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Poster Abstracts

Diversity of Tunicates (Class: Ascidiacea) in Different Shipwreck Habitat from Karimunjawa, Indonesia

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The purpose of this research was comparing the abundance and diversity of tunicates species living in two different habitats (wooden and metal shipwrecks) in Karimunjawa. The method used were morphological and molecular identification using DNA barcoding with COI gene target. The research resulted that there were significant differences in abundance as well as diversity of tunicates species living in both types of shipwrecks. The abundance of tunicates in metal shipwreck were respectively higher (41 and 35 individuals) than in wooden shipwreck (only 23 individuals). The diversity of ascidian species in metal shipwreck (25 and 20 species) also higher than in wooden shipwreck (10 species only). The total successfully identified ascidian species found from all sampling sites were 23 species, namely: Atriolum robustum, Atriolum sp., Botrylloides sp, Botrylloides simodensis, Botrylloides cf pannosum, Ciona robusta, Clavelina robusta, Clavelina arafuensis, Diazona sp., Didemnum sp, Didemnum concyliatum, Didemnum pseudovexillum, Didemnum vexillum, Didemnum cf. albopunctatum, Eudistoma plumbeum, Lissoclinum, Leptoclinides, Pycnoclavella communis, Polycitorella, Plebobranchia, Rhopalaea sp., Rhopalaea idoneta, and Trididemdum maragogi. The species identified using the DNA barcoding had similarity levels varying between 80-99.46% with the data in the gene bank. There are only two species found in all three locations, namely Atriolum sp. and Rhopalaea idoneta. Therefore, there is correlation between the type of substrate and species that are able to live, in this study concluded that ascidians found more diverse in metal shipwreck sites than wood. Further research is needed to determine the correlation between habitat and tunicates potential as natural product producer.

Polyethylene (PET) nanoplastics influence the behavior of immunocytes in the colonial ascidian Botryllus schlosseri

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Plastics are persistent large-scale pollutants that adversely affect humans, wildlife and ecosystems. Every year, more than 20 million tons of plastic waste leaks into aquatic ecosystems, polluting lakes, rivers and seas. Plastics less than 5 mm and 1 μm in diameter are defined as microplastics (MPs) and nanoplastics (NPs) respectively: they derive from the degradation and fragmentation of large plastic objects. NPs are able to enter cells and cross the blood-brain barrier, accumulating in vital organs of organisms and potentially influencing their physiology over long periods of exposure and accumulation. Ascidians are invertebrate chordates closely related to vertebrates. Their phylogenetic position render them ideal organisms to study the evolution of various biological processes, with particular focus on the invertebrate-vertebrate transition. Botryllus schlosseri is a colonial ascidian widely used in studies of innate immune responses. In this species, we are studying the behavior of immunocytes once exposed to polyethylene (PET) NPs. Haemocytes were directly exposed to NPs, or the latter were directly microinjected in the ampullae of the colonial circulatory system. Preliminary results indicate that the exposure of haemocytes to NPs negatively influences the phagocytosis of yeast (Saccharomyces cerevisiae) cells. This is accompanied by a modification of phagocyte morphology, probably related to cytoskeletal alterations. In addition, NPs have a negative effect also on the degranulation of morula cells, immunocytes with cytotoxic activity that represent the first step of the inflammatory response.

Study the link between the environment and asexual reproduction in salps using a cellular and molecular approach

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Salps are planktonic tunicates known to form vast seasonal blooms that heavily impact oceanic food webs and biogeochemical cycles. The main driving force behind the exponential blooms of salps is their asexual mode of reproduction, known as stolonial budding. In particular, the number of buds produced per individual and the speed of budding are mainly influenced by temperature and food availability (phytoplankton) and seem to change during the blooms.

In this study, we first aim to improve the morphological characterization of budding in the species Thalia democratica. Using confocal imaging, we are describing the mechanisms leading to stolon formation and bud development along the stolon. We notably focused on cell proliferation and identified several stem cell populations contributing to the different organs of the growing buds. These results will be used to conduct a single-cell RNA-seq analysis in order to reconstruct the dynamics of gene expression along the stolon.

A second approach aims to test the influence of environmental factors, i.e. temperature and food, on asexual reproduction in salps. Using longitudinal studies and archival plankton collections from the Bay of Villefranche-sur-mer, we have been able to correlate the number of buds produced with environmental conditions. In parallel, we are conducting experiments in the field and in our custom-built aquaria to test whether the rate of cell proliferation is influenced by food and temperature as well. Combining these observations with the morphological knowledge gained in the first part will help to better understand the link between salp abundance and budding mechanisms in response to varying environmental conditions.

Characterization of nickel-induced oxidative stress response in Botryllus schlosseri

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Botryllus schlosseri is a colonial ascidian species found globally in temperate coastal zones. Colonies consist of three generations: parent zooids, developing primary buds, and emerging secondary buds; all embedded in a shared tunic and organized into star-shaped systems. In B. schlosseri, weekly blastogenic generational succession, a form of stem-cell mediated asexual reproduction, is marked by the apoptosis and autophagy of zooid tissues as the primary buds mature into the next set of self-sustaining zooids. This cycle between degeneration and development makes B. schlosseri an excellent model for studies on regeneration, aging, and cellular biology. Despite decades of extensive research on optimizing culturing methodologies for B. schlosseri, a cell line has not been established. This study uses nickel (II) chloride to characterize toxicity responses, including oxidative stress pathways, in B. schlosseri. We aim to use in vivo data to inform future cell culture efforts, exploring nickel-induced mutagenesis as one potential method for cellular immortalization. Nickel genotoxicity induces DNA breakage via cellular accumulation of reactive oxygen species. As nickel toxicity in B. schlosseri was not characterized, we established the acute lethal concentration (LC50) for field-sourced adults (LC50 24-hour exposure = 864 mg L-1). Mortality was marked by the cessation of blood flow within the system and ampullae. A Superoxide Dismutase (SOD) assay will quantify the enzymatic antioxidant response to increasing concentrations of nickel. Future work will involve the application of RNA-sequencing and proteomics to analyze underlying altered molecular pathways of primary buds from sublethally nickel-exposed B. schlosseri. Funding provided by NSF MCB-2127517.

