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PERSPECTIVE

SPECIAL ISSUE
UNCOVERING DEVELOPMENTAL DIVERSITY

Extraordinary model systems for regeneration

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ABSTRACT

Regeneration is the remarkable phenomenon through which an organism can regrow lost or damaged parts with fully functional replacements, including complex anatomical structures, such as limbs. In 2019, Development launched its 'Model systems for regeneration' collection, a series of articles introducing some of the most popular model organisms for studying regeneration *in vivo.* To expand this topic further, this Perspective conveys the voices of five expert biologists from the field of regenerative biology, each of whom showcases some less well-known, but equally extraordinary, species for studying regeneration.

Apple snails: looking at regeneration with a new pair of eyes

Alice Accorsi

When I mention 'snails' people usually think about garden snails eating backyard lettuce, SpongeBob's friend, Gary, or skincare products made with snail mucus. Snails, with their characteristic spiral shells, are mollusks that come in a variety of forms and occupy diverse habitats. My favorite is *Pomacea canaliculata*, also known as golden apple snail, based on the color and the size of their shells (Fig. 1).

My journey alongside *P. canaliculata* started as a PhD student at the University of Modena and Reggio Emilia (Italy), where I studied the cellular composition of their immune system (Accorsi et al., 2013). When looking for a postdoc position, an exciting conversation with Alejandro Sánchez Alvarado piqued my interest in morphogenesis during both development and regeneration. Intending to investigate the regeneration of complex sensory organs, I joined his research group at the Stowers Institute for Medical Research, MO, USA. Inspired by Lazzaro Spallanzani's observation in the 18th century of snails regenerating their entire head (Carozzi, 1985), I discovered that apple snails can regrow several organs after injury, such as tentacles, portions of the nervous system, foot and shell. One of the most astonishing processes I observed was the full regeneration of their camera-type eyes, which are akin to eyes in humans and other vertebrates (Accorsi et al., 2024 preprint). Rebuilding both the structural components (cornea and lens) and the sensory component (retina) of the eye took only about a month, making them an extremely appealing research organism in which to investigate sensory organ regeneration.

Who would have guessed that an invasive freshwater snail, which many countries are trying to eradicate owing to the damage it causes to crops (de Brito and Joshi, 2016), might hold promising insights into regenerative biology and potential for human health? *P. canaliculata* are resilient to environmental fluctuations and produce many eggs all year round with a relatively short generation time (3-4 months). All

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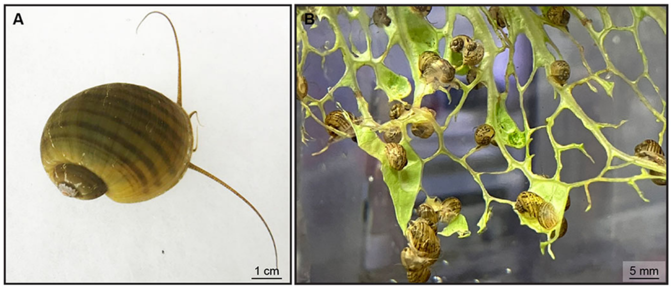

Fig. 1. The golden apple snail, *Pomacea canaliculata*. (A) Adult *P. canaliculata* with extended cephalic and oral tentacles. (B) Several *P. canaliculata* juveniles voraciously eating a lettuce leaf.

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Apart from being an invasive species, they are also the intermediate host for the rat lungworm *Angiostrongylus cantonensis*, a nematode that causes human eosinophilic meningitis (Lv et al., 2009). Thus, most studies on *P. canaliculata* have focused on ecological and parasitological aspects of this animal. However, these studies are hampered by the limited molecular and genetic tools. During my postdoc at the Stowers Institute for Medical Research, among other things, I developed protocols to micro-inject the embryos and develop stable mutant lines using the CRISPR/Cas9 technology (Accorsi et al., 2024 preprint). This new set of tools for genome manipulation, paired with optimized histological techniques and the sequenced genome (Liu et al., 2018), has opened the door to study gene function in golden apple snails. These tools not only bolster regeneration research but could potentially drive studies into myriad aspects of snail biology.

