-explain-and-re-explain-world/580681/

Zhang, W., Liu, H., Liu, W., & Xu, J. (2015). Polycomb-mediated loss of microRNA let-7c determines inflammatory macrophage polarization via PAK1-dependent NF-κB pathway. *Cell Death and Differentiation*, 22, 287-297.

Zuch, D. T., & Bradham, C. A. (2019). Spatially mapping gene expression in sea urchin primary mesenchyme cells. *Methods Cell Biol*, 151, 433-442.

Biography

Raymond Lance Allen (Niigaanigaabaw) received his Artium Baccalaureus from Ripon College in 2015 where he majored in Chemistry-Biology, minored in Latin and graduated *cum laude*. He has taken courses through Nicolet Area Technical College, University of St Andrews, and the University of Chicago's Marine Biological Laboratory. In graduate school, Allen was first author on a Zebrafish journal article titled, "A rotifer based technique to rear zebrafish larvae in small academic settings." He was a co-author on the following articles: "Live fate-mapping of joint-associated fibroblasts visualizes expansion of cell contributions during zebrafish fin regeneration" (Development); and "Introduction to Inclusion, Diversity, Equity, and Anti-racism in Biology Syllabus" (figshare). He was awarded a National Science Foundation Graduate Research Fellowship (2017), he was a recipient of the 2019 Dean's Award for Excellence in Mentoring (Duke University), and he was a 2020-21 Race and the Professions Fellow (Duke University).