PASEDB25 Abstracts

follistatin paralogs connect environmental to genetic control of wing dimorphisms in pea aphids

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Understanding how morphology evolves requires identifying the types of mutations that contribute to changes in development. Our focus is on wing dimorphism - the production of winged and fully wingless morphs within the same population - in pea aphids (*Acyrthosiphon pisum*). The species exhibits two wing dimorphisms, one which is induced by environmental conditions in genetically identical, asexual females, and one which is controlled by a single genetic locus in males. We previously showed that a *follistatin* gene is responsible for the male wing dimorphism. Here we describe the three *follistatin* paralogs that exist in the pea aphid genome and implicate one of these paralogs in control of the asexual female wing dimorphism. We also find that an ancestral *follistatin* gene likely had multiple promoters and that the *follistatin* duplicates that evolved wingless-specific expression retained only the ancestral wingless-specific promoter. Our work provides a roadmap for how gene duplication and subsequent divergence can enable the evolution of animal form.

Genetic underpinnings of the proximo-distal elaboration of pectoral appendages during the fin-to-limb transition

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The origin of the tetrapod limb remains an enigmatic puzzle in evolutionary biology. The elaboration of skeletal elements along the proximodistal axis is a hallmark of the fin-to-limb transition, marking the emergence of the tetrapod limb. While fish fins consist of proximal radials and distal fin rays, tetrapod limbs exhibit a distinct segmentation pattern, with three progressively specialized regions: a single proximal bone, two middle bones, and multiple distal bones. This segmentation pattern, absent in fins, defines tetrapod limb evolution. Despite recent discoveries in paleontology and embryology, the fundamental mechanisms governing skeletal elaboration remain elusive. We address this gap by analyzing the functions of *Hox* genes, homeobox transcription factors that play pivotal roles in establishing the proximodistal positional information during appendage development. Our initial transcriptomic analyses revealed that *Hoxa13*, which is indispensable for patterning distal extremities of pectoral appendages, represses a cohort of chondrogenic genes for patterning the distal bones of the zebrafish pectoral fin. Interestingly, Hoxd9a, a gene implicated in chondrocyte differentiation in tetrapods, emerged as a key player in this regulatory network. To determine the role of *Hox9* in fin patterning, we generated functional knockout of hox9 paralogs in zebrafish, and our preliminary analysis suggests that they play a role in patterning proximal elements of the fin endoskeleton. To further explore the interaction between *Hox9* and *Hox13* genes in zebrafish fin patterning, we generated a paralogous knockout of *Hoxa13* in combination with *Hoxd9a*. Our findings indicate that this combinatorial knockout leads to increased proximal to distal segmentation in the fin endoskeleton. This intriguing pattern may reflect a recapitulation of skeletal elaboration observed during the fin-to-limb transition. These newly obtained knowledge would lead to the molecular understanding of the proximodistal elaboration of the pectoral appendages coupled with Hox functional changes.

Insights into the origin of vision: uncovering a conserved photoreceptor cell differentiation mechanism

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Rhabdomeric and ciliary photoreceptor cells are the most commonly found photoreceptor cell types of animal visual systems. Both cell types are found in most animals that have been investigated, but typically only one type is used in a visual context. Light detection in rhabdomeric photoreceptor cells is facilitated by an r-opsin protein, and these cell types are typically associated with invertebrate visual systems. The rods and cones of vertebrate eyes are ciliary photoreceptor cells and utilize c-opsins to detect light. Understanding the gene regulatory network that guides the differentiation of these two photoreceptor cell types can lead to a deeper understanding of how vision evolved within animals. In this study, we use the tardigrade *Hypsibius exemplaris* to test a model of photoreceptor cell differentiation built on a series of mouse and fly studies. In this model of photoreceptor cell differentiation, orthologs of the transcription factors *orthodenticle* (*otd*), *pax6*, and *Six* family genes specify photoreceptor cell progenitors. Next, orthologs of *retinal homeobox* (*rx*) specify ciliary photoreceptors, and *atonal* (*ato*), *Barh*, and *prospero* (*pros*) specify rhabdomeric photoreceptors. Because *c-opsins* are not present in the genomes of many protostome species, including the popular model systems *Drosophila melanogaster* and *C. elegans*, the investigation of this mechanism in *H. exemplaris* is the first of its kind within Ecdysozoa, the molting animals. We used hybridization chain reaction in situ to analyze overlapping expression patterns of opsins and transcription factors of the model. We found that *He-otd*, *He-pax6*, and *He-six3* show overlapping patterns with r-opsins and c-opsins during tardigrade development, supporting a conserved role of these genes in early differentiation of photoreceptor cells. Additionally, preliminary results from the hypothesized type-specific transcription factors *pros* and *rx* suggest further conservation of some aspects of the model.

An *Sp6-9* ortholog is expressed in developing legs in the tardigrade *Hypsibius exemplaris*

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The transcription factor Sp6-9 is known for its role in patterning the developing appendages of arthropods. In some arthropods, sp6-9 functions to regulate outgrowth by activating expression of the transcription factor Distal-less (Dll). Expression patterns of sp6-9 and Dll have been found in the developing appendages of the onychophoran *Euperapotoides kanangrensis*, suggesting that sp6-9's role in mediating outgrowth through regulation of Dll might be an ancestral feature of these two closely related lineages. Here, we investigate sp6-9 orthologs in *Hypisibius exemplaris*, a representative of Tardigrada, a phylum of animals that is the sister group to the arthropod+onychophoran lineage. We uncovered two orthologs of sp6-9 in *H. exemplaris*. Expression of *He-sp6-9a* was found in the lateral ectoderm of tardigrade embryos at a stage of development before leg anlagen or *He-Dll* expression appears. Later, during the earliest stages of limb outgrowth, both *He-Dll* and *He-sp6-9a* are expressed. This overlapping pattern of *He-sp6-9a* and *He-Dll* in developing legs persists for the remainder of development. Our results suggest that *He-sp69a* functions to promote limb outgrowth in *H. exemplaris*, possibly through the regulation of expression of *He-Dll*, and that this function of *sp6-9* was likely present in the last common ancestor of Panarthropoda.

Evolution of dominance in color dimorphism in the *Drosophila montium* species subgroup is directly linked to evolution of phenotypic plasticity

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Allelic dominance and phenotypic plasticity influence how populations adapt by shaping the genotype-phenotype map. As there are many cases where discrete phenotypes flip at some threshold value when genotype or environment changes, dominance and plasticity can stem from the same features of development. However, direct connections between these two phenomena remain unclear. We investigated these connections using the Mendelian, female-limited color dimorphism found in many species of the *Drosophila montium* species subgroup. Those species have dark and light phenotypes, and the Dark allele is dominant in most species. However, in *D. bocqueti* and *D. jambulina,* the Light allele is dominant. By GWAS analyses, we revealed that independent regulatory mutations in the *POU domain motif 3* (*pdm3*) gene, a repressor of pigmentation, are responsible for the color dimorphism in multiple species. And selective genotyping showed that the dimorphism is linked to the pdm3 locus in both Dark-dominant and Light-dominant species. Then, we demonstrate that interspecific differences in dominance relationship between pdm3 alleles are due to interspecific changes in phenotypic plasticity. In Dark-dominant species, the Dark *pdm3* allele is dominant across all developmental temperatures. In contrast, Light-dominant species showed temperature-dependent dominance, with the Light pdm3 allele becoming increasingly dominant at higher temperatures. Those results showed that the evolution of dominance relationship between *pdm3* alleles is directly connected to the evolution of thermal plasticity. To understand the mechanism that creates phenotypic differences between dark and light phenotype by regulation of pdm3 gene, we conducted antibody staining and in situ hybridization. Those staining experiments did not show an obvious difference in the expression pattern of Pdm3 protein or mRNA between morphs. The results suggest that quantitative differences in gene expression levels may be responsible for creating discrete phenotypes. Taking these results together, we propose that threshold-like property of development connects dominance and plasticity.

tRNA Genes; Annotation of two Long-Read Chordate genomes

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Transfer RNAs (tRNAs) are a kind of non-coding RNAs (ncRNAs) that are involved in protein synthesis and genome regulation. Despite their importance, their genomic organization and duplication patterns remain under explored in non-model chordates. In this study a comprehensive annotation of tRNA genes is performed using long-read genome assemblies of *Latimeria chalumnae* (fLatCha1) and *Branchiostoma lanceolatum* (klBraLanc5), along with a short-read assembly of *L. chalumnae* (latCha1), to evaluate the impact of sequencing technology on ncRNA discovery. The raw gene Predictions from ARAGORN and tRNAscan-SE 2.0 identified over 30,000 tRNA gene candidates, but only 511 were shared by both tools, underscoring significant methodological variation. A pipeline was developed to unify predictions based on chromosomal coordinates and strand orientation, producing a non-redundant tRNA gene set for downstream analyses. Then initiator tRNAs (anticodon CAT) were classified using sequence-based hierarchical clustering (Levenshtein distances, ward.D2), revealing three cluster of genes classified like initiators, elongators and pseudogenes and also Genomic clustering based on spatial proximity (less or equal to 1 kb) showed that tRNAs often co-localize in conserved gene blocks. Functional clustering using isotype presence/absence matrices (Jaccard distances) further supported this modular organization. Additionally tandem duplications of tRNA genes within genomic clusters were detected particularly in L. chalumnae, suggesting recent local expansions. Chromosome-level density plots and Rfam annotations highlighted hotspots of ncRNA accumulation, especially in long-read assemblies. This work demonstrates the power of integrating predictive tools with clustering and functional analyses to uncover ncRNA dynamics at high resolution. The resulting dataset and pipeline provide a robust framework for exploring tRNA gene architecture and evolution across vertebrates and other chordates.

The Insatiable Sweet Tooth: How Evolution Rewired Insulin Signaling for Metabolic Resilience in Nectar-Feeding Bats

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Nectar-feeding bats thrive on a diet of simple sugars, rapidly lapping up floral nectar while hovering over flowers. This behavior routinely exposes them to postprandial blood glucose levels exceeding 750 mg/dL—levels that typically cause metabolic distress in mammals. Yet, these bats live 10-15 years, comparable to related species with more balanced diets. To understand how they tolerate extreme sugar intake, we analyzed gene expression and conducted ex vivo functional experiments in skeletal muscle across seven related bat species using phylogenetically informed comparisons. We found that nectar bat muscle shows impaired activation of the canonical insulin signaling pathway involving AKT and GLUT4, lacks glycogen storage, and

instead accumulates elevated lipid levels—features consistent with insulin resistance. Using primary muscle cells expressing fluorescently tagged GLUT4, we confirmed that while GLUT4 is present and capable of translocation, its activation via insulin–AKT signaling is impaired. This disruption is expected to relieve suppression of FOXO3a, a transcription factor associated with stress resistance and antioxidant defense. Despite reduced insulin-stimulated AKT-GLUT4 signaling, glucose can still enter cells through passive transporters such as GLUT1. Elevated blood lactate suggest a glycolytic shunt that diverts intracellular glucose toward lactate production, aiding NAD^+ regeneration and supporting redox homeostasis. This shift may also activate MAPK signaling, further stabilizing FOXO3a. Supporting this, proteomic and transcriptomic analyses revealed elevated antioxidant proteins and gene expression patterns consistent with enhanced redox buffering. Our findings suggest that nectar bats have evolutionarily rewired insulin signaling, resulting in impaired AKT-GLUT4 activation, extreme hyperglycemia, and reduced muscle glycogen. We propose that acute hyperglycemia functions as a short-term energy reserve to fuel hovering flight, similar to hummingbirds. By uncoupling chronic sugar intake from cellular damage, this adaptation promotes both glucose handling and metabolic resilience. Together, these features support life on an extreme sugar diet and offer insight into how mammals survive and adapt to metabolic extremes.

Glyco-EvoDevo: Chitin and the non-canonical uses and evolution of this highly abundant biopolymer

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Chitin is a major component of rigid biological structures such as the exoskeleton of arthropods and is the second most abundant polymer in the biosphere. The molecule is produced by myriad organisms using enzymes called chitin synthases. For decades it had been widely assumed that vertebrates do not produce endogenous chitin, but our laboratory unexpectedly discovered chitin synthase genes in the genomes of aquatic vertebrates (fishes and amphibians) and demonstrated that these genes were indeed responsible for chitin biosynthesis. The locations where chitin are found in the vertebrates are varied and include the integument, the gut, the electrosensory organs of the chondrichthyan fishes (sharks, skates, and rays), and other structures. Phylogenetic analysis indicates that chitin synthase genes have been lost in the lineage leading to amniotes (reptiles, birds and mammals). Biophysical studies of the structure of vertebrate chitin suggest that it exists largely in an unorganized and amorphous state, unlike that of rigid structures such as shrimp shells or cephalopod teeth. The lack of distinct crystallinity in many of the “soft matter” chitinous materials in nature suggests that chitin per se is much more malleable than previously thought and that it may have contributed to the emergence of novel structures in nature.

Profiling cellular diversity in sponges informs the evolutionary origin of neurons and coordinated behavior in animals

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The evolutionary origin of neurons and coordinated behaviors in animals is a major unresolved question. Using whole-body single-cell RNA sequencing in a sponge, an animal without nervous system and musculature, we identified 18 distinct cell types. These include myoepithelial cells responsive to nitric-oxide and secretory neuroid cells that reside in close proximity to digestive cells expressing postsynaptic scaffolding and receptor proteins. Visualizing neuroid cells by correlative x-ray and electron microscopy revealed secretory vesicles and cellular projections enwrapping digestive cell microvilli and cilia. Our data show a communication system that is organized around sponge digestive chambers, using conserved modules that became incorporated into the pre- and postsynapse in the nervous systems of other animals.

How to make a Jellyfish. Transcriptomic profiling of the chemically induced strobilation in the jellyfish *Aurelia sp.*

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The origin and the evolution of the basic body plan in bilaterians is an essential step in animal biology, and to decipher the degree of complexity in their direct ancestor should prove central to answer this question. Before bilaterian origins, the cnidarians (corals, sea anemones and jellyfishes) radiated and diversify in marine environments. there is phylogenetic evidence that Cnidarians and Bilaterians are a sister groups, so it is highly likely they have a common ancestor. Both taxonomical groups have striking morphological and genetic similarities during development. In nature, the cnidarian jellyfish, *Aurelia sp*, spend a long time as a tiny polyp attached to the sea floor, and environmental changes such as temperature, trigger their transformation to the free-swimming medusa stage through a process known as strobilation. However, the chemical compound 5-methoxy-2methyl-indole (5Me2MI) has been shown to induce strobilation five times faster than temperature and at concentrations as lower as 1 nM. We study 5Me2MI induced strobilation in Aurelia sp. looking to understand the molecular signaling and cellular events leading to the initial morphological transformations from a polyp to an ephyra (jellyfish juvenile stage). As a first step we carried out a transcriptomic analysis on the first days after 5Me2MI induction. We found that in just 24 to 48 hours after induction and before any morphological changes were apparent, about 250 genes have changed their expression. While close to 100 genes were downregulated, more than 150 genes became upregulated. Some of those genes were known to regulate morphogenetic events in bilaterians as *wnt* and homeobox genes, which opens the question if they have a similar function in *Aurelia sp*. Other genes changing their expression during strobilation induced by 5Me2MI were related to signal transduction, cell proliferation, oxidative stress, epigenetic regulation, metabolism and several other cellular and physiological processes.

Evolutionary morphospace of the *Batomorphi pectoral* fin: Swimming Innovations of Rays and Skates.

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Batoids (skates, rays and guitarfishes) are a highly diverse group of marine cartilaginous fishes with ample ecological adaptations and swimming modes. Paleontological findings and phylogenetic analyses suggest that convergence among batoids has happen multiple times. In this work we employed geometric morphometrics and phylogenetic comparative methods to characterize the evolutionary trends in the basal fin skeleton of extinct and extant batoids and dorsoventrally flattened sharks. We found that while Torpediniformes and Rhinopristitiformes have the highest disparity, the most speciose orders of batoids, Myliobatiformes and Rajiformes, display the lowest levels of morphological disparity. Differences in evolutionary rates by habitat indicate that both reef and freshwater species evolved faster than deep-sea and shelf-distributed species. Besides, we look at the differences based on swimming modes and found that Bateoids with oscillatory swimming exhibit higher evolutionary rates on their coracoid bar, and that specific groups underwent different rates of evolution on each element of the pectoral fin. To confirm this, we carry out modularity and integration analyses, that show differences in the covariation between structures among the analyzed groups. However, the convergence analysis does not support the resemblance between flattened sharks and batoids; however, we found convergence between extinct batoids and modern guitarfishes (also known as shovelnose rays). These findings suggest that habitat and swimming mode have shaped the pectoral fin evolution among batomorphi. Unfortunately, about one fifth of batoids are considered under extinction threat. We also analyzed the morphological disparity between species under different extinction threat to identify a possible trend towards homogenization of morphological variation, and we found that species richness between different areas does correlate with morphological disparity metrics. Some regions with less species richness present high morphological disparity, as in coral reefs. The species under extinction threat present the highest disparity, unmatched by the species at lower extinction risk.