Now at UC Davis, my team and I are taking full advantage of this foundational work to shed light on the regenerative potential of apple snails and the process of complex camera-type eye development and regeneration.

Leopard geckos as a long-lived model for aging and regeneration research

Longhua Guo

Regeneration of lost tissue occurs across phyla, but not in all species (Sanchez Alvarado and Tsonis, 2006), raising many questions regarding the mechanisms and evolution of regenerative processes. For example, why are some species better at regenerating complex structures? Can we improve human wound healing and tissue regeneration by learning about the fundamental rules of regeneration? In addition to traditional laboratory model organisms, comparative analysis of species across different phyla can help address these mysteries and reveal the evolutionary biology of regeneration.

Leopard geckos (*Eublepharis macularius*) are a promising reptile model for tissue regeneration research (Guo and Kruglyak, 2023) (Fig. 2). This is, first, because leopard geckos are charismatic animals with a gentle personality and are known to make good pets. In addition, they are slightly larger than an adult mouse, and crickets can serve as their regular diet, making them easy to maintain in captivity. At 32°C, eggs hatch in a month and hatchlings reach sexual maturity in 8-12 months. Second, a ~2.2 Gb leopard gecko genome has been assembled into chromosomes, allowing for comprehensive genetics and genomics research (Pinto et al., 2023), and inbred lines and outcrossing morphs have been established by the community of gecko breeders in recent decades (Guo et al., 2021). Third, and most importantly, leopard geckos can regenerate several tissues, such as the skin, without leaving scars (Peacock et al., 2015); an entire tail (Delorme et al., 2012), including the spinal cord (Gilbert and Vickaryous, 2018); and neurons in the brain (Austin et al., 2023) in 1-2 months. It won't be surprising if future research uncovers regenerative capacity in more tissues (e.g. heart).

Leopard geckos are charismatic animals with a gentle personality

In addition to their regenerative abilities, leopard geckos are relatively long-lived vertebrates with a maximal reported lifespan of 28 years. We and collaborators house a large colony of leopard geckos of various ages, making it possible to carry out comparative analysis of aging and regeneration with other species, such as mammals and planarians. For example, age-associated increase in fibrosis has been associated with impaired regeneration in mammals, such as house mice (Brack et al., 2007), but whether older geckos can regenerate their skin without scarring, like young adults, is an open question. Whatever the answer may be, it will provide meaningful information to promote scarless cutaneous wound healing. Although aging research in long-lived animals is challenging, learning the basic biology of aging in these species is necessary because it may provide unique insights into aging that are not readily evident in short-lived research models. For example, planarians are considered extremely long-lived (Sahu et al., 2017; Valenzano et al., 2017). Remarkably, however, inducing regeneration in aged planarians globally reverses the age-associated phenotypes (Dai et al., 2023). The findings in planarians are exciting because they not only reveal a naturally evolved solution to reverse aging, but also establish planarians as a long-lived laboratory model for aging research. It will be interesting to explore whether regeneration affects aging in other species, such as the vertebrate model of leopard geckos. Looking forward, we are excited to explore the potential of both invertebrate and vertebrate models to study aging, regeneration and rejuvenation.

Fig. 2. A leopard gecko (*Eublepharis macularius*).

Stentor coeruleus – the cell that lived

Wallace F. Marshall

Regeneration is a hallmark of life and the cell is the level at which life emerges from nonliving chemical matter. Therefore, understanding how regeneration occurs within cells is of fundamental importance to understanding the nature of life itself. However, most cells are extremely fragile and if you try to remove part of the cell, the whole thing bleeds out cytoplasm and dies, making regeneration hard to study.

