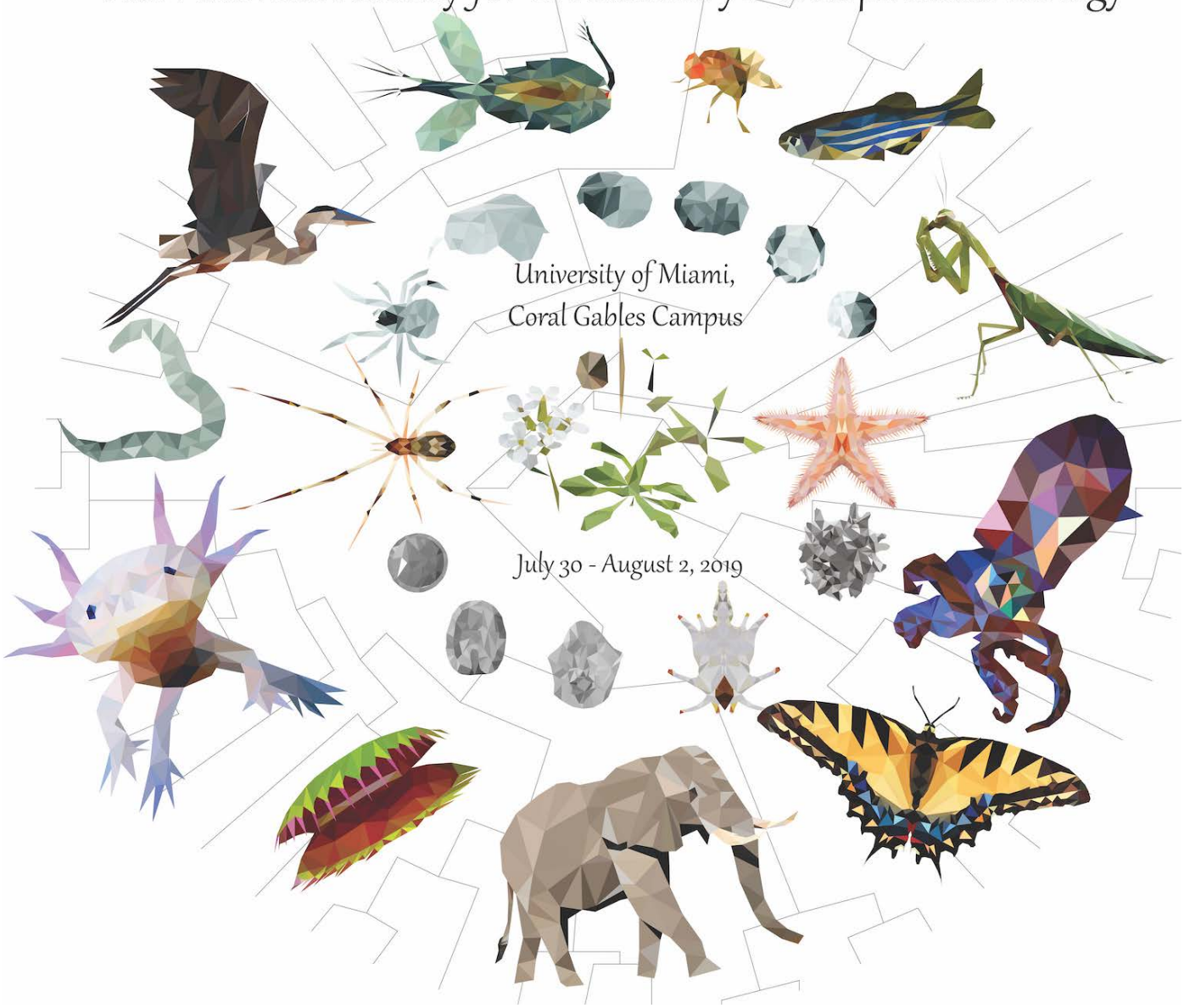
Abstracts & Information Booklet

Pan-American Society for Evolutionary Developmental Biology



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JEZ-B MOLECULAR AND DEVELOPMENTAL EVOLUTION

EVOLUTION & DEVELOPMENT

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Our Local Organizing Committee

Athula Wikramanayake
Billie J. Swalla
Leslie Pick
Chris Amemiya
Maria Puig Shah
and
Ebony Argaez & Katie Reding for designing our meeting logo (see 1st page of this booklet)

Meeting information can be found at our Meeting at a Glance site.

www.evodevopanam.org/meeting-at-a-glance.html

This includes:

- **Travel information & Directions**
- **Campus Map with the Cox Science Center, Shalala Student Center, Mahoney Dorm, Metrorail, and Parking highlighted**

Information about our invited speakers and awardees, including links to their research websites can be found at our Awards & Invited Speakers site.

http://www.evodevopanam.org/awards--invited-speakers.html

Opening Reception

Tuesday - July 30, 2019, 8:00 - 10:00 pm

Workshops

EvoDevo in Latin America
Wednesday - July 31, 2019, 5:00 - 7:00 pm
Cox Science Center Room 145
Organized by Federico Brown

People of Color in Science
Wednesday - July 31, 2019, 8:00 - 10:00 pm
Cox Science Center Room 145

EvoDevo Education Workshop
Thursday - August 1, 2019, 5:00 - 7:00 pm
Cox Science Center Room 126
Organized by Prashant Sharma

LGBTQ+ in Science Workshop
Thursday - August 1, 2019, 8:00 - 10:00 pm
Cox Science Center Room 126
Organized by Sofia Casasa & Tamara Franz-Odendaal

Women in Science Workshop
Friday - August 2, 2019, 5:00 - 6:00 pm
Cox Science Center Room 145
Organized by Tamara Franz-Odendaal

Closing Reception

Friday - August 2, 2019, 8:00 - 10:00 pm

Opening Night Tributes & Awards

Tuesday - July 30, 2019

6:00 - 8:00 pm

Rudy Raff Pioneers Award Presentation

Introduction by Ehab Abouheif

Tribute to Mary Jane West-Eberhard

Young Investigator's Award Presentation

Introduction by Julia Boughner

Tribute to Mary Jane West-Eberhard, PASEDB 2019 Rudy Raff Pioneer Award Winner

Invited Speaker

From alternative phenotypes to nature and nurture: Mary Jane West-Eberhard and her journey towards a true developmental *evolutionary* biology

Ralf J. Sommer

Dept Integrative Evolutionary Biology, Max-Planck Institute for Developmental Biology, Germany

Phenotypic plasticity, the property of a genotype to form distinct phenotypes in response to the environment, is increasingly recognized as major facilitator of evolutionary novelty and evolutionary diversification, although it was largely ignored in the Neo-Darwinian Synthesis in the 1930ies and 1940ies. Mary Jane West-Eberhard was among the first to recognize the importance of phenotypic plasticity and her 2003 monograph provided a solid theoretical foundation for plasticity and its role in evolution. Her theoretical contribution built on comparative studies on solitary hunting and social wasps and more generally, the significance of alternative phenotypes. I will review these theoretical aspects of phenotypic plasticity and the facilitation hypothesis before moving to a comprehensive case study – mouth-form plasticity in nematodes. My laboratory studies the free-living nematode *Pristionchus pacificus*, which we have established as model organism for integrative studies in evolutionary biology, by working at the interphase of developmental genetics, evo-devo, population genetics and ecology. *P. pacificus* is a potential predator of other nematodes, a novel behavioral trait that builds on the formation of a novel morphological structure, the formation of teeth-like denticles. These teeth occur in form of a dimorphism, an example of developmental plasticity that allows mechanistic insight. I will summarize our current understanding of the genetics and epigenetics of mouth-form regulation and will show how transgenerational effects and genetic assimilation can eventually result in evolutionary novelty.

PASEDB 2019 Early Career Award Winner
Invited Speaker

How (and why) the jerboa got its long feet

Kimberly Cooper

University of California San Diego, USA

Decades of mouse genetics and chick embryology have identified genes and pathways that are necessary for vertebrate limb development. Yet loss-of function mutations in the mouse or experimental manipulations in chick often result in dysmorphic and non-viable offspring that do not recapitulate the remarkable adaptive morphologies observed in nature. In an effort to tap into the naturally occurring genetic "selection experiment" that has been ongoing since the origin of the vertebrate limb, I developed the lesser Egyptian jerboa, *Jaculus jaculus*, as a new experimental system. The jerboa is closely related to the laboratory mouse, enabling direct comparison to an established model system with very similar genomic architecture and developmental staging, and yet extremely divergent in terms of its limb morphology. This desert-adapted bipedal rodent has extraordinarily long hindlimbs with three toes on its disproportionately large feet and normally proportioned forelimbs with five fingers. The three metatarsals of each foot have fused into a single bone, and adult jerboas have lost all intrinsic foot muscle. My laboratory takes a hierarchical approach to understand each aspect of this evolutionary transformation at the level of tissue architecture and cell behaviors to associated genes and the cis-regulatory modules that allowed the hindlimb to evolve independent of the forelimb. Toward this end, we are also developing advanced approaches to engineer the mouse genome in an effort to understand the genetics of complex trait evolution.

Plenary Session I

Wednesday - July 31, 2019
8:30 am - 12:00 pm

The *Journal of Experimental Zoology* (JEZ) and its role in the birth of Devo-Evo

A Symposium honoring Günter Wagner

Session Chair: Chris Amemiya

Günter Wagner Symposium
Invited Speaker

Developmental evolution as a mechanistic science 2.0

Manfred D. Laubichler

Arizona State University and Santa Fe Institute, USA

Twenty years ago, at the inaugural meeting of the newly established "Evo-Devo" section of the then American Society of Zoologists (now the Society for Integrative and Comparative Biology), Günter Wagner delivered one of the keynote lectures. Günter, together with Chi-Hua Chiu and myself then published "Developmental evolution as a mechanistic science," an expanded version of this keynote. This perspective then informed the orientation of JEZ: MDE under Günter's editorship. It also brought into the open a conceptual division within the evo-devo community: The distinction between "evo-devo" and the developmental evolution approach ("devo-evo"). This history serves as a backdrop for revisiting the tenets of the developmental evolution program and to ask how it has changed in light of newly available data and conceptual innovations.

Günter Wagner Symposium
Invited Speaker

Limb diversification in Microteiid lizards: morphology, ecology and development

Tiana Kohlsdorf

University of Sao Paulo, Brazil

The tetrapod limb comprises a synapomorphic feature, the autopodium, whose evolution is a key event in the evolution of vertebrates. After its single origin, this structure has morphologically diverged in several lineages, leading to a myriad of limb shapes and sizes. The evolutionary history of Tetrapoda comprises many cases of limb reduction encompassing digit loss, and non-pentadactyl autopodia are widespread among lineages. Recurrent events of digit loss are particularly notable in Squamata such as microteiid lizards (Gymnophthalmidae). In microteiids, non-pentadactyl autopodia independently evolved in two fossorial lineages: Cercosaurini and Gymnophthalmini. In addition, the microteiid literature also provides evidence for the reversal of digit loss. While published data describe a unique muscle anatomy in the autopodia of the Cercosaurini *Bachia*, with the arrangement of both the manus and pes being very similar, our CT-scan images suggest the autopodial bones are in fact distinct. Within Gymnophthalmini, there are two genera of monodactyl species each characterized by a styliform autopodium that resembles the single-digit phenotype of sonic hedgehog knockout mice. We characterized the osteology of these styliform autopodia using CT-scan imaging, and we sequenced the ZRS limb enhancer of *sonic hedgehog* in Gymnophthalmini, and compared these sequences with data from pentadactyl species. We conclude that: 1) in Cercosaurini species, the effects of reversals in digit loss are more extreme in muscles than in bones, 2) in Gymnophthalmini, transitions from the plesiomorphic pentadactyl limb morphology to a spine-like appendage dramatically affect the configuration of bones and muscles, and 3) the sequence patterns in the ZRS limb enhancer provide interesting insights about developmental pathways involved in the evolution of styliform autopodia in microteiids.

Günter Wagner Symposium
Invited Speaker

Hox cluster evolution in the ray-finned fishes

Chi-hua Chiu

Department of Biological Sciences, Kent State University, USA

Hox clusters and genes are an important genetic system for investigating genomic and developmental evolution. Teleost fishes have extra Hox clusters due to shared or lineage-specific genome duplication events and exhibit plasticity in cluster architecture and patterns of conservation of putative and known regulatory sequences. Our work on the Senegal bichir (*Polypterus senegalus*) suggests that Hox clusters in the earliest actinopterygians exhibit patterns of sequence evolution more dynamic than those observed in outgroups including shark, human, and coelacanth and that this trend is further exemplified in teleosts. This work provides insights into extrapolating between genome function of teleosts and outgroups such as human.

Günter Wagner Symposium
Invited Speaker

Reversion and deep homology in Dinosauria

Alexander O. Vargas

Universidad de Chile, Chile

Upon losing function, genes and body parts can degenerate or be entirely lost in evolution. It has been argued that once a body part is lost, it is very unlikely to re-appear (Dollo's Law). Well-documented cases may be considered rare, because most reversions have been disputed: significant structural differences with the lost trait are often present, suggesting the possibility of convergence or a merely superficial resemblance. Even if developmental similarities are present, they can be argued to reflect deep homology, by independent co-option of conserved developmental tool kits. To further this discussion, I will present examples from the evolution of development along the dinosaur-bird transition. The re-appearance of the pisiform in the wrist of birds (previously lost in Averostra) is a compelling case of reversion, being embryologically and anatomically indistinguishable to the ancient pisiform, and involving epigenetic mechanisms (embryonic muscular activity) as expected for a sesamoid. In other cases, such as the ascending process in the ankle of Dinosauriformes, and the false teeth of Pseudodontornithes, the adult structure shows many differences to lost traits, and could be considered entirely novel; however, there is evidence for an at least partial re-deployment of ancient developmental pathways. I will argue that such partial reversions can be considered special instances of deep homology (rather than being incompatible alternatives). Finally, I will discuss the loss of opposability of digit 4 in the foot of passeriform birds, a case in which the re-appeared trait is very similar to its ancient form, but nevertheless develops through a significantly different mechanism.
Funding: Anillo ACT172099 and Fondecyt 1190891, Conicyt, Government of Chile.

Günter Wagner Symposium
Invited Speaker

How the devil ray got its horns: the genetic basis of body plan remodeling in manta rays and their relatives

Karen Crow

San Francisco State University, USA

Compared to sharks, the skates and rays exhibit highly modified body plans that are adapted to life on the benthos, including dorso-ventral compression with pectoral fins that extend anteriorly and fuse to the head. Patterns of morphological evolution in the pectoral fins of most batoids are constrained due to their dual use in feeding and swimming. Skates use their pectoral fins to capture prey by "tenting", and employ undulatory swimming for locomotion. However, the manta rays and their relatives (Myliobatidae) have evolved distinct pectoral appendages that are functionally dedicated to feeding (cephalic lobes) or swimming (modified pectoral fins). Due to the presence of cephalic lobes, the mobulids have been referred to as the only vertebrate with three paired appendages. However, we found no evidence that cephalic lobes develop independently from pectoral fins. In an analysis of differential gene expression from comparative RNASeq data and *in situ* hybridization, we uncovered expression of several patterning genes that are shared between the anterior pectoral fin of skates and cephalic lobes. We found no evidence of independent posterior patterning by HoxD in cephalic lobes, and conclude that cephalic lobes are neither independent nor novel appendages. That said, both cephalic lobes and pectoral fins of myliobatids exhibit adaptations associated with specialized feeding or the evolution of the oscillatory swimming, including a redistribution of pectoral fin rays in the Myliobatidae and *Gymnura* that arose multiple times independently in association with pelagic flight and oscillatory swimming. Batoid fin rays are specified by *HoxA11* early in development, and unique domains in anterior pectoral fin and posterior pelvic fin are specified by *HoxA13*. Finally, we identified candidate genes that are likely associated with subtle changes in paired fin development that may be responsible for morphological disparity among batoids.

Günter Wagner Symposium
Invited Speaker

Revisiting the role of transcription factors in developmental evolution

Vincent Lynch

University of Buffalo SUNY, USA

The role of nucleotide substitutions in *cis*-regulatory elements and amino acid substitutions in protein coding gens in generating evolutionarily relevant phenotypic variation has been a long-standing debate in developmental evolution (and which may have out-lived its usefulness). Central to this argument is which substitutions generate phenotypic variation while minimizing the negative pleiotropic consequences of those substitutions. A large body of experimental studies supports the notion that morphology evolves through changes in both *cis*-regulatory elements and protein coding genes, while theoretical studies indicate both have evolved ways to limit, and can be constrained by, negative pleiotropy. Here I revisit the data supporting both models of developmental evolution and show that changes to *cis*-regulatory elements and proteins play an important role in phenotypic evolution. Next I trace the origin story of the '*cis*-regulatory paradigm' of developmental evolution, which is generally attributed to King and Wilson's classic "*Evolution at two levels in humans and chimpanzees*," to a misunderstanding of Wilson's definition of "regulatory gene" which Wilson used to mean both regulatory elements and regulatory proteins but has been misinterpreted to mean solely *cis*-regulatory elements (principally enhancers).