Aftermath of severe winter storms on a colonial tunicate invading the California outer coast

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San Francisco Bay is heavily invaded, but few known invasive species have ranges extending to the relatively untouched adjacent outer coast. Didemnum vexillum (Dvex) is a highly invasive colonial tunicate that has spread to fouling communities and beyond worldwide. Dvex is abundant at Point Bonita, a National Park site at the mouth of the Golden Gate, demonstrating the spread of invasive species to the outer coast from more common bay and fouling community sites. After back-to-back atmospheric rivers depositing heavy rainfall for five months during winter 2022-2023, Dvex at Point Bonita was significantly reduced; remaining colonies were thin, patchy, and heavily regressed. Data collected across survey trips before and after heavy winter storms reveal an apparent impact on Dvex population abundance at Point Bonita, likely due to salinity stress and mechanical removal of colonies on rolling boulders due to high wave energy. Surveys through spring 2024 documented a seasonal growth pattern consistent with Dvex found in other locations. Close monitoring of this site is necessary to investigate methods for management. Among management strategies discussed, spot treatments showed promise. As Dvex has potential to spread along the outer coast from this crucial site, intertidal and subtidal ecosystems could be smothered by this highly invasive species. Understanding how increasing storms interact with invasive species at sites like these will be useful towards management plans.

The impact of underwater noise pollution on the deuterostome invertebrates: the European project DeuteroNoise

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Noise pollution has emerged as a significant pollutant in recent years, driven by human activities, which disrupt the natural soundscape, impacting animal communication and behavior. The impact of anthropogenic noise has increased in recent decades, leading to a rise in studies; however, knowledge regarding the world of aquatic invertebrates is still lacking, despite noise having a significant impact on their activities. In fact, they possess receptors which are sensitive to water particles vibrations and could undergo alterations following exposure to prolonged noises.

DeuteroNoise is a three-year European project and aims to address this gap by investigating the impact of underwater noise generated by vessels on deuterostome invertebrates. The project, spanning across Italy, Spain, Norway, and Romania, focuses on various environments and deuterostome invertebrates. By studying sites such as the

Venice Lagoon, the North Adriatic, the Black Sea, the North Sea, and the Spanish coasts, DeuteroNoise aims to assess the physiological, behavioral, anatomical, and transcriptomic responses of these organisms to noise pollution.

The project involves extensive coordination among participating countries, including fieldwork, laboratory experiments, and data analysis. Fieldwork activities include measurements of soundscapes of the five different basins and animal sampling, while laboratory work involves reproducing noise conditions and monitoring organismal responses. Videos will document behavioral changes, and tests will evaluate alterations in receptor cell sensitivity. Transcriptomic analyses will be conducted to evaluate the effect of noise on gene expression.

Ultimately, DeuteroNoise seeks to develop innovative strategies to mitigate underwater noise pollution, thereby safeguarding marine ecosystems and their organisms.

Proteomic Profiling of Botryllus schlosseri During its Blastogenic Cycle

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Botryllus schlosseri, a small, invasive marine colonial chordate, serves as a key model in evolutionary biology and regeneration due to its unique asexual reproduction process called blastogenesis. This allows for potential exponential colony expansion, which is typically restricted by environmental factors in natural settings. We have optimized methods to rear B. schlosseri animals in controlled, landlocked laboratory environments that supports continuous and exponential growth through these blastogenic cycles. These cycles include four distinct stages, A, B, C, and Takeover, each represent various phases in the colony's lifecycle, with primary buds growing weekly among adult zooids and synchronously regressing during the Takeover stage. Using Liquid Chromatography/Tandem Mass Spectrometry (LC/MS) and data-independent acquisition (DIA) proteomics, we conducted the first extensive proteomic profiling of these stages. We quantified over 4000 proteins, shedding light on the molecular phenotypes of B. schlosseri and constructing protein networks related to regeneration and degeneration. Notably, during the Takeover stage, proteins linked to proliferative pathways like ribosomal protein synthesis and DNA replication were upregulated in buds, while pathways associated with degeneration such as protein digestion and absorption were enriched in regressing zooids. Additionally, we identified previously unannotated proteins with significant expression changes during the Takeover stage, opening new research avenues. This study enhances our understanding of in vivo tissue developmental pathways and potential in vitro cell immortalization strategies. The project is supported by NSF Grant MCB – 2127516.

Beta-galactosidase as a regional marker of the gastrointestinal tract

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Regionalisation along the gut is a key requirement for the digestion and absorption of food particles, along specialised sections carrying out these functions. Ciona intestinalis shows regionalisation along the gastrointestinal (GI) tract and we further expand the repertoire of biomarkers for regionalisation with the enzyme beta-galactosidase.

Within a variety of ascidian species, we characterise the expression of beta-galactosidase in the adult GI tract. We further characterise the beta-galactosidase orthologues found in ascidians, using a variety of bioinformatic and phylogenetic analyses. The expression patterns of beta-galactosidase points to it being an ancestral regional marker in the digestive tract at least across tunicates and likely more widely.

From high-content image-based CRISPR screening to provisional biomolecular network models for heart progenitors in Ciona

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The use of model organisms is essential for understanding animal development and evolution of developmental diversity. Forward genetic screens have identified regulators of various developmental processes. Recent advancements, such as RNA interference in C. elegans1,2 and the CRISPR/Cas93,5 system, have enabled discovery of genes regulating conserved developmental processes.

Our lab studies the specification and development of the cardiopharyngeal cell lineage6,7 using the ascidian Ciona robusta, a model organism ideal for systems and cell biology. We have profiled the cardiopharyngeal transcriptomes4 and are now investigating the function of zygotically expressed and lineage-specific genes in cardiopharyngeal development.