Specification of the primary embryonic axis in metazoan embryos: Insights from sea star development

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A critical early event during embryonic development in metazoans is the specification of the primary embryonic axis, the anterior-posterior (AP) axis in bilaterians. AP axis formation is strongly influenced by the maternally established animal-vegetal (AV) axis of the oocyte. A conserved mechanism for AP axis specification is through the localized activation of Wnt/β-catenin (cWnt) signaling in blastomeres derived from the vegetal pole. Studies in echinoderms have demonstrated that Dishevelled (Dvl), a central cWnt regulator, is required to activate this pathway in vegetal pole-derived blastomeres. Dvl protein localizes to puncta throughout the cortex in oocytes of the seastar *Patiria miniata*, and during maturation, these puncta vanish, and new

puncta form at the vegetal pole. The maternal mechanisms that regulate Dvl activity in the early embryo are unknown. To identify the maternal factors that regulate Dvl and cWnt activation in vegetal blastomeres, I conducted RNA-seq and mass spectrometry on isolated Patiria oocyte animal and vegetal halves and found several mRNAs, long non-coding RNAs, and proteins that are differentially enriched at the two poles. After validating the asymmetric enrichment of these factors to either the vegetal or animal pole, I will experimentally test the hypothesis that these factors specify the AV axis and regulate AP axis formation by establishing cytoarchitectural polarity in the oocyte and/or activating cWnt signaling at the vegetal pole. This work will provide valuable insights into the maternal mechanisms that specify the AV axis in echinoderms and into the evolution of this fundamental polarity in animal eggs.

Identification of non-coding elements associated with the evolutionary loss of digit 1

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Modern tetrapods evolved pentadactyly (five digits on each limb) from ancestors with more than five digits. However, despite the fact that tetrapods have never re-evolved more than five digits, digit loss has evolved many times. Within mammals, digit 1 (thumb on the forelimb or hallux on the hindlimb) is the most frequently lost digit, and is typically lost before any other digits in evolution. Moreover, digit 1 is usually lost independently between the fore- and hindlimb. Evidence from numerous studies of limb development suggests that digit 1 development is at least partially independent of the other digits. However, specific genetic mechanisms driving the loss of digit 1 have not been uncovered. Using a suite of bioinformatic tools, genomes, and RNAseq data, we have identified a set of conserved non-coding elements which tend to evolve in concert with the loss of digit 1. These elements are enriched in proximity to genes with important roles in digit 1 development. Furthermore, we find that elements with accelerated rates of evolution in digit-reduced species show significant loss of binding sites for transcription factors that are crucial to limb development and chondrogenesis. These results suggest that regulatory elements important for the development of digit 1 are evolutionarily accelerated in mammalian lineages that have undergone digit 1 loss.

Genetic mechanisms regulating sucker development in cephalopod limbs

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The limbs of coleoid cephalopods (octopus, squid, and cuttlefish) are characterized by specialized suckers, a morphological innovation used for hunting, locomotion and chemo-sensation. Sucker development begins with the emergence of the sucker ridge that subdivides to form discrete sucker buds. The sucker buds then further divide (in some species) and undergo morphogenetic remodeling to form the cup-shaped suckers. While genetic mechanisms regulating sucker field induction and patterning remain unknown, morphological and gene expression studies suggest that conserved regulatory mechanisms might be involved in sucker development. We have characterized the molecular development of suckers in the dwarf cuttlefish, *Sepia bandensis*. Previously, we found that induction of the sucker field is coordinated with development of the three main axes of the limb, which are regulated by the same gene networks that act in insect and vertebrate limbs. Now we show that developing suckers express conserved regulators of developmental boundaries, suggesting that conserved morphogenetic mechanisms are involved in sucker patterning.

Gut patterning in the tardigrade *Hypsibius exemplaris*

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A tripartite through-gut is a prominent feature of the bilaterian body plan. The tripartite through-gut includes foregut, midgut, and hindgut sections, bookended by a mouth and anus. Despite an incredible diversity of gut anatomies and morphogenetic processes underpinning gut development, gut patterning is highly conserved across Bilateria. Tardigrada is the outgroup of the Onychophora + Arthropoda clade within Panarthropoda. Features of the tardigrade foregut — a protrusible mouth cone, triradiate pharyngeal lumen, pharyngeal armature, an anterior-terminally positioned mouth, and stylets — closely approximate features of ancestral panarthropods. By contrast, tardigrades have a highly secondarily miniaturized and compact body plan, and the consequences of these modifications on gut anatomy and development are unclear. Therefore, elucidating gut patterning in Tardigrada could provide insight into the conservation and diversification of the gut in Panarthropoda. We investigated the expression patterns of several canonical gut patterning genes in the tardigrade *Hypsibius exemplaris*. We resolved a molecular code for all parts of the tardigrade gut. Our results indicate that tardigrades retain foregut, midgut, and hindgut regions that are directly homologous to their counterparts in other panarthropods and more distantly related bilaterians. Furthermore, our results indicate that the tardigrade midgut was reduced in length relative to the foregut and hindgut during miniaturization and compaction of the body plan. Additionally, our results reveal that the homeodomain transcription factor coding gene *orthodenticle* (*otd*) played a broad pattering role in the foregut in the last common ancestor of Panarthropoda, a role that is retained in modern tardigrades, but has been reduced or lost in some other panarthropod lineages.

Investigating the link between the wound response and initiation of regeneration in Ctenophora

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The ability to regenerate missing body parts lost due to injury is broadly distributed in the animal kingdom. However, the origin and evolution of this trait remains a mystery to this day. Comparative studies of the cellular and molecular mechanisms that regulate regeneration can provide insight into the evolution of regeneration by revealing unifying principles. In animals like Hydra, acoels, planarian, and flies, successful regeneration requires a wound induced gene regulatory network consisting of mitogen-activated protein kinase (MAPK), Immediate early genes (IEG), and the Wnt pathway. Previous work in the early branching Ctenophora demonstrated upregulation of

IEGs immediately following injury and upregulation of Wnt components after wound healing. Here, we investigate MAPK signaling during the wound healing and regeneration in the ctenophore *Mnemiopsis leidyi* by using an antibody against phosphorylated extracellular signal-regulated kinases (ERK). Using hybridization chain reaction (HCR), we also follow gene expression of *jun*, *fos* and components of the Wnt pathway through wound healing and the initiation of regeneration. We detected an increase in ERK signal and expression of transcription factors *jun* and *fos* at the injury site immediately after injury. Additionally, after wound healing is complete, we detected an increase in the expression of β-catenin and transcription co-factor *tcf*. Our data suggests that a conserved MAPK-IEG-Wnt gene regulatory network may also play a role in regeneration in ctenophores as seen in other lineages, although further functional characterization is needed.

Redefining the development of the freshwater sponge canal system using 3D optical coherence microscopy

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The ability to create and maintain a biological tubular system is a feature found in nearly all animals. These tubular systems are composed of either epithelial or endothelial cells, transporting essential liquids and gases throughout the body. Though the size and function of these systems varies greatly within and between animals, they are formed through defined methods of tubulogenesis that possess shared molecular, cellular, and morphological processes. We leveraged sponges (phylum Porifera), an early-branching lineage of animals built around a tubular canal system, to gain insight into the evolutionary origins of tubulogenesis in metazoans. The freshwater sponge *Spongilla lacustris* canal system is composed of morphologically distinct incurrent and excurrent portions that are joined by digestive chambers for filter feeding. Water enters through small pores on the surface and into a large incurrent lumen. From this lumen, water is pumped through incurrent canals into digestive chambers. Water exits into the excurrent canals, where it is then expelled from the sponge through the osculum. Despite the animal's simple body plan, the development of the complete canal system has remained largely uncharacterized due to limited volumetric imaging approaches. Here, we use the optical coherence microscope, a novel method of non-invasive and label-free 3D imaging, to define the developmental morphology of the canal system in gemmule-hatched *S. lacustris*.

Unveiling the role of maternal factors in regulating egg polarity and Wnt/β-catenin signaling during early embryogenesis in the cnidarian *Nematostella*

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Oocytes from most animals have a primary polarity referred to as the animal-vegetal (AV) axis that is established during oogenesis by the asymmetric distribution of maternal determinants. In many taxa the AV axis strongly influences the specification and patterning of the primary embryonic axis through the localized activation of the Wnt/β-catenin (cWnt) signaling pathway by maternal determinants that are asymmetrically distributed along the primary egg axis. In the cnidarian *Nematostella vectensis*, localized activation of the cWnt signaling pathway in animal

pole-derived blastomeres specifies endomesodermal cell fates at this pole and initiates patterning of the primary embryonic axis, the oral-aboral axis. In contrast, in bilaterians such as echinoderms, localized activation of cWnt signaling at the vegetal pole regulates endomesoderm specification and initiates patterning of the anterior-posterior (AP) axis. This observation has led to the hypothesis that both endomesoderm specification and gastrulation evolved at the animal pole in pre-bilaterians by the asymmetric activation of Wnt signaling, whereas these processes have been shifted to the vegetal pole during bilaterian evolution. Identification of upstream maternal regulators of Wnt signaling will be crucial to design experiments to test this hypothesis. To this end, I carried out mass-spectrometry proteomics of isolated animal and vegetal halves from *Nematostella* eggs. This analysis identified proteins that are potentially differentially enriched along the AV axis and are involved in specifying egg polarity and cWnt pathway activation during *Nematostella* embryogenesis. Preliminary functional tests of the roles of these factors in regulating egg polarity and cWnt activation will be presented.

β-catenin localization in the ctenophore *Mnemiopsis leidyi* suggests an ancestral role in cell adhesion and nuclear function

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The emergence of animal multicellularity marks a pivotal evolutionary event which was enabled by molecular innovations in the way cells adhere and communicate. β-catenin is significant to this transition due to its dual role as both a structural component in the cadherin-catenin complex and as a transcriptional coactivator involved during Wnt/β-catenin signaling. However, our knowledge of how this protein functions in ctenophores- the earliest diverging extant metazoan taxon- is limited. To study β-catenin function in the ctenophore *Mnemiopsis leidyi*, we generated affinity-purified polyclonal antibodies targeting Mlβ-catenin. This reagent was used to observe β-catenin protein localization in *Mnemiopsis* embryos. Evidence of consistent β-catenin protein enrichment at cell-cell interfaces in *Mnemiopsis* embryos was discovered, suggesting that Mlβ-catenin has a role in cell adhesion. Additionally, β-catenin was found enriched in some nuclei, particularly restricted to the oral pole around the time of gastrulation, suggesting Mlβ-catenin may have nuclear function in *Mnemiopsis* embryos. We now aim to use these antibodies to identify proteins that interact with Mlβ-catenin through co-immunoprecipitation and mass spectrometry. Additionally, by combining in silico AI-based protein interaction prediction tool like AlphaFold with in vitro transcription-translation assays, we aim to better understand how these proteins are interacting. Our ultimate goal is to conduct functional studies in *Mnemiopsis leidyi* to unravel the regulation of embryonic β-catenin in this lineage, providing insights into the evolution of animal multicellularity.

Hybrid incompatibility emerges at the one-cell stage in interspecies *Caenorhabditis* embryos

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Intrinsic reproductive isolation occurs when genetic divergence between populations disrupts hybrid development, preventing gene flow and reinforcing speciation. Over the past two decades, researchers have identified molecular mechanisms underlying a few dozen cases of hybrid incompatibility in animals. Much of this work has focused on mismatches in zygotic gene regulation, but other mechanisms have also emerged, including symbiont-driven incompatibilities, nucleoporin mismatches affecting nuclear-cytoplasmic transport, and divergence in centromeric or heterochromatic regions and their regulatory proteins which can lead to the inability of the oocyte cytoplasm to segregate sperm-derived chromosomes. Since studies to date have focused on a limited number of species, uncovering mechanisms across diverse taxa will be important for understanding broader patterns of hybrid incompatibility. In this talk I will describe the mechanistic basis of hybrid incompatibility in *Caenorhabditis* nematodes. Within this group, most species pairs do not mate, and hybrids typically die during embryogenesis in those that do. Although individual embryos within a cross arrest at variable timepoints throughout development, I will show that incompatibilities typically originate between fertilization and the 4-cell stage, prior to the onset of zygotic transcription. In *Caenorhabditis,* like most animals, sperm deliver chromatin and centrioles into the oocyte. After remaining quiescent during oocyte meiosis, the sperm chromatin acquires a nuclear envelope, and centrioles initiate centrosome formation. Centrosomes remain tethered to the sperm pronucleus, which positions them near the cortex to establish anterior-posterior polarity. There are two key processes that are destabilized in hybrids: (1) the ability of oocytes to control sperm-derived pronuclear expansion, and (2) successful polar body formation. When sperm pronuclear expansion is delayed, centrosomes detach, which leads to defects in polarity establishment. Hybrid embryos typically experience one or more stochastic failures of early developmental events that accumulate and eventually kill them.

Reconstructing ancient synaptic architecture with single-cell RNA-seq and deep learning

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Proteins act as cores of animal cells where they orchestrate distinct functions. However, how protein-protein interactions diversified across cells in animal evolution is poorly understood. Here, we focus on reconstructing interaction profiles of synaptic proteins across different animals to uncover the conserved and divergent constituents of early animal synapses throughout evolution. To do this, we integrate full proteomes and single-cell transcriptomic data across diverse species, into a unified analytical pipeline. First, we use human synaptic gene modules as templates to locate homologous modules across a broad range of species, from vertebrates to invertebrates. We comprehensively identify known synaptic homologs across animals using sequence matching-based and protein language model-based homology detection tools. Then, to refine our list of potential synaptic protein interaction partners for each cell type we perform co-expression analysis using published single-cell data and identify co-expressed synaptic proteins as well as potential novel interacting partners. A novel deep learning model is developed to predict the protein interaction profile of each candidate. Lastly, we will perform experimental validation to examine the predicted modules. The results of this study will reveal a clear map of the evolutionary path of synaptic gene modules and uncover novel mechanisms and potential new types of synapses.

Notch expression during ctenophore development gives insight into its ancestral function

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The canonical Notch pathway is a juxtacrine signaling module with widely conserved roles maintaining progenitor cell populations, promoting binary cell fate decisions, and establishing tissue boundaries. This pathway is likely a Metazoan innovation, although genomic evidence in single-celled Eukaryotes suggests that domain shuffling led to the formation of Metazoan Notch. Given that the vast majority of Notch studies focus on Bilaterians, it is unclear when or how Notch gained its developmental signaling functions. To clarify the ancestral function of Notch, we turned to an early branching Metazoan — the ctenophore *Mnemiopsis leidyi* — and conducted structural analyses of putative Notch components to evaluate predicted signaling potential. We characterized gene expression of these components with in situ hybridization and show they are expressed in highly proliferative zones during late embryogenesis. Using HCR (hybridization chain reaction), we examine MlNotch expression relative to canonical transcriptional targets and putative stem cells and identify differential expression patterns at a cellular resolution. We are also troubleshooting knockdown methods to evaluate MlNotch activity during late embryonic development. These characterizations suggest that Notch evolved its signaling potential by the Metazoan divergence and was likely involved in regulating progenitor cell populations and coordinating developmental fate decisions.

Resolving the polygenic basis of regulatory polymorphisms in abdominal pigmentation between *D. yakuba* and *D. santomea*

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Examples of adaptations, especially in animal form, are at least as countless as there are species. But we know surprisingly little about which types of genetic variation form the substrate from which new morphological traits arise, which evolutionary forces govern their fate, and ultimately, how these microevolutionary processes lead to the macroevolutionary patterns we see between species. The inherent difficulty of linking segregating genetic variants in a population to developmental variation in a focal trait requires precise molecular approaches that can both measure and validate the functional effects of causal variation. Here, the core goal is to leverage the tractability of abdominal pigmentation differences in the sister species *Drosophila yakuba* and *D. santomea* to precisely recapitulate the polygenic basis of their regulatory differences. To validate candidate cis-regulatory element polymorphisms between these species, I use common landing site reporter constructs, hybridization chain reaction in situ (HCR), and scarless homology-directed repair approaches. Together, I can validate and refine the minimal changes required to recapitulate *D. santomea* differences into *D. yakuba* using a novel targeted introgression approach that can uncover the more subtle masking epistatic effects between loci. This type of thorough, molecular genetic analysis is a necessary first step in understanding how population genetic processes can generate the types of species differences we see everywhere we look.