Selected Abstracts 1

Oral Presentations

Wednesday - July 31, 2019
1:30 - 3:00 pm

EvoDevo of Marine Invertebrates

Session Chair: Athula Wikramanayake

Circulating stem cells, transient niches, and coloniality in tunicates

Juan Jiménez[1], Isadora Santos de-Abreu[2], Laurel S. Hiebert[1], David Soares[1], Silvana Allodi[2], Stefano Tiozzo[3], Cintia M. de-Barros[2], Federico D. Brown[1]

[1]*Universidade de Sao Paulo, Brazil*
[2]*Universidade Federal do Rio de Janeiro, Brazil*
[3]*Sorbonne University, France*

Upon adverse environmental conditions, ascidian colonies can adjust in shape to grow into spatial refuges, or can arrest in time by entering morphologically distinct dormancy stages. In various ascidian species, circulating putative stem cells (CPSCs) have been documented to be involved in regeneration and adult colony asexual propagation. Most studies of these cell population(s) have focused in stem cell parasitism or whole body regeneration in colonial species, but few have investigated the evolutionary origins of CPSCs or occurrence in solitary species. We report the presence of CPSCs in the hemolymph of the solitary *Styela plicata* and identified the intestinal submucosa (IS) as a site of origin for CPSCs. Using imaging flow cytometry of the hemolymph, we found one population of aldehyde dehydrogenase (ALDH+) cells with low granularity, suggesting a stem-like state. To find the origin of CPSCs, we searched for hemoblast-like cells in different tissues by immunohistology using a marker for undifferentiated cells (Piwi) and a marker for mitotic cells (pH3). We observed the expression of these markers in the IS. Ultrastructural analyses confirmed the presence of small cells that resembled hemoblasts close to elongated glandular cells suggesting ongoing signaling in the IS. Altogether, all features presented here of the IS currently support this site as the origin of CPSCs observed in the hemoplymph. Our results support the hypothesis that CPSCs were already present in the solitary styelid ancestor and that these diverged functionally to acquire new roles of asexual reproduction or whole body regeneration in the colonial styelids. A highly dynamic nature of both cellular migration and homing observed in ascidians may serve as an adaptive developmental mechanism to withstand environmental perturbations, as well as to facilitate the repeated evolutionary transitions between solitary and colonial life histories in ascidians.

Regrowing your head: regeneration of the seastar anterior nervous system

Minyan Zheng, Olga Zueva, Greg Cary, Andrew Wolff, Veronica Hinman

Carnegie Mellon University, USA

Regeneration is a fascinating process by which animals are able to reform lost tissues following dramatic injury. Of particular interest is whether regeneration is a genuinely homologous process or has evolved independently in multiple lineages. We will present our recent work seeking to understand how sea star larvae are able to regenerate their anterior nervous system. We have established an innovative BAC-reporter based cell lineage tracing system in these larvae that allows us to determine the origin of regenerated neurons. We show how evolutionary conserved wounding processes establish respecification of ectodermal lineages, re-establish anterio-posterior patterning and then recapitulate an embryonic neurogenesis pathway. We present our findings within comparative framework of these processes in other animals to highlight similarities and differences in this process.

Tracing the evolutionary origin of chordate somites in the indirect-developing hemichordate *Ptychodera flava*

Cindy Chou, Che-Yi Lin, Tzu-Pei Fan, Kuang-Tse Wang, Yi-Hsien Su, Jr-Kai Yu

Institute of Cellular and Organismic Biology, Academia Sinica, Taipei, Taiwan

Somites are a novel character of the chordate body plan and their evolutionary origins remain unclear. In vertebrates, paraxial mesoderm of the trunk region becomes segmented somites, which are further subdivided into specialized compartments: the sclerotome gives rise to the vertebral and rib cartilage and the dermomyotome gives rise to skeletal muscle and the dermis of the back. To elucidate the deuterostome origin of somites and the ancestral mechanism of mesoderm patterning, it is useful to study the chordate's closest sister group, Ambulacraria, which includes hemichordates and echinoderms. In this study, we utilized the indirect-developing hemichordate *Ptychodera flava* to investigate the genetic conservation of mesoderm developmental mechanisms within the deuterostome lineage. First, we used a candidate gene approach where we identified homologues of chordate mesoderm and somite markers in *P. flava* and observed their expressions during embryogenesis. Our data show that many of these candidate genes are also expressed in the hemichordate mesoderm, suggesting that these genes may play conserved roles in mesodermal development. Second, we employed an RNA-seq approach to comprehensively screen for genes associated with mesodermal development in the *P. flava* embryo, which is positively regulated by FGF signaling. In the tornaria larva, we found 244 genes to be concurrently upregulated under FGF signaling activation and downregulated under FGF signaling inhibition. Using *in situ* hybridization, we verified most of these genes were indeed expressed in mesodermal tissue. Our results show that some genes identified in this study may be part of an evolutionarily conserved toolkit for mesoderm development in deuterostomes. Moreover, the mesoderm of the hemichordate also displays compartmentalization, suggesting that regionalization of the mesoderm is an ancestral deuterostome trait. Our results set a foundation for further studies tracing the development of mesodermal tissues in the hemichordate and elucidating the deuterostome origin of somites.

Sex change in marine snails: leveraging ecotoxicology to identify developmental mechanisms

Maryna P. Lesoway, Jonathan Q. Henry

University of Illinois Urbana-Champaign, USA

Sequential hermaphroditism, whereby an individual changes sex over the course of its lifetime, is relatively rare but phylogenetically widespread, providing a useful case study for understanding the evolution of diverse sexual systems. The calyptraeid gastropods, which change sex once from male to female (protandry), have been known to be sequential hermaphrodites over a century. Transitioning males resorb the external penis and grow external female genitalia and accessory structures in addition to shifting from production of spermatocytes to oocytes. The precise timing of this transition is environmentally mediated, primarily by contact with conspecifics. However, the molecular mechanisms controlling sexual development and sex change remain unknown in this group. Taking advantage of the effects of the organotin compound, tributyltin (TBT), which causes growth of male reproductive organs in females of various marine molluscs (known as imposex), we confirm that TBT induces growth of the external penis in *Crepidula atrasolea* and other calypraeids, both in immature juveniles and in mature females. Several mechanisms of action have been suggested for TBT, including binding with the retinoid X receptor (RXR). We therefore tested the effects of various pharmacological agents that disrupt RXR and other components of the retinoic acid (RA) pathway. Penis growth is induced by RA agonists, as well as the RAR and RXR receptor antagonists tested. Dual drug treatments (i.e., TBT and RA antagonists) show reduced penis growth compared to animals treated with TBT alone. These results point towards a role of retinoic acid signaling in inducing male sexual development, and suggest areas of further study in the evolution of sexual development in molluscs.

Rotifer neurogenesis: so simple and yet complex

Evgeny Ivashkin[1,2], Elena E. Voronezhskaya[3], Kristin E. Gribble[1]

[1] *Marine Biological Laboratory, Woods Hole, USA*
[2] *Severtsov Institute of Ecology and Evolution RAS, Russia*
[3] *Koltsov Institute of Developmental Biology RAS, Russia*

Rotifers are microscopic invertebrate animals found in nearly all aquatic habitats. They are thought to be early-diverging Lophotrochozoa, and recent studies place rotifers in the Gnathifera, along with other enigmatic animals which evolution and development are cloudy. Understanding rotifer nervous system development could shed light on the evolutionary history of Gnathifera, but rotiferan neurogenesis is poorly investigated. To fill this gap, we undertook investigation of nervous system development in the monogonont rotifer *Brachionus manjavacas*. We visualized an array of specific neuronal markers in combination with the pan-neuronal marker acetylated alpha-tubulin in developing *B. manjavacas* embryos to characterize neuronal differentiation. *In vivo* nuclear tracing allowed us to see the earliest stages of the neuronal development. Rotifer neurogenesis starts in a solid anterior and two smaller posterior ectodermal neurogenic areas at the gastrula stage. Neuroblast proliferation occurs prior to ingression, and all division spindles are oriented strictly in the plane of the epithelial layer. The anterior zone expands to about half of dorsal surface by late gastrula and later generates cerebral and mastaxal ganglia. Posterior zones merge in the process of foot differentiation and form caudal ganglion. Neurons appear simultaneously within each ganglion and their differentiation proceeding from anterior to posterior. Surprisingly, neurons start to express neurotransmitters (RFamide-like peptides, serotonin, dopamine and acetylcholine) only after complete ganglia formation and neurite outgrowth. Eleven flask-shaped cells emerge very early at the front margin of the anterior neurogenic area, shift dorsally by swelling of the corona, and become sensory dorsal antenna of adults. Such developmental features of the rotifer dorsal antenna may represent homology with the apical organ of mollusks and annelids. Our results demonstrate that differentiation of the *B. manjavacas* central nervous system differs from that of flatworms which are often considered the closest group to rotifers. Anterior neurogenic events look similar to cerebral ganglion formation in directly developing polychaetes, but the rotifer posterior neurogenic zone is unique, disproportionately diminished, and restricted to the foot region. Such a mixture of patterns may suggest a body plan rearrangement and secondary simplification in a Gnathifera ancestor. Comparative analysis of neurogenesis-related gene expression is necessary to clarify this question.
The work was supported by RFBR grant № 19-04-01181 and the Owens Family Foundation.

Ctenophore phagocyte behavior in response to microbial challenge

William E. Browne[1], Lauren E. Vandepas[2], Abigail C. Dieter[1], Grace A. Snyder[3], Nikki Traylor-Knowles[3]

[1]*University of Miami, USA*
[2] *National Oceanic and Atmospheric Administration, USA*
[3]*University of Miami Rosenstiel School of Marine an Atmospheric Science, USA*

Innate immunity is an ancient defense mechanism that operates in multicellular organisms to detect and eliminate pathogens and distinguish self from non-self. When challenged by pathogens, the innate immune response is activated in specific cell types. Activation of these cells is typically triggered by the detection of pathogens at the cell surface by specialized membrane proteins that initiate a signaling cascade, producing a broad immune response that can include engulfment of foreign pathogens. While innate immunity has been studied in many different marine organisms including molluscs, crustaceans, and cnidarians, pathogen defense mechanisms have not been characterized in the ctenophores - a non-bilaterian lineage that diverged early during metazoan diversification. We have used cell biological and biochemical approaches to directly observe ctenophore phagocyte cell type behaviors and signaling responses when challenged by microbial pathogens. We also identify putative gene homologs in several ctenophore species, supporting the presence of innate immunity components necessary for surveillance at the cell surface, intracellular signaling and transcriptional response to foreign pathogens. Our findings in the ctenophore lineage provide a unique opportunity to explore both conserved and novel aspects of pathogen defense mechanisms associated with the evolution of the animal immune system.

Plenary Session II

Thursday - August 1, 2019
8:30 am - 10:00 am

Evolution of Developmental Gene Networks

Session Chair: Rodrigo Nunes da Fonseca

Invited Speaker

Social regulation of a rudimentary organ generates complex worker-caste systems in ants

Rajendhran Rajakumar, Sophie Koch, Mélanie Couture, Marie-Julie Favé, Angelica Lillico-Ouachour, Travis Chen, Giovanna De Blasis, Arjuna Rajakumar, Dominic Ouellette, Ehab Abouheif

McGill University, Canada

The origin of complex worker-caste systems in ants perplexed Darwin and has remained an enduring problem for evolutionary and developmental biology. Ants originated approximately 150 million years ago, and produce colonies with winged queen and male castes as well as a wingless worker caste. In the hyperdiverse genus Pheidole, the wingless worker caste has evolved into two morphologically distinct subcastes—small-headed minor workers and large-headed soldiers. The wings of queens and males develop from populations of cells in larvae that are called wing imaginal discs. Although minor workers and soldiers are wingless, vestiges or rudiments of wing imaginal discs appear transiently during soldier development. Such rudimentary traits are phylogenetically widespread and are primarily used as evidence of common descent, yet their functional importance remains equivocal. Here we show that the growth of rudimentary wing discs is necessary for regulating allometry—disproportionate scaling—between head and body size to generate large-headed soldiers in the genus Pheidole. We also show that Pheidole colonies have evolved the capacity to socially regulate the growth of rudimentary wing discs to control worker subcaste determination, which allows these colonies to maintain the ratio of minor workers to soldiers. Finally, we provide comparative and experimental evidence that suggests that rudimentary wing discs have facilitated the parallel evolution of complex worker-caste systems across the ants. More generally, rudimentary organs may unexpectedly acquire novel regulatory functions during development to facilitate adaptive evolution.

Invited Speaker

Emerging models of duplication and loss of eye structures: the Amazonian four eyed fish (*Anableps anableps*) and the subterranean catfish (*Phreatobius cisternarum*)

Patricia Schneider

Universidade Federal do Pará, Brazil

Vertebrate eyes share the same general organization however, many species have developed individual specializations that improve their visual perception of the environment. The subterranean catfish *Phreatobius cisternarum* lives in a phreatic environment, under these conditions, this species presents anatomical (miniaturization) and molecular adaptations at the level of their visual perception. *Anableps anableps* consists in a unique model organism to study the plasticity of eye development. The eye of *A. anableps* is partially divided and composed of duplicated pupils and corneas, through which the light stimuli from under and above the waterline enters the eye at the same time. Moreover, the adult fish has a retina that is divided into dorsal and ventral regions with asymmetric expression of photoreceptor proteins, which allows for simultaneous aerial and aquatic vision. In this work, we have combined histological, molecular and RNA-seq analysis to understand the visual and sensory adaptations to the phreatic environment of *P. cisternarum* and the partial eye duplication in *A. anableps*.

Invited Speaker

Evolution and development of floral zygomorphy

Wenheng Zhang

Department of Biology, Virginia Commonwealth University, USA

Studies of developmental biology have suggested that macroevolutionary patterns are rooted primarily in properties of development, which are thought to restrict variation and to bias transformations. The independent evolution of similar characters (also known as homoplasy) could therefore reflect constraints in development as well as selection by similar environmental pressures. One of the most prominent homoplastic traits in angiosperms is floral zygomorphy. Zygomorphic (i.e., bilaterally symmetrical, monosymmetric) flowers that possess only a single plane of symmetry have originated from actinomorphic- (i.e., radially symmetrical, polysymmetric, regular) flowered ancestors that have three or more planes of symmetry repeatedly during angiosperm evolution (at least 199 times). These shifts in floral symmetry are believed to reflect responses to selection by insect and later by vertebrate pollinators and result in specialized plant-pollinator interactions. Recent work in developmental genetics has begun to reveal the molecular mechanisms underlying the development of the diverse zygomorphic flowers in angiosperms. While this has advanced our knowledge of the evolution of floral symmetry, how the particularities of development in individual clades influence the course of morphological evolution of zygomorphy in those clades has rarely been investigated. We recently analyzed patterns of floral organ initiation and displays of zygomorphy, representing 405 taxa in 330 genera, covering 83% of orders (30 out of 36) and 37% of families (116 out of 313) in angiosperms in the context of a phylogeny using ancestral state reconstructions. We have demonstrated that development indeed constrains the processes that give rise to floral zygomorphy, while phylogenetic distance allows relaxation of these constraints, which provides novel insights on the role that development plays in the evolution of floral zygomorphy.

Selected Abstracts 2

Oral Presentations

Thursday - August 1, 2019
10:30 - 12:00 pm

EvoDevo Mechanisms

Session Chair: Tamara Franz-Odendaal

Evolutionary Developmental Biology (Evo-Devo-Path): linking evolution, development, anatomical variations and anomalies, and medicine

Rui Diogo

Howard University, USA

Since the rise of Evo-Devo few authors have attempted to combine the increasing knowledge obtained from the study of model organisms and human medicine with data from comparative and evolutionary biology in order to investigate the links between development, pathology and macroevolution. Fortunately, this situation is slowly changing, with a renewed interest in Evolutionary Developmental Pathology (Evo-Devo-Path). However, this interest and the new data obtained from it, as well as their main implications for evolution and medicine, have not been the object of a synthesis to the broader scientific community and wider public. Here I will provide such a synthesis, including 1) a brief historical account on the study of the links between evolution, development and pathologies; 2) case studies from the recent work done by me and other colleagues on subjects related to Evo-Devo-Path; and 3) a general discussion on the broader anatomical, developmental and macroevolutionary implications of these case studies and of research recently done by other authors. An important aim is to contribute to the understanding of the links between the phenotype and genotype, within both normal and abnormal development. Therefore, I will also include the results of work I have been doing with physicians and surgeons who are interested in directly applying and/or collecting Evo-Devo-Path data to their own medical interventions, as part of an effort to focus on the applications and implications of these data for direct medical use. My primary aim is to highlight the strength of studying developmental anomalies within an evolutionary framework to understand morphological diversity and disease by connecting these recent works with the research done and broader ideas proposed by authors such as Étienne Geoffroy Saint-Hilaire, Waddington, Goldschmidt, Gould and Per Alberch, among many others, to pave the way for further and much needed work regarding abnormal development and macroevolution.