We aim to generate a provisional biomolecular network model for cellular behaviors in multipotent cardiopharyngeal progenitors. We designed a pilot library of guide RNAs targeting genes relevant to cardiopharyngeal development based on microarray data and RNA-sequencing results8, creating a resource for tunicate researchers. We developed a pipeline for high-content image analysis to screen for phenotypes associated with CRISPR-mediated loss-of-function of target genes in the cardiopharyngeal lineage, analyzing over 2,000 image stacks. By targeting each gene and analyzing the effects using confocal microscopy, we extracted computationally morphometric features relevant to cardiopharyngeal development. Computational analysis of high-content phenotypic profiles allowed us to generate models of the biomolecular networks underlying cardiopharyngeal cell behavior, as previously done in C. elegans phenotypic screens9.

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A genomic journey through sea squirt diversity: production of high-quality reference genomes for several European ascidians, with a focus on colonial species

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Ascidians play a central role in the ecology of marine benthic communities, with several species able to colonize natural and artificial substrates, and therefore potentially invasive. However, the overall ascidian biodiversity is currently underestimated, as also shown by the numerous cases of ascidian taxa recently recognized as species complexes based on integrative taxonomy or on genetic and genomic data. In the framework of the BGE project (https://biodiversitygenomics.eu/) and the ERGA (European Reference Genome Atlas) initiative, we aim to push the application of genomic science to ascidian biodiversity research by increasing the production of high-quality reference genomes for European ascidians, particularly targeting colonial species and known cases of cryptic species. Indeed, only few colonial ascidians are currently used as models for studying processes such as allorecognition, regeneration, stemness, and asexual development, and for investigating the evolution of colonial lifestyle in chordates. Here we present the whole project, detailing the sequencing strategy, mainly based on Nanopore long reads, and the assembly pipeline. The analysed species belong to five families and two orders (Styelidae and Pyuridae as Stolidobranchia; Clavelinidae, Polyclinidae, and Didemnidae as Aplousobranchia): all specimens were sampled in the field and some of them preserved in sub-optimal conditions for HMW-DNA extractions. Our preliminary results show that the optimization of the DNA extraction protocols, together with the refinement of the assembly pipeline, allow reaching an average genome coverage between 135x and 400x, thus obtaining high quality contig-level and scaffold-level assemblies matching ERGA quality standards. The genomic resources provided by this project will increase the knowledge on ascidian biodiversity and help investigate the genes/pathways/regulatory mechanisms involved in the colonial lifestyle in ascidians.

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The BGE project has received funding from the European Union under the European Union’s Horizon Europe research and innovation programme, co-funded by the Swiss Government and the British Government.

An understanding of the pH-dependent elevation of egg coat in *Phallusia philippinensis.*

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Eggs of Phallusia philippinensis, living in warm sea, exhibit the phenomenon called vitelline coat (VC) elevation. This phenomenon occurs when eggs are released from the oviduct into the seawater. The elevation of egg coat is well known as a change to the fertilization envelope after fertilization in sea urchin and starfish. However, VC elevation of Phallusia philippinensis occurred regardless of fertilization. Previous research revealed that the pH in the oviduct is approximately 5.7, while the pH of the seawater is 8.2. Considering the pH difference between internal environment and seawater, we analyzed whether VC elevation depends on the surrounding pH. Therefore, eggs were placed in seawater with various pH (5.0 ~ 9.0) and VC diameter was measured. It was revealed that VC elevation is faster in high pH and slower in low pH. Moreover, VC elevation was suppressed by adding high polymer in the seawater. Comparison of VC structure before and after elevation using microscope revealed that the space between the follicle cells, where sperm bind and penetrate through, is wider after elevation. To understand the importance of VC elevation in fertilization and embryogenesis, non-elevated VC eggs were inseminated and the cleaved eggs were counted. These results indicate that there was no significant difference between elevated and non-elevated egg in fertilization. Hence, our research suggests that VC elevation is dependent on the ambient pH and osmotic pressure. Moreover, it indicated that this phenomenon is not necessary for fertilization.

DNA replication-dependent regulation of gene expression in the Ciona embryo

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Timing of gene expression is strictly regulated in the ascidian embryo. The acetylcholinesterase-encoding gene (AChE) is expressed mainly in the muscle-lineage cells from the gastrula stage. The DNA replication inhibitor, aphidicolin, influences the expression of AChE. Continuous treatment of Ciona embryos with aphidicolin from the 32-cell stage suppressed AChE expression even after the gastrula stage. In contrast, aphidicolin treatment from the 64-cell stage did not affect the AChE expression. Expression of Mrf, encoding a transcription factor that activates AChE expression, was also suppressed by aphidicolin treatment starting from the 32-cell stage. These findings suggested that 6 rounds (or the 6th round) of DNA replication were required for these genes to be ready for expression. Another possibility was that a side-effect of aphidicolin somehow inhibited the expression of factors responsible for transcriptional activation of AChE and Mrf. We detected the expression of Zic-r.b in embryos treated with aphidicolin starting from the 32-cell stage. Other transcription factors activating Mrf expression are already present before the 32-cell stage. Therefore, DNA replication is indeed important for transcriptional activation of Mrf. We also tested DNA replication inhibitors with mechanisms of action different from that of aphidicolin. Inhibitors of Proliferative Cell Nuclear Antigen (PCNA), T2AA and PCNA-I1, did not completely block DNA replication but appeared to cause developmental arrest. We are currently looking at the expression of Mrf and AChE in the embryos treated with these drugs.

Investigating the roles of Claudin proteins in Ciona neurodevelopment

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In vertebrates, claudins are integral membrane proteins crucial to the structure and function of tight junctions between cells. They regulate the passage of ions and small molecules between epithelial and endothelial cells, thus maintaining the selective permeability of barriers like the blood-brain barrier and intestinal lining. Alterations in claudin expression or function are implicated in diseases such as cancer and inflammatory bowel disease. It is generally thought that tight junctions, specifically characterized by the presence of claudin proteins, are unique to vertebrates and that invertebrates generally lack true tight junctions with claudins. However, it has been reported that tunicates have tight junctions and claudin-encoding genes. Of note, one such claudin gene, Claudin.j (KH.C10.182), is expressed in migrating bipolar tail neuron (BTN) precursors. Here we report our recent investigations into the potential role of Claudin.j in BTN collective migration, as well as potential roles for the claudin family in Ciona development in general.