The evolution of the Cadherin-Catenin-Complex in the ctenophore species *Mnemiopsis leidyi*

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The Cadherin-Catenin-Complex (CCC) is a crucial component of adherens junctions in epithelia for both maintaining homeostasis, normal embryonic development, and cell-cell adhesion. The CCC allows epithelial cells to adhere to their neighboring cells through a mechanical interaction between classical cadherins and the actin cytoskeleton through the interaction between β- and α-catenin. Despite this importance in all animals surveyed, the CCC has been poorly characterized in ctenophores, the sister lineage to the rest of animals. Using a combination of Hidden Markov Models and clustering using CLANS, we revealed that they possess a full set of CCC genes. Nevertheless, a previous bioinformatic study showed that *Mnemiopsis leidyi* cadherins don't possess a β-catenin binding site, casting doubt on the conservation of the β-catenin function in cell-to-cell adhesion in ctenophores. However, we previously showed that β-catenin was localized at the cell-cell junctions during M. leidyi embryonic development, suggesting that the role of β-catenin in cell-to-cell adhesion might be conserved in M. leidyi. To better understand the CCC in ctenophores, we are currently developing hybridization chain reaction on the 3 CCC genes to see if they are co-expressing in vivo. Finally, to test if interactions within the CCC are conserved, we plan to carry out both co-immunoprecipitation coupled with mass spectrometry, and Yeast 2-Hybrid screening. Further experiments are necessary to fully characterize the CCC in the ctenophore *M. leidyi.*

TGF-Beta and non-canonical Wnt signaling interactions coordinate anterior-posterior and dorsal-ventral axis formation in sea urchin embryos

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In deuterostomes (chordates, hemichordates and echinoderms), the specification and patterning of the anterior-posterior (AP) and dorsal-ventral (DV) axes overlap in time and space. However, we lack a clear understanding of the explicit molecular mechanisms that control these axes as well as the extent of their interactions in any developmental model organism. In sea urchin embryos an integrated network of canonical (Wnt/β-catenin) and non-canonical (Wnt/JNK and Wnt/PKC) signaling pathways specify and pattern the early germ layers (endoderm, mesoderm, and ectoderm) along the AP axis. During this progressive AP patterning process, ventrally localized Nodal signaling initiates DV axis specification, establishing opposing Nodal and BMP2/4 signaling gradients that activate DV GRNs in all three germ layers. We have previously shown

that non-canonical Wnt16-Fzd1/2/7 signaling is broadly active during early AP and DV axis specification, playing a critical role in the sea urchin AP Wnt signaling network. Here, we use functional knockdown experiments to show that non-canonical Fzd1/2/7 signaling and a different broadly expressed Wnt ligand, Wnt6, are also necessary early Nodal signaling activity, as well as the transcription of bmp2/4 and the dorsal ectodermal GRN. Unexpectedly, our data indicate that nodal transcription is normal in Wnt6 and Fzd1/2/7 knockdown embryos, but phosphorylation of its downstream target Smad2/3 is perturbed. Furthermore, we show that dorsal BMP2/4 signaling is necessary for the activation of a secreted Wnt ligand antagonist, Wnt inhibitory factor1 (Wif-1), in dorsal-anterior blastomeres. Wif-1 functional knockdown assays indicate that Wif-1 is essential for the positioning of the anterior neuroectodermal gene regulatory network around the anterior pole during AP patterning. Together, our results illustrate that in sea urchin embryos there are direct interactions among components of the early Wnt signaling network and Nodal-BMP2/4 signaling pathways that govern AP and DV formation, respectively.

Conserved canonical and non-canonical Wnt signaling mechanisms govern anterior-posterior axis formation in Ambulacrarian embryos

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One of the first events to occur in animal development is the formation of the Anterior-Posterior (AP) axis. This fundamental step in embryogenesis is largely controlled by a deep evolutionarily conserved posterior-to-anterior gradient of Wnt/β-catenin signaling that patterns the early germ layer (endoderm, mesoderm, ectoderm) gene regulatory networks (GRNs) along this axis. In the sea urchin, AP specification and patterning is controlled by an integrated Wnt signaling network of canonical (Wnt/β-catenin) and non-canonical (Wnt/JNK and Wnt/PKC) signaling pathways. Here we use mRNA overexpression and siRNA knockdowns to show that many components of the sea urchin AP Wnt signaling network are also critical for AP axis pattering in the in the hemichordate, *Saccoglossus kowalevskii.* In sea urchins Wnt8-Fzd5/8-JNK signaling is critical for the restriction of the anterior neuroectoderm (ANE) GRN to a territory around the anterior pole. Our functional perturbations show that Wnt8, Fzd5/8 and JNK are necessary for ANE restriction in Saccoglossus. We also show that knockdowns of the Wnt antagonist Dkk1 leads to the elimination of the ANE GRN. Finally, our data indicate that Fzd1/2/7 in Saccoglossus does not antagonize the Wnt/JNK restriction mechanism, as it does in sea urchins, but rather functions synergistically with Fzd5/8 to restrict the ANE GRN around the anterior pole. Echinoderms and Hemichordates are members of the Ambulacrarian phyla that forms the immediate sister group with chordates. Thus, collectively these data indicate that interactions among canonical and non-canonical Wnt signaling pathways during AP axis formation were present in the last common chordate/ambulacrarian ancestor.

Genetic Regulation of Fin Ray Formation

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Fin development is regulated by the collinear expression of *hox* genes, with *hox13* genes expressed distally to pattern fin rays. We hypothesize that *hoxa13* promotes fin ray formation by regulating genes involved in mesenchymal cell migration and proliferation into the prospective fin ray domain. To test this, we generated *hoxa13a*; *hoxa13b* double knockout zebrafish, which showed a severe reduction of fin rays in pectoral, pelvic, dorsal, and anal fins. Conversely, we are overexpressing *hoxa13* in developing fins using transgenic systems to upregulate *hoxa13* in a timely and spatially restricted manner. Preliminary results suggest changes to fin development and mesenchymal cell migration, confirming the critical role of *hoxa13* in fin ray development. We are currently identifying the downstream target genes of *hoxa13* utilizing comparative genomics and immunofluorescence. These analyses will reveal the evolutionary developmental mechanisms of fin rays, which are critical for fish locomotion and evolutionary predecessors of tetrapod digits.

mTOR Signaling Inhibition Disrupts Macrophage Crosstalk with Epithelial and Connective Tissue Cells During Fin Regeneration

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Although mTOR signaling has been extensively studied across various biological contexts, its role in appendage regeneration has only recently been elucidated. A recent study demonstrated that axolotls utilize an evolutionarily divergent, hyperactivated mTOR protein to rapidly initiate protein translation during limb regeneration. The Senegal bichir (*Polypterus senegalus*) has emerged as a promising fish model for appendage regeneration due to its ability to regenerate entire fins following amputation at the endochondral skeleton level. We previously showed that regenerating Polypterus fins and axolotl limbs share a core transcriptional program. Pathway enrichment analysis identified mTORC1 signaling as one of the significantly enriched pathways in the regenerating Polypterus fin transcriptome. Although the Polypterus mTOR protein lacks the unique modifications found in axolotls, we observed a rapid increase in protein synthesis following fin amputation. Pharmacological inhibition of mTOR signaling with rapamycin completely blocked fin regeneration. In this study, we employed single-nucleus RNA sequencing to investigate the impact of mTOR inhibition on the cellular and transcriptional landscape of Polypterus fin regeneration. Despite sustained expression of several early regeneration markers in rapamycin-treated samples, we observed significant transcriptional alterations, particularly within the macrophage population. These included reduced expression of *mcf2l, hmox1*, *ctsk*, and several *HLA* genes, alongside a notable shift in *il18r1* expression from macrophages to epithelial cells. These changes suggest impaired macrophage functions related to inflammation resolution—such as reduced migration, antigen presentation, and debris clearance—and indicate dysregulated immune-epithelial crosstalk. Together with existing data from axolotl, our findings support a conserved role for mTOR signaling in coordinating a rapid and effective regenerative response in successful appendage regeneration models.

Investigating endogenous function of the conserved neuropeptide RFamide in cnidarian sea anemone *Nematostella vectensis*

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Originally discovered in molluscs and subsequently found in diverse phyla across the animal kingdom, RFamide is an example of a highly conserved neuropeptide — the ancient signalling molecules present in all animals with nervous systems. Within the group Bilateria, known functions of RFamides include involvement in feeding, reproduction, and nociception in vertebrates, with diverse and overlapping functions like feeding also present in invertebrates (e.g. insects, nematodes). Cnidaria (e.g. sea anemones, jellyfish, corals) is the sister group to Bilateria and shares a common ancestor. RFamides have been found in all cnidarian classes, and spatiotemporal expression analysis along with external application of synthetic peptides suggests functions remain diverse, affecting processes like feeding in some species and phototaxis or developmental timing in others. The role of endogenous RFamide in cnidarians has not yet been characterized, and alongside existing bilaterian data it could provide new insights on the evolutionary origins of neuropeptide signalling. To this end, we have utilized CRISPR-Cas9 to generate knockout mutant *Nematostella vectensis* lines. We also developed an anti-RFamide antibody for *N. vectensis* and validated its specificity by in situ hybridization and immunohistochemistry performed on mutants. We are currently using the antibody alongside other markers to examine where RFamide is being expressed at different points in development relative to identifiable cell types and other functional genes. RFamidergic neurons are initially more diffuse but become relatively concentrated in pharynx and tentacular regions of the animal during development from late planula stages to polyp. Given these patterns of expression, our ongoing monitoring and analysis of RFamide knockout mutants includes assays to identify possible perturbations in feeding behaviour.

RTK signaling, but not transcription, is necessary for dpERK induction, D quadrant organizer specification, and dorsal ventral axis patterning in the marine gastropod, *Crepidula atrasolea*

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During metazoan development, body axes are patterned in part by organizing centers. Both vertebrates and Spiralia have organizers that specify the dorsal/ventral (DV) axis. While pharmacological inhibitors have been used to probe pathways controlling this process in Spiralia, the molecular underpinnings remain unclear. Deeper study will help clarify body axis evolution and ancestral states of signaling pathways involved in organizer specification and axis formation. We established *C. atrasolea* as a model for organizer specification by identifying the 4d micromere as the organizer and showing that dpERK activation is necessary for its formation. Inhibition of receptor tyrosine kinases (RTKs) using SU5402 reduced dpERK activation and blocked both organizer specification and DV axis formation. To assess the role of RTK-mediated dpERK activation, we sequenced SU5402-treated embryos at the 25-cell stage. Differential gene

expression analysis revealed few changes, suggesting that transcription is not required for organizer formation. This was further validated when the 4d micromere was born and the DV axis was maintained under a transcriptionally repressed state induced by DRB treatment. To identify RTKs upstream of dpERK, we analyzed a developmental RNA-seq time course and found three FGFRs highly expressed during early development. However, dominant-negative knockdowns (DN-KD) of these FGFRs had no effect on organizer cell birth. Proteomic analysis of 8-cell embryos identified four additional RTK family members. Ongoing experiments will test their roles in organizer specification and axis formation to identify the target of SU5402. Together, these findings establish *C. atrasolea* as a tractable model for dissecting Spiralian organizer mechanisms. We have shown that RTK mediated dpERK activation is necessary for organizer formation, a conserved trait between molluscs and equal cleaving annelids. Our work suggests alternative RTKs mediate organizer function, and demonstrate RTK/dpERK does not act on transcription and transcription is not necessary for organizer cell birth.

Gene Network Logic Underlying Butterfly Wing Patterning

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Lepidoptera exhibit extraordinary diversity in wing shapes and color patterns, traits that are not only visually striking but also play crucial adaptive roles in survival and reproduction. The relatively simple tissue architecture of lepidopteran wings, combined with their morphological complexity, positions them as an exceptional model for studying the developmental and evolutionary mechanisms underlying ecologically significant traits. Over the past decade, research on the evolution of butterfly color patterns has made remarkable strides, particularly in mapping phenotypic variation to discrete genomic loci. Notably, three loci, the ligand WntA, the non-coding RNA *mir-193/ivory*, and the transcription factor Optix, have repeatedly emerged as key players, or “hotspots,” in the evolution of wing pattern diversity across multiple species. This repeated association raises fundamental questions: Why do these loci serve as recurrent focal points for evolutionary change? What features of the regulatory networks in which they function confer such evolutionary flexibility? Our lab is working to gain a mechanistic understanding of the gene regulatory networks that connect these loci and underlie the evolution of the vast array of butterfly wing patterns. I will share our recent findings, which utilize a combination of genetic perturbations and spatiotemporal expression analyses of key genes to elucidate the feedback mechanisms, gene interactions, and temporal dynamics that define wing patterns in two butterfly species.

Comparative embryogenesis and neuroanatomy of the sister spionids *Streblospio gynobranchiata* and *Streblospio benedicti*

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The marine polychaetes *Streblospio gynobranchiata* and *Streblospio benedicti* are sister species, with different modes of development that offer an opportunity to explore evolutionary divergence in embryogenesis and neural development. *S. benedicti* is a rare poecilogonous species that produces distinct larval morphotypes: planktotrophic larvae are obligately feeding and originate from small eggs (100mm) and lecithotrophic larvae are on-feeding and originate from large eggs (200mm). Conversely, *S. gynobranchiata* has recently diverged and has only planktotrophic development. By comparing the poecilogony of *S. benedicti* and the monomorphic development of *S. gynobranchiata*, we can investigate the features of embryogenesis and neural architecture that are conserved across planktotrophic larvae, and isolate species-specific patterns. We compare embryonic stages of *S. gynobranchiata* to *S. benedicti*, specifically looking at cell division timing and the cell volume differences between the developmental modes. In addition, we also show the neural structures associated with the different larval types of both species. The larval neural structures of *S. benedicti* appear dimorphic: lecithotrophic larvae have an increased number of peripheral neurons in each trunk segment (a feature more typical of juvenile stages) – while planktotrophic larvae lack these but possess a distinct neurite near the larval foregut. To examine if neural traits are associated with development mode, we examined the neuroanatomy of *S. gynobranchiata* larvae. Our comparative analysis provides insight into how neural architecture may evolve in response to shifts in developmental mode. Future directions include developmental transcriptomic analysis of *S. gynobranchiata* to further compare gene expression with its *S. benedicti* larval counterparts.

Functional analysis of the *Nematostella* Wnt/β-catenin destruction complex provides insight into the evolution of a critical regulatory module in a major metazoan signal transduction pathway

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The genesis of signaling pathways likely drove metazoan evolution and diversification, but how these pathways evolved and acquired signaling activity is poorly understood. To this end we studied the Wnt/β-catenin (cWnt) pathway destruction complex (DC) - which regulates cytoplasmic β-catenin levels - in the cnidarian Nematostella. Cnidarians are the closest outgroup to bilaterians and in bilaterians β-catenin binding to Axin and APC proteins, and Axin-APC heterodimerization are required for DC function. Experimental analysis showed that both NvAxin and NvAPC-like regulate cWnt signaling in *Nematostella* embryos, but bioinformatic predictions showed that all non- bilaterian Axin and APC-like homologs lack domains required for bilaterian DC activity questioning if non-bilaterians have a functional cWnt DC. We found that Nematostella Axin (NvAxin) interacts with NvGSK-3β and NvAPC-like interacts with β-catenin, and NvAxin and NvAPC-like can heterodimerize to form a functional DC. Unexpectedly, we noted that NvAxin binds β-catenin weakly despite lacking the bilaterian β-catenin-binding motif (βcatBM). Using AlphaFold, we identified two βcatBM-like sequences in NvAxin, one within the Axin-RGS domain and another near the C-terminus, and a single βcatBM-like sequence in Axin-RGS of placozoans, poriferans, and ctenophores. Our results suggest that the bilaterian βcatBM evolved from a low-affinity βcatBM-like sequence within the Axin-RGS in early metazoans and this sequence was duplicated in the cnidarian-bilaterian ancestor. The Axin-RGS copy was lost in the bilaterian lineage and the other acquired higher β-catenin affinity. We suggest that scaffolding proteins in

ancestral metazoan signaling pathways had promiscuous activity that increased pathway evolvability during their broad deployment in bilaterians.

Unraveling the evolution of cis-regulatory landscapes that control limb skeletal proportion

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The tetrapod limb has highly conserved homologous skeletal elements that diverged in shape and size over hundreds of millions of years, shaping an extreme diversity of morphology and function. Given that each limb bone grows using a largely shared set of developmental toolkit genes, their diverse proportion is likely governed by the evolution of cis-regulatory elements (CREs) that locally modulate the expression of these genes in different bones and different species. What are the CREs that control bone growth, and how has their evolution diversified limb skeletal proportion? To tackle these questions, we employ a comparative genomics approach using mouse and jerboa, a bipedal rodent characterized by extraordinarily elongated hindlimbs, especially the feet. To identify CREs that drive jerboa foot bone elongation from the vast complexity of the genome, we performed integrative analyses of chromatin accessibility and underlying sequence evolution of mouse and jerboa cis-regulatory genome. Using a multiple whole-genome alignment of 72 rodent species, we identified a subset of jerboa putative CREs that experienced accelerated nucleotide substitution specifically in the jerboa lineage. We demonstrated that these jerboa-accelerated CREs and their orthologous counterparts in mice are associated with distinct chromatin accessibility landscapes during development of the foot bone. Further, the genes near these sequences are enriched for epigenetic modifiers, including members of the polycomb repressive complex 2 (PRC2) that represses transcription through histone methylation. In mice, loss of Ezh2, the catalytic subunit of PRC2, causes skeletal growth defects due to aberrant upregulation of growth-inhibiting genes, and mutations in most PRC2 genes cause overgrowth syndromes in humans. Together, our results highlight putative causative CREs and their distinct evolutionary and regulatory profiles between mouse and jerboa, suggesting that evolution of the regulatory control of epigenetic modifiers, and downstream consequences, may have contributed to the evolution of skeletal proportion.