Evolution of MEIS transcription factor activity during metazoan neural development

Pavel Federenchik, Dale Frank

Department of Biochemistry, Technion - Israel Institute of Technology, Israel

Antero-posterior (AP) axis formation in bilaterians requires Homeobox transcription factor (TF) proteins. Meis family homeodomain and Hox TFs interact to induce neural AP pattern in vertebrates. In *Xenopus*, the Meis3 TF controls specification of hindbrain and neural crest fates. Meis orthologues have two highly conserved domains: (1) Meis-specific box (2) Homeobox. Hox and Meis proteins are also expressed in radial Cnidarians, and in single-celled eucaryotes like amoeba and algae. *Xenopus* and *Drosophila* Meis TFs both induce posterior neural tissue (hindbrain and spinal cord) in *Xenopus* embryos and explants. We asked: When during metazoan evolution did Meis proteins acquire the ability to induce the vertebrate-specific hindbrain? We initially analysed activity of Cnidarian (*Nematostella*) Meis proteins in *Xenopus*. By dissecting the Meis TF into various domains, we found that the highly conserved Meis-box and Homeobox DNA-binding domain suffice to induce spinal cord, but not hindbrain. Bilaterian and Cnidarian Meis TFs share a functionally conserved transcription activation domain in the C-terminus that induces hindbrain. Meis proteins from unicellular algae like *Chalmydomonas* and *Volvox* also induce hindbrain in *Xenopus*. These proteins have a homeodomain, but lack the Meis-box. A truncated *Xenopus* Meis3 protein containing the homeodomain+C-terminus domain, but lacking a Meis-box efficiently induces hindbrain, like Meis proteins from algae. Early in metazoan/eucaryotic evolution, the ability to induce "vertebrate" structure was already intrinsic to Meis protein activity, suggesting that the basic building-blocks regulating neural AP axis formation in vertebrates pre-date the structures themselves. Early eucaryotes started off with powerful TFs (Meis and Hox) that were later utilized along the evolutionary pathway to create our most advanced and vertebrate specific neural structures, the hindbrain and neural crest.

Evolution of a nuclear receptor-mediated mechanism regulating developmental plasticity

Sofia Casasa, Erik J. Ragsdale

Indiana University, USA

Developmental plasticity, or the ability of developmental systems to vary with the environment, is a widespread phenomenon in nature. Genetic mechanisms for developmental plasticity have started to be revealed in a few model systems, although how these mechanisms have evolved to give rise to a wide diversity of plastic responses remains poorly understood. The nematode *Pristionchus pacificus*, like several other members of its taxonomic family (Diplogastridae), exhibit a mouth polyphenism in response to starvation and crowding. Low bacterial-food availability and high population densities induce development of a predatory (eurystomatous) morph that feeds on other nematodes, whereas high bacteria and low population densities increase the production of the microbivore (stenostomatous) morph. The developmental genetics underlying this polyphenic response is increasingly well understood and involve a switch-like mechanism composed of multiple genetic factors that can be manipulated in the lab. Given both the wide diversity of plastic responses in the family, including secondary loss of plasticity, and the ability to perturb genes that control them, this system provides a unique opportunity to track the evolutionary history of the mechanisms underlying developmental plasticity. By using a comparative RNA-sequencing approach paired with genetic mutants, we aim to better understand 1) the mechanisms regulating polyphenic development downstream of the switch-like mechanism and 2) whether and how these mechanisms have diversified across species.

Evolution of anterior-posterior axis specification and patterning: insights from the sea urchin embryo

Susan Gautam[1], Stephanie Burr[2], Ryan C. Range[1]

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The early specification and patterning of cell fates along the primary body axis of many metazoan embryos relies on a gradient of Wnt signaling. In most embryos this patterning mechanism depends primarily on high levels of localized canonical Wnt/Beta-catenin signaling around one pole of this embryonic axis, which will form endoderm/endomesoderm, and localized Wnt signaling antagonists around the opposite pole that typically aid in specifying the ectodermal and neuroectodermal territories. We have recently shown for the first time in any embryo that the deuterostome sea urchin integrates information from three different Wnt signaling branches (the canonical Wnt/Beta-catenin as well as non-canonical Wnt/JNK and Wnt/PKC pathways) to specify and pattern early regulatory states along the embryonic anterior-posterior (AP) axis. Our functional evidence indicate that these pathways interact at through several extracellular Wnt signaling modulators (e.g. Wnt1, Wnt8, Wnt16, and Dkk1), receptors (Fzl5/8 and Fzl1/2/7), intracellular transduction molecules (e.g. PKC, NFAT, and ATF2) and the transcriptional gene regulatory networks they activate. Here, we present new evidence that the transcription factor Sp5 is activated by the non-canonical Wnt1/Wnt8-Fzl5/8-JNK signaling pathway that is essential to position the anterior neuroectoderm (ANE) gene regulatory network (GRN) around the anterior pole of the embryo. Our functional data indicate that SP5 acts downstream of Wnt1/Wnt8-Fzl5/8-JNK to downregulate the ANE GRN from more posterior equatorial ectoderm cells. Importantly, evidence from vertebrate studies indicates that Sp5 also works downstream of early AP Wnt signaling to position the ANE GRN around the anterior pole. Together with our results, these data suggest that the Wnt/Sp5 regulatory cassette represents a fundamental AP patterning mechanism conserved among deuterostome embryos.

Evolution of Axin function in the Wnt/β-catenin signaling pathway in metazoans: Insights from the cnidarian *Nematostella vectensis*

Hongyan Sun, Kurt Statz-Geary, Athula Wikramanayake

Department of Biology, University of Miami USA

Axin is a critical scaffolding protein in the Wnt/β-catenin (cWnt) pathway that binds several components of a destruction complex (DC) that regulates β-catenin stability. In bilaterians, Axin interacts with β-catenin through a conserved binding domain (βcatBD) and targets it for degradation. APC, another major scaffolding protein in the DC also has multiple β-catenin binding domains distinct from the βcatBD in Axin. In bilaterians, binding of β-catenin to both Axin and APC is important for its phosphorylation and subsequent degradation via the proteasome. Intriguingly, Axin proteins in all four non-bilaterian taxa lack the βcatBD, questioning a conserved role for this protein in regulating the signaling pool of β-catenin in all metazoans. To determine Axin function in the DC in the non-bilaterian *Nematostella,* NvAxin was overexpressed in *Nematostella* embryos by RNA injection, and Axin and APC were knocked out using CRISPR/Cas9. Overexpression of NvAxin did not produce any obvious cWnt-dependent phenotypes nor did it affect endomesoderm gene expression. However, CRISPR/Cas9-mediated knockout of *NvAxin* and *APC* produced ectopic oral structures consistent with a role for the Axin/APC complex in negatively regulating cWnt signaling in *Nematostella*. To determine if NvAxin could regulate cWnt signaling in a bilaterian we overexpressed it in sea urchin embryos. We saw that while overexpression of sea urchin Axin in sea urchin embryos downregulated cWnt signaling and severely anteriorized embryos, overexpression of NvAxin in sea urchin embryos had no effect. Our results suggest that Axin forms an essential part of the DC in *Nematostella*, but NvAxin alone is not sufficient to downregulate cWnt signaling. We propose that unlike in bilaterians where β-catenin binds to both Axin and APC, in *Nematostella* β-catenin binds to APC and GSK3β binds to Axin thereby forming a functional DC. These results are providing insight into the early evolution of the β-catenin DC in metazoans.

Metabolic adaptation and resiliency in cavefish

Nicolas Rohner

Stowers Institute for Medical Research, USA

Adapting to extreme environments requires drastic changes to an animal's metabolism. Adaptation to the total darkness of caves can be particular challenging. One hallmark of cave environments is a shortage of internal food sources, which requires drastic morphological, behavioral, and physiological adaptations. *Astyanax mexicanus* is a promising research organism to unravel the genetic basis of such changes, as surface and cave morphs of the same species are available for comparison. Additionally, both morphotypes remain interfertile and can be bred outside their natural environments. We have previously shown that cavefish evolved impressive physiological adaptations such as increased appetite, starvation resistance, and altered feeding behaviors in part due to mutations in *mc4r*. In addition, we found that cavefish display elevated blood sugar levels and insulin resistance caused by a mutation of the insulin receptor. In contrast to human patients, carrying similar mutations, cavefish do not display common markers and symptoms of metabolic diseases and high blood sugar. Furthermore, cavefish develop large amounts of hypertrophic visceral adipocytes without obvious signs of inflammation due to reduced amounts of pro-inflammatory cytokines. Taking together, our work suggests that cavefish develop these phenotypes as part of their starvation resistance and have evolved resilience phenotypes that allow them to tolerate stark deviations from what would be considered normal physiology in other vertebrates.

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Oral Presentations

Thursday - August 1, 2019
1:30 - 3:00 pm

On the Wings of EvoDevo

Session Chair: Sofia Casasa

Evo-Devo of butterfly wing patterns

Jeffrey Marcus

University of Manitoba, Canada

The evolution and development of lepidopteran wing patterns are a valuable system for understanding cellular development and differentiation of phenotypes with clear ecological functions. Butterfly wings are only two cells thick, with single epithelia making up the dorsal and ventral wing surfaces. The color patterns found on lepidopteran wings are formed from a mosaic of scale cells containing pigments or with structural coloration caused by the reflection of particular light wavelengths by ridges found on scale surfaces. Butterfly color patterns are laid down on top of a pre-existing developmental genetic architecture that directs wing development and which determines the shape of each wing, allows for the functional specialization of forewings and hindwings and of dorsal and ventral wing surfaces, and which specifies the positions of the longitudinal veins on the wing. This architecture also provides a mechanism by which different regions on the wing can be regulated independently of one another. The Nymphalid groundplan is an archetype that can be used to compare and homologize color patterns from different Lepidoptera species. Many genes related to the determination of many color pattern elements within the Nymphalid groundplan have been identified, and for border ocelli (or eyespot) patterns in particular, a large genetic regulatory network for pattern formation and differentiation has been assembled. Many Lepidoptera model species have contributed to our understanding of butterfly color pattern development and evolution, but recent comparative genomic work examining of intraspecific and interspecific variation in *Heliconius* has identified new genes with important roles in pattern development. The gene products produced by the genes *WntA, cortex, optix,* and *aristaless1* appear to be responsible for much of the intraspecific and interspecific wing color pattern variation in *Heliconius.* These gene products also feature in color pattern development in butterfly species that adhere more closely to the Nymphalid ground plan.

An evo-devo laboratory course for undergrads using CRISPR in butterflies and frogs

Arnaud Martin

The George Washington University, USA

"*The things we hear about in other biology courses about genome editing were actually performed in this class and we got to see real organisms with the result of the injections we did* [...]. *This class revealed how important it is for us to contribute to the right sort of conversation in scientific innovations as we graduate from college.*" *~Anonymous student feedback*

CRISPR offers new opportunities for biology students to perform inspiring Evo-Devo research that explore the gene-to-phenotype relationship in depth. It is also crucial to introduce the future generation of biologists, practitioners and other protagonists to the technical and societal aspects of gene editing. In this talk, I will share my experience leading an undergraduate laboratory class for the past 5 semesters, where biology senior undergraduates formulated hypotheses regarding the roles of candidate genes, designed guide RNAs targeting them, and each injected CRISPR in hundreds of embryos, targeting developmental genes such as *abdominal-A, STAT92E, WntA, optix*, and *slc45a2*, in two strategically chosen vertebrate and invertebrate organisms: the *Vanessa cardui* butterfly and *Xenopus laevis* frog. Both frogs and butterflies are commercially available are outstanding teaching tools as they provide scalable numbers or readily fertilize, large eggs that can be injected using cheap microinjection devices. Each semester, students consistently generated spectacular mosaic knockout morphological phenotypes with key insights in developmental biology and functional genomics. The class also includes discussions, readings, student presentations and essays on the Bio-Ethics of Genome Editing. Student feedback and subsequent applications to PhD programs indicated the approach fostered the student interests for research careers.

Genetics of scale development and sexual dimorphism on butterfly wings

Brian Counterman, Luis-Fernando Rodriguez-Caro, Shivam Bhardwaj, Jared Cole, Jennifer Fenner

Department of Biological Sciences, Mississippi State University, USA

Butterfly wings provide an excellent opportunity to study the evolution and development of morphological diversity. The colors of butterfly wings are the result of pigments and cytoskeletal structures in scales that are finely organized across the wing surface. Arrays of these differently colored scales collectively produce the striking color patterns found on butterfly wings. Some of the most remarkable variation in wing color patterns is found between different sexes of the same species, with males often displaying bright, dazzling colors that influence their chances of mating success. The Dogface butterfly, *Zerene cesonia*, offer an example of this sexual dimorphism in color pattern, with males having bright ultraviolet (UV) reflective wing patches that are not present on the female wings. This bright UV reflectance results from tightly organized nanostructures across the surface of wing scales that are lacking on female scales. Here, we use an RNA-seq approach to characterize gene expression patterns in developing wings of males and females. The color patterns of male and female *Z. cesonia* have similar pigment patterns, but clearly differ in scale nanostructures responsible for UV reflectance. We use these data to characterize the gene networks associated with butterfly scale development and to demonstrate the developmental homology of butterfly wing scales and wing sensory bristles in Drosophila. Next, we explore genes with sexually dimorphic expression to identify candidate gene networks involved in the development of UV reflecting structures on male wings. Lastly, we explore the role of *doublesex (dsx)*, a gene known to be involved in sexually dimorphic development across insects, in the development of the sexually dimorphic patterns on *Zerene* wings. We present several candidate genes involved in the development of UV reflecting structures and explore the evolution of a novel *dsx* isoform involved in wing development.

Characterizing co-option in the evolution of the treehopper helmet, a novel body wall outgrowth

Cera R. Fisher, Jill L. Wegrzyn, Elizabeth L. Jockusch

University of Connecticut, USA

How novel traits and characters originate remains a central question in evo-devo. In arthropods, new characters have repeatedly arisen as outgrowths of the body wall, and the helmet structure of treehoppers (Hemiptera: Membracidae) is a particularly remarkable example of such a novelty. Anatomically, the treehopper helmet is a 3-dimensional projection of the pronotum (dorsal body wall of the first thoracic segment), which has been molded by natural selection into myriad elaborate forms. The presence of the helmet distinguishes treehoppers from their close relatives the leafhoppers (family Cicadellidae), which retain the ancestral condition of the pronotum—small and flat, similar to its serial homologue the mesonotum. We tested three hypotheses for the origin of the novel treehopper helmet by comparing transcriptomes of specific body regions in a leafhopper and a treehopper. In leafhoppers, pronotal gene expression is most similar to that of the mesonotum. By contrast, in treehoppers, helmet gene expression is most similar to that of wings, supporting the wing-network co-option hypothesis for the origin of the helmet. To understand the relationships between the differentially regulated genes that produced the co-option signal, we constructed gene modules with weighted gene co-expression network analysis (WGCNA). In performing this analysis, we found that WGCNA is sensitive to sample-specific gene expression, and developed methods to detect and rule out such effects. We did not find a module that correlated significantly with the treehopper helmet and wings, highlighting the fact that, co-option notwithstanding, the treehopper helmet is a very different anatomical structure from wings. However, we were able to recover co-expressing modules of genes that correlated strongly with treehopper and leafhopper wings, and a separate module associated with treehopper helmets. The hub genes of all of these modules included genes associated with wing development, further supporting wing-network co-option.