Mechanical regulation of body axis establishment in ascidian Phallusia mammillata

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Embryo patterning and the establishment of embryonic body axes are fundamental processes during embryogenesis. In ascidian zygotes, large-scale cytoplasmic reorganization takes place upon fertilization, resulting in pre-patterning of the embryo before the first cleavage. This provides a unique system for understanding the establishment and evolution of vertebrate-like body plans. Although it is known that the relocalization of a cortical polarity domain - the myoplasm and cortical ER - depends on the microtubule cytoskeleton, the exact regulation of this mechanically driven process remains to be elucidated. In this study, we aim to understand the force-generating mechanisms of myoplasm repositioning to the future dorsal-posterior pole of the embryo by employing quantitative approaches, including live embryo time-lapse imaging and Brillouin microscopy. Our preliminary observations reveal that the myoplasm exhibits greater stiffness compared to the rest of the embryo cytoplasm. Additionally, the inhibition of either actin or microtubule polymerization during the second phase of cytoplasmic reorganization leads to the lack of myoplasm relocalization, despite the preservation of material properties within this domain. These findings suggest that both actin and microtubule dynamics are crucial for the proper relocalization of the myoplasm. This study aims to shed light on the intricate interplay between mechanical forces and molecular components in orchestrating the embryonic patterning in ascidians. By elucidating these mechanisms, we hope to contribute to a deeper understanding of the fundamental principles governing embryogenesis and the evolution of chordate body plans.

The stem cell niches in the colonial ascidian *Botryllus schlosseri*

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In many adult animals, stem cells are hosted in stem cell niches, micro-environments which maintain the cells and provide the signals for self-renewal and differentiation.

Here we review the features of the stem cell niches in Botryllus schlosseri, involved in development and regeneration. These niches are transient and located in cycling individuals.

The endostyle niche, in the anterior ventral region of the endostyle, has been identified as a key site harboring somatic stem cells. Flow cytometry and transcriptome analysis revealed that this niche host hematopoietic stem cells (HSCs) which share similarities with mammalian HSCs, indicating a conserved function.

Cell islands (CIs) are aggregates of phagocytes, germinal and somatic stem cells, positive for Vasa, Oct4, and Pl10. They are in the ventral body wall in two rows flanking the endostyle. Labelled cells from the endostyle niche migrate to CIs in the next generation of adult zooids and flow cytometry identified populations expressing germ line stem cell markers homing into CIs.

The gonad niche was proposed as a budlet niche. This niche contains primordial germ cells expressing Vasa and Oct4. Germline stem cell precursors migrate from the bud into the budlet at the double vesicle stage, guided by a Sphingosine-1-phosphate gradient.

Orthologs of Yamanaka factors (YF), including Myc, SOXB1, Pou3, and Pou2, are expressed in the endostyle niche, cell islands, germline cells, underscoring the pluripotent capabilities within these niches. Notably, the YF are expressed also by candidate stem cells in ampullae, a putative stable colonial niche.

MorphoNet : How to Segment and curate 3D + t dataset in a few clicks

Tao Laurent

MorphoNet is an interactive morphodynamic web browser and standalone application designed to help scientists, teachers and students share, analyze and visualize the large 3D morphological datasets that can be generated by modern imaging technology, ranging from live light sheet microscopy of cells and embryos to X Ray tomography of fossils.

MorphoNet is created so that biologists can interact, without programming skills, with the 3D + time datasets. We have developed a system to integrate powerful image processing plugins and segmentation tools (such as Cellpose) without requiring any programming knowledge or a programming environment on a computer. In a similar way as genomic browsers display genetic features and epigenetic or gene expression data as traces onto the primary genome sequence, quantitative and qualitative information can be imported and projected onto individual or grouped segmented objects in MorphoNet. These "morphological augmentations" can be saved and shared with other users (and used in publications), respecting the FAIR philosophy.

Moreover, MorphoNet offers more flexibility than genome browsers. While the DNA base pair is the universal unit of information in genome browsers, the relevant units of information can vary within imaging datasets, from complex organs down to molecular complexes. The choice of the unit of information is therefore left to the user. Objects can also be hidden, made translucent or hierarchically grouped: spatially (e.g., by tissue), temporally (e.g., by cell lineages), by imaging channels (e.g., nuclei and plasma membranes), and identified with specific color labels.

In this workshop, we propose to train participants to a new open-source tool dedicated to 3D and 3D+t microscopic images.

We have developed a new way to interact with 3D images using the surface meshes of the segment objects. We use the powerful rendering and interactivity of meshes while each segmentation plugin is directly applied to the original images.

During this workshop we will first introduce the concept of the MorphoNet platform and explain classical usage of 3D (or 4D) interactions. And secondly train the participants to several plugins of 3D segmentation that we integrated in the MorphoNet software. Cherry on the cake, participants will be able to learn how to visualize and interact with their own data with a Virtual Reality Headset.

At the end of this workshop the participants will be able to load their 3D microscope images in MorphoNet in order to perform interactive segmentation.

First chromosome-level haplotype-resolved genome assembly and annotation of the colonial tunicate *Botryllus schlosseri*

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The colonial tunicate Botryllus schlosseri has long been studied for its multiple developmental pathways and regenerative abilities and its genetically determined allorecognition system based on a polymorphic locus that controls chimerism and cell parasitism phenomena. We present the first chromosome-level genome assembly from an isogenic colony of B. schlosseri clade A1, which was generated using a combination of long and short reads and scaffolded with Hi-C technology.

We assembled both a collapsed haploid version and a phased diploid version of this genome. While the collapsed haploid assembly spans 533 Mb, the two haplotypes in the phased diploid version exhibit slight differences in sizes: 480Mbp (N50= 29Mbp), and 464Mbp (N50= 28.9Mbp), respectively. All three assemblies exhibit a BUSCO completeness score of over 90.6% (including 1.2% of duplicated BUSCO markers) and were scaffolded into 16 chromosomal pseudomolecules.

These high-quality, chromosome-scale genome assemblies of Botryllus schlosseri represents a valuable resource for the scientific community, providing a foundation for future investigations into the molecular mechanisms underlying coloniality, regeneration, histocompatibility, and the immune system in tunicates.