Evolution and development of sex-specific trait exaggeration in dung beetles

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Exaggerated traits, used as weapons and ornaments in male-male competition and female choice play a major role in morphological diversification. The horns of male dung beetles are one of nature’s most charismatic innovations and provide a powerful system to study the developmental mechanisms underpinning the evolution of morphology. Sex-limited head horns are used by

males during fights for access to females and have rapidly diversified. Their development has been studied extensively to understand the origins of innovation, exaggeration, and the developmental mechanisms driving sex-specific trait variation and its evolution. However, head horns are evolutionary novelties and, as such, might represent special cases of exaggeration and diversification and it is unclear whether similar mechanisms act in other traits. In addition to head horns, forelegs of dung beetles are also exaggerated. Females evolved broad forelegs adapted for digging underground brood chambers, while males of most species rarely dig and use their forelegs for mating. In addition to being sex- and species- specific, their exaggeration also depends on larval nutrition and traits are more pronounced when developing beetles are well-nourished. Providing an opportunity to explore the mechanisms driving trait diversification and plasticity. Here, we trace the evolutionary history of nutritional plasticity and sexual dimorphism in foreleg length in 12 species that vary in size and diverged more than 45 million years ago. Species not only differed strongly in overall size but also the degree to which males and females differed in relative foreleg length. Surprisingly, strong sex differences in exaggeration and nutritional plasticity have been lost multiple times independently, suggesting the convergent reduction of sexual dimorphism. We discuss putative mechanisms underlying the evolution of sex-specific scaling relationships.

Investigating neuropeptidergic regulation of life cycle transition in the sea anemone cnidarian *Nematostella vectensis*

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Conserved neuropeptides, known as Wamides, promote life cycle transition from a larva into a juvenile/adult form across Cnidaria (e.g. hydroids and sea anemones) and Bilateria (e.g. annelid worms), consistent with an evolutionarily ancient role for neuropeptides in regulating development. However, the underlying molecular mechanisms are poorly understood. To address this knowledge gap, we investigate how cnidarian Wamide neuropeptides - GLWamides - accelerate life cycle transition from a free-swimming planula larva into a juvenile polyp in the sea anemone *Nematostella vectensis*. We find that synthetic GLWamide primarily reacts with endodermal cells at both the planula and polyp stages, indicating that GLWamide receptors are expressed in the endoderm. In addition, using RNA-seq, we show that an ETS transcription factor is upregulated in response to GLWamidergic signaling at life cycle transition. Analyses of spatio-temporal expression patterns of ets by meganuclease-mediated transgenic reporter assay and in situ hybridization reveal that ets expression is activated throughout the endoderm at life cycle transition. In light of these results, we suggest that GLWamide neuropeptides induce the endoderm to express an ETS transcription factor, which may be critical for initiating life cycle transition in the sea anemone. We are currently analyzing the developmental function of *ets* by using knockout mutant lines we developed by CRISPR-Cas9-mediated reverse genetics. Furthermore, we are carrying out ligand-based capture of receptors followed by mass spectroscopy to determine the identity of GLWamide receptors.

Induction of reproductive fate in the pea aphid

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The pea aphid, *Acyrthosiphon pisum*, exhibits remarkable developmental plasticity in response to seasonal changes in photoperiod. In spring and summer, aphids reproduce asexually and are viviparous, yielding large numbers of genetically identical female offspring. The longer nights accompanying the fall induce these asexual aphids to produce sexual males and females, which mate to lay frost-resistant eggs. These eggs diapause through the cold winter months and hatch into asexually reproducing females in the spring, founding new clonal populations. Among other aspects of this reproductive polyphenism, we are interested in the process by which aphid mothers specify the asexual versus sexual fate of their progeny during embryonic development. One hypothesis is that an asexual-promoting substance produced by mothers under short nights, known as “virginoparin,” specifies her embryonic progeny as asexual. Although juvenile hormone (JH) is clearly sufficient to specify asexual fate under long-night conditions, we present evidence that maternal JH does not mediate this process and is thus not virginoparin. Nevertheless, we present evidence that JH signaling may still play a role specifying reproductive fate, perhaps in coordination with insulin signaling.

The Role of Homeobox Gene Cluster Reorganization in the Evolution of Medusozoan Life Cycles

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Medusozoa, which includes the jellyfish lineages Scyphozoa (true jellyfish), Cubozoa (box jellyfish), Staurozoa (stalked jellyfish), and Hydrozoa (hydras and hydromedusae), are a group of cnidarians characterized by complex life cycles, alternating between a benthic polyp stage and a pelagic medusa (jellyfish) stage. This life cycle is distinct from their cnidarian counterpart, the anthozoans (corals and sea anemones), which lack a medusa stage in their life cycle. Although medusae are ancestral for Medusozoa, hydrozoans have lost the medusa stage several times in their evolution. Here we implicate homeobox gene cluster organization and homeobox gene expression in the origin, development and evolutionary loss of the jellyfish life cycle stage in medusozoans. In particular, we find a significant proportion of ANTP and PRD class genes differentially expressed between life cycle stages in scyphozoans and hydrozoans, including upregulation in the developing medusa. Using a comparative genomic approach, we find that many of these life cycle specific homeobox genes arose through the expansion of ancestral homeobox clusters. Furthermore, medusa-specific homeobox genes exhibit a pattern of independent losses in hydrozoan lineages that have lost the medusa life cycle stage. By contrast, anthozoan ANTP and PRD homeobox clusters are highly conserved and more closely resemble that of their bilaterian relatives. These patterns of genome structure and homeobox gene expression support the assertion that the evolution of complex life cycles in Medusozoa was accompanied by homeobox gene clusters expansion and cluster reorganization. Following cluster expansion, homeobox genes were co-opted for patterning different life cycle stages, and

homeobox gene loss within hydrozoans were responsible for the independent losses of the medusa stage within this group.

Distributed neural computation and the evolution of the first brains

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The transition from diffuse nerve nets to centralized brains was a major event in animal history: it enabled entirely new lifestyles, and likely caused an evolutionary arms race responsible for the origins of modern animal diversity. We do not know how the first brains were organized, how they could compute, or how they subsequently evolved the abilities integral to our cognitive experience. Although the earliest brainy animals are long extinct, some living lineages retain ancient features of the first brains. The most informative is the acoels: the evolutionary sister lineage to all other brainy animals. To understand how the first brains could have been organized, I am studying a lab-tractable acoel, *Hofstenia miamia*. *Hofstenia*'s brain consists of a dense network of neurite bundles that wraps around the head under the skin. I found that these brains lack regionalization and stereotypy: every brain has a different structure that morphs over time. Neural cell types, despite having distinct functions, are always distributed across the brain. Complex behavior, such as hunting, is robust to amputation of large, arbitrary brain regions - showing that neural computations (for sensory integration, decision-making, motor control, etc.) happen in most or all brain regions. Together, these data suggest that the *Hofstenia* brain is composed of tessellating circuit tiles that are each computationally pluripotent - an organization resembling a much-elaborated nerve net, but unlike typical brains. Further behavioral analyses show that more tiles allow greater perceptual resolution, and that they likely interact, allowing computations to emerge from their collective activity. It is possible that the first brains were organized similarly. In this view, nerve nets were first concentrated in early bilaterian heads, with local circuit tiles interacting to allow distributed computation. Stereotyped and regionalized circuits likely evolved later, establishing a groundplan for the future evolution of complex brains.

Inferring 'the sister to the stinger' using phylogenetic methods at the cell-type level

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The stinging cells (cnidae) of cnidarians (corals, jellyfish, and anemones) are a defining evolutionary novelty of the clade. These cells contain explosive structures called cnidocysts, which rapidly evert a tubule within a pressurized capsule upon activation, often delivering a cocktail of toxins known as venom. The morphology, contents, and function of cnidae are impressively diverse, but the precise evolutionary and developmental origin of this diagnostic character of cnidarians has remained uncertain. Cnidae and neurons share developmental pathways mediated by transcription factors. This, in combination with the shared presence of sensory structures and electrophysiological activity, suggests that cnidae and neurons could have furcated into sister cell types from a common ancestral cell type. Single-cell transcriptomics has provided a wealth of information about animal cell type diversity. This has given rise to comparative methods that aim to disentangle the relationships between different cell types. One such approach, by Mah and Dunn 2024, infers cell phylogenies by using the principal components (PCs) from single-cell RNA sequencing datasets (scRNA seq) as phylogenetically informative characters. We have adapted this workflow to investigate the evolutionary history of cnidae with respect to other sensory and secretory cell types in Cnidaria. After compiling published scRNA seq datasets of several cnidarian species, we are generating a matrix of phylogenetically informative PC's and testing multiple models to infer a cnidarian cell phylogeny. Here we report the results of this analysis to disentangle the relationships between sensory and secretory cell types across cnidarian cell atlases. This phylogenetic approach to investigate novel cell types can help to better understand how novelty evolves and provide a new framework to test fundamental questions in cell biology.

A Phylogenetic Analysis of Cnidarian Cell Types

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Phylogenies transformed the way we think about species: they revealed that the evolutionary history of species is branching. However, evolutionary individuals also exist at other biological levels of organizations, including genes and cells. Here, I develop a method to infer a cell phylogeny, and present a phylogeny of cell types from three hydrozoan cnidarian species: *Hydra vulgaris*, *Hydractinia symbiolongicarpus*, and *Clytia hemisphaerica*. Cell phylogenies, a phylogeny where the taxa are cells instead of species, describe the branching evolutionary history of cell types. This phylogeny reveals a striking relationship between potency and cell evolutionary history, with deep evolutionary implications: stem cells and germ cells segregate into one clade, and all differentiated somatic cells another. However, ectodermal and endodermal epithelial stem cells in Hydra may have evolved convergently from their respective tissues, rather than share ancestry with the main stem cell clade. Additionally, a close evolutionary affinity between gland cells and epithelial cells emerges, despite their distant developmental relationship. This explicit phylogenetic framework drives new hypotheses and illuminates deep evolutionary relationships in the evolution of hydrozoan cell types.

Maternally-derived toxin HsyPTx1 as a putative defense mechanism against predation in larval Hydractinia symbiolongicarpus

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Hydractinia symbiolongicarpus, like others in the phylum Cnidaria, have a wide range of toxins for prey capture and defense. Across cnidarians these toxins are often delivered through stinging nematocysts, and have typically only been studied in adult animals. Less is known about toxins present in cnidarian embryos and larvae, which are presumably used for defense. Prior research in the sea anemone *Nematostella vectensis* found a potassium channel blocker toxin (NvPTx1) that is maternally deposited in oocytes and later expressed in planulae as a potential defense against predation. A homolog of this gene (HsyPTx1) was found expressed in female reproductive polyps of *H. symbiolongicarpus*, presumably in developing oocytes. *H.*

symbiolongicarpus naturally grows on the shell of the hermit crab Pagurus longicarpus, which we have observed to reject *Hydractinia* oocytes and embryos. We hypothesize that this toxin may be important for larval defense against hermit crab predation. We are establishing a HsyPTx1-knockout line through CRISPR-Cas9 injection, and homozygous knockout embryos will be offered to P. *longicarpus* to observe if predation will occur without HsyPTx1 toxin. This work will explore the potential role of a maternally-produced toxin in protecting offspring from predation and potentially uncover an ancestral cnidarian toxin-defense mechanism.

Matthew_K_Travert,Paulyn_Cartwright

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Temporal control of muscle and skeletal development by thyroid hormone shapes the adult pectoral fin

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Precise timing of endocrine and paracrine signals coordinates cell, tissue, and organ-level organization during development. Among these, thyroid hormone regulates larval-to-adult transitions in amphibians and fishes, including metamorphosis, stimulating the growth and differentiation of pectoral fins and limbs. In teleost fishes, the pectoral fins are the last fins to develop and undergo a ventral repositioning and rotation from a vertical, midbody orientation to a horizontal, ventral orientation in adults. Here, we show that thyroid hormone regulates pectoral fin development in zebrafish by promoting ossification and muscle growth, enabling the fin to reorient to its adult position. We generated constitutively hypothyroid zebrafish by genetic ablation of the thyroid follicles during embryogenesis. These fish began to exhibit reduced fin length and delayed ossification and muscle formation around 7 mm standard length, coinciding with the normal larval-to-adult transition. Hypothyroid fish developed pectoral fins with enlarged scapulae, fewer muscle fibers, a ventral displacement of the origin site of the posterior adductor superficialis muscle—the main elevator of the fin—, and retained their larval vertical orientation. These morphological changes parallel features seen in acanthomorphs—the most diverse evolutionary radiation of teleosts—which also retain a midbody, vertical fin. We propose that shifts in thyroid hormone metabolism or signaling may have provided a substrate for teleost evolutionary diversification by regulating the timing when the pectoral fin develops. Furthermore, our findings support the hypothesis that heterochrony, potentially mediated by endocrine regulation, alter the relative rates in which different organs develop, leading to changes in biomechanical stress. This endocrine-biomechanical model may represent a key developmental mechanism underlying the evolutionary disparity of fins and limbs across vertebrates.

Opening a can of 'worms' – Molecular mechanism underlying the specification of the central nervous system in the annelid *Capitella teleta*

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The central nervous system (CNS) is a bilaterian innovation that enables better information processing and response. Although it is present across multiple bilaterian taxa, its origin is still debated. Unraveling developmental mechanisms across bilaterian taxa can help address this question. One major group where this information is still lacking is Spiralia. This clade is characterized by a division program called spiral cleavage, which is present in multiple phyla (e.g., annelids, molluscs). In our lab, we study the annelid *Capitella teleta* as its phylogenetic position in Sedentaria represents a good comparison with other annelids and it has a fully sequenced genome and a fate map for major tissues/organs including the CNS. We previously showed that the 1a–d and 2d micromeres can form neural tissue, brain and ventral nerve cord (VNC), respectively, in isolation. However, in the presence of other blastomeres, VNC formation can be repressed or rescued. This led us to hypothesize that a combination of inherited determinants and signaling pathways is necessary for VNC specification. To address this question, we performed blastomere isolations that led to the autonomous formation of the brain, and repression/rescue of the nerve cord, and performed RNA-sequencing on those isolates. In our dataset, we identified components of the canonical Wnt signaling pathway (cWnt) in multiple pairwise comparisons suggesting a pro-neural role. Additional pharmacological experiments supported the role of cWnt in neural fate specification in the VNC. Regarding transcription factors, we found lineage-specific TALE homeobox genes, which have a suggested role in lineage specification in other spiralians. Overall, this data shows that blastomeres that form the CNS could be specified via lineage-specific homeobox genes and neural fate in the VNC also relies on cWnt signaling. For future directions, we plan to further characterize the function of these homeobox genes and Wnt signaling in early fate specification.

Fish tongues and other oddities: Using zebrafish to examine the development and evolution of trophic novelties

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Cypriniforms are a diverse group of freshwater fishes characterized by several trophic novelties that include: kinethmoid-mediated premaxillary protrusion, a muscular palatal organ on the roof of the oral cavity, and epibranchial organs used to aggregate small food particles. In this talk I will discuss our findings regarding the anatomy, early development, and function of these trophic novelties. As a cypriniform zebrafish can be used to study the development of some of these trophic novelties. While functional morphologists have long studied the functional implications of suites of trophic structures, the evolutionary origin and morphogenesis of novelties has received less attention. However, the developmental biology community has also not examined such trophic novelties in a functional and evolutionary context. One issue that confounds the evolutionary study of novelties is that these complex features often originate all at once, thus examining selection for piecemeal evolution of such novelties is impossible. When such complex novelties first originate they must become incorporated into an integrated system to be rendered fully functional. Thus, morphological integration is key at the outset of acquisition of some morphological novelties, such as that of their novel jaw protrusion. Other novelties, such as the palatal organ, must be fully incorporated into the existing trophic machinery to be able to function properly. Importantly, the palatal organ is derived from cardiopharyngeal mesoderm and this mesodermal stem cell population is a neglected source of great morphological diversity within fishes.

***otk* is an accessory member in nymphalid wing color patterning**

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Butterfly wing color pattern is a diverse phenotypic readout of intricate molecular patterning mechanisms that differs greatly between species. As a key component of this process, the WntA ligand orchestrates the position and color of various pattern elements across Nymphalidae (Brush-foot butterflies). Despite its importance, the detailed molecular pathway of WntA signal transduction is poorly understood. Here, we show that *Off-track* (*otk*), a conserved transmembrane kinase and co-receptor of Wnt ligands, is an accessory component of the WntA signal transduction pathway. In the Painted Lady butterfly, *Vanessa cardui*, *otk* is required for the local refinement of Central Symmetry System (CSS) patterns on its wings. This mechanism is likely mediated through a positive feedback loop that further refines WntA expression upon signal co-receptance by *otk*. The endogenous expression of otk is anti-correlated with WntA expression in the CSS of *V. cardui* pupal wings, likely due to local transcriptional repression of otk through WntA-mediated signaling. In contrast, *otk* of the Gulf Fritillary, *Agraulis vanillae*, is functionally dispensable in instructing color patterns, showing no visible wing phenotype among the G0 crispants. Intriguingly, the expression of *otk* in *A.vanillae* pupal wing is fully complementary to the WntA expression, which is entirely inverse of the *otk* expression pattern seen in *V. cardui.* Overall, we show that *otk* is an accessory component in the WntA signaling network, performing a species-specific role in color pattern instruction.