Social regulation of insulin signaling and the evolution of eusociality in ants

Vikram Chandra[1], Ingrid Fetter-Pruneda[1], Peter Oxley[2], Amelia Ritger[3], Sean McKenzie[4], Romain Libbrecht[5], Daniel Kronauer[1]

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[4]*University of Lausanne, Switzerland*
[5]*Johannes Gutenberg University, Germany*

How complex animal behavior evolves is not well understood. The origin of eusociality, a major evolutionary transition in individuality, is an extreme instance of such behavioral evolution: fundamentally, it requires the emergence of an obligately sterile, altruistic worker caste, and the evolution of a colony-level developmental program for caste differentiation. Social evolutionary theory has explained how this can be an adaptive strategy, but its proximate molecular mechanisms have been obscure. Ants evolved eusociality roughly 140 million years ago from subsocial wasps that had reproductive cycles. The queens and workers that comprise eusocial ant colonies are thought to be homologous to the reproductive and non-reproductive phases of this ancestral cycle. To identify conserved candidate regulators of the eusocial division of labor, we conducted a comparative transcriptomic screen across seven ecologically- and phylogenetically-diverse species. We discovered that insulin expression is always upregulated in the brains of queens and other reproductive ants across the ant phylogeny. In most animals, insulin directly regulates ovary activation and oocyte development, and is thus a good candidate regulator of the reproductive division of labor. We studied the expression and function of insulin in the relatively-tractable queenless clonal raider ant. We found that insulin is primarily produced in a cluster of 15 neurons in the brain. Signals from the larvae suppress adult insulin expression independently of nutritional state, inhibiting the adults' reproduction and inducing stereotyped colony reproductive cycles that are reminiscent of ancestral subsocial cycles. Increasing insulin peptide levels (either by experimentally injecting synthetic insulin peptide, or by increasing access to nutrition during development) overrides larval suppression, producing obligately reproductive ants. These data lend themselves to a simple model for the evolution of eusociality via nutritionally-determined reproductive asymmetries potentially amplified by larval signals.

Evo-Devo of Wing Shape in the Lepidoptera

H. Frederik Nijhout, Kenneth Z. McKenna

Department of Biology, Duke University, USA

The Lepidoptera (butterflies and moths) comprise some 150,000 species, each with a distinctive wing shape and color pattern. Both shape and color pattern are determined during the last larval instar and the pupal stage. Larval imaginal disks show discrete patterns of expression of a set of morphogens that divide the wing into four compartments along the antero-posterior axis. We found that wing shape in the adult determined by differential proximo-distal and antero-posterior growth in these four compartments. Species-characteristic differences in adult wing shape can be explained by species-specific differential growth in the four compartments. In many species, the color patterns also show distinct discontinuities at the compartment boundaries.

Plenary Session III

Friday - August 2, 2019
8:30 am - 10:00 am

EvoDevo of Innovations

Session Chair: Leslie Pick

Invited Speaker

Genetics, development, and evolution of phenotypic diversification, integration, and innovation in monkeyflowers

Yaowu Yuan

Department of Ecology and Evolutionary Biology, University of Connecticut, USA

Understanding the genetic bases and developmental mechanisms underlying phenotypic diversification and innovation is one of the central aims of evo-devo and, justifiably, has attracted considerable research effort in the past decades. However, the genetics and development of phenotypic integration — another important aspect of phenotypic evolution – – have been substantially less explored. In this talk I will lay out a long-term plan to address all three problems with a single system — a group of closely related monkeyflowers (*Mimulus*), including one bumblebee-pollinated, two hummingbird-pollinated, and one self-pollinated species. Despite being dramatically different in flower and leaf phenotypes, these species can be readily crossed with hand-pollination to produce fertile offspring. In addition, they have several features that greatly facilitate genetic and developmental analyses, including high fecundity (up to 1,000 seeds per flower), short generation time (2.5-3 months), small genomes (~450 Mb) that have been assembled into chromosomes, and amenability to routine transgenic manipulations. I will discuss my strategies to locate the genes that cause the phenotypic variation among these species, to dissect the role of pleiotropy, linkage (including pseudo-linkage created by chromosome rearrangements), and epistasis in the evolution of phenotypic integration, and to reveal the developmental pre-patterns that enable the emergence of phenotypic novelty.

Invited Speaker

Parallel evolution of novel cell types at the base of the animal tree of life

Leslie S. Babonis[1], Mark Q. Martindale[2]

[1]*Whitney Lab for Marine Bioscience, University of Florida*
[2]*Whitney Lab for Marine Bioscience, Department of Biology, University of Florida*

The origin of novel traits is an important driver of speciation. Ctenophores (comb jellies) are unified by their possession of a novel cell type: the colloblast, an adhesive cell found only in the tentacles. To understand the origin of colloblasts, we examined tentacle development in the model ctenophore *Mnemiopsis leidyi*. Multiple lineages of embryonic micromeres contribute cells to the tentacles during early development. In the larval (cydippid) stage, the cells comprising the tentacles are generated constantly from a population of stem cells found in the base of the tentacle (the tentacle bulb). Treatment of cydippid larvae with a reversible inhibitor of cell proliferation (hydroxyurea) resulted in loss of tentacles in *M. leidyi*. One lineage of ctenophores (Beroida) lacks tentacles completely, feeding exclusively on other ctenophores. The phylogenetic position of Beroida suggests that tentacles (and therefore, colloblasts) were secondarily lost in this lineage. We used transcriptomes from 36 ctenophore species to identify gene losses that occurred specifically in lineages lacking colloblasts. We cross-referenced these colloblast-specific candidate genes with temporal RNA-Seq during embryogenesis in *Mnemiopsis leidyi* and confirm that candidates are preferentially expressed during tentacle morphogenesis and are upregulated in the tentacle bulb of adults. Both sets of candidates were enriched for an N-terminal signal peptide and protein domains associated with secretion, consistent with their inclusion in the regulated secretory pathway. Finally, using cell lineage tracing, we demonstrate that colloblasts and neurons share a common progenitor, suggesting the evolution of colloblasts involved co-option of a neurosecretory gene regulatory network. Together these data offer an initial glimpse into the genetic architecture underlying ctenophore cell-type diversity.

Invited Speaker

Disruption of canonical Wnt signaling in an arachnid reveals a non-canonical response of the *Wnt8-caudal* cassette in posterior segmentation

Prashant P. Sharma, Emily V. W. Setton

University of Wisconsin-Madison, USA

Segmentation is a key characteristic of the phylum Arthropoda that is linked to the evolutionary success of this lineage. The formation of segments, both along the antero-posterior axis of the body and along the proximo-distal appendage axis, requires the activity of the Wnt family of secreted proteins, as inferred from functional data in a handful of insect model organisms, but comparable data are limited for Chelicerata. Here we examined the inhibition of canonical Wnt signaling in the cobweb spider *Parasteatoda tepidariorum* using parental RNA interference (pRNAi) against the Wnt-1 co-receptor *arrow* (*arr*; vertebrate homolog: *LRP5* and *LRP6*), a key member of the canonical Wnt-signaling pathway in holometabolous insects and vertebrates. Toward a more refined characterization of the ensuing segmentation phenotype, we sequenced transcriptomes of embryos displaying loss-of-function phenotypes and quantified gene expression by mapping reads to the recently sequenced genome of *P. tepidariorum*. Here we show that knockdown of *Ptep-arr* results in systemic disruption of segmentation, in tandem with reduced expression of almost all the Wnts. Intriguingly, *Ptep-arr* loss-of-function phenotypes undergo overexpression of the canonical Wnt gene *Wnt8* and its target *caudal* (*cad*), while retaining trunk and posterior Hox expression. By contrast, knockdown of *arr* homologs in two hemimetabolous insects recapitulated the phenotype previously known from holometabolous insects, wherein anterior segments are properly formed but posterior segments are truncated. Whereas *Wnt8* homologs not affected by *arr* knockdown in the posterior growth zone, the overexpression of *cad* occurs only in the spider, suggesting a lineage-specific regulation of *cad* by Wnt signaling in this arachnid. This work underscores the diagnostic power of differential gene expression tools in categorizing catastrophic phenotypes and unveiling developmental systems drift.

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Oral Presentations

Friday - August 2, 2019
10:30 - 12:00 pm

Chordate Evolution and Development

Session Chair: Daniel Medeiros

Mesoderm formation in lampreys and the evolution of the vertebrate head

Takayuki Onai[1], Fumiaki Sugahara[2], Noritaka Adachi[3]

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[2]*Department of Anatomy, Hyogo College of Medicine, Japan*
[3]*Division of Biology, Aix-Marseille Université, IBDM, France*

Vertebrates, jawless and jawed, have a distinct head comprising the cranium, brain, cranial nerves, special sense organs and head muscles. With respect to the muscular tissue of vertebrate heads — especially anterior to the otic vesicle — there has long been discussion about its evolutionary and developmental origin, as well as its possible segmental nature. For the better studied gnathostomes, the presence of pre-otic segments has been denied by anti-segmentalists, but championed by segmentalists (who focus sometimes on marginally detectable somitomeres and sometimes on more obvious head cavities). Resolving these arguments has been hampered by the relative paucity of relevant work on the head mesoderm in agnathan vertebrates (hagfishes and lampreys). Here, we examined cranial development in lampreys by fine-grained optical sectioning of their embryos using confocal laser scanning microscopy. This technique revealed no mesodermal cavities in the pre-otic paraxial head mesoderm. Further, ancillary gene expression mapping showed that the anterior head mesodermal region of lamprey embryos does not transcribe key genes involved in the development of the unequivocal, more posterior somites of vertebrates in general. This indicates that the anterior paraxial head mesoderm of vertebrates is a novelty of the group with no clear antecedent in the complete rostrocaudal segments of amphioxus or, by extension, in ancestral invertebrate chordates.

Revealing the hidden foundations: phylogenetic identity of the vertebral axis imparted by notochordal signals

Brianna Preskin[1], Katrin Henke[2], Stephen Treaster[2], Michel Bagnat[1], Gloria Arratia[3], Matthew P. Harris[2]

[1]*Department of Cell Biology, Duke University Medical Center, USA*
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[3]*Department of Systematics and Evolutionary Biology, University of Kansas, USA*

The development and patterning of the spine is a fundamental aspect to vertebrate body plan and skeletal adaptation. Although a shared trait among all vertebrates, there is considerable variation in how vertebrae form such that comparisons across vertebrate classes such as fishes and mammals remain difficult. In part this is due to divergence in early patterning and different role and contribution of somatic mesoderm in forming pattern of the spine and form of the vertebrae. Recently the notochord has been shown to be critical in regulating the meristic pattern of the vertebral centra in fishes in a manner independent of somatic boundaries. However, the relevance of notochord signaling in regulating the spine in vertebrates more broadly remains unclear. Here, we describe the identification of a new zebrafish mutant, *spondolous (spod)*, showing a specific shift in morphology of the forming centra of the vertebrae. Unlike any other adult stages of living teleost fishes, *spod* forms hemicentra as well as periodic diplospondyly, or two centra per vertebral segment. Using detailed sampling and reconstruction of paleontological evidence of stem teleosts and outgroups, we demonstrate a specific transition in spinal patterning and vertebral morphology in ray finned fish evolution that mirrors the phenotype revealed in *spod*. We find that *spod* is due to a gain-of-function mutation in an extracellular protein, *calymmin*, expressed exclusively in the notochordal sheath. We find that *spod* affects early developmental specification and patterning across the notochordal sheath leading to a dramatic shift in the way the somatic mesodermal contributes to the forming vertebrae that is similar to developmental programs observed in amniotes. The *spod* phenotype closely resembles early ray-finned fishes, holosteans and the oldest fossil teleosts suggesting that signals from the notochord specifically shift developmental programs in a manner that imparts class-level identity to the forming spine in vertebrate evolution.

Nodal and Hedgehog cooperate in gill slit formation during development of the cephalochordate *Branchiostoma floridae*

Hiroki Ono[1], Demian Koop[2], Linda Z. Holland[3]

[1]*Oki Marine Laboratory, Shimane University, Japan*
[2]*Department of Anatomy, University of Sydney, Australia*
[3]*University of California San Diego, USA*

The larval pharynx of the cephalochordate *Branchiostoma* (amphioxus) is asymmetrical. The mouth is on the left and endostyle and gill slits are on the right. At the neurula, Nodal and Hedgehog (Hh) expression becomes restricted to the left. To dissect their respective roles in gill slit formation, we inhibited each pathway separately for 20 min at intervals during the neurula stage, before gill slits penetrate, and monitored effects on morphology and expression of pharyngeal markers. The results pinpoint the short interval spanning the gastrula/neurula transition as the critical period for specification and positioning of future gill slits. Thus, reduced Nodal signaling shifts the gill slits ventrally, skews the pharyngeal domains of Hh, Pax1/9, Pax2/5/8, Six1/2, IrxC towards the left and reduces Hh and Tbx1/10 expression in endoderm and mesoderm, respectively. Nodal autoregulates. Reduced Hh signaling does not affect gill slit positions or Hh or Nodal expression but down-regulates the Hh target, Gli in the presumptive gill slits. Thus, during the neurula, Nodal and Hh cooperate in gill slit development—Hh mediates gill slit formation while Nodal establishes their left-right position.

Organization and evolution of skeletal cell GRNs

Brian F. Eames

Department of Anatomy, Physiology & Pharmacology, University of Saskatchewan, Canada

The majority of the vertebrate skeleton is produced by three related cell types, but the organization and evolution of the gene regulatory networks (GRNs) driving skeletal cell differentiation are not well-understood. While osteoblasts produce bone throughout the body, two types of chondrocyte are differentiated by the fact that some chondrocytes progress through a developmental transition termed maturation, when they undergo hypertrophy and express additional genes. To understand the organization and evolution of the GRNs underlying osteoblast, immature chondrocyte, and mature chondrocyte differentiation, we used laser-capture microdissection of homologous embryonic skeletal cells in various animal clades and compiled their transcriptomes. Our analyses of mouse skeletal cells confirmed that a Sox9 GRN operates in immature chondrocytes, a Runx2 GRN characterizes osteoblasts, and both GRNs are used in mature chondrocytes. Furthermore, discrete sets of genomic loci show evidence of GRN interaction in mouse mature chondrocytes. Some loci average the expression levels seen in immature chondroctyes and osteoblasts, while other loci, including *Col10a1*, the classic gene marker of mature chondrocytes, exhibit synergistic expression levels. Preliminary analyses of homologous mouse, chick, and gar skeletal cells illuminate how complex GRNs evolve. Interestingly, in mouse and chick, there is a strong negative correlation between gene expression in the Sox9 vs. Runx2 GRNs, but gar skeletal cells don't exhibit this cross-inhibition, revealing a potential tetrapod synapomorphy during evolution of skeletal cell GRNs. Current efforts use these data to test hypotheses for the mechanistic origins of the osteoblast, a vertebrate invention.

Using computer modelling to examine the role of developmental innovation in the origins of complex mammalian teeth

Aidan M.C. Couzens[1], Karen E. Sears[1], Martin Rücklin[2]

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The extent to which major evolutionary transitions reflect developmental biases or constraints is poorly understood but potentially important for predicting sequences of trait evolution. Classically, morphological variation has been viewed as 'copious and continuous' but evo-devo studies suggest that development is biased towards generating particular forms of variation. The origin of mammals is a classic example of a major evolutionary transition associated with the acquisition of important anatomical innovations, a key example being complex, multicusped teeth required for chewing. Using a computer simulation of tooth development, we examined how tinkering with genetic and cellular parameters influences transitions from simple to complex teeth. We find that tooth morphospace is partitioned into simple, gradually varying tooth morphologies, and more complex, highly disparate tooth morphologies. Due to an early shift in tooth cusp patterning in the model, teeth with laterally separated cusps become much more complex than teeth where cusps are arranged longitudinally or in triangles. Based on comparison with the fossil record of mammalian dentitions we hypothesise that this early cusp patterning shift was important for the derivation of complex mammalian teeth from the much simpler amniote pattern. Our results suggest that early and late developmental shifts can produce starkly different patterns of morphological variation and highlight how computer modelling of development can provide insights into major evolutionary transitions.

Conserved gene signaling and a derived patterning mechanism underlies the development of avian footpad scales

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Vertebrates possess a diverse range of integumentary epithelial appendages, including scales, feathers and hair. These structures share extensive early developmental homology, as they mostly originate from a conserved anatomical placode. In the context of avian epithelial appendages, feathers and scutate scales are known to develop from an anatomical placode. However, our understanding of avian reticulate (footpad) scale development remains unclear. Here, we demonstrate that reticulate scales develop from restricted circular domains of thickened epithelium, with localised conserved gene expression in both the epithelium and underlying mesenchyme. These domains constitute either anatomical placodes, or circular initiatory fields (comparable to the avian feather tract). Subsequent patterning of reticulate scales is consistent with reaction-diffusion (RD) simulation, whereby this primary domain subdivides into smaller secondary units, which produce individual scales. In contrast, the footpad scales of a squamate model (the bearded dragon, *Pogona vitticeps*) develop synchronously across the ventral footpad surface. Widely conserved gene signalling underlies the initial development of avian reticulate scales. However, their subsequent patterning is distinct from the footpad scale patterning of a squamate model. Therefore, we suggest reticulate scales are a comparatively derived epithelial appendage, patterned through a modified RD system.

