The crustacean Parhyale

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Creatures Column

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How did the crustacean Parhyale move from tropical intertidal shores and mangroves into the lab, and what does it bring to biological research?

Parhyale (pronounced like *par-high-alley* or *par-ee-alley*) was first brought to the lab in 1997, almost by serendipity, when Bill Browne visited a public aquarium searching for interesting crustaceans to study on his doctoral research. *Parhyale* was not one of the species on display, but an intruder growing in the seawater filtering system of the aquarium. Bill adopted them for his research project[1] reasoning that, as aquarium pests, they would breed rapidly and require minimal care. Since that time, basic genetic tools, genome resources and imaging approaches have been developed in *Parhyale*, making this animal an attractive experimental system for studying embryonic development and regeneration[2]. Researchers from different fields are also starting to adopt *Parhyale* to study circadian and tidal rhythms, sensory systems, the breakdown of cellulose/wood, and for monitoring pollutants in the environment.

Parhyale are amphipod crustaceans resembling the sand hoppers that we sometimes encounter on the beach. They belong to a larger group (called malacostracan crustaceans) that also includes crabs, lobsters and krill. *Parhyale hawaiensis* live among the rocks, gravel and algae in shallow marine habitats –intertidal areas, estuaries, mangroves– in many tropical areas around the world including the Pacific, India and Brazil.

Male and female couples produce broods of embryos, which are carried by the females in a ventral brood pouch. After completing embryonic development (in ~10 days) juveniles are released from the brood pouch and start their life among algae and stones in the shallow sea bed, feeding among the detritus. They reach sexual maturity within a few months. They keep growing through successive molts to a size of ~1 cm, reproducing year-round and giving birth to multiple broods per year.

Most laboratory populations descend from the culture established by Bill Browne in Nipam Patel's lab in 1997. The geographical origin of that population remains unknown.

Among the diverse animals that populated biology labs in the 1990s, only few – *Drosophila*, *C. elegans*, mice and zebrafish – flourished as 'model' organisms. Their rise to stardom the 1970s and 80s was driven by innovative genetic and molecular approaches, and the conviction that genes and mechanisms discovered in these animals will be sufficiently

conserved to provide insights on the biology of all animals, including humans. In the 1990s, however, a new need started to emerge: curiosity about how organisms evolve pushed many researchers to study development in new species. The motive was to discover genetic and developmental *differences* among species that might explain how evolution has generated the diverse forms that we find in nature. Crustaceans were particularly attractive for such studies, because we can find very diverse forms among crustacean species (e.g. think of the different arrays of specialised legs, claws and swimming appendages that we find in lobsters, sand hoppers, brine shrimp and water fleas). Researchers studying animal relationships had also come to the conclusion that insects are most closely related to crustaceans, and probably evolved from crustacean ancestors. That was a big bonus, because *Drosophila* (an insect) was our most valuable source of knowledge on how genes control development of the body. Knowledge from research in *Drosophila* provided insights on how change in specific genes (for example *Hox* genes[3]) could underpin evolutionary changes in body plans. The birth of *Parhyale* as an experimental system, in the lab of Nipam Patel, occurred in that wider context.

Early studies in *Parhyale*, at the turn of the century, established methods for manipulating (isolating, killing, or microinjecting dyes into) cells in the early embryo in order to determine their fates and plasticity during development[4-7], as well as methods for studying gene expression and knocking down gene function[8,9]. These studies focused on how early embryos develop based on a stereotypic cell lineage, and how they generate their segments and limbs. Comparative studies on *Hox* genes and leg patterning genes helped to elucidate how different types of appendages (antennae, mouthparts, legs, and swimmerets) are organised in different parts of the body, and how they evolved from the ancestors of today's crustaceans and insects[9-13].

Our ability to label cells and manipulate genes in *Parhyale* received a strong boost with the introduction of transgenesis and CRISPR-mediated gene editing. Transgenesis was established by Tassos Pavlopoulos, who used the *Minos* transposon as a vector for inserting foreign DNA stably in the genome of *Parhyale*[14]. CRISPR brought an efficient means of inactivating genes[12,13]. These approaches have opened the door to developing a wide range of experimental tools in *Parhyale* (see Box). The development of transgenic lines to label cells using fluorescent proteins, combined with the transparency of embryos and adult legs, have opened unique opportunities for studying development and regeneration by live imaging[15,16].