A new transcription factor binding site near *pdm3* underlies the elaboration of butterfly eyespots

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How morphology evolves at the gene regulatory level remains an open question in evolutionary developmental biology. The butterfly eyespot is an ideal system to address this question because it is genetically tractable and because this pattern has diversified across nymphalid butterflies. Here, we combine ChIP-seq data across species and developmental stages to identify a new binding site for the transcription factor spalt associated with the evolution of eyespots. This binding site is located in an intron of the gene *pdm3*. We find that this cis-regulatory element is only necessary for the development of the outer rings of hindwing eyespots using CRISPR-Cas9 in *Junonia coenia*. ATAC-seq data confirm that this cis-regulatory element is specifically accessible during early pupal wing development. We also confirm that *pdm3* is expressed in the eyespot rings during early pupal development using in situ hybridization, and CRISPR-Cas9 knockout of the *pdm3* gene affects multiple wing pattern elements. This work was supported by NSF GRFP DGE-2139899 to JMCM and NSF IOS-2128164 and DEB-2242865 to RDR.

Searching for the Pieces: Finding the Pair-Rule Genes in *Oncopeltus fasciatus*

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Insects exhibit a conserved segmented body plan defined by a hierarchical regulatory gene system that gives rise to segment identities. A key group within this hierarchy is the pair-rule genes (PRGs), initially identified in *Drosophila melanogaster* through mutant screens that resulted in the loss of alternating body segments. PRGs encode transcription factors that regulate downstream genes essential for segment formation and development. Although some orthologs of *Drosophila* PRGs are conserved in other species, the milkweed bug, *Oncopeltus fasciatus*, does not possess these PRGs. Emerging as a model system for the Hemiptera order, two novel Oncopeltus genes with pair-rule (PR) function have been identified, demonstrating that PR-patterning is conserved across insects despite differing gene usage. This discovery facilitates the exploration of the evolutionary divergence of PRGs and the re-wiring of regulatory networks. Our lab conducted an RNA-seq experiment based on the temporal patterns of the previously identified Oncopeltus PRG, *Of-E75*. A novel PRG, *Of-Blimp1*, was identified through an in situ hybridization screen of transcription factors expressed similarly to *Of-E75*. We successfully generated *Of-Blimp1* loss-of-function mutants and are working on inducing a loss-of-function mutation for *Of-E75*. To uncover additional novel genes from this RNA-seq experiment, I am performing a functional screen of genes encoding transcription factors expressed in similar temporal patterns. Using parental RNA interference (RNAi), I am injecting double-stranded RNA matching candidate genes into female Oncopeltus and scoring offspring for changes in morphology and downstream gene expression. This approach aims to identify segmentation genes that may have been overlooked by in situ hybridization screening. Preliminary results indicate potential PRG-like function, validating our approach. By analyzing the PRGs in *Oncopeltus* and comparing them across *Hemiptera*, we will deepen our understanding of PRG evolutionary dynamics, providing valuable insights into insect development.

Zelda regulates body length by coordinating embryonic cell division in hemimetabolous insects

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Zelda is known as an Maternal-to-Zygotic Transition (MZT) pioneer factor in *Drosophila melanogaster,* where its essential function is to drive zygotic genome activation by modifying chromatin architecture. In addition, Zelda plays roles in *D. melanogaster* regeneration, neuroblast formation, and abdominal patterning. However, little is known about Zelda's function in hemimetabolous insects. In this study, we used two hemimetabolous model species to discover novel zygotic functions of Zelda. First, we analyzed the spatiotemporal expression patterns during embryogenesis and found that *zelda* is expressed segmentally both in the cricket *Gryllus bimaculatus* and in the milkweed bug *Oncopeltus fasciatus*. Next, we performed embryonic RNAi and CRISPR experiments, resulting in various segmental fusions in both species. Our detailed analyses of early embryos revealed that *Gb-zelda* CRISPant embryos were smaller and shorter

than wild type embryos, even though expression patterns of many segmentation genes were normal. We quantified cell division frequency and orientation and found that *G. bimaculatus zelda* CRISPants had significantly more dividing cells than controls. However, whereas wild type embryos have most spindles oriented parallel to the anteroposterior axis, spindle orientations in *zelda* CRISPants were more randomly distributed. Our findings highlight a possible ancestral function of Zelda, providing new insights into the mechanisms underlying axial elongation in short germ insects and the evolution of insect metamorphosis.

The hypothesized roles of phagocytosis genes in kleptocnidy in the gastropod nudibranch *Berghia stephanieae*

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Understanding the emergence of novel phenotypes—such as nematocyst sequestration—is critical for elucidating drivers of diversification. Such phenotypes can often result from subfunctionalization or neofunctionalization of more conserved processes. Phagocytosis one such conserved physiological process in eukaryotes with many important biological functions. It has been modified in nudibranch gastropods for a process called kleptocnidy (or nematocyst sequestration), which is specialized for the uptake of venomous stinging organelles stolen from their cnidarian prey in structures called cerata. This process is highly selective, but we know little about how these organelles might be targeted. We used spatial transcriptomics data collected in the emerging model nudibranch species *Berghia stephanieae* to investigate whether genes known to be involved in phagocytosis, particularly those related to target reception, are expressed in sequestering tissues. Whole-animal and cerata-only samples were cryosectioned and mounted on barcoded Visium slides, then stained with hematoxylin and eosin for morphological context. After library preparation and sequencing, we used 10x Genomics Cell Ranger to map reads to the polished *B. stephanieae* genome and analyzed tissue clusters with Seurat in R. In both sample types, transcriptionally distinct clusters aligned with morphologically distinct tissues. Comparing phagocytic tissues—including the digestive gland and cnidosac—revealed that C-type lectin macrophage mannose receptor 1 is expressed in the cnidosac, and we also identified additional C-type lectins and immune genes with cnidosac-specific expression. Moreover, numerous C-type lectins were expressed outside the cnidosac, indicating that only a subset of this conserved family is involved in nematocyst sequestration. Future work combining Visium Spatial Gene Expression with single-cell RNA-sequencing will clarify how phagocytic cells within the cnidosac recognize nematocysts. Collectively, our results provide new morphological and transcriptomic insights that advance knowledge of kleptocnidy in nudibranchs and inform broader investigations of novelty and evolution.

The Role of Transcription Factor FOXO in the Regulation of Developmental Plasticity in Horned Beetles

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Variation in nutrition is one of the most common metabolic challenges during organismal development. One mechanism organisms use to cope with variable environmental conditions is phenotypic plasticity, which allows organisms to develop distinct phenotypes in response to varying environmental conditions. The horned beetle Onthophagus taurus is a model system in the study of developmental plasticity. Males of this species develop large horns in response to high nutrition conditions, yet remain hornless under low nutrition conditions. Previous studies demonstrated that the evolutionarily conserved transcription factor Forkhead box O (FOXO) is a major horn growth modulator in Onthophagus beetles. This transcription factor acts by inhibiting horn growth in a low nutrition environment and promoting horn growth in high nutrition conditions. Yet, a major question that remains is how does FOXO interact with its target genes? And, which downstream effectors are regulated by FOXO to confer tissue-specific nutritional plasticity during beetle development? Here, we first utilize CUT&RUN in pupal horn tissue to identify the direct targets of FOXO across low and high nutrition conditions. We also use RNA sequencing to compare the differential gene expression profile of FOXO knockdown animals with control individuals across nutritional conditions. We aim to identify both direct and indirect downstream effectors that are modulated by FOXO to regulate horn plasticity in distinct nutritional conditions. Taken together, this project will shed light on the mechanisms of developmental plasticity revolving around the evolutionarily conserved transcription factor FOXO.

Leveraging Killifish Biodiversity and Genomics to Explore Developmental Gene Regulation in Extreme Environments

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Annual killifishes are models to explore the genetic intersection of ecology, evolution, and development (Eco-Evo-Devo). Annual killifishes have repeatedly evolved to survive in desiccated pools. When the ephemeral pools dry up, adults die, but embryos survive buried in the substrate with specialized egg envelopes, three distinct diapause stages, and hatching that is triggered by submersion in water during the next habitat inundation. The third diapause is used to time hatching and is the only vertebrate diapause known to occur after organogenesis. We develop the new killifish laboratory research models to study the integration of genetic and environmental cues that control dormancy and hatching. We leverage comparative genomic methods to examine the how these traits evolve across annual and non-annual killifish species. Thus, we aim to illuminate the genetic basis arrested development, environmentally-cued hatching, and how the integration of genetic and environmental signals controls development in extreme environments.

A Blast from the Past: 'Living Fossil' Fishes Unravel the Developmental Evolution of Major Transitions in Vertebrates

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The macroevolution of vertebrates has been accompanied by gains and losses of genes, gene regulatory elements, and developmental processes contributing to major evolutionary transitions and novelties such as the origins of the jawed and bony vertebrates, the emergence of tetrapods from fishes, and the emergence of the teleost fishes with their unmatched biodiversity. Vertebrate diversity, however, is not fully represented by the few classic, but very derived vertebrate models (zebrafish, frog, chicken, mouse). To broaden our understanding of the genomic basis of macroevolutionary transitions leading to the ~70,000 extant vertebrate species, we are developing the holostean fishes, i.e., gars and bowfins used by Darwin to coin the term "living fossils", as new model species for comparative genomics and Evo-Devo. Holosteans occupy a key phylogenetic position within the ray-finned fishes and possess slowly evolving and thereby highly informative genomes. Their archaic body plans feature numerous ancestral morphologies, including a respiratory gas bladder, enamel-like ganoid coating on scales, an ancestral-like caudal region, and the ability to regenerate body parts. Among living species, holosteans are thus uniquely suited to infer the gene regulatory networks governing ancestral bony vertebrate genome functions and body plans. Our evolutionary-developmental analyses using holosteans as genomic "bridge species" connect the disparate sets of developmental gene functions and morphologies among distant bony vertebrate lineages such as tetrapods and teleosts. Transcriptional and epigenomic profiling through gar and bowfin development inform the gene regulatory basis of major transitions during vertebrate evolution such as the origin of limbs from fins in tetrapod fishes. Furthermore, our studies of gene regulation in the holostean central nervous system provide a key comparative data set to understand the diversification of brain functions in the teleost fishes by sub- and neofunctionalization following a whole genome duplication in the teleost ancestor.

VitelloTag, a tool for high-throughput delivery into oocytes

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Delivering molecular tools into oocytes is essential for developmental and reproductive biology. Microinjection, the conventional method, is equipment-intensive, often technically challenging, and low-yield, and is impractical in species with delicate oocytes or restricted spawning seasons. To overcome these limitations, we developed VitelloTag, a cost-effective, high-throughput system using vitellogenin-derived fusion proteins to enable efficient cargo delivery via receptor-mediated endocytosis. We demonstrate its utility by delivering Cas9/sgRNA complexes in two distantly related species for gene knockout.

Uncovering the Genetic Basis of Snake Body Elongation

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Snakes are vertebrates and are included in the class Reptilia. Snakes in particular, have many vertebrae and have lost their limbs during evolution. During embryonic development, snakes undergo a longer period of axis elongation compared to other vertebrates. The elongated body is divided into vertebrae, resulting in the formation of numerous vertebrae in snakes. The molecular mechanism responsible for snakes developing a longer body remains unclear. In vertebrates, the embryonic trunk is formed by the presomitic mesoderm (PSM) which repeats cell division and extends posteriorly during development. The anterior PSM differentiate somites, the precursors of vertebrae, although the posterior PSM (pPSM) maintain undifferentiation. Maintenance of the pPSM is required to support ongoing axis elongation. We obtained early-stage snake embryos and identified genes highly expressed in the pPSM by comparing their expression levels with those in somites. Similarly, we identified these genes in the pPSM and somites of vertebrates with shorter bodies than snakes, such as mice, chickens, and turtles. Next, we identified four species orthologs to compare gene expression levels across the four species. Using these orthologs, we compared the expression levels of orthologs expressed in the pPSM of the four species and identified pPSM-highly expressed genes with differences in gene expression levels across the four species. Then we focused on genes that showed a correlation between gene expression levels in the pPSM of the four species and the number of somites. No genes directly involved in axis elongation were detected. However, we identified genes with transcription factor functions that are highly expressed in the snake and genes involved in cell division. Additionally, among genes that are highly or rarely expressed in the snake, many genes with functions related to ECM reorganization were detected. These genes may specifically function in axis elongation in snakes.

Biodiversity is more than meets the eye: Segmentation is conserved despite variation in the underlying gene network

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Much of the Evo-Devo field is driven by the goal of identifying genetic underpinnings of trait variation between species. Morphological or phenotypic differences between species are observed and traced back to identify accompanying and possibly causal genetic variation. Conversely, when morphologies or phenotypes are not different between species, we tacitly or explicitly assume that genetic underpinnings are similarly conserved, often reflecting constrained and thus conserved roles of genetic toolkit members. In 2001, True and Haag published a landmark paper presenting examples of biological phenomena that are more stable during evolution than the genes that control them. They termed this “developmental systems drift” noting that gene network changes in whole systems, notably sex determination, appear to vary without providing selective advantage. The Pick lab and other have, in recent years, found that the genes controlling segmentation vary across insect species, despite the fact that all insects are segmented. We have used the more neutral term “phenotypic stability” to describe this

phenomenon for variations we observe in the segmentation gene network. Specifically, by taking a gene-centric approach, we have examined the cohort of pair-rule genes required to establish segments in phylogenetically diverse insect species. This approach did not rely on tracing morphological or phenotypic differences and thus, one prediction was that there would be no change in the set of pair-rule genes. However, we found examples of loss, gain, and change in function of these genes in different insect lineages. In Hemiptera, a sister group to the holometabolous insects, we find that none of the *Drosophila* pair-rule orthologs have pair-rule expression or function. However, Oncopeltus fasciatus, a model system for this order, do develop via a pair-rule, using at least two genes that have no role in segmentation in *Drosophila*. Thus, the phenotypic outcome of pair-rule-patterning is more highly conserved than the cohort of genes directing it. I will present three short vignettes highlighting the extent of genetic variation in segment patterning across insect radiations and discuss approaches to understand how regulatory genes, which are required for survival, can change during evolution.

Two novel craniofacial genes linked to extreme jaw evolution in pupfishes revealed by tissue-specific transcriptomics and speciation genomics

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Gene expression changes are critical drivers of phenotypic diversity among related species. Despite progress in identifying conserved developmental toolkits across taxa, the role of novel and species-specific gene expression changes in craniofacial divergence between young radiating species remains largely unexplored. Here, we leverage the striking adaptive radiation of Cyprinodon pupfishes endemic to San Salvador Island, Bahamas—which show extreme craniofacial divergence—to uncover the genetic changes underlying jaw morphological evolution. All three dietary specialists exhibit dramatic craniofacial differences that evolved from a common generalist ancestor within the last 10,000 years. Using bulk RNA-seq, we compared transcriptomic profiles of the developing craniofacial region—a hotspot of morphological evolution—to the relatively conserved caudal region across five Cyprinodon species and six populations. Focusing on the hatching stage (8 days post-fertilization), the earliest stage when morphological differences are apparent, we identified a high proportion (>80%) of craniofacial-exclusive differentially expressed genes (DEGs) in specialists. In contrast, generalists showed a relatively equal proportion of caudal- and craniofacial-DEGs. To link expression divergence with underlying genetic variation, we intersected specialist-specific and craniofacial-exclusive DEGs with highly differentiated SNPs (Fst > 0.95) and genomic regions showing strong evidence of hard selective sweeps in each specialist lineage. This approach revealed fourteen candidate genes across all specialists –including pycr3 and atp8a1–which had not previously been implicated in craniofacial development. Using in-situ mRNA hybridization, we confirmed their differential spatial expression in craniofacial structures between species, including the pharyngeal arches and jaw muscles, underscoring their likely functional relevance in divergent craniofacial development. This integrative framework highlights a powerful strategy to identify novel genetic regulators of craniofacial divergence, with implications for understanding human craniofacial variation and

congenital birth defects. Funding: NSF CAREER 1749764 & NIH 5R01DE027052-02 (CHM), NIH 1K99DE034058-01 (MFP)

Cellular mechanisms of bacterial recognition and response in sponges

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Sponges are filter-feeding invertebrates renowned for their intimate associations with bacteria. Like other animals, they must defend against opportunistic pathogens, but like protists they feed (in part) by direct phagocytosis of bacterial prey. Many species also harbor dense and complex microbial communities within their body cavity, not relegated to external mucosal surfaces or the lumen of the gut, as in other animals. This ecological context imposes a unique immunological challenge: the distinction between innocuous microbes and infectious threats cannot rely solely on the detection of conserved microbial features (MAMPs) in privileged body compartments. Still, we find that some bacterial cues, including select LPS variants, activate NF-kB signaling in the freshwater sponge model, *Ephydatia muelleri*. A partial explanation may be that sponges have a diverse catalog of pattern recognition receptors that enable nuanced discrimination between different microbes. To this end, we describe a novel class of candidate glycan-binding receptors potentially involved in bacterial recognition. Finally, we introduce a new sponge–Legionella infection model to investigate responses to intracellular pathogens. This work represents a transition from decades of genomic surveys documenting sponge-bacterial associations toward experimental approaches that reveal the cellular and mechanistic basis of these interactions. By integrating molecular immunology with a novel infection model, we aim to uncover how sponges maintain microbial homeostasis and how immune signaling pathways first evolved during the emergence of animal multicellularity.