Enhancing Proteomic Analyses of *Botryllus schlosseri*: Optimizing Computational Approaches for DIA Data Processing and Curation.

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Proteins are essential cellular components crucial for shaping organismal traits, particularly in response to environmental stressors. Their regulation offers mechanistic insights into organism and ecosystem resilience. Recent advancements in mass spectrometry have revolutionized proteomics, enabling the simultaneous quantitation of thousands of proteins in a single analysis. Specifically, the Data-independent mode of acquisition (DIA) has emerged as a powerful method, providing comprehensive proteomic insights with precision. However, analyzing DIA data remains challenging due to sample variability and algorithmic complexities. This study addresses these challenges by meticulously evaluating cutting-edge algorithms tailored for DIA proteomic data processing and curation. Using samples with varied ratios of Botryllus schlosseri, HeLa, and bovine serum albumin (BSA) proteins, we compared the mProphet and Avant Guard algorithms for data curation and validation of precursors, alongside MSstats and DirectLFQ algorithms for protein quantitation and normalization. Additionally, various chromatographic gradients and columns were explored to optimize DIA analysis. The assessment of algorithmic performances and chromatography conditions enabled by this research provides insights into the accuracy, precision, and adaptability of computational methodologies to maximize accurate quantitative information about Botryllus schlosseri protein regulation. The results of this study facilitate and guide the implementation of optimal strategies for DIA proteomic analyses of botryllid tunicates, focusing specifically on advancing our comprehension of Botryllus schlosseri proteome dynamics in response to environmental stimuli. This research was funded by NSF grants MCB-2127516 and MCB-2127517 (D.K. and, A.M.G, respectively).

Germline stem cell migration in *Botryllus schlosseri*

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A conserved feature of germline development is the migration of germline progenitors to the germline niche (genital ridge) following segregation from somatic cells, and has been characterized from Drosophila to humans. In most organisms, this occurs once during embryogenesis. However, in the colonial ascidian, Botryllus schlosseri, germline migration happens every week. Botryllus grows by regenerating new bodies (called zooids) in a process called blastogenesis. Germline regeneration is due to the presence of embryonically specified, long-lived germline stem cells (GSCs), which migrate between niches during this process. Migration occurs at a defined time in the blastogenic cycle, during which the GSCs leave the old niche, and migrate to the newly developing body. Once in the new niche, they will settle, a subset will differentiate into gametes, others will self-renew and wait for signals that instruct them to migrate to the niche in the next generation. GSCs migrate as germ/follicle cell clusters, identified as clusters of vasa+ and TGFbeta+ cells. In previous data using in situ hybridization, we found that during cluster migration, these cells appear in the circulation during a 48 hr window. In addition, the mixing of circulating GSCs in the vasculature between colonies either through parabiosis or transplantation have found that GSCs from some genotypes possess an intrinsic advantage over those from others, allowing them outcompete others for migration and niche occupancy. It was shown that winner GSCs possess greater migratory capabilities and increased notch signaling may be a factor contributing to their increased clustering. Altogether, circulating GSCs both within and between colonies likely undergo critical changes that determine their ability to compete for and home to the developing secondary buds. We have performed scRNA seq on Botryllus blood from the A1 and B2 stages of the asexual cycle. GSCs are only in the circulation during B2, consistent with previous studies. Our current goals are to identify differences between migrating vs non-migrating cells and empirically validate the expression of differentially expressed genes in circulating vs non-circulating GSCs, as well as from different competitive genotypes. Our goal is to characterize the molecular and cellular mechanisms that influence germ cell niche occupancy and parasitism.

Toward understanding what might have caused the diversification of transcriptional repression mechanisms in urochordate early embryonic germlines.

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Germline formation is an essential and common process during embryogenesis for sexually reproducing animals. However, mechanisms of germline formation are known to be diversified even between closely related species. For example, in a wide range of animals, RNA polymerase II (RNAPII)-dependent global transcription is repressed to protect germline cells from somatic differentiation, and this transcriptional quiescence is established by taxon-specific molecules, such as Pgc in Drosophila, PIE-1 in C. elegans and Pem in ascidians, with no orthologs outside of each group. The addition of new molecules to the mechanisms contrasts with many other developmental programs known to have changed without altering the outcomes, in which such changes are often achieved by alterations of enhancer sequences and thus of gene regulatory networks. How might the molecular mechanisms regulating the germline formation have been able to diversify so easily?

Closely related urochordate species could be ideal model organisms to address this question for the following reasons. First, embryonic-developmental processes including cell fate maps and germline formation are similar among some urochordate species. Second, Posterior end mark (Pem) has been identified as a molecule responsible for transcriptional repression in several ascidian species, Halocynthia roretzi, Ciona intestinalis and Ciona savignyi but we have revealed that the functional domains required for transcriptional repression are different between Pems in H. roretzi and C. savignyi, suggesting that molecular mechanism can be diversified within the molecule among different ascidian species. Finally, Oikopleura dioica, a larvacean belonging to Urochordata and taking the preformation mechanism for early embryonic germline formation as ascidians, shows transcriptional repression in the germline cells but does not have Pem in the genome. Therefore, comparative analyses on germline development among those urochordate species could lead us to understand detailed molecular processes of such diversification during evolution.

We are currently conducting the following experiments. First, we are carrying out cross-species microinjection of Pem mRNAs as well as functional domain swapping experiments to determine whether the different functional domains found between Pem orthologs act on different mechanisms leading to the transcriptional silencing or they, despite their amino acid sequence differences, have a same function acting on the same mechanisms in different species. We are also analyzing to look for common

properties in the amino acid sequences between the different Pem functional domains. Finally, we are conducting a functional screening using O. dioica embryos to identify a molecule(s) that regulates transcriptional repression in the germline. In this presentation, we would like to introduce to you some of the results obtained so far and discuss them with you.