Selected Abstracts 5

Oral Presentations

Friday - August 2, 2019
1:30 - 3:00 pm

Genome EvoDevo

Session Chair: Veronica Hinman

Odyssey of strange fish: investigating 'ancient fish' genomes and development to illuminate vertebrate evolution

Ingo Braasch[1], Andrew W. Thompson[1], Mauricio Losilla[1], Allyse Ferrara[2], The Gar Genome Consortium, John H. Postlethwait[3]

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[2]*Biological Sciences, Nicholls State University, USA*
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Ray-finned fishes – and among them particularly teleost fishes such as zebrafish – are commonly used to investigate the genomic basis of vertebrate development and evolution. However, teleosts are derived from a teleost-specific genome duplication that had major impact on their genome and gene function evolution. Together with the earlier two rounds of vertebrate genome duplication, this complicates macroevolutionary comparisons across vertebrates: the 'big bang' of genome duplications led to lineage-specific genome reshuffling and gene losses, obscuring the distinction of orthologs vs. paralogs and hiding the origins of vertebrate gene functions and developmental processes. Here we show that so-called 'ancient fishes' of the holostean lineage (gars and bowfin), the sister lineage to teleosts, have highly informative genomes and body plans that provide unique opportunities to address wide-ranging comparative investigations among vertebrates. Holosteans serve both as an 'unduplicated' outgroup to the 30,000 living teleost species as well as an outgroup to lobe-finned vertebrates including coelacanth, lungfish, and 30,000 extant tetrapod species. Using examples from diverse developmental pathways and processes, we demonstrate that comparative genomic, developmental, transcriptomic, and epigenomic analyses using holosteans as "bridge species" are indispensable for connecting the often disparate sets of genes, gene regulatory elements, and morphologies among distant vertebrate lineages such as tetrapods and teleost fishes. The structure and content of the genome of our main holostean model species, the spotted gar (*Lepisosteus oculatus*), is representative of the bony vertebrate ancestor and retained numerous genes differentially lost in other lineages. The 'evolutionary inertia' of holostean genomes particularly facilitates the identification of *cis*-regulatory elements, revealing hidden orthology of regulatory elements across vertebrate lineages. Rearing gars in the laboratory in comparison to zebrafish and medaka, we developmentally test hypotheses about the evolutionary origins of vertebrate gene functions. Holosteans are thus powerful Evo-Devo models that illuminate vertebrate evolution, development, disease, and regeneration.

Evolution and plasticity of the Gene Regulatory Network underlying trichoblast formation in *Arabidopsis thaliana*: a survey of 850 worldwide accessions

Daniela Sosa-Peredo[1,2], Adriana Garay-Arroyo[2,3], Aaron Castillo[2,3], Elena R. Álvarez-Buylla[2,3], Alma Piñeyro-Nelson[1,2]

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[2]*Centro de Ciencias de la Complejidad, Universidad Nacional Autónoma de México. 3er Circuito Exterior, Ciudad Universitaria, Coyoacán, México*
[3]*Departamento de Ecología Funcional, Instituto de Ecología, Universidad Nacional Autónoma de México. 3er Circuito Exterior, Ciudad Universitaria, Coyoacán, México*

Plants are sessile organisms that contend with environmental changes through the dynamic modification of their physiology, gene expression and morphology. One of the most plastic organs in plants is the root, which undertakes fundamental functions such as water and cation/anion exchange, while providing physical anchorage to the substrate. Nutrient and water intake are performed primarily by the root hairs, whose disposition and density can vary both due to genetic and environmental changes. Given that a Gene Regulatory Network (GRN) considered necessary and sufficient for root hair formation has been previously put forward, we here explore its plasticity through the survey of mutational changes across homolog genes from 850 accessions of *Arabidopsis thaliana* distributed worldwide. Additionally, this GRN was expanded from 10 to 19 genes in order to include genes regulated by different hormonal and nutritional (phosphate) cues. While general analyses show overall genetic conservation across all accessions analyzed, detailed study detected that out of the 19 genes analyzed, 5 have non-synonymous substitutions in some accessions. These changes could potentially impact gene function and its role in epidermal morphogenesis in the *Arabidopsis* root. The potential impacts on the underlying GRN given the roles of these genes in it, as well as potential morphological changes and effects on local populations carrying such substitutions are discussed.

A large insertion containing a duplicated follistatin gene underlies the pea aphid male wing dimorphism

Binshuang Li[1], Ryan D. Bickel[1], Benjamin J. Parker[1], Neetha Nanoth Vellichirammal[2], Jean-Christophe Simon[3], David L. Stern[4], Jennifer A. Brisson[1]

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[2]*University of Nebraska, USA*
[3]*INRA-Rennes, France*
[4]*Janelia Research Campus, USA*

Wing dimorphisms have long served as models for examining the ecological and evolutionary tradeoffs associated with alternative morphologies, yet the genomic regions responsible are unknown. We investigated the developmental genetic basis of the pea aphid (*Acyrthosiphon pisum*) male wing dimorphism, wherein males exhibit one of two morphologies that differ in correlated traits that include the presence or absence of wings. We mapped trait differences to a single genomic region and used long-read sequencing to reveal the presence of a large (~120kb) insertion on the wingless allele. In this insertion, we found a *follistatin* gene duplicate that is expressed across the pea aphid developmental stages only in the wingless morph. This duplicate is experiencing purifying selection. Based on the fact that it is expressed and under selection, and because of the known role of follistatins in tissue-specific regulation, this duplicate is likely the causative gene underlying the wingless relative to winged phenotype. We further analyzed sequence variation at this locus across pea aphid lineages that began diverging ~16,000 ya and discovered that both alleles were present prior to lineage diversification, and that the insertion likely occurred millions of years ago. Most lineages harbor individuals with both alleles, although some have entirely fixed the newer, wingless allele, indicating a possible selective advantage of that phenotype. Overall, our study shows that dramatic, intraspecific morphological differences can be caused by structural genetic variation. This study represents an important breakthrough in understanding the molecular evolution of wing dimorphisms, which have evolved repeatedly in insects.

Studying GRN evolution at divergence times beyond the point of alignable genomes

Isabella Schember, Hasiba Asma, Marc S. Halfon

University at Buffalo-State University of New York, USA

Studying GRN evolution requires knowledge of homologous *cis*-regulatory modules (CRMs) in multiple species. CRM identification is challenging, especially where genomes have diverged past where they can be aligned. We have developed two powerful resources to facilitate CRM discovery and GRN evolution studies in insects. *REDfly* is a comprehensive database describing >24,000 validated *Drosophila* CRMs regulating more than 1600 genes. It includes detailed information about the spatio-temporal patterns of gene expression regulated by each CRM. This serves as input for *SCRMshaw*, a computational method for CRM discovery. *SCRMshaw* uses the *Drosophila* data for CRM discovery in diverse insect species including mosquitoes, beetles, and bees, and can identify homologous CRMs for orthologous genes, a decided advantage for evo-devo studies. Several compelling examples have already been identified. In particular, we are using these tools to study GRN evolution during development of the central nervous systems of both *Drosophila melanogaster* and the mosquito *Aedes aegypti*. The two species show significant divergence in a set of genes coexpressed in the midline of the *Drosophila* ventral nerve cord, including the "master regulator" *single minded (sim)*. In contrast to the midline expression seen in *Drosophila*, in *A. aegypti* these genes are instead co-expressed laterally in the nerve cord. This suggests that in *A. aegypti* this "midline GRN" has been redeployed in a new location. Using SCRMshaw, we identified CRMs from both *A. aegypti* and *D. melanogaster*, and have shown that the altered gene expression observed in *A. aegypti* results primarily from *trans*-dependent redeployment of the GRN; this stems from *cis*-mediated changes in the expression of *sim* and as-yet unidentified regulators. These results illustrate a novel "repeal, replace, and redeploy" mode of evolution in which a conserved GRN moves to a new site while its original function is co-opted by a different GRN.

Genomic logic underlying morphological divergence

Riccardo Papa[1], Steven Van Belleghem[1], Heriberto Carbia Gutierrez[1], Edgardo Santiago-Rivera[1], Carolina Concha[2], Owen McMillan[2], Brian Counterman[3]

[1]*University of Puerto Rico-Rio Piedras, Puerto Rico*
[2]*Smithsonian Tropical Research Institute, Panama*
[3]*Mississippi State University, USA*

Characterizing the genomic modifications that control morphological variation and link these changes with alteration in gene regulation, expression and interactions is crucial to our understanding of evolutionary processes underlying morphological diversity in animals. Thus, understanding the molecular underpinning of genome to phenome requires the genomic characterization of the developmental mechanisms controlling organismal morphology. Adaptive radiations harbor incredible diversity that can be utilized as a test tube to study the causal networks of interacting genes that link changes in the genomes to the final phenome. The adaptive radiation in the color patterns of *Heliconius* butterflies wings is a great system to explore the logic of developmental evolution underlying phenotypic changes. Their wing color patterns result from adaptation of many different mimicry complexes that provide mutualistic protection from avian predators. While these colorations are controlled by few genes, their regulatory architecture remain mostly unexplored. I will present our latest ATAC-seq and CRISPR/Cas9 data that begins to unfold the complex regulatory architecture and its relative function during wing development. Finally, I will demonstrate a novel computational approach to interpret these biological data that fill in gaps in understanding how a genotype translates to a phenotype. Comprehensively, our work has the potential to generate a new understanding of the common molecular features that underlie rapid morphological diversification.

Dissecting the shared and divergent genetic architectures of a novel male genital structure and a novel female genital structure.

Eden W. McQueen[1], William J. Glassford[2], Peter Andolfatto[2], Mark Rebeiz[1]

[1]*University of Pittsburgh, USA*
[2]*Columbia University, USA*

Investigating the genetic origination and evolution of novel morphologies is fundamental to our understanding of biological complexity. Animal genitalia are complex structures that evolve rapidly, and it is common to see species-level differences in both male and female genitalia. In many systems male and female genitalia are known to share some developmental programming, yet differ in final morphology and function. Comparing the developmental networks of male and female genital traits is an opportunity to investigate how developmental networks deployed in rapidly evolving morphologies operate under the constraints of pleiotropy. We investigated the genetic underpinnings of two genital structures in the *Drosophila melanogaster* subgroup. Males in this subgroup possess a novel genital outgrowth called the posterior lobe. Females of this subgroup have a novel feature on their ovipositors called the oviscapt pouch. The posterior lobe and oviscapt pouch correlate in size across species, suggesting a genetic or functional association.
Previously, the gene network required for posterior lobe formation was found to have been co-opted from a larval structure. Using gene knockdown, *in-situ* hybridization, antibody staining, and enhancer analysis, we investigated whether this network is shared between the posterior lobe and the oviscapt pouch. We discovered that patterning genes from the posterior lobe network, and even their lobe enhancers, are also involved in the patterning of the oviscapt pouch during this structure's development. We next performed a quantitative trait locus analysis using two closely-related species in this group with divergent genital morphologies to identify shared and unique loci of diversification for the male and female structures. We found evidence that some loci associated with diversification across species may be shared between these two structures. Our discovery of pleiotropy in this system highlights the need to consider the role of genetic linkage in the evolution of novelty and structural complexity.

Closing Night Tributes & Awards

Friday - August 2, 2019

6:00 - 8:00 pm

PASEDB Past President Award Presentation

Introduction by Jeffrey Marcus

PASEDB Service Award Presentation

Introduction by Billie Swalla

PASEDB 2019 Past President Award
Invited Speaker

Developmental origin and evolution of bats

Karen Sears

University of California Los Angeles, USA

Comprising 20% of mammals, bats (Order Chiroptera) are the only mammals capable of powered flight. Bats achieves powered flight through specializations in their forelimbs, including novel wing membranes. Once in the air, bats experienced a massive adaptive radiation into diverse ecological niches. As a result, today's bats have more highly diverse diets and, in association, highly diverse phenotypes in systems such as teeth and eyes. We are investigating the developmental mechanisms through which bats arose and then diversified. In regard to bat origins, our studies suggest that the novel aspects of the bat wing membrane arose not through a redeployment of a limb-like outgrowth program, but rather through novel expression of other genes. In regard the subsequent radiation, we have found that development of both the teeth and eye systems is highly plastic in bats, and this provided the raw material necessary for adaptive radiation by natural selection.

PASEDB 2019 Service Award Winner
Invited Speaker

Holobiont Evo-Devo: The importance of symbiosis to evolutionary trajectories

Scott F. Gilbert

Swarthmore College, USA

Evolutionary developmental biology has concentrated on the formation of new structures through changes in gene expression. However, whereas the human genome contains some 22,000 genes, it receives over eight million different genes from its microbial symbionts. The differential expression of microbial genes may be critical in producing new anatomical, physiological, and behavioral phenotypes. Moreover, both vertically and horizontally transmitted microbes have been shown to alter development to produce in selectable adaptations. Recent research proposes that microbial symbionts are necessary for the development of particular organs of certain species, for the variation of selectable traits within a species population, and for the emergence of particular social behaviors. This research also suggests that some evolutionary transitions have been facilitated by symbiotic microbes. Herbivory, the complex of anatomical, physiological, and behavioral traits allowing animals to eat plants, is one of those transitions. Herbivory will be discussed from the point of view of holobiont evolutionary developmental biology, wherein specific adaptations (such as the rumen), are seen as being induced by microbes, and the behavioral and physiological manifestations of herbivorous phenotypes need to be preceded by the successful establishment of communities of symbiotic microbes that can digest plant cell walls and detoxify plant poisons.

Poster Session I

Wednesday - July 31, 2019
3:00 - 5:00 pm

Presenters of Odd-Numbered Abstracts

Poster Session II

Thursday - August 1, 2019
3:00 - 5:00 pm

Presenters of Even-Numbered Abstracts

Poster Session III

Friday - August 2, 2019
3:00 - 5:00 pm

Presenters of Odd-Numbered Abstracts 3:00 - 4:00 pm
Presenters of Even-Numbered Abstracts 4:00 - 5:00 pm

Odd-Numbered Posters

Sub-sections

I. Skeletal Elements, Limbs, Skulls & Feathers
II. Arthropod Innovations, Allometry, Wing & Horn Development
III. Marine & Freshwater Invertebrate EvoDevo

P.1 (Skeletal Elements, Limbs, Skulls & Feathers)

Embryological and functional perspectives of understanding the diversity of scleral ossicles

Tamara A. Franz-Odendaal

Mount Saint Vincent University, Canada

Most vertebrates have an ocular skeleton that is composed of cartilage and/or bone. There is enormous diversity in the bone elements, the scleral ossicles, and many examples of secondary losses and gains throughout the evolutionary history of vertebrates. The scleral ossicles are induced by epithelial placodes in avians (and other reptiles) but not in teleost fishes. Analyses of developmental mechanisms in chicken embryos has uncovered signaling pathways that are common to the development of other serial placodal structures. Furthermore, comparative analyses of the development and growth of the eyeball and its ocular skeleton in chicken and barn owl embryos and hatchlings, has revealed that there are distinct shifts in the ossification of the scleral ossicles in these bird species despite similar early developmental patterning. These differences shed light on the functional importance of the scleral ossicle ring of bones in avians, and possibly other vertebrates.

P.3 (Skeletal Elements, Limbs, Skulls & Feathers)

Modulation of skull shape by synchondroses in East African cichlids

Pierre Le Pabic[1], Brian P. Heubel[1], Yuan Tao[2], Anthony D. Long[2], Tom F. Schilling[2]

[1]*University of North Carolina Wilmington, USA*
[2]*University of California Irvine, USA*

How do phenotypically integrated structures evolve? We explore this question using the fish skull - a composite structure of some 100 bones articulated into a functional whole able to support various activities including feeding and breathing. Morphological changes affecting one skull bone are intuitively predicted to negatively impact the functionality of the whole, yet the extreme morphological diversity of fish skulls does not support such constraint. To explore the developmental and genetic mechanisms underlying fish skull evolution, we focus on two cichlid species from Lake Malawi with distinct skull morphologies – *Dimidiochromis compressiceps* (DC) and *Copadichromis azureus* (CA). DC is an ambushed predator with an elongated and laterally compressed head adapted to suction-feeding. CA is a benthic species with a smaller head adapted to biting. Our comparative analysis of skull development in CA and DC indicates that several species-specific morphological differences arise during post embryonic development through differential growth of particular cartilage-derived skull bones. We demonstrate by histological staining, in situ hybridization and immunostaining that growth of these particular bones takes place at synchondroses that function as bidirectional endochondral growth zones. Differences in synchondrosis size indicative of greater activity in DC than in CA are first observed at early larval stages and persist into adulthood, suggestive of a potential role for embryonic patterning in setting up initial differences that later translate into drastically different adult morphologies. A mapping cross between DC and CA was performed and genetic mapping in the F2 generation is underway to identify the loci underlying differences in synchondrosis activity.