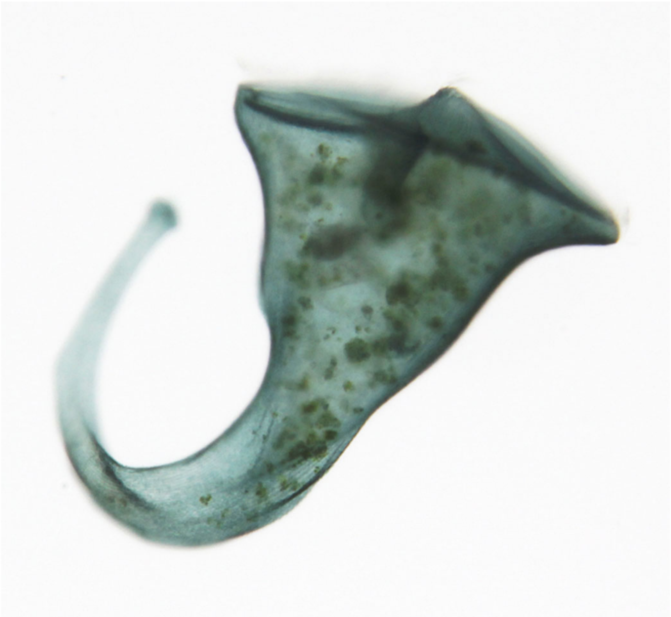

Fig. 3. *Stentor coeruleus.* Image of a single *Stentor coeruleus* cell using brightfield microscopy. (Image by Mark Slabodnick.)

To learn how cells regenerate, we have turned to *Stentor coeruleus*, a unicellular organism that lives in ponds, because of its amazing ability to recover from wounds that would kill most other cells (Marshall, 2021) (Fig. 3). *Stentor* is a ciliate, similar to *Paramecium* or *Tetrahymena*. But, unlike those more well-known ciliates, a single *Stentor* cell can be up to 2 mm in size. *Stentor* can be cut in half and both halves regenerate. If smaller pieces are removed, they too regenerate, which allows a wide range of surgical perturbations to investigate how cells respond to the loss of particular structures, such as the ring of cilia located at one end of the cell. In addition to its ability to survive wounding, *Stentor* has a visible and stereotyped cell pattern with the surface of the cell covered with parallel blue stripes, which provide a reference to determine whether it regenerated the correct structure.

Why is *Stentor* so good at surviving being cut into pieces? One possible reason may be its large size – it can tolerate the loss of more cytoplasm and metabolites before the wound is healed, simply because it has so much more to start with compared to other cells. *Stentor* is also highly polyploid, containing a large nucleus with around 50,000 copies of every gene (Slabodnick et al., 2017) that runs the length of the cell. Therefore, however you cut the cell, the pieces contain enough genome copies to survive and regenerate.

> **Why is *Stentor* so good at surviving being cut into pieces? One possible reason may be its large size ...**

We are not the first to study *Stentor* regeneration; this was an important area of investigation about 100 years ago. But, although early pioneers provided a wealth of descriptions of *Stentor* recovering from rearrangements and grafts (Tartar, 1961), we still do not understand the molecular and biophysical mechanisms that allow a S*tentor* cell to 'know' when a particular structure is missing, and then re-assemble it. These are the questions that we are trying to address in my lab. I am perhaps most interested in understanding how *Stentor* cells detect rearrangements of their components, which induces regeneration of replacements. How do cells detect changes in geometric relations on the size scale of hundreds of microns?

With few exceptions, the regeneration of missing structures in *Stentor* requires gene expression, meaning we can use RNA sequencing to analyze the transcriptome that accompanies regeneration (Sood et al., 2022). RNA interference works well in *Stentor* (Slabodnick et al., 2014), allowing us to test candidates obtained from transcriptomic analysis. In this way, we are now piecing together some of the pathways involved. Remarkably, some highly conserved genes, such as E2F and Pumilio, which play roles in multicellular animals, also play important roles in *Stentor* regeneration (Sood et al., 2022). With an initial set of pathways in hand, the idea now is to discover how they are activated.