Cis*-regulatory interfaces reveal the molecular mechanisms underlying the notochord gene regulatory network of *Ciona

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Abstract Tissue-specific gene expression is fundamental in development and evolution, and is mediated by transcription factors (TFs) and by the cis-regulatory regions (enhancers) that they control. Transcription factors and their respective tissue-specific enhancers are essential components of gene regulatory networks responsible for the development of tissues and organs. Although numerous transcription factors have been characterized from different organisms, the knowledge of the enhancers responsible for their tissue-specific expression remains fragmentary. Here we use Ciona, a marine invertebrate chordate, to study the enhancers associated with ten transcription factors expressed in the notochord, an evolutionary hallmark of the chordate phylum. We employed CRISPR/Cas9-mediated genome editing and mis/over-expression experiments to gain insights into the function of Lmx1-r, a notochord TF that is evolutionarily conserved among chordates. Our results illustrate how two evolutionarily conserved transcription factors, Brachyury and Foxa2, coordinate the deployment of other notochord transcription factors. Through the analysis of Lmx1-r CRISPant and transgenic embryos, we have gained a first insight into the function of this TF, which seems likely to be mainly involved in the regulation of the shape and movements of developing notochord cells. The results of these detailed cis-regulatory analyses delineate a high-resolution view of the essential notochord gene regulatory network of Ciona, and provide a reference for studies of transcription factors, enhancers, and their roles in development, disease, and evolution.

Movement disturbance, neuronal dysfunction and the role of RAB39 and PARK7 in tunicates

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Parkinson's disease (PD), a neurodegenerative clinical syndrome with a range of causes and clinical presentations, has been induced in some organisms after injection of different neurotoxins, such as rotenone (ROT), which affect mitochondrial complex I. Due to the conserved characteristics of ascidians, these animals constitute an interesting model for comparative and genetic studies of neurodegenerative diseases. In this study, we investigated the effects of ROT on the ascidian nervous system, evaluating apoptosis, catecholaminergic enzymes, behavioral deficits and mitochondrial dysfunction. Furthermore we investigated the neuron development after RAB39 and PARK7 knockout, important genes related to PD. The study revealed morphological disorganization that induced vacuolation in the ascidian brain. Neuronal death was confirmed by elevated transcriptional levels of caspase-3 and intense staining for caspase-3 by immunofluorescence. In addition, there was weaker staining for dopa-descarboxylase (DDC), which is involved in dopamine biosynthesis. Furthermore, the mitochondria showed a dysfunction in their membrane potential, followed by a decrease in the hydrolytic activity of ATP synthase and high transcriptional levels of ubiquitin. Finally, after administration of the drug l-3,4-dihydroxyphenylalanine (L-DOPA), there was a recovery of motor movements revealed by behavioral tests. Besides that, after CRISPR/cas9, the results showed that RAB39 gene is present in Ciona robusta neurons and epidermis. Overall, the current research attempts to provide new data on ascidian brain degeneration, serving as a useful and relevant model for parkinsonism.

Development of novel nuclear markers for ascidian phylogenomics

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Class Ascidiacea lacks appropriate molecular markers for phylogenetics, which presents a major roadblock in studying the systematics and evolution of these species. Using anchored hybrid enrichment, we are developing 100-200 de novo nuclear markers for each of the following four Families: Didemnidae (Order Aplousobranchia), Ascidiidae (Order Phlebobranchia), Pyuridae (Order Stoliobranchia), and Styelidae (Order Stolidobranchia). We have selected these four families because they are the most speciose families in each order, allowing us to develop markers that amplify both deeply (number of species per family) and broadly (across the entire class). We have sequenced the genomes necessary to design the markers, and are currently in the process of developing these markers. We have collected 250 genomic DNA samples, largely focused on ascidians from Belize. After sequencing libraries enriched for the markers, we will construct phylogenomic trees for each of the four families. Our sampling will yield trees that represent all major clades in each of these families and will address knowledge gaps in families with no published phylogenetic tree (Ascidiidae), trees based on morphology only (Didemnidae), and trees based on a single locus (Pyuridae).

Cellular remodeling and JAK inhibition promote zygotic gene expression in the *Ciona* germline

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During development, remodeling of the cellular transcriptome and proteome underlies cell fate decisions and, in somatic lineages, transcription control is a major determinant of fateful biomolecular transitions. By contrast, early germline fate specification in numerous vertebrate and invertebrate species relies extensively on RNA-level regulation, exerted on asymmetrically inherited maternal supplies, with little-to-no zygotic transcription. However delayed, a maternal-to-zygotic transition is nevertheless poised to complete the deployment of pre-gametic programs in the germline. Here, we focused on early germline specification in the tunicate Ciona to study zygotic genome activation. We first demonstrate that a peculiar cellular remodeling event excludes localized postplasmic mRNAs, including Pem-1, which encodes the general inhibitor of transcription. Subsequently, zygotic transcription begins in Pem-1-negative primordial germ cells (PGCs), as revealed by histochemical detection of elongating RNA Polymerase II, and nascent transcripts from the Mef2 locus. Using PGC-specific Mef2 transcription as a read-out, we uncovered a provisional antagonism between JAK and MEK/BMPRI/GSK3 signaling, which controls the onset of zygotic gene expression, following cellular remodeling of PGCs. We propose a 2-step model for the onset of zygotic transcription in the Ciona germline, which relies on successive cellular remodeling and JAK inhibition, and discuss the significance of germ plasm dislocation and remodeling in the context of developmental fate specification.

Scrambled genomes in *Oikopleura dioica*, tunicates and the whole Tree of Life.

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The appendicularian Oikopleura dioica was thought to be a globally distributed species since its description by Hermann Fol in 1872. However, cryptic speciation started to be suspected when the first O. dioica genome was published in 2010, reporting strong nucletotide divergence between Atlantic and Pacific isolates.

We searched for morphological markers that could differentiate between the populations sampled near Okinawa (Japan) and those sampled near Barcelona (Spain) or Osaka (Japan), and only found egg diameter to be statistically different. Nevertheless, we also found that the crosses between Okinawa and Osaka were infertile, and that molecular markers such as ribosomal RNA internal transcribed spacer, or cytochrome oxidase 1, displayed sequence divergence levels that are indicative of speciation. Surprisingly, these sequence analyses suggested the Barcelona and Osaka population to be closer to each other than to Okinawa, which counters geographical intuition, but may be explained by the strong oceanic current separating Okinawa from the main Japanese islands.