P.5 (Skeletal Elements, Limbs, Skulls & Feathers)

The origins and co-evolution of neuromuscular systems of paired appendages in batoids

Tetsuya Nakamura

Department of Genetics, Rutgers University, USA

Appendage patterning and evolution have been active areas of inquiry for the past two centuries. While most work has centered on the skeleton, particularly that of amniotes, the evolutionary origins and molecular underpinnings of the neuromuscular diversity of fish appendages have remained enigmatic. The fundamental pattern of segmentation in amniotes, for example, is that all muscle precursors and spinal nerves enter either the paired appendages or body wall at the same spinal level. The condition in finned vertebrates is not understood. To address this gap in knowledge, we investigated the development of muscles and nerves in unpaired and paired fins of skates and compared them to those of chain catsharks. During skate and shark embryogenesis, cell populations of muscle precursors and associated spinal nerves at the same axial level contribute to both appendages and body wall, perhaps representing an ancestral condition of gnathostome appendicular neuromuscular systems. Remarkably in skates, this neuromuscular bifurcation as well as colinear *Hox* expression extend posteriorly to pattern a broad paired fin domain. In addition, we identified migratory muscle precursors (MMPs), which are known to develop into paired appendage muscles with *Pax3* and *Lbx1* gene expression, in the dorsal fins of skates. Our results suggest that muscles of paired fins have evolved via redeployment of the genetic program of MMPs that were already involved in dorsal fin development. Appendicular neuromuscular systems most likely have emerged as side branches of body wall neuromusculature and have been modified to adapt to distinct aquatic and terrestrial habitats.

P.7 (Skeletal Elements, Limbs, Skulls & Feathers)

Deep evolutionary origin of limb and fin regeneration

Sylvain Darnet[1], Aline C. Dragalzew[1], Danielson B. Amaral[1], Josane de Freitas Sousa[1], Andrew W. Thompson[2], Amanda N. Cass[3], Jamily Lorena[1], Eder S. Pires[4], Carinne M. Costa[1], Marcos P. Sousa[5], Nadia B. Fröbisch[6], Guilherme Oliveira[4], Patricia N. Schneider[1], Marcus C. Davis[3], Ingo Braasch[2], Igor Schneider[1]

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Salamanders and lungfishes are the only sarcopterygians (lobe-finned vertebrates) capable of paired appendage regeneration, regardless of the amputation level. Among actinopterygians (ray-finned fishes), regeneration after amputation at the fin endoskeleton has only been demonstrated in polypterid fishes (Cladistia). Whether this ability evolved independently in sarcopterygians and actinopterygians or has a common origin remains unknown. Here we combine fin regeneration assays and comparative RNA-seq analysis of *Polypterus* and axolotl blastemas to provide support for a common origin of paired appendage regeneration in Osteichthyes (bony vertebrates). We show that, in addition to polypterids, regeneration after fin endoskeleton amputation occurs in extant representatives of two other non-teleost actinopterygians: the American paddlefish (Chondrostei) and the spotted gar (Holostei). Further, we assessed regeneration in four teleost species and show that, with the exception of the blue gourami (Anabantidae), three species were capable of regenerating fins after endoskeleton amputation: the white convict and the oscar (Cichlidae), and the goldfish (Cyprinidae). Our comparative RNA-seq analysis of regenerating blastemas of axolotl and *Polypterus* reveals the activation of common genetic pathways and expression profiles, consistent with a shared genetic program of appendage regeneration. Comparison of RNA-seq data from early *Polypterus* blastema to single-cell RNA-seq data from axolotl limb bud and limb regeneration stages shows that *Polypterus* and axolotl share a regeneration-specific genetic program. Collectively, our findings support a deep evolutionary origin of paired appendage regeneration in Osteichthyes and provide an evolutionary framework for studies on the genetic basis of appendage regeneration.

P.9 (Skeletal Elements, Limbs, Skulls & Feathers)

Exploring the evolution of the amniote forelimb musculature by studying its embryology

Daniel Smith-Paredes, Bhart-Anjan S. Bhullar

Geology of Geophysics Department, Yale University, USA

In Amniotes, cells from the dorsal portion of the somite, the dermomyotome, delaminate and migrate to invade the limb bud, in contrast to non-tetrapods in which at least some somitic extension takes place. Reaching the limb bud, these cells aggregate into a Dorsal and Ventral mass of pre-muscular cells. These two masses subdivide, forming smaller divisions that will eventually split into individualized recognizable muscles of the adult. The pattern of cleavage of the shoulder and arm muscles has been described only in a handful of species, representing urodeles, lizards, turtles, marsupials and birds. Since the time of these seminal investigations, the embryonic pattern of muscle development has been used some times as a tool for understanding homologies across amniotes, but we remain limited to the information provided by the few species investigated and the constrains of the available technologies at the times of these studies. Half a century later, we have a clearer picture of the phylogenetic relationships among clades and new tools for studying and visualizing developing anatomy. We studied the development of closely spaced series of embryos of mammals, archosaurs, lizards and turtles, comprising most major groups of amniotes alive, and studied the embryology of forelimb muscles, along with the developing nerves and skeleton, by using fluorescent immunostaining and confocal microscopy. Our data reveals a sequence of early events and muscle division much more conserved across amniotes than previously described, also placing these early divisions in the context of the rest of the developing anatomy of the arm. We also tracked and followed the embryonic origin of each adult muscle, comparing it with their supposed homologues across different clades. Based on our results, we propose a reconsideration of some assumed homologies and provide new important information regarding the development and evolution of the amniote forelimb musculature.

P.11 (Skeletal Elements, Limbs, Skulls & Feathers)

Transcriptional differences that uncouple the evolution of vertebrate forelimb and hindlimb skeletal proportion

Aditya Saxena, Virag Sharma, Mai Tran, Haydee L. Gutierrez, Amanda Birmingham, Joel M. Erberich, Michael Hiller, Kimberly L. Cooper

University of California San Diego, USA

The variety of limb skeletal proportions is a striking aspect of species diversity that includes such extreme modifications as the short and thick digits of the burrowing mole hand and the elongated fingers that support powered flight in bats. Despite the critical importance of skeletal proportion for limb form and function, little is known of the molecular mechanisms that determine the different lengths of individual limb bones in any animal or how skeletal proportion evolves independently in the forelimbs or hindlimbs. Here, we take advantage of the close evolutionary relationship of the laboratory mouse (*Mus musculus*) and bipedal jerboa (*Jaculus jaculus*), the similarity between forelimbs of the two species, and the accelerated rate of jerboa foot elongation to identify differentially expressed genes that are associated with the evolution of hindlimb proportion uncoupled from the forelimb. By directly comparing homologous skeletal elements of the two species, we show that expression levels of a majority of orthologous genes diverged over approximately 55 million years since the last common ancestor. However, differential expression of only about 10% of the genome is associated with the accelerated rate of foot growth relative to the forearm. Among the most striking of these differences, downregulation of a BMP signaling inhibitor, *Mab21L2*, an inhibitory TGFβ ligand, *Gdf10*, and upregulation of a retinoic acid antagonist, *Crabp1*, suggest that the evolutionarily increased growth rate of the jerboa foot likely occurred in part by releasing latent growth potential. The genes we identified here provide a framework to understand the modular genetic control of skeletal growth and the extraordinary evolutionary malleability of the vertebrate limb.

P.13 (Skeletal Elements, Limbs, Skulls & Feathers)

Developmental similarities and differences in convergent phenotypes of metatarsal fusion between jerboa and chicken

Rio Tsutsumi, Haydee Gutierrez, Kimberly L. Cooper

Department of Biological Sciences, University of California San Diego, USA

Fusion of metapodials into single cannon bone is one of the convergent phenotypes that are commonly seen in cursorial vertebrates. What is the developmental basis of metatarsal fusions? and are there developmental similarities in the convergent phenotypes? In this study, we studied cellular processes of metatarsals fusion using bipedal rodent, lesser Egyptian jerboas and chickens, as model species of comparative developmental analysis. In jerboa, we classified the process of fusion into three steps: First, more less cylinder metatarsals are reshaped into transverse arch so that the adjacent metatarsals interface at flat surface. Second, bone minerals are deposited bridging the interface between adjacent metatarsals. Third, the bridged septum is removed and finally three metatarsals share single marrow cavity. We showed that during the bridging, the anabolic activity of osteoblasts is temporarily upregulated at the interface of metatarsals. When the bridged septum is removed, the catabolic activity of osteoclasts is temporarily upregulated at the septum of metatarsals. The results suggested that the locally biased activity of osteoblasts and osteoclasts might be evolutionary acquired developmental mechanisms for the metatarsal fusion. In chickens, the process of metatarsal fusion undergo similar three steps as what we observed in jerboas at tissue levels. However, our analysis on osteoblasts and osteoclast activities revealed that although there is some similarity in the osteoblast activity at the metatarsal interfaces, the pattern of osteoclast activity is distinct from that of jerboa. Our comparative analysis provide potential models to understand cellular basis that reshape cursorial limb structures in development and evolution.

P.15 (Skeletal Elements, Limbs, Skulls & Feathers)

From the depths: deep comparative phylogenomics in fishes to identify genetic mechanisms of evolution, development and disease

Jacob M. Daane[1], Alex Dornburg[2], Thomas J. Near[3], Matthew P. Harris[4], H. William Detrich III[1]

[1]*Northeastern University, USA*
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[4]*Harvard Medical School, USA*

Advances in sequencing technologies have led to an explosion of genetic information that has transformed our understanding of evolution and development. However, taxonomic sampling is inevitably limited by the costs and computational demands of genome assembly. As a result, the resolution of genome evolution throughout a phylogeny is often limited and the majority of biodiversity remains unexplored. To circumvent these restrictions, we have focused on systematic targeted sequencing of protein coding exons and conserved non-coding elements across diversifying clades. The resulting datasets enable detailed dissection of protein coding and putative gene regulatory evolution within a phylogenetic context and without requiring assembly of reference genomes. Here, we discuss application of this approach to understand the adaptive radiation of Antarctic notothenioid fishes. This suborder provides one of the few examples of radiation following a major climate event, thereby facilitating retrospective analysis of the genetic mechanisms of adaptation. We show that notothenioids, contrary to typical expectations of adaptive evolution, experienced a punctuated burst of genomic diversification and evolved key skeletal modifications *long before* the onset of polar conditions in the Southern Ocean. We further show that diversifying selection in pathways associated with human skeletal dysplasia facilitated ecologically important variation in buoyancy among Antarctic notothenioid species. Finally, we demonstrate the sufficiency of altered *trip11, col1a1a* and *col1a2* function in zebrafish to phenocopy skeletal reduction in Antarctic notothenioids. Our combined phylogenomic and experimental approach enables broad analyses of vertebrates to explore the genetic causes for natural variation and the roles of this variation in human disease. Knowledge of the historical and environmental contexts for the origin of key traits in adaptive radiations not only permits the reconstruction of events that result in evolutionary innovation but also provides a framework for forecasting the effects of climate change on the stability and evolvability of natural populations.

P.17 (Skeletal Elements, Limbs, Skulls & Feathers)

Exploring molecular fingerprints of chondrichthyan skeletal cells to reaffirm conservation of bone in vertebrates: do skates have osteoblasts?

Oghenevwogaga J. Atake, Patsy Gomez-Picos, Katie Ovens, David M.L. Cooper, Ian McQuillan, Brian F. Eames

University of Saskatchewan, Canada

Only vertebrates can make bone, and despite the fact that chondrichthyans (e.g., sharks, skates) clearly evolved from an ancestral bony vertebrate, the chondrichthyan skeleton is believed to lack bone. Multiple reports of mineralized bone-like tissues in tesserae, neural arches, and centra of sharks, however, challenge this belief. Recently, we reported the same bone-like features in mineralized tissues of skates, suggesting a novel shared trait among elasmobranchs. Relying upon histological staining, candidate gene expression, and cell morphology, these reports failed to classify chondrichthyan mineralized tissues conclusively as bone, due to largely overlapping characteristics of bone and mineralized cartilage. Just like other cell types, however, osteoblasts and chondrocytes express characteristic sets of genes, termed molecular fingerprints. Using laser capture microdissection coupled to RNA-sequencing, we identified molecular fingerprints of osteoblasts in bone and chondrocytes in mineralizing cartilage of mouse and chick. Osteoblasts in mouse and chick had similar gene expression profiles. Conversely, molecular fingerprints of osteoblasts and mineralizing chondrocytes were distinguishable in both of these terrestrial vertebrates. Expanding this approach to skeletal cells of aquatic vertebrates, such as gar and skate, we are testing the hypothesis that skate bone-like tissue is homologous to bone. Although osteoblasts of aquatic vertebrates, such as gar and zebrafish, express chondrogenic markers (e.g., col2), an unbiased comparative transcriptomic approach can discriminate more easily than candidate approaches. These studies can unravel homology between skeletal tissue types, and can help to rewrite current knowledge about bone in chondrichthyans.

P.19 (Skeletal Elements, Limbs, Skulls & Feathers)

Functional assessment of a putative *Satb2* enhancer under convergent accelerated evolution in the thylacine and wolf

Laura E. Cook[1], Charles Feigin[2], Axel Newton[1], Andrew J. Pask[1]

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Despite having sequenced thousands of genomes, it is still not well understood how the similarities and differences in these genomes underpin the diversity in form we observed within the animal kingdom. The naturally occurring phenomenon of convergent evolution can help answer questions about the evolution of specific phenotypic traits as we can look at whether distantly-related species use the same or different molecular mechanisms to produce shared phenotypes. The best-known example of morphological convergence in mammals is the thylacine-canid comparison. Despite last sharing a common ancestor approximately 160 million years ago, the thylacine and canids share remarkable similarities in their body plan, in particular their skull morphology. We have computationally identified 283 non-coding regions under accelerated evolution in both the thylacine and wolf (TWARs). Comparison of transcription factor binding sites (TFBS) in these convergent species to outgroup species have identified TWARs which have convergently gained or lost TFBS and may indicate potential targets underpinning convergence. One of these elements sits upstream of the critical craniofacial patterning gene *Satb2.* This convergent element is ultra-conserved and has multiple DNA-binding sites along with convergently gained TFBS for two key TFs: Sox10 and Lef1. This element also overlaps with predicted active enhancer markers in bone precursor cells. Here we examine if the sequence convergence observed in this element between the thylacine and wolf results in functional convergence in the spatial, temporal and expression level driven by this element. We have examined this both *in vivo* in transgenic reporter mice and *in vitro* in cell assays. These data will provide novel information on the regions of the genome driving convergent craniofacial development and the loci that are targets of selection in evolution.

P.21 (Skeletal Elements, Limbs, Skulls & Feathers)

Uncovering the developmental and molecular basis of craniofacial convergence between the thylacine and canids

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The marsupial thylacine and placental canids represent one of the best cases of convergent evolution seen within mammals. Their similarities are especially evident in their craniofacial morphology, specifically between bones of intramembranous origin derived from the neural crest. This example provides an excellent model to examine the underlying genetic and developmental mechanisms that drive their convergent morphologies. To examine this, we identified 283 putative craniofacial regions under accelerated evolution in the thylacine and wolf lineages (TWARs) which are strong candidates behind their craniofacial convergence. Of these, we identified a previously uncharacterized enhancer upstream of the neural crest / craniofacial TGF-β family receptor *ACVR2A*. Functional assays showed that this enhancer drives expression in the developing mouse facial complex and is active in neural crest cells and osteoblasts, suggesting this newly identified element is a novel craniofacial enhancer and evolutionary target of skull convergence. Together, our model and functional pipeline provide a powerful system to examine the underlying basis of adaptive and convergent morphological evolution.

P.23 (Skeletal Elements, Limbs, Skulls & Feathers)

Morphological variation and molecular origination of Chiropteran wing membranes

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Through the evolution of novel wing structures, bats became the only mammal to achieve powered flight. This achievement led to an unprecedented adaptive radiation and diversification in membrane structure, such that today bats employ diverse flight styles and account for over 20% of all mammalian species. However, despite the importance of the evolution of the bat wing to the group's success, the mechanisms that drove the origination and subsequent diversification of the novel components of the wing remain largely unknown. Bat wings are comprised of several, novel membranous structures that are supported by elongated forelimb and digit bones. Our research specifically investigates the evolutionary origination and diversification of two novel membranes of the bat wing: the plagiopatagium which connects the 5th digit to the body and hind limb in all bat species, and the uropatagium which connects the hind limbs in many species. We characterize differences in adult membrane phenotype among bat species with diverse flight styles. This is achieved by performing geometric morphometrics on specimens housed at the American Museum of Natural History (AMNH). Additionally, we seek to determine when during development and from what tissue sources the membranes initially form, and when differences in membrane form arise among species. The geometric morphometric approach is also extended to embryonic and juvenile bats from a range of developmental stages. Embryonic specimens are obtained from AMNH fluid collections and newly captured bats. Lastly, we establish a general cellular and molecular framework for plagiopatagia and uropatagia development in bats, and establish how this framework differs among species with divergent membrane development. This is achieved by visualizing cellular processes, gene expression, and protein localization in developing embryos.