Timing is Everything: Temporal Expansion of Abd-B Expression Underlies Evolved Pigmentation in *Drosophila*

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The roles of *Hox* genes in post-embryonic contexts have been largely overlooked, especially with respect to how these roles have evolved. The segmental abdominal pigmentation of *Drosophila* represents an ideal model to study this phenomenon. The *Drosophila Hox* gene *Abd-B* is known to control segmental abdominal pigmentation patterns in species within the melanogaster group. To initiate the expression of pigmentation genes, *Abd-B* is expressed segmentally during pupal development. However, it remains unclear how *Abd-B* evolved control over the pigmentation program within this clade. Therefore, we investigated the spatiotemporal expression of *Abd-B* in multiple species both within and outside the melanogaster group. To visualize the spatial and temporal expression of *Abd-B* during abdominal development, we used Hybridization Chain Reaction (HCR) to probe gene expression during metamorphosis. We found HCR to be a reliable technique for visualizing the expression of lowly expressed genes, especially transcription factors, in the developing abdomen of multiple *Drosophila* species. Time-course analyses of nascent transcription during pupal development showed that in *D. melanogaster*, *Abd-B* is actively

transcribed later into metamorphosis, whereas in the unpigmented outgroup species *D. willistoni*, transcription terminates much earlier. Given that the pigmentation program is not active until the late stages of pupal development, these results suggest that for *Abd-B* to evolve a direct role in regulating pigmentation genes, a temporal expansion of its expression must have evolved within the melanogaster group. This represents a key example of a *Hox* gene evolving its role in controlling late developmental processes.

Regulatory Variation in BarH2 Drives Yellow–White Color Polymorphism in Anatolian Bumble Bees

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Bumble bees continue to captivate researchers due to their diverse coloration patterns and the evolutionary insights they offer. In Anatolia, multiple species of bumble bee converge on a white banded color form, often from yellow banded sister lineages, as a result of mimicry. This presents a replicated system to study the genetic basis and of phenotypic convergence and diversification. To determine the genetic basis of one of these yellow-white polymorphic species, *Bombus niveatus*, we performed a genome-wide association study. This revealed a fixed association peak located in the promoter region of the developmental homeobox transcription factor gene *BarH2*, a gene known to lead to thoracic patterning in *Drosophila*. We examine the evolutionary history of each mimetic yellow to white Anatolian transition with respect to alleles at this locus. Additionally, the discovery of a bilateral gynandromorph specimen—displaying both color forms with corresponding sex-specific traits—offers a powerful natural experiment to decipher the genetic and developmental mechanisms driving this color switch.

Mimetic bumble bees repeatedly employ a *Hox* hotspot to promote modular color pattern diversity

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Bumble bees exhibit an exceptional diversity of aposematic body color patterns that involve modular color combinations by body segment. This global diversity is largely driven by extensive divergence and convergence onto local mimicry patterns. This phenomenon leads to parallel color switches across species spanning mimicry regions, providing replicates ideal for informing evolutionary genetic principles. We utilized GWAS to examine the genetic basis of a red-black pattern transition in three co-mimetic bumble bee species in the western United States and determined the origin of implicated alleles using a phylogenetic approach. We discovered these closely related species each acquired their color forms independently, but did so by targeting the same cis-regulatory locus in an abdominal Hox gene. We connect genotype to phenotype using cross-development and cross-species transcriptomics data to show this gene and associated ncRNAs trigger a core network of genes that ultimately impact several pigment enzymes. We build a model for guiding color diversity in bumble bees involving Hox gene prepatterning and homeotic transformations that create new patterns by shifting colors along the body axis.

The cellular and genetic changes underlying the pectoral girdle transformation from water to land.

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The morphological transformation of the pectoral/shoulder girdle is fundamental to the water-to-land transition in vertebrate evolution. Although previous studies have resolved the embryonic origins of tetrapod shoulder girdles, those of fish pectoral girdles remain uncharacterized, creating a gap in the understanding of girdle transformation mechanisms from fish to tetrapods. We recently identified the embryonic origins of the pectoral girdle in basal and derived teleost species, including the cleithrum as an ancestral girdle element lost in extant tetrapods. Our combinatorial approach of photoconversion and genetic lineage tracing demonstrates that cleithrum development combines four adjoining embryonic populations. A comparison of these pectoral girdle progenitors with extinct and extant vertebrates highlights that cleithrum loss, indispensable for neck evolution, is associated with the disappearance of its unique developmental environment at the head/trunk interface. We also found that gli3, a transcription factor gene in the Hedgehog signaling pathway, regulates osteoblast differentiation in the cleithrum. Overall, our study establishes an embryological and genetic framework for pectoral/shoulder girdle formation and provides evolutionary trajectories from their origin in water to diversification on land.

Investigating cis-regulatory elements near and within the dorsally restricted nr2f2 gene in the retina of the Four-eyed Fish, *Anableps anableps*

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The dorsal-ventral axis of the vertebrate neural retina is compartmentalized through the restricted expression of genes, allowing for the accommodation of different light input across the visual field. Like most vertebrates, the four-eyed fish (*Anableps anableps*) retina contains a ventral and dorsal region that process light from aerial and aquatic sources, respectively. In the four-eyed fish, the ventral region is notably expanded to accommodate a greater degree of aerial light that this fish observes at the surface of the water. During development, spatially restricted gene expression leads to the differential expression of region-specific genes in either retina region. While asymmetric gene expression is well-characterized in the four-eyed fish retina, little is understood about the cis-regulatory landscape controlling the expression of these genes. RNA-seq identified *nr2f2* as one of the highest ranked differentially expressed genes in the dorsal retina of the four-eyed fish. HCR-FISH confirms a dorsally restricted expression pattern for nr2f2 in the outer and inner nuclear layers of the adult four-eyed fish retina. We next performed ATAC-seq on bisected dorsal and ventral retinas and revealed differentially represented peaks in the dorsal or ventral retina. ATAC-seq peaks around *nr2f2* were highly represented in the dorsal retina of the four-eyed fish, suggesting a potential role in controlling *nr2f2* expression. We selected three peaks near or within an intron of the *nr2f2* gene to test their role as putative cis-regulatory elements (CREs). Furthermore, we profiled the activity of the selected CREs using zebrafish transgenesis. Our preliminary results suggest that these elements are active in the retina of the zebrafish. Altogether,

our results suggest that we have identified a potential enhancer cluster with activity similar to the expression pattern of *nr2f2*, indicating that these enhancers could be regulating *nr2f2* gene function in the dorsal patterning of the retina.

Cnidarian striated muscle cell evolution and development revealed through scRNAseq of polyps and medusae of the hydrozoan *Podocoryna americana*

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The hydrozoan Podocoryna americana possesses a complex life cycle featuring both sessile colonial polyp and free-living medusa (jellyfish) life stages, each with distinct morphological and functional characteristics. To determine the molecular basis of life stage-specific traits, such as striated swimming muscles in the adult medusa stage, we performed a comprehensive single-cell transcriptomic analysis of dissected Podocoryna polyps, reproductive (budding) polyps with developing medusae, and liberated adult medusae. Downstream analysis identified distinct cell types including pluripotent stem cells, neurons, digestive gland cells, epithelial cells and smooth and striated muscle cells with different gene expression profiles across life stages. Striated swimming muscle cells were uniquely present in the medusa and specifically expressed homologs of the muscle structural proteins myosin heavy chain, myosin regulatory light chain, tropomyosin and tropomodulin and the myogenic transcription factors Msx and Otx. Gene expression trajectory analysis resolved the development of striated muscle cells from multipotent progenitor cells in budding polyps, highlighting the sequential expression of myogenic transcription factors and muscle structural proteins in developing muscle cells. Phylogenetic analysis of muscle cell marker genes uncovered independent origins and lineage-specific gene duplications of hydrozoan muscle structural proteins from bilaterian homologs, indicating that striated muscle cell types evolved independently in cnidarians and bilaterians. Together, these results reveal the molecular basis for striated muscle development in early animal evolution and create new opportunities to characterize the function, differentiation trajectories, and regeneration potential of muscle cells across all animals.

Species-Specific O-GlcNAcylation Profiles in Arthropod Support Different Mechanisms for Reproductive Success

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O-GlcNAcylation (O-linked β-N-acetylglucosamine) is a dynamic intracellular post-translational modification (PTM) that regulates several cellular processes. This modification is catalyzed by O-GlcNAc transferase (OGT), which covalently attaches GlcNAc to serine/threonine residues, and

removed by O-GlcNAcase (OGA). Although O-GlcNAcylation is essential across metazoans, functional studies in Arthropoda have been largely restricted to the model organism Drosophila melanogaster. Here, we explore the O-GlcNAcylation dynamics throughout arthropods, and how its disbalance regulates their fitness. First, we observed that OGT expression in *Tribolium castaneum* (*Tc*), *Gryllus bimaculatus* (*Gb*) and *Drosophila melanogaster* (*Dm*) are highly dynamic and conserved, with high levels during early embryogenesis. In addition, OGT KD in Tc and Gb reduces egg laying over time, with a significant impact in egg hatching and Tc oogenesis. Comparative analyses of arthropod ovary O-GlcNAcylation suggest different profiles, with a broader distribution in basal organisms, such as Ixodes scapularis, *Parhyale hawaiensis* and *G. bimaculatus*, whereas in *Tc* and *Dm* it is more tightly localized to somatic cells than to germ cells. Single-cell transcriptomic analysis of the Dm ovary further uncovers a higher OGT/OGA expression ratio in somatic cells, underscoring cell-type–specific roles for this PTM. Finally, targeted OGT KD in Dm somatic versus germ cells elicits distinct phenotypes, where only somatic KD accelerates oogenesis and increases egg production over time. Taken together, these findings suggest that O-GlcNAcylation is an important regulator of animal fitness, modulating reproductive capacity and developmental timing through spatially and temporally controlled mechanisms conserved across arthropod evolution.

Conservation and Innovation in the Insect Small ORFome: Functional and Comparative Insights from *Tribolium castaneum* and *Rhodnius prolixus*

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Small open reading frames (sORFs; <100 codons) are increasingly recognized as important genomic elements capable of encoding functional micropeptides involved in diverse biological processes. In this study, we present a multi-omic analysis of the sORFome in the red flour beetle Tribolium castaneum, identifying 454 high-confidence sORFs with unique features, including elevated GC content, an average length of 58 codons, and deep conservation across arthropods and even non-metazoan taxa. Functional annotations revealed numerous protein-binding motifs and conserved domains, while transcriptomic and ribosome profiling data supported both broad and tissue-specific expression, particularly in embryonic and reproductive tissues—highlighting developmental roles and the potential for evolutionary novelty. To explore the functional relevance of these findings in an evolutionary context, we draw on our previous work in the hemimetabolous insect *Rhodnius prolixus*, a vector of Chagas disease. There, we characterized the smORF-containing gene *mille-pattes* (*mlpt/pri/tal*), showing that it plays conserved roles in embryonic segmentation, leg patterning, and head development—paralleling its functions in *Tribolium*. We also identified a hemipteran-specific peptide, smHemiptera, and predicted conserved interactions between Mlpt micropeptides and the Svb transcription factor. More recently, we demonstrated that mlpt regulates gut physiology, hormonal signaling, and vector competence in *R. prolixus*, linking smORF function to Trypanosoma cruzi development and disease transmission. By integrating comparative data from a holometabolous model (*Tribolium*) and a hemimetabolous vector species (*Rhodnius*), we reveal the evolutionary conservation and functional diversification of smORFs across insects. The shared and lineage-specific roles of mlpt and associated micropeptides underscore smORFs as dynamic components of gene regulatory networks. This comparative approach highlights the power of combining evolutionary, developmental, and functional genomics to uncover hidden layers of genome biology with broad implications for insect development and vector-borne disease research.

RNAi Gene knockdown in the marine annelid *Streblospio benedicti* by delivering dsRNA

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The marine annelid *Streblospio benedicti* is a model system for studying developmental evolution and evolutionary genetics, but to date no methods for gene expression manipulation have been established. This annelid has two distinctively different offspring morphs that differ dramatically in their ontogeny, life-history, and morphology. As a model for developmental evolution, *S. benedicti* is easily cultured in the lab; although one major drawback for functional genetic manipulations is that the timing of fertilization cannot be controlled. Typical methods for transgenesis and gene expression manipulation involve microinjections of early embryos, but this approach is most suitable for animals with large brood sizes and established fertilization protocols so that early embryos can be attained. For species where microinjections are difficult, delivery of dsRNA through other means becomes more necessary. Using RNAi methodology, we were able to knock down gene expression in planktotrophic larvae by feeding dsRNA, and in juvenile worms through microinjection of in vitro transcribed dsRNA. We tested two genes: R-opsin (expressed in eyespots and involved in light sensing pathways) and F-actin (ubiquitously expressed in the cellular cytoskeleton). For both larval and juvenile stages, we validated RNA knock-down efficiency by qPCR (quantitative PCR measures amount of gene transcription). For planktotrophic larvae we also used Hybridization Chain Reaction (HCR) in situs, which label RNA, to visualize the reduction in gene expression. Our study constitutes the first time that a gene knockdown by RNAi is developed in a marine annelid species.

Finding the genes that underly life-history and evolutionary developmental differences in the marine polychaete *Streblospio benedicti*

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The process of evolutionary divergence involves changing gene expression during development. We investigate gene expression difference between two alternative larval types of the polychaete *Streblospio benedicti*. This worm can produce two distinct offspring types that drastically differ in egg size, embryogenesis, and larval development. Despite these heritable genetic differences, *S. benedicti* is a single species and represents the earliest evolutionary stage of developmental divergence. We uncover genes whose expression is linked with one of the two developmental morphs and find candidate genes that dramatically change larval morphology. We focus on the chitin-synthase pathway because chaetae are chitin based and are a key morphological structure in polychaetes. We find the first set of genes that contribute to morphological differentiation within a species that has multiple developmental modes.

Spatial resolution of gene expression in the duplicated cornea of the four-eyed fish *Anableps anableps*

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The *Anableps* eye is composed of duplicated corneas, a single pyriform lens and a sub-functionalized retina. Aerial light stimuli travel through the dorsal cornea and reach the ventral retina, while light stimuli from below the waterline, cross the ventral cornea and reach the dorsal retina. With a single optic nerve to receive and process visual inputs, the four-eyed fish has developed adaptations to accommodate simultaneous aerial and aquatic vision. Among the principal modifications that evolved in the *Anableps* lineage are the advent of a dorsal cornea adapted to aerial vision and the regionalization of the retina into distinct dorsal and ventral photoreceptor gene expression domains. The subdivided cornea is a key adaptation of the *Anableps*, yet the molecular programs that distinguish aerial and aquatic cornea remain poorly understood. To obtain high throughput gene expression data and positional information, we have deployed spatial RNA-seq technology using the Visium Spatial platform (10x Genomics). This technique yielded ~2000 spatial 'spots' at a spatial resolution of 55μm, corresponding to ~5–10 cells per spatial spot. Detection of gene expression patterns that correspond to the correct cell populations was confirmed by assessing known markers of the cornea (*krt5*), lens (*cryaa*), and retina (*crx*). Our findings demonstrate that spatial RNA-seq is effective in delineating gene expression territories and identifying distinct clusters in the ventral and dorsal corneas. Our group has established spatial transcriptomics as an invaluable tool for research using non-model species, where the number of available specimens can be a limiting factor.

Cis-Regulatory Control of Dorsal-Ventral Retina Patterning in the Four-Eyed Fish, *Anableps anableps*

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The vertebrate retina establishes dorsal-ventral (D-V) boundaries through spatially restricted gene expression, enabling differential light processing from distinct environments. In the surface-dwelling four-eyed fish (*Anableps anableps*), the dorsal retina receives light from underwater, while the ventral retina detects light from above the waterline. This species has evolved a unique expansion of the ventral retina, allowing for simultaneous aerial and aquatic vision. Despite observations of asymmetric gene expression in the *Anableps* retina, the regulatory mechanisms governing these distinct territories remain poorly understood. Here, we performed bulk RNA sequencing (RNA-seq) to identify differentially expressed genes in the dorsal and ventral retina. Additionally, ATAC-seq in bisected retinae revealed open chromatin regions and putative cis-regulatory elements (CREs) associated with D-V patterning. Notably, we identified a gene desert highly enriched in the dorsal retina, containing a potential cluster of cis-regulatory elements, also

known as super-enhancers. We analyzed nearby genes and identified a dorsally restricted gene positioned near this genomic region. Comparative genomic analysis using mVISTA indicates that this region is evolutionarily conserved, suggesting a critical regulatory role in retinal D-V patterning. Spatial transcriptomics and Hybridization Chain Reaction (HCR) confirmed the dorsally restricted expression of this gene, while single-nucleus RNA sequencing (snRNA-seq) localized its expression primarily to the inner nuclear layer (INL) of the dorsal retina. Altogether, our results suggest that the dorsal-specific expression of this gene is regulated by the identified super-enhancer. Finally, Hi-C analysis demonstrated significant chromatin interactions between the candidate gene and the putative super-enhancer, placing both within the same topologically associating domain (TAD). These findings provide novel insights into a conserved mechanism underlying dorsal-ventral patterning in the retina, offering broader implications for the regulation of vertebrate visual adaptations.