Using whole genome sequence and annotation representing each of these populations, our molecular clock analysis confirmed that Okinawa is an outgroup. Chromosomal-level scaffolding of the assemblies revealed an extreme level of structural variation, essentially scrambling most of the gene order between the populations to an extent that was never reported even for closely related species. Application of the tools used for comparing O. dioica genomes to selected species in the Drosophila, Ciona and Caenorhabditis genera suggested that the extent of scrambling observed in O. dioica is exceptional. I will report on the pipelines in which we have streamlined the tools, and on how we apply them to the whole Tree of Life to screen for similar cases, or their absence.

This ITM is a good opportunity to talk together on how to materialize O. dioica’s diversity in the taxonomy. It is essential to represent the genomic diversity of O. dioica in bioinformatics databases. For experimental research, genomes need to be matched to the correct population, which is not straightforward when only a single taxonomic ID is available. Moreover, environmental DNA (eDNA) studies would also benefit from more precise taxonomic assignment. We have recently requested the creation of new taxonomic IDs in the NCBI database, allowing us and others to signal that sequences are more related to the O. dioica found near Okinawa or Osaka than to the ones found near the Atlantic Ocean and Mediterranean Sea. These IDs are at the moment marked “unclassified Oikopleura”, so that our community can later decide at which taxonomic rank to graft them on the Tree of Life.

Investigating the role of DEPDC1 in Asymmetric and Oriented Division of the cardiopharyngeal progenitors of *Ciona*

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In Ciona, the asymmetric oriented division of cardiopharyngeal progenitors, aka trunk ventral cells, after migration is essential for the first heart vs. pharyngeal muscle fate choice. This study focuses on validating the biomolecular networks that control these divisions, as predicted from a broad CRISPR/Cas9 phenotypic screen, with a specific focus on DEPDC1, a RhoGAP protein. Knockdown experiments via CRISPR/Cas9 reveal that DEPDC1 deficiency leads to a shift from asymmetric and oriented divisions to symmetric and misoriented divisions. Our investigation extends to partners of Depdc1 that integrate polarity cues and mediate spindle positioning, including force distributions. To probe these functional modules, we are developing a suite of molecular tools, which includes optogenetic activators and inhibitors, custom nanobodies and biosensors. Additionally, we are developing tension sensors as a novel tool to directly measure these mechanical forces, enriching the quantitative analysis of spindle dynamics during division. These advancements will not only elucidate the role of DEPDC1 but also refine the genetic engineering toolkit available for Ciona. This research will provide crucial insights into the mechanical and genetic factors influencing asymmetric cell division in Ciona, with broader implications for understanding developmental processes and cellular mechanics.

The Effect of Temperature on Early Embryonic Development of Ascidia sp.

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Invasive invertebrate organisms can outcompete native species for resources, leading to disruption in local ecosystems. Solitary tunicate introductions occur across a broad variety of thermal regimes. Here, we investigate flexibility in reproductive and developmental timing of invasive solitary tunicates at different temperatures, and in comparison to their field habitats. We are focused on invasive tunicates at contrasting dock locations: within San Francisco Bay and in a small coastal harbor (Half Moon Bay, HMB) with less variable conditions. In the lab, we are carrying out self- and cross-fertilizations to compare variation in timing and success to early developmental stages. In Ascidia sp., collected at HMB, we found self-fertilization is limited at low (10℃) and high (19℃) temperatures and most successful at an intermediate 14℃. Some individuals at the lowest temperature (10℃) appeared completely unsuccessful at self-fertilization up to 5 hours. Additionally, at 14℃, cross-fertilizations demonstrated a higher percentage of 2-cell stage at 2 hours (~76%), in comparison to self-fertilizations (~11%) at 2 hours. Overall, selfing at the higher temperatures showed faster initial cleaving while the lowest temperature demonstrated a delay, and cross-fertilizations experienced a higher average of initial cleaving than self-fertilization. Early developmental stages are sensitive to low and high temperature effects within the ranges found in their invasive habitat. And self-fertilization is less successful at more stressful low temperatures. These temperature sensitivities in early development could potentially limit or slow the establishment of new populations in some locations.

Hox3 and Cyp26 are required for heart development

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The ascidian heart has a tubular structure with pacemakers at both ends (PH at the hypobranchial end and PV at the visceral end). The direction of blood flow is periodically reversed. We have identified genes predominantly expressed in the PH and/or PV regions of Ciona robusta heart by means of RNA-Seq analysis. These genes included Hox3 and Cyp26.

Hox3 is the most strongly expressed gene among genes predominantly expressed in the PH and PV regions. In situ hybridization of isolated hearts revealed that Hox3 was expressed in non-muscle cells near the border between the heart tube and the blood sinus. We attempted a TALEN-mediated heart-specific knockout of Hox3. Expression of TALEN genes under the control of a Mesp regulatory region resulted in individuals with irregularly swollen heart tubes. In these individuals, the heart was beating, but the rhythm of the heartbeat was abnormal.

Cyp26 is another gene predominantly expressed in the PH and PV regions. In situ hybridization visualized a gradient of Cyp26 mRNA, with the strongest staining at both ends of the heart tube. Cardiac-specific knockout of Cyp26 resulted in individuals with no beating heart. These results suggest that Hox3 and Cyp26 are involved in pacemaker development.

DRESS, a tunicate-specific synaptobrevin/VAMP, is responsible for the initiation and the evolution of tunicate-specific metamorphosis

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Acquiring novel traits is essential for driving evolution. Among chordates, the tunicates, other than appendicularians, are characterized by their vase-like adult shape. This shape is constructed by complete, holometabolic metamorphosis from a tadpole larva. How the ancestor of tunicates acquired this mode of metamorphosis is a central question in relation to chordate evolution that, due to the complexity of metamorphic events, remains elusive.