P.25 (Skeletal Elements, Limbs, Skulls & Feathers)

Shining synchrotron light onto the question of molar/jaw evo-devo integration using a mouse model

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The evo-devo processes that integrate the morphogenesis of mammalian teeth and jaws remain unclear, including the extent to which surrounding jaw tissue constrains the developing tooth organ including its onset time. We sought to compare retromolar space with molar onset and length. We hypothesized that first(M1), second(M2) and third(M3) molars would initiate only as retromolar space lengthened via jaw growth. We aimed to directly visualize in 3D earliest molar onset to crown completion, and to measure molar, total jaw (TJL) and retromolar (RML) lengths. At the Canadian Light Source synchrotron, we micro-computed-tomography(µCT) scanned (8.75µm resolution) a cross-sectional series of PFA-fixed, silver-stained wild-type(C57BL/6J) mice aged embryonic-day(E) 10 to postnatal-day(P) 32 (n≤3 specimens/stage). Using Amira software (FEI), we visualized these µCT scan-sets in 3D and, using sagittal, coronal and transverse planes, measured upper and lower molar crypt and crown mesio-distal lengths, TJL and RML. Measurement data were analyzed in Excel (Microsoft). From E14 to P32, in upper and lower jaws, for every stage/age there was space distal to the last-initiated molar. TJL rapidly grew from E12-E18 (spanning M1, M2 onset), slowed from P0/birth-P18 (spanning M3 onset), spiked at P21, and stabilized from P23-P32. Upper/lower molars followed a trend of M1>M2>M3, with lower molars closer to M1>M2≥M3. Until P3, RML≈M1 length in lower and upper jaws. After P6, lower RML>M1 length, while upper RML≤M3 length. Space immediately distal to the last-initiated molar was available throughout the period of M1-M3 onset and development. Spatial relationships were comparable between upper and lower molars/jaws; however, upper RML was shorter by ~20%. Jaw space does not appear to constrain molar onset; rather conditions within the dental lamina likely exert greater influence on onset time. In an evo-devo context, onset of dental development does not appear to be influenced by jaw shape and size.

P.27 (Skeletal Elements, Limbs, Skulls & Feathers)

Mid-Cretaceous amber embedded feathers illuminate the evolutionary complexity of rachis

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Modern feather rachises, composed of a dense external cortex and spongy internal medulla, are flexible, yet stiff enough so the body surface can be streamlined and/or able to withstand large aerodynamic forces encountered during flight. The lack of knowledge about rachidial morphology in early feathers prevents an understanding of how this complex architecture evolved. This has led to controversy in understanding the early evolution of bird flight. Feather morphotypes previously unknown in extant birds, called “rachis dominated feathers” (RDFs) have been reported in several lineages of non-avian and avian theropods. A recent study demonstrated that these feathers consist of only the rachidial dorsal cortex, which challenges the existing models depicting the early evolution of feathers and raise the question of how these rachidial morphotypes have evolved. We show the rachidial dorsal cortex, rachidial ridge, medulla and ventral cortex are formed sequentially in extant birds and demonstrate that diverse Mesozoic feathers documented in fossil records are not distinct feather morphotypes. Instead, they represent feathers with limited differentiation and/or keratinization of the posterior half of the cylindrical rachis. These feathers with rachises consisting only of the dorsal cortex are not as strong as those with a cylindrical rachis and may have played limited aerodynamic functions.

P.29 (Skeletal Elements, Limbs, Skulls & Feathers)

Chitin synthase gene evolution in vertebrates

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The polysaccharide chitin is commonly used in nature in the tough exoskeletons of arthropods and in the rigid cell walls of fungi. The molecule is synthesized by glycosyltransferase enzymes called chitin synthases. Recently, our research group could demonstrate that chitin is endogenously produced in vertebrates in a variety of tissues, such as the epidermis of diverse fishes and salamanders, the eye and gut lumen of ray-finned fishes and the ampullae of Lorenzini of cartilaginous fishes. We carried out comparative studies of vertebrate genomes and transcriptomes to identify chitin synthase sequences and construct a sequence-based phylogeny of vertebrate chitin genes (*CHS*). We show that *CHS* genes are common across vertebrates, including jawless vertebrates, cartilaginous fishes and bony fishes. However, they seem to have been lost from land-living tetrapods. Within bony fishes, we identified ancestral duplicate *CHS* genes, *CHS1* and *CHS2*, that likely arose through a tandem gene duplication. Both genes are found in ray-finned fishes, including chondrosteans, holosteans and teleosts. Lungfish and amphibians preserve only *CHS1*. The greatest diversity of *CHS* genes could be found in teleost fishes: The *CHS2* gene duplicated in the basal teleost whole genome duplication, but only basal teleost lineages preserve both copies. There have also been multiple lineage-specific tandem duplications of *CHS2* genes in teleosts. In cartilaginous fishes there was a separate ancestral tandem duplication, followed by an additional tandem duplication in rays and skates, raising the number of *CHS* genes in this lineage to three. We show that the evolution of *CHS* genes in vertebrates has been marked by a dynamic process of gene duplication and loss in different lineages. The relative ubiquity and diversity of *CHS* genes in vertebrates, as well as the presence of chitin in a variety of tissues, suggests important and diverse role for the chitin biomolecule in vertebrate physiology.

P.31 (Arthropod Innovations, Allometry, Wing & Horn Development)

Orthology and phylostratigraphy unravel the contribution of new genes in the development of chelicerates

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Phenotypes often arise from complex coordination of several genes during development. Even when genomes and expression data of a species of interest are available, it is hard to establish *de novo* the genetic mechanism of specific phenotypes. Of particular interest is the role that new genes may have in the origin of traits considered evolutionary novelties or drivers of diversification for specific groups. A key step towards unraveling this question is obtaining an understanding of the relative age of origin of the genes. We implemented a phylostratigraphy method, using a collection of 29 reference genomes spanning all cellular organisms, to elucidate the relative origin of genes in arachnids whose genomes have recently been sequenced. We present the results of this new bioinformatic approach using transcriptomic datasets derived from appendages of the scorpion *Centruroides sculpturatus* (Arachnida: Scorpiones) during limb formation and different developmental stages of the spider *Parasteatoda tepidariorum* (Arachnida: Araneae). We also show that our orthology-based approach to estimating phylostrata outperforms traditional methods that rely only on sequence similarity. The distinction of orthologous and paralogous relationships in the estimation of phylostrata pinpoints the deployment of phylostratigraphically young genes along the AP axis of these arachnid exemplars, toward identification of selector genes that pattern morphological structures unique to this lineage.

P.33 (Arthropod Innovations, Allometry, Wing & Horn Development)

The chemical, behavioural and developmental basis of caste regulation in ants

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Considering the central role of gene-environment interactions during organismal development, our research uses ants to investigate the effects of the ecological environment on organismal plasticity and diversity. Colonies of the ant genus Pheidole have evolved two types of workers – small headed minor workers and big headed soldiers, which exist in a 95:5 ratio respectively. This innovation led to their ecological success as a dominant and hyperdiverse genus. Multiple factors affect caste ratios, but adult soldiers play a primary role by producing an inhibitory pheromone that inhibits the soldier developmental pathway when there are too many soldiers. Previous work in the lab has shown that adult soldiers are also able to affect the size and allometry of soldiers, thus their caste identity, and give rise to intermediate individuals that do not naturally occur. Preliminary data shows that exposure to soldiers cuticular hydrocarbons causes similar effect, suggesting that the inhibitory pheromone is composed of cuticular hydrocarbons that are transmitted by passive and selective interactions. Future studies aim to investigate naturally occurring intermediates in other ant genera to understand how dimorphic caste systems might have evolved. Overall, our work aims to understand how environmental cues can influence organisms at various scales by regulating major evolutionary transitions such as the worker caste polyphenism in Pheidole ants that shift colony structures and influence their ecological success. By looking at multiple functional scales our work elucidates the role of the social environment in shaping the development and evolution of complex systems such as ant colonies.

P.35 (Arthropod Innovations, Allometry, Wing & Horn Development)

It's a horn, a wing, a helmet: the role of wing serial homologs in insect innovations

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Understanding the origin of novel complex traits is a foundational challenge in evolutionary biology. The most commonly used definition of novelty entails the absence of homology to ancestral traits. This definition, however, is increasingly difficult to reconcile with empirical findings across diverse taxa, which instead emphasize the role of differential re-purposing of conserved developmental modules outside their traditional developmental context as a dominant route to innovation. Yet how descent with modification in developmental evolution may lead to morphological innovation remains poorly understood, including the role of pre-existing gene networks in enabling, biasing, or hindering such innovation. Here we employed RNA interference mediated analysis of wing gene function with Hox-gene mediated transformations of body segments to investigate the origin of the thoracic horns of scarabaeine beetles, one of the most dramatic classes of secondary sexual traits in the animal kingdom. We find (1) that thoracic horns derive from bilateral source tissues that fuse to form a single medial outgrowth during development, (2) that diverse wing genes are functionally required for instructing this process, (3) and that in the absence of Hox-input thoracic horns transform into ectopic wings. Combined our results provide strong evidence for the serial homology between thoracic horns and insects wings, and raise the possibility that other insect innovations may similarly derive from wing serial homologs.

P.37 (Arthropod Innovations, Allometry, Wing & Horn Development)

Investigating the regulatory network of *optix* in its repeated co-option for multiple wing pattern traits across nymphalid butterflies

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The *optix* gene encodes a homeobox transcription factor responsible for driving convergent evolution in *Heliconius* butterflies. Surprisingly, recent studies have also shown that *optix* plays a deeply ancestral role as a master regulator of all wing color in the major butterfly family Nymphalidae. Given the limited variation in the amino acid sequence of *optix* across different nymphalid species, and the fact that *optix* has been evolutionarily deployed in multiple novel developmental contexts, we use *optix* as a model for exploring cis-regulatory architecture of co-option and morphological adaptation. We hypothesize that the *optix* locus is an input-output node that integrates various combinatorial trans-regulatory inputs into a variety of phenotypes across different species in nymphalid butterflies. My work will delves into how this transcription factor is regulated both in terms of cis- and trans-regulation and how the *optix* network has evolved over different evolutionary time scales to regulate novel color pattern traits. It will also try to address the question of when a transcription factor is co-opted for a new function, to what extent does it control its ancestral repertoire of target genes.

P.39 (Arthropod Innovations, Allometry, Wing & Horn Development)

Investigating morphological integration and modularity of membracid pronota

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Although a relatively small family of insects made up of approximately 3,300 species, membracids are unique in that they develop elaborately large pronotal ornaments that mimic a wide range of natural structures from sticks, dried leaves, and petals, to ants, spiders, and wasps. How such tremendous diversity has arisen in this group of insects remains a mystery. Here, I approach membracid pronotal diversity through the concepts of integration and modularity. Specifically, I am interested in the degree of morphological integration between the membracid pronotum and other constituent parts of the organism. It has been suggested that the pronotal tissue shares developmental mechanisms with wing tissues, namely, they have been observed to express known wing-patterning genes, as well as morphological similarities with wings. I hypothesize that the membracid pronotum is more modular (less integrated) compared to other morphological structures and this decoupling of developmental regulation from other body parts could allow for the diversity we observe. However, the pronotal tissue itself may be comprised of developmental modules, or compartments similar to Anterior-Posterior compartments of holometabolous insect wings, borrowing a wing-gene development program as a template for changes in pronotal shapes. While this approach does not get at the molecular mechanisms leading to diverse pronotal forms, it would begin to address the question of how much variability selection is acting on and whether the novelty of the peculiar membracid pronotum is not the structure itself, but the level of integration and modularity of it.

P.41 (Arthropod Innovations, Allometry, Wing & Horn Development)

Genetic underpinnings of novel trait development within a beetle-fungal mutualism

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The Ambrosia Symbiosis describes several subfamilies of weevils (Family: Curculionidae) that have evolved obligate nutritional mutualisms across many phyla of fungi. This ecological adaptation to mycophagy is tightly associated with the development of novel structures (mycangia) used to store and transport symbionts between tree hosts. Mycangia are diverse between species in location and structural form, varying from simple to complex. Despite a lack of conservation and specificity in their fungal mutualists, the parallel evolution of mycangia suggests nutritional mutualisms may prompt the development of these structures through similar gene regulatory networks. In order to study the genetic underpinnings of novel symbiosis-associated trait development, we are utilizing *Euwallacea validus* as a model to study pre-mandibular mycangia. To obtain detailed 3D, in situ structural information of mycangia, we utilized micro-computed tomography. We will report current results of our candidate gene approach by using RNAi to assess the role of regulatory and patterning genes on mycangial development.

P.43 (Arthropod Innovations, Allometry, Wing & Horn Development)

Transitions in allometry reveals evolution of worker caste polymorphism in leaf-cutter ants

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Worker caste polymorphism evolved independently several times in ants. Worker polymorphism is phenotypically plastic, which describes the ability of a genotype to produce different phenotypes in response to distinct environmental cues. Morphological differences between worker subcastes arise mainly by allometric changes of the head with respect to body size, where a proportional or disproportional scaling determine the shape differences among subcastes. This allometric differentiation which occurs during development, provides insight into the mechanisms that colonies use to generate polymorphism and the alloethism (size-based task specialization) which follows. Evolution of different patterns in head-body allometry have been classified mainly in four types: (1) monophasic; (2) diphasic; (3) triphasic; and (4) dimorphic. This classification of head-body allometries in ants reveals developmental and evolutionary transitions from complete monomorphism to complete dimorphism. Yet, the transitions between these different types of worker polymorphism remains poorly understood. In order to investigate the developmental, ecological, and evolutionary transitions of worker caste polymorphism ants, we are focusing on the Attini, a promising group called the 'fungus-growing' ants. Firstly, we have established the allometry regression line of body length and head width using current techniques in order to find their allometric relationships. The preliminary results show a monophasic allometry for *Acromyrmex echinator*, *Acromyrmex Coronatus* a triphasic allometry for *Atta cephalotes*, while for their sister group a non-leaf cutter species *Trachymyrmex cornetzi* a monomorphic allometry. This preliminary results show a phylogenetic progression of head-to-body allometry in these species. Therefore, this group will provide insights into the developmental mechanisms underlying allometry and that explain the diversity of worker caste polymorphism in ants.

P.45 (Arthropod Innovations, Allometry, Wing & Horn Development)

Exploring the role of insulin signaling in relative growth: a case study on wing-body scaling in Lepidoptera

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Adult forms emerge from the relative growth of the body and its parts. Each appendage and organ have a unique pattern of growth that influences the size and shape it attains. This produces adult size relationships referred to as static allometries, which have received a great amount of attention in evolutionary and developmental biology. However, many questions remain unanswered, e.g. What sorts of developmental processes coordinate growth? And how do these processes change given variation in body size? It has become increasingly clear that nutrition is one of the strongest influences on size relationships. In insects, nutrition acts via insulin/TOR signaling to facilitate inter- and intra-specific variation in body size and appendage size. Yet, the mechanism by which insulin signaling influences the scaling of growth remains unclear. Here we will discuss the potential roles of insulin signaling in wing-body scaling in Lepidoptera. We analyzed the growth of wings in animals reared on different diet qualities that induce a range of body sizes not normally present in our laboratory populations. By growing wings in tissue culture, we survey how perturbation and stimulation of insulin/TOR signaling influences wing growth. To conclude, we will discuss the implications of our findings for the development and evolution of organismal form

P.47 (Arthropod Innovations, Allometry, Wing & Horn Development)

A simple developmental model explains emergent tissue patterns and wing morphologies from a wide diversity of extant and fossilized insects

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In many species of insects, their wings resemble stained glass windows. They have a translucent surface that is supported by thickened struts called veins. Over the last 300 million years, these veins have evolved into diverse geometric patterns across insects. For many species, even the left and right wings from the same individual have veins with unique geometric arrangements. It is completely unknown about how these fingerprint-like patterns form. We begin by presenting a quantitative study of the wing veins of 200+ species of dragonflies and damselflies—a group with particularly elaborate vein patterns. We characterize the geometric arrangements of veins and develop a simple developmental model of fingerprint vein patterning. We show that our model is capable of recapitulating the vein geometries of species from other distantly-related insect clades. This includes the wings of grasshoppers, lacewings, and those of giant, long-extinct species whose fossilized wings are quite different from anything that is currently alive. Next, we quantify intraspecific wing variation in two newly collected, large-scale datasets of the wings from Orthopteran and Hemipteran agricultural pests. Finally, we use these data to make specific, falsifiable hypotheses about the developmental mechanisms that underlie intraspecific variation and wing patterning in diverse species.