Unraveling the origins of complete metamorphosis: A comparative analysis of metamorphic regulatory gene interactions in hemimetabolous and holometabolous insects

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The evolutionary origin of complete metamorphosis in insects remains one of the most enduring puzzles in evolutionary developmental biology. A key, unresolved question is how the genetic regulation of development diverges between hemimetabolous and holometabolous lineages to produce distinct life history stages. Chronologically inappropriate morphogenesis (Chinmo) and Abrupt (Ab) are conserved transcription factors known to regulate juvenile identity and metamorphic timing in holometabolous insects, making them ideal candidates for comparing developmental regulation across hemimetabolous and holometabolous lineages. To address this, we compared the roles of Chinmo and Ab in holometabolous and hemimetabolous insects. Specifically, we used RNA interference to observe how post-embryonic development is regulated by these genes in the holometabolous beetles *Tribolium castaneum* and *Tenebrio molitor* and in the hemimetabolous milkweed bug Oncopeltus fasciatus. We saw that Chinmo and Ab synergistically suppress Broad (Br) expressions, the pupal specifier, to regulate larval development in *T. castaneum*. In contrast, *chinmo* and *br* are co-expressed and act antagonistically to balance progressive nymphal morphogenesis in O. fasciatus. Current work investigates the roles of Chinmo, Ab, and Br in *T. molitor*. These results suggest that in holometabolous insects, Br has conserved its role as pro-growth factor, while Chinmo appears to have gained a novel role in repressing postembryonic expression of broad. We propose that the temporal separation of Br and Chinmo may have enabled Br to adopt a novel pupal-specifying role while allowing the absence of Chinmo to compress the nymphal stages into one pupal stage. This work provides insight into how changes in the timing of gene expression and interactions may have contributed to the evolutionary emergence of complete metamorphosis.

The regulation of insect metamorphosis: Investigating the role of the leg-gap gene, Distal-less, in *Tribolium castaneum* and *Tenebrio molitor*

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The regulation of developmental timing has important implications for both the survival and reproductive fitness of individuals and their ecological interactions with other organisms. Metamorphosis, characterized by a dramatic transformation from a larva to an adult, results from a complex interaction between various genes and hormones, with juvenile hormone (JH) as an established key regulator. Previous research has suggested the appendage-patterning gene, *Distal-less* (*Dll*), to be an influencing factor in this process, where the knockdown of its expression in the red flour beetle, *Tribolium castaneum*, resulted in appendage malformities and supernumerary molts. Here, we continued the examination of *Dll* in a separate beetle species, the yellow mealworm *Tenebrio molitor*, to better understand the process by which the leg development gene could regulate metamorphic timing. *Dll* knockdown in *T. molitor* resulted in supernumerary molts of the larva. However, the molts were not accompanied by any observable appendage abnormalities, which were previously present in *T. castaneum*. The results propose a potential neuroendocrine role of *Dll* beyond appendage patterning. This aligns with the function of its mammalian counterpart, the *Dlx* gene, which has been shown to be involved in vertebrate brain development, suggesting a potentially important and conserved role of *Dll* in the CNS and JH regulation.

Sea robins as a model for evolutionary innovations

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While advancements in genetics and genomics have transformed evolutionary biology, much of this research has focused on traits that are reduced or lost. In contrast, the molecular mechanisms underlying new or gained traits in wild species remain less understood. Moreover, classic questions about whether evolutionary change results from a few genes of large effect or many genes of small effect are still being debated. To address these questions, we have been developing sea robins as a model for studying dramatic skeletal, sensory, and nervous system innovations in vertebrate evolution. Sea robins are saltwater fish that exhibit numerous evolutionary innovations, including expanded, wing-like pectoral fins and free fin rays (called “legs”) that are separated from the pectoral fin. We crossed two readily accessible sea robin species with notable trait differences, including significant pectoral fin size variation, to produce viable hybrids and explore the genetic basis of species-specific differences. We performed RNA-sequencing and allele-specific expression (ASE) analysis on the pectoral fins of both species and F1 hybrids. We found that the gene *aldh1a2*, which is involved in retinoic acid synthesis, was significantly upregulated in the species with larger pectoral fins. ASE analysis indicated that this expression difference likely results from cis-regulatory changes. Notably, *aldh1a2* has putatively been linked to increased forelimb size in other species, while reduced expression is associated with smaller wings in the flightless emus. When we disrupted *aldh1a2* using CRISPR-Cas9 genome editing, we found that sea robin larvae developed without pectoral fins. Future work will

involve identifying potential species-specific enhancers of *aldh1a2*. Our work with sea robins illuminates the genetic basis of morphological innovations in wild species while exploring the role of key developmental genes in driving evolutionary change.

Enhancer Evolution Underlying Pea Aphid Wing Dimorphisms

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Understanding the origins of phenotypic novelty is a primary conundrum in evolutionary-developmental biology. For instance, we are not sure if novel phenotypes first evolve as a response to the environment, before becoming genetically determined and fixed in a population (genetic assimilation). Pea aphids are an excellent model for studying genetic assimilation, as they produce a novel wingless phenotype via two means: environmental induction in females (polyphenism), and genetic determination in males. Separate paralogs of the gene *follistatin* (*fs*) are involved in each mode of induction. *fs-2* is involved in the female polyphenism, while the allelic presence or absence of the *fs-3* insertion underlies the male wing dimorphism. The female wing polyphenism is ancestral, suggesting that genetic assimilation occurred via fs gene duplication and divergence. Determining changes in enhancers (small regions of DNA that control gene expression) between fs paralogs will provide major insight into how this transition from environmental to genetic activation took place. We have identified multiple enhancers in paralog-specific sequence active in specific cell types of the fly brain, in which fs paralogs are expressed in aphids. The activity of these enhancers provides the first peek into how regulation of these paralogs might have changed post-duplication. Continuing efforts to characterize the regulation of these fs paralogs will help shed light not only on the evolution of pea aphid wing dimorphisms, but also the role of genetic assimilation in the evolution of novel phenotypes.

Separation of church and state: dynamic developmental changes of the cloaca and the formation of an anus

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For most vertebrates the urogenital and digestive tracts terminate in a shared exit opening in the body wall called the cloaca. However, placental mammals and ray-finned fishes have evolved a derived configuration wherein the digestive tract ends in a distinct anus separated from the urogenital system. Despite the importance of these exits and their malformation in human conditions such as persistent cloaca, the developmental genetic controls that underlie cloaca formation and the changes in these controls that lead to an independent anus are poorly understood. We are investigating these mechanisms using zebrafish, which exhibit a pseudo-cloacal arrangement where the hindgut (HG) and pronephric duct (PND) exit the body through adjacent but distinct openings of the vent. Using knock-in endogenous reporter lines, we show that *Hox13* genes are expressed in the terminal regions, with different *Hox* clusters exhibiting different tissue specificity. We find that *hoxa13b* is expressed in more superficial gut layers and

the hindgut sphincter, while *hoxd13a* is present in the intestinal lining and cloacal membrane. Combinatorial loss of *Hox13* paralogs result in the fusion of the HG and PND prior to leaving the body through a single hole, recapitulating the cloaca phenotype also observed in mouse *Hox13* mutants. Next, we assessed the effect of activating mutations in the Vav2/Wasl pathway, which modulates Hox activity in the fins, on cloacal development. Shockingly, we discovered that waslb gain-of-function resulted in the formation of a diastema separating the HG and PND exits. This morphology effectively phenocopies the independent anus of placental mammals. Altogether our findings reveal a conserved role of *Hox13* genes in regulating terminal tube configurations across vertebrates and highlight the Vav2/Wasl pathway as a candidate mechanism in the evolution of independent urogenital opening and anus in mammals.

Manipulation of a polyphenism-switch network reveals the molecular canalization of a threshold trait

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How molecular mechanisms channel environmental signals to predictable developmental decisions remains an enduring question in evolutionary biology. Such decisions are exemplified by developmental polyphenism, whereby environmental cues are translated through a threshold to canalized, alternative forms. In some models for polyphenism, such as the nematode Pristionchus pacificus, genes that regulate a switch between forms have been identified. However, how such regulators toggle between stereotyped forms has yet to be described at the molecular level. Here, we show the transcriptional effects of a suite of mutants that, through their perturbation of different parts of a polyphenism mechanism, either fix one of the two wild-type morphs or one of a range of intermediates. After identifying the transcriptionally critical period of the polyphenism, we found that one of the genes promoting a wild-type morph reverses the polarity of its switch-like activity across the critical window, indicating that polyphenism switching is more dynamic than previously assumed. Also, despite the intermediate morphologies of some mutants, these regulators share a common set of ultimate target genes with wild-type constitutive mutants, suggesting that they together form a bistable switch mechanism. However, the polarity of gene expression in intermediate mutants was unexpected: the transcriptional profiles of Eu-like intermediates resembled those of St-constitutive lines, while St-like intermediates resemble Eu-constitutive lines, showing a discordance between morphology and transcription. Upon investigating this discordance, we identified genes with both strong differential expression between fixed morphs and high variability among intermediates, uncovering a core set of morph-distinguishing genes that are differentially misregulated in intermediates, thereby explaining observed developmental differences. Together, our findings reveal that the P. pacificus polyphenism switch is both temporally dynamic and transcriptionally bistable, and that canalized mouth development depends on the regulation of a conserved gene set.

Towards rapid generation and ready access of monoclonal antibody reagents

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Monoclonal antibodies (mAbs) are invaluable reagents for our community and are used extensively in basic biological research and in the biomedical industry. mAbs comprise a multi-billion-dollar industry annually, but the generation of these reagents is a very expensive and labor-intensive proposition for most academic researchers. An expedient and inexpensive platform to produce robust and stable monoclonal antibodies that can be distributed using an open-source model is highly desirable. To this end, we submit that we can exploit the lamprey immune system, innovative molecular cloning methods, and synthetic biology to rapidly and inexpensively generate robust lamprey-based antibodies (lampribodies) to protein and glycan targets. Lampreys have evolved a convergent adaptive immune system compared to that of the jawed vertebrates (e.g., mouse, human) that employs leucine-rich repeats rather than VDJ segments for building their antibody repertoire. It is estimated that a staggering 10^14 unique lamprey antibodies are theoretically possible, similar to that of the mouse antibody repertoire. We are developing a novel system that expresses lampribodies on the surface of E. coli cells that should greatly facilitate screening and purification for specific antigen binders. Purified lampribodies will then be conjugated to oligos for use in immunohistochemistry-hybridization chain reaction (IHC-HCR). This platform would be much more expedient than the current methods for generating monoclonal antibodies and would provide a useful and affordable tool for various areas of research and medicine. Funding: Internal funding from UC Merced

Are vertebrates constrained to two sets of paired appendages?

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Holocephalans exhibit appendages called pre-pelvic claspers (PPCs) that are located anterior to the pelvic fins, while pelvic claspers (in male cartilaginous fishes) are pelvic fin modifications located posteriorly as modified metapterygia. Articulation points of the PPCs have not previously been imaged, or evaluated in a comparative context, therefore they may represent modified pelvic fin structures if they articulate with the propterygium. Alternatively, they could represent the only example of an independent third set of paired appendages in an extant taxon, if they articulate independent from the pelvic fin basal cartilages, challenging the current paradigm that extant jawed vertebrates are constrained to two sets of paired appendages. We evaluated PPC developmental growth rates, morphology, and articulation pointes in spotted ratfish (*Hydrolagus colliei*, Holocephala) and compared variation among representatives of the three extant holocephalan families. Both the pre-pelvic and pelvic claspers exhibit a dramatic surge in growth at sexual maturity, suggesting synchronous development via shared hormonal regulation and puberty. MicroCT scans revealed that PPCs are not modified propterygia, nor do they articulate with the propterygium. They articulate with the anterior prepelvic process on the anterior puboischiadic bar (or pelvic girdle), and express independent posterior Hox patterning. Taken together, these data suggest that while they are associated with the pelvic girdle, PPCs may indeed represent a third, independent set of paired appendages in extant holocephalans.

Amplification of collagen gene copy number in the elongated and slow-growing nematode, *Caenorhabditis inopinata*

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Understanding the genomic drivers of morphological change is a central goal of evolutionary developmental biology. *Caenorhabditis inopinata* is an exceptional member of its genus, with adults exhibiting dramatic increases in length and maturation time compared to its close relatives (including its sister species, *C. elegans*). Two previous transcriptomic studies reported extensive divergence in collagen gene expression among *C. inopinata* and *C. elegans*. Additionally, some collagen genes regulate body size and shape in *C. elegans*, and a body size TGF-β signaling pathway also regulates the activity of many cuticle collagens in *C. elegans*. Collagen genes may be important evolutionary substrates of body shape and developmental rate divergence. To address this possibility, we estimated the number of collagen genes among the genomes of 54 Caenorhabditis species. *C. inopinata* had 219 collagen genes (nearly >2σ from the mean (178 collagen genes)). Inference of orthologous groups of collagen genes, in tandem with an examination of the genomic location of collagen genes in *C. inopinata*, revealed potential lineage-specific duplication events. PaRerns of synteny suggest the existence of a highly-conserved and ancient casseRe of three collagen genes that are homologous to *col-144*, *col-145*, and *col-10* in *C. elegans*. This three-gene casseRe is largely stable across phylogeny, found in eleven Caenorhabditis species with high quality assemblies. In *C. inopinata*, this three- gene casseRe has expanded to ~13 copies, likely enabled via transposable element activity. Additionally, at least one additional collagen gene casseRe (*col-167, col-168, col-169, col-170*, and *col-171*) has expanded from five copies in C. elegans to ten copies in C. inopinata. RNA-seq data reveal these homologous genes to have divergent transcriptional dynamics between *C. elegans* and *C. inopinata*. To understand the role of these genes in body size regulation, we generated a 5,941 bp deletion removing the entire col-144/145/10 casseRe in *C. elegans*. Preliminary observations suggest this mutation promotes slow growth. Ongoing reverse genetic work that directly manipulates collagen copy number in *C. elegans* and *C. inopinata* will also be discussed. This work suggests the existence of a lineage-specific amplification of a highly- conserved and ancient collagen gene casseRe in a species with a novel body shape and slow rate of growth, and changes in collagen gene copy number may be connected to the evolution of body size and developmental rate in this group.

Can development predict macroevolution? Insights from dung beetle horns and fly wings

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Why do some traits evolve quickly, while others seem stuck in place? Part of the answer may lie in the hidden architecture of development. Developmental variability—how sensitive traits are to developmental perturbations—has long been seen as a generator of phenotypic diversity. But could it also predict how evolution unfolds across many million years? Here, I summarize several recent studies that integrate quantitative genetics, functional genetics (RNAi), and comparative phylogenetic approaches in flies and dung beetles to ask whether developmental variability can

predict both micro- and macroevolutionary divergence. First, using dung beetle horns, we show that developmental architecture channels variation into specific phenotypic dimensions—dimensions that also evolve most rapidly across populations and species. Extending this framework to fly wings, we find that patterns of developmental variability strongly predict heritable variation within populations as well as macroevolutionary divergence over 185 million years. Remarkably, these alignments between development and evolution are consistent across systems, suggesting that developmental variability may be a surprisingly robust predictor of biological diversification. I will discuss the potential mechanisms behind this predictive power and the broader implications for understanding evolvability.

The role of Fat-Hippo signaling in the origin and diversification of beetle horns

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The origin of novel traits is one of the foundational questions in evo-devo, yet the underlying mechanisms driving their evolution remain poorly understood. One possible evolutionary mechanism is the co-option of existing genetic modules into new developmental settings. Here, we investigated the function of the Fat-Hippo signaling pathway in the development of the head horns in Onthophagus beetles, a highly diversified and developmentally plastic novel trait. We focused on this pathway for three reasons: (i) it is an evolutionarily conserved regulator of organ size from vertebrates to arthropods; (ii) our previous analyses of chromatin dynamics indicated enrichment of the binding motif of a key pathway member - Scalloped (Sd) - in open chromatin regions (OCRs) differentially accessible in the horns of male beetles; and (iii) earlier work on other beetle species suggested that this pathway may act as a regulator bridging environmental conditions and plastic trait formation. Using RNA interference we have begun to systematically access gene function of key pathway members. We find - consistent with work on diverse other organisms - that Hippo signaling regulates the relative sizes of appendages such as legs and wings. In addition, however, we find that knockdown of different pathway members affects the relative size, but also as shape as well as orientation of horns relative to the head. Moreover, these functions appear conserved across multiple horned beetle species separated by ~40 million years, suggesting Hippo signaling was recruited very early in beetle horn evolution. We use our findings to explore when and how deeply conserved signaling pathways are able to fuel the origin and diversification of novel traits.

Comparative Analysis of Gene Regulatory Programs in Vertebrate Appendage Regeneration.