The ~3.6 Gb genome of *Parhyale* has been sequenced at high coverage from a single individual[17] (see Box). A growing number of chromatin and transcriptional profiling data are available, covering embryonic and adult stages, as well as circadian and molting cycles[17-21].

As new tools and resources become established, new research questions are becoming experimentally tractable in *Parhyale*. A notable example is the study of regeneration. Throughout their lifetime, *Parhyale* can regenerate appendages (legs, antennae, swimmerets) lost though severe injury. Leg regeneration is complete within approximately one week and the regenerated legs appear to be perfect functional replicas of the original structures[19]. This striking ability, which was first explored by Nikos Konstantinides in our team[22], raises two questions that we are now trying to address. First, how are the different types of cells that make up a leg (epidermal, muscle, neural, glial, etc.) remade? Are they made from stem cells that are set aside for this purpose, or from already differentiated cells

that retain a degree of plasticity? *Parhyale* offer a unique opportunity to address this question, because in these animals we are able to observe the entire process of leg regeneration at high resolution, based on a method developed by Frederike Alwes[15]. This is quite unprecedented, as in other regenerating species (salamanders, fish, flatworms etc.) we are unable to immobilise regenerating animals under the microscope for a long enough period.

Second, given the high fidelity of regeneration in *Parhyale*[19], we wonder to what extent leg regeneration mirrors leg embryonic development, or follows distinct mechanisms that converge on the same outcome. Thus far, comparing the temporal dynamics of gene expression during leg development and regeneration suggests that regeneration does not mirror development[20].

Beyond development and regeneration, *Parhyale* represents an attractive system for studying biological phenomena that have not been genetically tractable (or do not exist) in other animals. These include sensory adaptations in the visual system[23], the ability to digest and extract energy from cellulose or wood[17] (studied by Tassos Pavlopoulos' team), and the interplay between tidal and circadian rhythms[21] (studied by Patrick Emery and Joshua Rosenthal's teams). *Parhyale* is also considered as a test species for monitoring environmental pollution in coastal tropical ecosystems (studied by the team of Gizela Umbuzeiro).

The *Parhyale* research community is small, numbering no more than 30 people. While there is still much work to be done to extend and refine our experimental toolkit in this organism, key genetic approaches (transgenesis, CRISPR) and resources (genome sequence, chromatin and transcriptional profiling) are already established. Live imaging provides unique opportunities to observe the entire time courses of development and regeneration at single cell resolution.

Acknowledgements

I thank the *Parhyale* research community, including past and present members of my team, for working together to build *Parhyale* as an experimental system. Apologies for papers I could not cite due to space constraints. Our research on *Parhyale* regeneration is supported by the European Research Council of the E.U. (grant ERC-2015-AdG #694918).

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Box: Experimental approaches and resources available in *Parhyale*
(reviewed in ref. 2)

Genetic approaches and live imaging

- Gene silencing and CRISPR-mediated gene knock-out[9,12.13]
- Stable transgenesis, gene trapping, and CRISPR knock-in[11,14,17,24]
- Conditional gene expression though heat shock[10]
- Mosaic expression by early blastomere injection or cell transplantation[4,6,7,14,10,22]
- Specific promoters for muscle[14], central nervous system[22], photoreceptors[23]
- Long-term live imaging of embryos and regenerating adults[7,15,16]

Genomic resources

- 3.6 Gb genome assembly[17] (latest assembly contig N50 = 10 kb, scaffold N50 = 20 Mb, scaffold L50 = 42; https://www.ncbi.nlm.nih.gov/assembly/GCA_001587735.2/)
- RNAseq data from embryos[17,18], regenerating legs[20], circadian[21] and molting cycles[20]
- Single nucleus RNAseq of diverse adult cell types[19]
- Chromatin (ATACseq) profiles from embryos[18] and adult legs