Intra-species comparisons in *Astyanax mexicanus* bring a novel angle to regeneration research

Mathilda T. M. Mommersteeg

The Mexican cavefish, *Astyanax mexicanus*, is a single small fish species comprising cave-dwelling and surface populations from the Sierra de El Abra region of Mexico (Gross et al., 2015; Hubbs and Innes, 1936) (Fig. 4). During the rainy season, surface fish flood from rivers into the many subterranean caves in the area and, as some river levels have retreated over time, a number of these caves have become isolated from the rivers. Trapped surface fish managed to survive in the dark caves by evolving key traits (Gross, 2012; Jeffery, 2019). The fish lost their eyes and pigment, redundant in the absence of light (Krishnan and Rohner, 2017; O'Gorman et al., 2021). Instead, they developed other features necessary for surviving in the cave environment, such as greater resistance to starvation, highly sensitive taste buds and vibration-sensitive lateral line systems specialized in finding food in the dark (Berning and Gross, 2023; Jeffery, 2009; Yoshizawa et al., 2014). Despite these evolutionary adaptations, the surface fish and cavefish are still one species and this makes them a powerful comparative model for regeneration research (Potts et al., 2021). The first regenerative experiments on *Astyanax* were performed by Priscilla Rasquin in 1949, who showed that the surface fish can regenerate their optic nerve (Rasquin, 1949). Since then, we have shown that surface fish can regenerate their heart completely after injury, whereas cavefish cannot and form a permanent fibrotic scar, similar to the human injury response after a heart attack (Stockdale et al., 2018). A similar

Fig. 4. A silver-eyed *Astyanax mexicanus* river surface fish (left) together with an albino, eye-less cavefish (right) from the Pachón cave. Both fish were raised in the laboratory setting. (Image by Colin Beesley, Department of Physiology, Anatomy & Genetics, University of Oxford, UK).

differential response seems present between surface and cavefish skeletal muscle based on gene expression differences in the first days after injury (Olsen et al., 2023). In contrast, fin regeneration occurs in both fish (Stockdale et al., 2018).

Astyanax mexicanus provide the distinctive advantage of being able to directly compare a natural regenerative and scarring response within one species, without having to correct for inter-species differences. Comparing injury-responsive transcriptomes of, for example, regenerating zebrafish to a non-regenerative mouse model is complicated by their completely different physical characteristics, which need to be corrected for, such as warm- versus cold-bloodedness. Instead, using *Astyanax* allows us to immediately zoom in on the mechanisms exclusively regulating regeneration versus scarring (Potts et al., 2021). The advantages of *Astyanax mexicanus* as a research model over other models extend even further when taking into account other unique experiments that are possible because the cavefish and surface fish are still one species. The fish can still interbreed, allowing the possibility of forward genetic screening to identify loci associated with specific phenotypic changes using quantitative trait loci analysis (Carlson et al., 2014; Protas et al., 2006). This approach circumvents problematic biases arising with candidate gene approaches and provides a powerful approach to identify loci and molecular pathways crucial to regeneration.

> **Using *Astyanax* allows us to immediately zoom in on the mechanisms exclusively regulating regeneration versus scarring**

During their adaptation to the cave environment, the cavefish have adapted processes that are also key to regeneration, such as metabolism and the immune system (Aspiras et al., 2015; Peuß et al., 2020; Riddle et al., 2018). Directly comparing these altered processes with their equivalents in the regenerative setting will help tease out the cell type-specific responses and molecular pathways in surface fish that are essential to regeneration and determine the difference between regeneration and scarring. It will be interesting to see whether the muscle and other organs, such as the liver and spinal cord, respond in a similar way to the heart. Mapping the inter-organ regenerative differences between cavefish and surface fish will further enhance the power of this model to advance our understanding of regeneration.