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Some vertebrates possess the remarkable ability to regenerate paired appendages after injury. Fossil evidence suggests that this regenerative capacity is an ancient trait among tetrapods. However, salamanders are the only known extant tetrapods capable of regenerating their limbs, indicating that regeneration has been lost in other limbed lineages. Comparative studies of evolutionarily shared genetic mechanisms may offer insights into how genetic programs for regeneration evolved. We explored this by comparing bulk RNA sequencing, spatial transcriptomics, and ATAC sequencing of highly regenerative species—*Ambystoma mexicanum* (axolotl), *Protopterus annectens* (African lungfish), and *Polypterus senegalus* (Senegal bichir)—with the non-regenerative lizard limb of Anolis carolinensis. Bulk RNA-seq at 3 days post-amputation (dpa) revealed genes commonly upregulated in all four species, such as Junb, which has been previously associated with an early response to injury and regeneration. Bulk RNA-seq also unveiled genes upregulated only in the regenerators, such as *Sox11*, with their expression either downregulated or unchanged in lizards. We then investigated the spatial distribution of candidate genes. Spatial transcriptomics revealed distal expression of *Palld* and *Sox11* regenerating species, in contrast to the more proximal or absent/undetectable expression observed in the lizard limb at 3 dpa. Lastly, ATAC-seq revealed differentially accessible regions genome-wide when comparing intact versus regenerating limbs and fins. The combined bulk ATAC-seq and RNA-seq data revealed putative Tissue Regeneration Enhancer Elements (TREEs) near genes differentially expressed during regeneration. Through zebrafish transgenesis, we have begun assessing candidate TREEs for their potential to induce gene expression during regeneration. Ultimately, these findings can advance our understanding of the genetic programs driving vertebrate limb and fin regeneration and provide insight into the evolutionary loss of regenerative capacity in lineages such as mammals.

The role of ontogenetic integration in correlated evolution: Aposematism in poison-dart frogs

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It is common for multiple traits to evolve in a correlated fashion, resulting in complex, integrated phenotypes. Although correlated evolution can, in principle, arise solely due to selection on specific combinations of independently evolving traits, if the correlated traits share a common ontogenetic basis, then selection can more effectively produce concerted phenotypic change by directing the evolution of developmental modules rather than individual traits. The developmental relationships between traits can therefore play a major role on the evolutionary dynamics of complex phenotypes. Although development has been identified as an important player in the evolution of traits with obvious ontogenetic connections, such as different limb or skull bones, its role in less obviously connected suites of traits has not received as much attention. In this talk I present results from ongoing work to understand the role of ontogeny in the correlated evolution of predator avoidance traits in poison-dart frogs. To date, we have found evidence pointing to ontogenetic integration of at least some of these traits via evolution at relatively pleiotropic pathways, such as HIPPO and MAPK, which we are currently working to test functionally.

Developmental and genomic insights on the evolution of dispersal-fecundity polyphenism in soapberry bugs

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Colby College

Phenotypic plasticity is thought to evolve through changes in development that alter the integration of environmental cues. In many insects, polyphenism affecting the development of flight trades dispersal ability for increased fecundity. The soapberry bug *Jadera haematoloma* (Hemiptera: Rhopalidae) exhibits wing polyphenism in response to juvenile nutritional resources and cohort density. Our lab has documented the evolution of polyphenic thresholds in different host-adapted populations, and the role of insulin, EGF and other signaling pathways in wing morph determination and in the development of wing shape. Transcriptomic analysis has provided a functional view of this environmentally-dependent growth control. Assembly and annotation of a genome sequence for *J. haematoloma* provide insights into the evolution of heteropteran chromosomes and, through comparisons with two other rhopalid species lacking wing morphs, allow analysis of gene family expansion and reduction in this polyphenic species. Our lab group is now focusing on the regulation of muscle development and its atrophy, which occurs in soapberry bugs over a matter of 2-3 weeks.

Innovation and diversification of and through sexual dimorphism: insights from horned beetles

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Sexual dimorphism represents one of the most significant dimensions of intraspecific variation, with the potential to constrain or facilitate the diversification of novel traits. Yet, rather than exhibiting equal sexual dimorphism across all traits, organisms are mosaics of tissues that vary in the degree to which they exhibit dimorphism. While the ecological and fitness relevance of sex-dependent development is often well characterized, the genetic and developmental mechanisms underlying the origins of sexual dimorphism and its subsequent evolution are not. Using dung beetles that have evolved multiple evolutionarily novel head horns, my work assesses the regulatory mechanisms that instruct sex-biased development along two critical axes of diversification: divergence across different body regions and macro-evolutionary divergences across species, with the ultimate goal of identifying genomic mechanisms by which sexual dimorphisms originate and diversify. Among others, I aim to test the hypotheses that (i) evolutionary elaboration of sexual dimorphism is made possible through the rapid acquisition of novel cis-regulatory elements and/or the elaboration of sex- and trait-specific transcriptomes, and (ii) that the evolution of novel secondary sexual traits is facilitated by the evolution of novel regulatory interactions, rather than the re-use of preexisting regulatory machinery. I discuss my results in light of fundamental questions regarding gene regulatory network evolution: are the networks that instruct the formation of a trait the same as those that facilitate its diversification, or is context-responsiveness decoupled from trait formation in development and evolution?

Cis-regulatory dimensions of inter- and intraspecific variation in beetle horn development

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Nutrition-responsive and sexually dimorphic traits are among the fastest evolving phenotype classes, yet the contribution of cis-regulatory elements and associated gene networks in facilitating, biasing, or hindering such diversification is largely unexplored. This work aims to deepen current insight into the plasticity and evolvability of developmental gene networks in generating organismal diversity using beetle head horns. Using a combination of comparative genomics and ATAC-sequencing in the genus Onthophagus, we find largely discrete regulatory networks underlie two types of intraspecific variation in horn development, nutritional plasticity and sexual dimorphism, potentially permitting the extreme elaboration of this trait over evolutionary time. Moreover, we identify three distinct classes of cis--regulatory modifications associated with horn diversification within and between species: changes in 1) accessibility and 2) sequence of conserved regulatory elements and 3) acquisition of lineage-specific regulatory elements. We then focus on the exaggeration and loss of plasticity in *O. taurus* and *O. sagittarius* horn development, respectively, and identify an evolutionarily dynamic cis-regulatory landscape nearby candidate genes involved in horn development. Lastly, we validate our predictions by perturbing the function of BMP-signaling regulatory proteins in developing horns to show this signaling pathway has been co-opted during the diversification of this novel trait. More broadly, our results highlight the significance of cis-regulatory elements in mediating gene network interactions responsible for transducing genetic and environmental signals toward alternative or novel phenotypes.

Genomic parallelism defines repeated evolution of an inducible offense

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A common response to resource competition is an inducible offense, the facultative predation of competitors. At its extreme, this response involves alternative phenotypes, or polyphenism. However, the genetic architecture and adaptive potential of polyphenism before new challenges is unknown. Using replicated experimental evolution, during which starved nematodes could consume nematode competitors, we investigated heritable shifts in the induction of a predatory morph. The genetic response showed high parallelism, including repeated selection for a specific cis-regulatory, transcription-factor variant. To explore constraints on polyphenism evolution, we also artificially selected directly for morphology and drove the induced morph near to fixation, revealing a trade-off between morph induction and whole-organism performance. Our results set a genetic expectation for how polyphenism that mediates direct competition evolves amid scarce resources.

Unlocking tooth formation: functional genetic approaches alter cell fates and regeneration rates

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Organogenesis is regulated by extracellular signals that govern both primary organ position and their later replacement or renewal through regeneration. Some organs, including epithelial appendages like teeth and hair, are organized as fields of iterated organ units that often regenerate in a programmed fashion. Spurred by observations of dogs lacking most of their teeth and hair, it was first postulated by Darwin in On the Origin that these disparate epithelial appendages may be homologous. Lab-generated mutants and human disease phenotypes have since revealed immense overlap in genes and signaling pathways that are required for the normal development of multiple epithelial organ field types, providing evidence for Darwin's hypothesis. However, less is known regarding the temporal sufficiency of these same pathways towards driving organ specification and regeneration. Using transgenic stickleback fish and zebrafish that allow temporally controlled gene overexpression, we tested whether Eda and Wnt10a are sufficient to affect tooth field establishment or tooth regeneration rates. In both stickleback and zebrafish, upregulation of Eda and Wnt10a contributed to faster tooth regeneration rates, while only Eda was sufficient to expand tooth fields and initiate ectopic teeth, including facial teeth in sticklebacks. Next, we tested the hypothesis that BMP signaling plays an opposite, inhibitory role on tooth regeneration rate, as occurs during mammalian hair regeneration. In both fish species, upregulation of the BMP inhibitor Grem2a resulted in grossly similar phenotypes as Wnt10a upregulation, suggesting specific effects on tooth regeneration for these two secreted factors. Conversely, upregulation of Bmp6 or Dkk2, a Wnt pathway inhibitor, reduced tooth regeneration rates and tooth number overall by inhibiting the formation of all new teeth, consistent with the known opposing roles for BMP and Wnt signaling during hair regeneration. Together these data support shared genetic responses for the initiation and regeneration of teeth and hair

Epigenetic changes underlying the pea aphid wing plasticity

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Phenotypic plasticity refers to the ability of one genotype to develop different phenotypes in response to varying environmental conditions. In the pea aphid *Acyrthosiphon pisum*, asexual females give birth to genetically identical daughters that develop into either winged or wingless morphs, depending on environmental cues. To investigate the epigenetic mechanisms underlying this wing plasticity, we examined chromatin accessibility in brain and embryo tissues using ATAC-seq, alongside gene expression profiles obtained through RNA-seq. Our analysis identified 50,935 consensus ATAC-seq peaks enriched for transcription factor binding motifs, suggesting these peaks are likely bearing gene-regulation function. Integration of ATAC-seq and RNA-seq data revealed that genes associated with promoter-like accessible regions exhibited elevated

expression levels, indicating a potential regulatory role for these regions in transcriptional activation. Further analysis of differentially accessible regions and differentially expressed genes identified three overlapping candidate genes that need particular attention, as they may play key roles in regulating wing polyphenism in the pea aphid.

Function of Wnt/beta-Catenin signaling during neurogenesis in the annelid Capitella teleta

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In animals, neurons are generated through the process of neurogenesis, which relies on the tightly regulated balance between proliferation of neural precursor cells (NPCs) and production of post-mitotic, differentiated neurons. Studies in vertebrates and the annelid *Platynereis dumerilii* indicate that Wnt/beta-Catenin signaling regulates NPC proliferation and differentiation during neurogenesis, although in different, context-dependent ways. Whether canonical Wnt signaling ancestrally regulated neurogenesis in animals remains an unanswered question, largely due to the paucity of studies outside of vertebrates. In this study, we investigated the role of Wnt/beta-Catenin signaling during neurogenesis in the ventral nerve cord (VNC) of the spiralian annelid *Capitella teleta*. Based on evidence in *P. dumerilii*, we hypothesized that canonical Wnt signaling promotes the transition from proliferating NPCs to differentiated neurons in the developing VNC of *C. teleta*. To test this, we incubated *C. teleta* larvae in the Wnt antagonist iCRT-14 or Wnt agonist azakenpaullone. Following drug treatment, we evaluated changes in the overall architecture of the VNC and characterized the expression of various neurogenic markers. We found that downregulation of Wnt signaling resulted in increased expression of Ct-neurogenin, a marker for proliferating NPCs in *C. teleta*, and decreased expression of Ct-neuroD and Ct-elav1, markers for differentiating neurons. This observation supports our hypothesis that Wnt signaling is required for NPCs to differentiate. In contrast, upregulation of Wnt signaling led to a reduction in the expression of all three neurogenic markers, which may have occurred due to the premature differentiation of NPCs. Future directions include performing cell proliferation assays to evaluate changes in the number of proliferating NPCs and characterizing the expression of additional neurogenic markers, as well as components of the Wnt pathway. Our findings thus far suggest that the involvement of Wnt/beta-Catenin signaling during neurogenesis may be ancestral to at least annelids.

Assessing homology of the ventral midline in the bilaterian annelid *Capitella teleta*

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A synapomorphy of Bilateria is a bilaterally-symmetric body plan, which relies on formation of dorsal and ventral midlines during development. In some taxa including chordates and pancrustaceans, midline cells are genetically and morphologically distinct, and they pattern surrounding tissues. Annelids, within Spiralia, have bilateral symmetry at multiple life history stages, and some annelid larvae have a specialized ciliary band at the ventral midline called the neurotroch. Ciliary bands are transient and are specific to spiralian larvae, expressing genes found only in this clade. We hypothesize that the neurotroch evolved in spiralians and became inserted into the middle of the ancestral bilaterian midline. To test homology of the neurotroch and surrounding cells in the annelid *Capitella teleta*, we characterized expression of *Ct-sim*, a homolog of a transcriptional regulator of midline identity, and *Ct-slit* and *Ct-netrin*, homologs of secreted midline proteins. All three genes are expressed in bilaterally-symmetric domains on either side of the neurotroch in *C. teleta*, although *Ct-sim* is also expressed in other tissues. This bilateral expression contrasts with the midline expression seen in insects and vertebrates. Interestingly, a gradient of BMP signaling regulates dorsal-ventral axis formation including midline formation in some bilaterians, but does not regulate dorsal-ventral axis formation in *C. teleta*. To further explore this, we incubated *C. teleta* embryos in BMP protein and assayed midline formation. Ectopic BMP resulted in loss of the neurotroch and a ventral collapse but not loss of genes expressed on either side of the midline. Our gene expression results suggest that cells on either side of the neurotroch may be homologous to signaling midline cells in other bilaterian animals. However, BMP signaling does not regulate formation of these cells, and future functional studies are required to understand evolution of the midline in annelids.

Immune cell behaviors and gene expression in the ctenophore *Mnemiopsis*

William Browne
University of Miami

Understanding the evolution of metazoan cell types that mediate self/non-self recognition is essential for understanding the origins of metazoan multicellularity. Ctenophora are one of the earliest diverging extant metazoan phyla and are thus well positioned to provide valuable insights regarding the early evolution of innate immune mechanisms and cell types. Our efforts to broadly characterize *Mnemiopsis leidyi* innate immunity have included morphological and behavioral analyses of amoeboid cell types to assess their role in mediating specific innate immune responses. Our characterizations have also included analyses of gene expression from isolated *Mnemiopsis* phagocytes. Our results suggest an early diversification of metazoan innate immune cell types.

Developmental Origins of Novelty in Molluscan Body-Plans

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As one of the largest, most diverse, and visually striking groups of marine animals, molluscs have been used by humans for centuries, valued for materials like pearls and as culinary staples such as pulpo, escargot, and moules. Marine molluscs have also been pivotal in biological research. Notably, studies on a squid species led to the 1963 Nobel Prize in Physiology or Medicine for elucidating the ionic mechanisms of action potentials, and work with a sea slug species contributed to the 2000 Nobel Prize for discovering the biological mechanisms of memory storage. Despite these contributions, biomedicine has since increasingly shifted its focus to cellular and genetic levels, and no mollusc species has joined the pantheon of established "model" organisms like the fly, roundworm, zebrafish, or mouse. This is largely because commonly studied mollusc species were typically large, challenging to breed in laboratory settings, and, critically, their embryos were not amenable to genetic transformation—meaning exogenous DNA and RNA molecules could not be effectively delivered to their eggs, a prerequisite for techniques like CRISPR-Cas9 genome editing. In this talk, I will describe recent advancements to establish genetically tractable species. Using a comparative approach to understand the developmental processes that influence molluscan diversity, I will share recent insights into the evolutionary origins of conserved and novel features.

Early events in annelid regeneration suggest lineage-specific cell dedifferentiation

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Annelids include some of the most remarkable regenerators of the animal kingdom. Like many other animals, annelids regenerate by forming a blastema, a mass of undifferentiated cells at the wound from which new structures develop. However, how their blastema is initially formed is poorly understood. Other animals with extensive regenerative properties such as flatworms employ migrating pluripotent stem cells to carry out regeneration, but the cellular source for annelid regeneration is poorly understood. To better understand where and when blastemal cells arise, we performed a fine-scale time series of cellular and molecular assays after head amputation in the freshwater oligochaete Pristina leidyi. We show that cell proliferation at the wound begins in distinct differentiated tissue layers starting around 9 hours post-amputation (hpa) in the gut epithelium, followed by the mesodermal blastema (12hpa) and then the epidermis (18hpa). Blastemal proliferation begins as soon as the ventral nerve cord extends into the wound site, immediately adjacent to neural termini, supporting nerve dependence. We used pulse-chase experiments to show that dividing cells pre-amputation are not preferentially contributing to the blastema, inconsistent with the previously proposed notion that Pristina regenerates through resident cycling stem cells like flatworms. RT-qPCR and in situ hybridization reveals a sharp de novo upregulation of pluripotency markers in differentiated tissue at the wound within just 3 hours of amputation, long before proliferation begins. These results imply that the same pre-existing differentiated cells at the amputation site transcriptionally take on a stem cell-like identity before dividing. Together, these data support the hypothesis that, like limb regeneration in vertebrates, head regeneration in annelids involves nerve-dependent dedifferentiation and proliferation of somatic cells.