The emergence of group-specific genes is a simple mechanism for acquiring novel traits. In the ascidian Ciona, we have shown that group-specific genes or isoforms, such as those encoding cellulose synthase, serine racemase, and trimeric G-protein, contribute to metamorphic events. In this presentation, we will report that a novel protein DRESS plays a key role in tunicate metamorphosis and posit that the acquisition of DRESS facilitated the evolution of tunicate metamorphosis.

We recently demonstrated that the sequential activation of three types of G-proteins initiates Ciona's metamorphosis. Among them, Gq synthesizes IP3 and diacylglycerol (DAG) as the second messenger molecules. We found that DAG is a substantial inducer of metamorphosis. A primary target of DAG is known to be protein kinase C (PKC). We searched for PKC expressed in the adhesive papillae, the central organ for metamorphosis initiation. One PKC homolog is abundantly expressed in the papilla region compared to the remaining trunk. However, the protein encoded by this gene has a synaptobrevin-like domain and a transmembrane domain instead of a kinase domain. We named this protein DRESS, after Diacylglycerol-RESponsive Synaptobrevin. Knockdown with an antisense morpholino oligonucleotide showed that dress is necessary for metamorphosis. The predicted 3-dimensional structure and biochemical analyses indicate that DRESS interacts with syntaxin and SNAP25, which are significant components of the SNARE complex, suggesting that DRESS functions as a synaptobrevin/VAMP.

Phylogenetic analyses indicate that DRESS is a tunicate-specific protein, present in both ascidians and the thaliacean Dolioletta gegenbauri. However, DRESS is not found in the Oikopleura dioica genome. These data, together with its necessity for metamorphosis, suggest that the emergence of DRESS may have contributed to the acquisition of metamorphosis in the tunicates.

Adapting to the future: Salinity and temperature tolerance of the non-indigenous ascidian *Phallusia nigra*

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Ascidians are important members of fouling communities worldwide, transporting between different environmental conditions with the essential ability to cope with wide environmental conditions. Phallusia nigra is an example for a widely distributed solitary ascidian native to the Red Sea, and considered non-indigenous in the Mediterranean Sea and Singapore. Here, we aimed to compare the ability of non-indigenous and native populations to adapt to changing environmental conditions. For this, a comparison between three different populations of P. nigra were done, from native and introduced ranges. Initially, artificial fertilizations were done from all populations to culture P. nigra under laboratory conditions, a protocol designed for this study. Later, using lab-cultured juveniles, a multi-factorial stress experiment was conducted for a month, composed of three different salinities: 35, 40, 43 [PSU] and three different temperatures: 16, 25, 31 [°C]. Survivability was evaluated three times a week, and blood-flow current direction was measured once a week. Results show that the most significant factors for survival were salinity for the Mediterranean Sea population and temperature for the Red Sea population. The Singapore population presented notable survival rates throughout the experiment at 25 and 31 °C, regardless of the salinity treatment. Low temperature had a significant effect on the survival of the Mediterranean and Red Sea populations.

These results will contribute to creating a worldwide species distribution model of P. nigra under different global change scenarios and will actively contribute to the global efforts to protect marine environments.

Characterization of nickel-induced oxidative stress response in Botryllus schlosseri

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Botryllus schlosseri is a colonial ascidian species found globally in temperate coastal zones. Colonies consist of three generations: parent zooids, developing primary buds, and emerging secondary buds; all embedded in a shared tunic and organized into star-shaped systems. In B. schlosseri, weekly blastogenic generational succession, a form of stem-cell mediated asexual reproduction, is marked by the apoptosis and autophagy of zooid tissues as the primary buds mature into the next set of self-sustaining zooids. This cycle between degeneration and development makes B. schlosseri an excellent model for studies on regeneration, aging, and cellular biology. Despite decades of extensive research on optimizing culturing methodologies for B. schlosseri, a cell line has not been established. This study uses nickel (II) chloride to characterize toxicity responses, including oxidative stress pathways, in B. schlosseri. We aim to use in vivo data to inform future cell culture efforts, exploring nickel-induced mutagenesis as one potential method for cellular immortalization. Nickel genotoxicity induces DNA breakage via cellular accumulation of reactive oxygen species. As nickel toxicity in B. schlosseri was not characterized, we established the acute lethal concentration (LC50) for field-sourced adults (LC50 24-hour exposure = 864 mg L-1). Mortality was marked by the cessation of blood flow within the system and ampullae. A Superoxide Dismutase (SOD) assay will quantify the enzymatic antioxidant response to increasing concentrations of nickel. Future work will involve the application of RNA-sequencing and proteomics to analyze underlying altered molecular pathways of primary buds from sublethally nickel-exposed B. schlosseri. Funding provided by NSF MCB-2127517.

Epigenetic landscape of the scrambled genome of Oikopleura dioica

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Oikopleura dioica is a species of pelagic tunicate that is present in all oceans in the world. It is used as a model for developmental studies due to its fast life cycle and ease of culture. We have shown that O. dioica obtained from different regions of the world have highly rearranged genome, despite being morphologically indistinguishable, and that gene expression of orthologs are highly correlated. In this research, through experimental and computational approaches, we aim to describe the epigenomic landscape of O. dioica and compare different populations to investigate the conservation of gene regulation across highly rearranged genomes. We found that O. dioica has a small number of enhancers, mostly located within the intronic regions. We also found that inference of transcription factor binding correlates with gene expression and might explain gene expression conservation across populations.

Extended Embryo Retention in Chordates

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Ascidians, invertebrate chordates that are closely related to vertebrates, provide a unique opportunity to study the evolution of viviparity. Comparative studies with ascidians may reveal critical insights into viviparity, highlighting similarities with placental animals, such as reduced egg count, elongated gestation, and complex extraembryonic membranes. Hypsistozoa fasmeriana, a marine colonial ascidian endemic to Aotearoa, New Zealand, is a remarkable case study of viviparity. This species nurtures its offspring for approximately 5.5 months, relying on the parent for sustenance. Despite originating from a small, low-yolk egg (25 µm), the resulting tadpole grows to 6-8 mm. Extended embryo retention has long been recognised as pivotal in transitioning from oviparity to viviparity. We sequenced the H. fasmeriana genome in the first step to understand how this ascidian provides for its young for such an extended period of time.