Evolutionary insights into animal regeneration from tiny jellyfish

Yu-ichiro Nakajima

Animal regenerative mechanisms vary widely among species. Despite recent progress in identifying key molecular and cellular components required for regeneration, many questions remain unanswered, particularly regarding the evolution of these mechanisms: which mechanisms have been retained from common ancestors and which are species-specific acquisitions? To address these issues, comparing regeneration machinery in extant distantly related animals is necessary. Cnidarians, including sea anemones, corals and jellyfish, are examples of early-branching metazoans and, as the sister group to bilaterians, are useful for an evolutionary perspective on diverse biological phenomena. Whereas polyp-type cnidarians, such as *Hydra* and *Nematostella vectensis*, are capable of whole-body regeneration, most medusae species seemingly possess only organ-level regenerative capacity (Fujita et al., 2021). This significant difference remains to be explained but might be due to the distinct distribution and potency of stem cells as well as varying regenerative responses. Aside from the established model, *Clytia* (Houliston et al., 2022), there are no jellyfish models for studying regeneration.

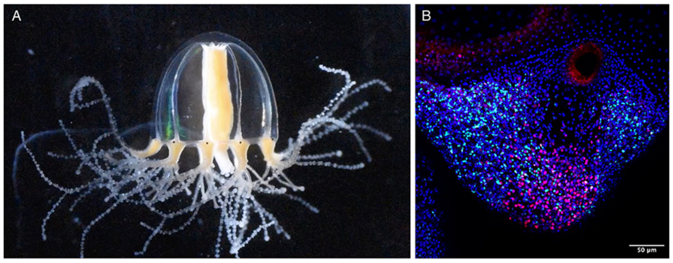

Fig. 5. The hydrozoan jellyfish *Cladonema pacificum* and its blastema formation during tentacle regeneration. (A) The medusa of *Cladonema pacificum* possesses tentacles with a characteristic branching pattern. (B) Repair-specific proliferative cells (red) form a blastema whereas resident stem cells (green) are localized at the basal side of the tentacle (tentacle bulb).

I was introduced to jellyfish relatively recently. After my PhD course, I used the fruit fly *Drosophila* to investigate the mechanisms controlling epithelial homeostasis and environmental responses. As a postdoc in Matt Gibson's lab (Stowers Institute for Medical Research, MO, USA), I observed my colleagues establishing *Nematostella* as a fascinating research model (Ikmi et al., 2014). Although deeply invested in the *Drosophila* system, I was also seeking an opportunity to work with different models once I started my research team. After returning to Japan as an Assistant Professor at Tohoku University, I met Ryusaku Deguchi (Miyagi University of Education, Japan), who kindly introduced me to *Cladonema pacificum*, a hydrozoan jellyfish species found along the coast of Japan (Takeda et al., 2018) (Fig. 5A). *Cladonema* is easily maintained in the lab without special equipment, allowing for monitoring of its entire life cycle, and the size of *Cladonema* medusa is small, ranging from approximately 1 to 10 mm, which is important for establishing animals as research models.

> ***Cladonema* is notable for its branching tentacles**

Among many interesting biological features, *Cladonema* is notable for its branching tentacles, which continue to grow and branch during maturation (Fujiki et al., 2019; Hou et al., 2021). We found that, after amputation, functional tentacles regenerate within a few days when the bulb is left intact (Fujita et al., 2019). Given the manageable size of the tentacles and their rapid and robust regeneration capacity, we anticipated that *Cladonema* tentacle regeneration would be a useful model for studying appendage regeneration. We characterized the distribution of proliferative and stem cells in the medusa tentacle and discovered that repair-specific proliferative cells with stem cell characteristics appear at the injury site and form the blastema (Fujita et al., 2023) (Fig. 5B). Interestingly, these repair-specific proliferative cells preferentially differentiate into epithelial cells, whereas resident stem cells continuously generate all the cell types that constitute the tentacle. Repair-specific proliferative cells are analogous to lineage-restricted stem/progenitor cells observed in bilaterian appendage regeneration, such as salamander limbs (Sandoval-Guzman et al., 2014). Considering the distant ancestry between cnidarians and bilaterians, we expect that each group has independently acquired a similar mechanism of blastema formation. Using *Cladonema* as an emerging jellyfish model, we are currently investigating stem cell heterogeneity and potential cell plasticity, aiming to provide evolutionary insights into regeneration and uncover hidden biology during these fascinating processes.

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Competing interests

The authors declare no competing or financial interests.

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