P.49 (Arthropod Innovations, Allometry, Wing & Horn Development)

From Flying Pansies to *rosa* Forms: How plasticity shapes the development of pigment and structural coloration on Dogface butterfly wings

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The colors of an organism can have a tremendous impact on their lives. The amazing diversity of colors found in nature largely results from a combination of pigmentation and structural features. Butterflies, and their wing colors, are an excellent model to study how these colors can impact the development and success of an organism. Studying factors that influence variation in butterfly color pattern offers great promise in advancing our understanding of basic biological processes, such as pattern development and adaptive evolution, that can have broad implications. In butterflies adult color pattern variations are often the result of plastic responses during development to varying environmental conditions. The Dogface butterfly, *Zerene,* provides a fantastic system for studying the environmental impacts on the development of both pigment and structural coloration. *Zerene* exhibits environmentally induced plasticity in a sexually dimorphic structural color, Ultraviolet, as well as a pink pigment based color. Through a combination of conditional rearing and genetic manipulations, we have characterized the developmental changes resulting from both seasonally and nutritionally induced plasticity in the production of pigments, pterin and melanin, and a structural color, UV, in the *Z. cesonia.* We show that changes in scale structures and overall organization can be environmentally induced. Furthermore, these changes can be partially recapitulated by knocking out the gene *spalt.* These results suggest a shared environmental and genetic trigger that led to scale ultra-structural changes, resulting in color pattern changes in both the pigment and structural colors.

P.51 (Arthropod Innovations, Allometry, Wing & Horn Development)

cis-regulatory architecture of butterfly wing pattern evolution

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The origin and evolution of body shapes is often achieved by changes in gene expression during development. A mechanism behind expression divergence is the modification of regulatory elements, such as promoters and enhancers. Characterization of the genetic drivers of morphological variation, i.e. genes, and their regulatory architecture, is a fundamental goal in the field of evo-devo. Here we used butterfly wing patterns to study the mechanism behind morphological pattern evolution. Specifically, we have tested the function of the gene *WntA* in butterflies of the highly diverse family Nymphalidae. CRISPR/Cas9 loss-of-function experiments demonstrate that this signaling ligand is required for determination of multiple major wing pattern elements. These results identify WntA as a key signal for the pre-patterning of a biological system of exuberant diversity and illustrate how shifts in the deployment and effects of a single developmental gene underlie morphological change. Color pattern evolution is hypothesized to be linked to changes in the cis-regulatory architecture of WntA. Using a combination of phylogenetic analysis, ATAC-seq chromatin profiling, and CRISPR/Cas9 we have functionally characterized several *WntA* cis-regulatory elements that control butterfly wing patterning. Our results imply a complex interaction between regulatory elements, with some individual elements replicating the effect of *WntA* knockout and also driving other pleiotropic effects apart from wing patterns. These results provide a new model of the cis-regulatory architecture of a key morphological adaptation gene, where individual enhancers are both pleiotropic and have major phenotypic effects.

P.53 (Arthropod Innovations, Allometry, Wing & Horn Development)

Vestigial patterning provides insights into the evolutionary diversity of insect wing size

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Insect wings dramatically vary in size and shape, but the mechanisms that drive this diversity are largely unknown. Previous work in our lab shows that in the fruit fly, *Drosophila melanogaster*, the pattern of the wing selector gene, Vestigial (*Vg*) grows faster than the wing imaginal disc itself. Furthermore, this overexpansion of the *Vg* pattern contributes to about one fifth of the adult wing size in this species. Here, we investigate whether this overexpansion is an evolutionary conserved signature of other *Drosophila* species. We performed a quantitative time-course analysis of *Vg* patterns in wing discs of four Drosophila species: *Drosophila melanogaster, Drosophila simulans, Drosophila ananassae* and *Drosophila virilis.* We compare the amount of *Vg* overexpansion with differences in wing sizes relative to the size of the whole organism in these species. We propose that the size of insect wings evolve by tuning the mechanisms of *Vg* patterning during development.

P.55 (Arthropod Innovations, Allometry, Wing & Horn Development)

Polyphenic growth and patterning in the wing of the soapberry bug, *Jadera haematoloma*

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Phenotypic plasticity is thought to evolve through changes in development that alter the integration of environmental cues. The red-shouldered soapberry bug *Jadera haematoloma* (Hemiptera: Rhopalidae) exhibits polyphenism in which a non-linear response to juvenile nutrition, producing distinct morphs that specialize in dispersal versus fecundity. Our lab has previously shown that host adapted ecotypes in this species have evolved differing nutritional thresholds for morph specification, which is mediated, in part, via the insulin-like signaling pathway. These differences have evolved only within the approximately 80 years since the introduction of a novel host plant, and these insects have spread across temperate North America as a result to increased host availability. Soapberry bugs are a promise model for the study of plasticity, integrating ecological, evolutionary and developmental genetic approaches. Here, we present evidence from genomic studies, gene expression, functional genetic tests, and geometric morphometric analysis for the involvement of EGF signaling in wing morph specification and patterning.

P.57 (Arthropod Innovations, Allometry, Wing & Horn Development)

Genetic basis of ommochrome pigmentation in butterfly wings

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Wing pattern diversity in butterflies is well documented and its adaptive significance is known, yet the genetics of novel pigmentation traits is poorly understood. One such family of pigments, ommochromes, are documented throughout the eyes of insects, however only in butterfly wings are they observed in an expanded palette of hues and patterns. I identified candidate genes underlying the novel expansion of ommochromes into butterfly wings through comparing RNA sequencing of ommochrome driving regulatory gene knockouts and of various pupal developmental stages. Using CRISPR Cas9 mediated genome editing, I produced mutations in 5 conserved ommochrome pathway genes (*KF1, cinnabar, cardinal, scarlet, white*) and 3 novel candidate genes (*MFS1, MFS2, ABCC2*) in three species of nymphalid butterflies, *Vanessa cardui*, *Junonia coenia*, and *Heliconius erato lativitta*. Here, I characterize eight putative ommochrome genes as they function in butterfly wing, eye, and larval pigmentation. My results suggest conservation of the enzymatic ommochrome pathway in both wing and eye pigmentation, with *cinnabar* mutations producing wing phenotypes as expected from their role in *Drosophila melanogaster*. Additionally, my knockouts of *MFS1, MFS2,* and *ABCC2* revealed novel function of these genes in ommochrome production and suggest that cooption of transport genes underlies, in part, the expansion of the ommochrome pathway in butterfly wings. These results collectively suggest a new model ommochrome pathway in butterfly wings which has diverged from the pathway as described in *D. melanogaster*.

P.59 (Arthropod Innovations, Allometry, Wing & Horn Development)

Understanding the nutritional underpinnings of phenotypic plasticity in *O. taurus* through metabolomic analysis

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Phenotypic plasticity is an organism's ability to develop into different morphs based on environmental conditions. An extreme form of phenotypic plasticity is known as polyphenism, in which an organism has the option of developing into one of several distinct phenotypes. The dung beetle, Onthophagus taurus, exhibits polyphenic horn development, with the males developing long horns under optimal nutrient conditions and small horns under sub-optimal nutrient conditions. Nutrients obtained from the environment affect the beetle's physiological conditions and ultimately alter developmental pathways to create distinct phenotypes. To understand this, we measured metabolite composition in O. taurus under different nutrition levels. Four beetle groups (large male, small male, large female, and small female) were used for unbiased metabolomic analysis utilizing liquid chromatography mass spectrometry. We will then use this information on metabolomic composition to better understand the developmental pathways that are being activated in response to nutrition and are influencing horn development in O. taurus.

P.61 (Arthropod Innovations, Allometry, Wing & Horn Development)

Role of palmitate and cholesterol modifications in the protein Hedgehog for horn development of *Onthophagus taurus*

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The Hedgehog signaling pathway is a major developmental pathway responsible for anterior-posterior axis patterning during early animal development. In the beetle species *Onthophagus taurus*, it also plays a crucial role in head horn development. In this case, *hedgehog*, as well as the insect sex-determining gene, *doublesex*, have been co-opted in this evolutionarily newer context. Horn development in *O. taurus* is a nutrient-responsive polyphenic trait. Nutrition during the early larval stage determines whether the small or large horned phenotype is expressed in adult males. In this work, we investigate the nutrients that are directly tied to Hedgehog signaling (mainly the lipids that modify the protein) to see how they influence horn development and determine how conserved their roles in Hedgehog function are in the context of horn development. This is done through investigating potential intermediates between nutritional availability and genetic regulation, specifically palmitate and cholesterol, which modify Hedgehog. Two genes important for each modification are: *rasp*, an acetyltransferase responsible for the addition of palmitate onto Hedgehog and *lpR*, a gene coding for the receptor of lipophorin which facilitates cholesterol transport. We knocked-down both *rasp* and *lpR* to reduce Hedgehog's access to palmitate or cholesterol modifications respectively. Inversely, we are also feeding larvae an excess of cholesterol to observe the how increasing Hedgehog's access to it impacts horn development. Thus far, *rasp* knock-down results have shown an increase in horn growth at smaller body sizes, similar to that previously reported *hedgehog* knock-down phenotypes. Roughly 30% of animals have exhibited malformed elytra which has also been observed in the preliminary results of *lpR* knock-downs. Collectively, our results suggest that modifications onto Hedgehog are vital for proper signaling in the context of horn development.

P.63 (Arthropod Innovations, Allometry, Wing & Horn Development)

Developmental genetics of *Photuris* firefly lanterns

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Elucidating the developmental genetic basis of novel biological structures remains an important challenge in evo-devo. Traits that represent a qualitative departure from ancestral morphology are inherently interesting as their formation implies a radical reorganization of developmental programming. We are investigating the genetic underpinnings of lantern formation in *Photuris* fireflies. Lanterns are auto-luminescent structures that lack homology to any traits found outside of a relatively small clade of luminescent beetles. Combining a traditional candidate gene approach with differential transcript expression analysis and followed by functional assays via RNA interference, we have identified several genes that have been recruited in various aspects of lantern formation and/or function. Among these are genes specifying regional identity on the anterior-posterior axis, genes that mediate sexual dimorphism and signaling molecules that function in dorsal-ventral patterning. We are currently investigating genes known to play roles in pigmentation patterning in other insects to determine the means by which pigment deposition is disrupted in lantern-associated cuticular regions. Through this research, we hope to shed light on general mechanisms through which developmental repatterning might lead to the origin of novel morphology.

P.65 (Arthropod Innovations, Allometry, Wing & Horn Development)

Unravelling complex gene interactions underlying the evolution and development of form using haploid wasp genetics

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Complex interactions within large networks of genes underlie the consistent and repeatable development of form. The identity of the participants and the nature of theinteractions within the networks are largely obscure, but can be revealed by the phenomenon of epistasis, where the novel combination of alleles leads to a phenotype significantly different from the sum of the phenotypes of the alleles in isolation. In a preliminary QTL study, we identified a complex set of epistatic interactions that govern whether a developmental defect occurs in hybrid male progeny between the wasps Nasonia vitripennis and N. giraulti. These interactions are conceptually reminiscent to the negative epistatic interactions that lead to hybrid sterility in other insects, but are unique in affecting the outcome of a complex developmental process. Studying epistatic interactions in Nasonia is advantageous due in large part to the haploidy of males. This eliminates the dominance interactions that make generating and identifying the appropriate hybrid genotypes difficult in traditional diploid systems. Viable and fertile hybrids between Nasonia species can be made, and recombinant haploid F2 males are readily obtainable. With genome sequences available for the relevant species, these features make Nasonia a powerful system for evolutionary genetic analysis of epistatic interactions. Here we describe the introgression of two of the relevant alleles from N. giraulti into N. vitripennis, which each exhibit fully penetrant clefting in both males and females. We also describe preliminary work in understanding of the developmental basis of the defective phenotype, and in mapping the causative alleles. Finally, we discuss the importance of dissecting networks of interacting genes using epistasis in order to fully understand the structure of developmental networks and the constraints on their evolution.

P.67 (Marine & Freshwater Invertebrate EvoDevo)

Embryonic cis-regulatory modifications during a life history switch in sea urchins

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The evolution of lecithotrophic (non-feeding) from planktotrophic (feeding) larval development is a repeated occurrence found among many marine invertebrate taxa. Perhaps the best studied example of this life history switch is the lecithotrophic sea urchin *Heliocidaris erythrogramma*, which produces large, lipid-rich eggs and undergoes rapid larval development. Embryological and transcriptomic assays have revealed extensive modifications to morphogenesis and developmental gene expression in this species relative to the ancestral, planktotrophic state found in most other sea urchin species. Among the most prominent of these changes is altered reprogramming of embryonic cell fate specification, simplified larval morphology, and abbreviated time to metamorphosis. However, the genomic basis for this derived developmental program remains poorly understood. To better characterize the gene regulatory mechanisms driving these changes, we have conducted ATAC-sequencing on six successive embryonic cell cycles (4th-9th cleavage), blastulae, gastrulae, and larvae in three sea urchin species: *H. erythrogramma*, its planktotrophic congener *H. tuberculata*, and the well-studied planktotroph *Lytechinus variegatus*. Differences in developmental chromatin accessibility are often species-specific, but we still find many changes associated with planktotrophic or lecithotrophic development. Importantly, a number of differentially accessible loci between *H. erythrogramma* and the two planktotrophic species are upstream or near transcriptional start sites of key developmental genes. We also measure significant increases and decreases in chromatin accessibility at specific genomic loci through development, including early cleavage, which may reflect regulatory state dynamics during blastomere and tissue fate specification. These results demonstrate variation in chromatin accessibility, including possible cis-regulatory modifications, may be driving changes in gene regulation between these species during a critical developmental period in which many embryonic cell fates are specified. These regulatory shifts could play an important role the evolution of altered blastomere specification and derived pre-metamorphic development in the lecithotroph *H. erythrogramma*.

P.69 (Marine & Freshwater Invertebrate EvoDevo)

Echinobase: a community resource for BAC-based gene expression reporters in echinoderms

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Echinobase is a Model Organism Database (MOD) that supports an international community of echinoderm researchers by providing two types of services: 1) *computational resources*, including databases of genome and transcriptome sequences; and 2) *experimental resources*, including the large-insert BAC libraries that were originally constructed to assemble the original sea urchin genome sequence. These BACs serve distinctive experimental functions by serving as platforms for generating reporter constructs. BAC vectors support large inserts of genomic DNA (>100 kb), that typically contain sufficient regulatory information to recapitulate spatiotemporal expression of endogenous genes. Here, we outline several strategies for homologous recombination to efficiently manipulate BAC clones for tracing gene expression *in vivo*, rescuing protein function, and identifying *cis*-regulatory modules. We have developed BAC reporters that express an array of fluorescent proteins, including photoconvertible proteins, and tagged proteins that exhibit specific subcellular locations. When microinjected in echinoderm embryos, BAC DNA is stably incorporated in the host genome during cell division. This provides long-term, stable expression of reporter proteins, allowing for detailed cell lineage tracing experiments. BAC clones also provide a stable genetic background for experimental manipulation, an important advantage when working with outbred animals. Even as genome editing technologies continue to improve, BAC-based reporter constructs will continue to have invaluable roles for interrogating the mechanisms that regulate gene expression.

P.71 (Marine & Freshwater Invertebrate EvoDevo)

Cell junctions of unique molecular composition in a sponge (Porifera)

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The integrity and organization of animal tissues depends upon specialized protein complexes that mediate adhesion between cells with each other (cadherin-based adherens junctions), and with the extracellular matrix (integrin-based focal adhesions). Reconstructing how and when these cell junctions evolved is central to understanding early tissue evolution in animals. We examined focal adhesion protein homologs in tissues of the freshwater sponge, *Ephydatia muelleri* (phylum Porifera). We found that sponge homologs of focal adhesion proteins co-precipitate as a complex and localize to cell junctions in sponge tissues. These data support that the adhesion roles of focal adhesion proteins evolved early, prior to the divergence of sponges and other animals. However, in contrast to the spatially partitioned distribution of cell junctions in epithelia of other animals, focal adhesion proteins were found to be co-distributed with the adherens junction protein Emβ-catenin in sponge tissues; both at certain cell-cell and cell-extracellular matrix adhesion structures. Sponge cell junctions were found to be unique in other ways, too. The basopinacoderm (substrate-attachment epithelium) lacks typical polarity in that focal adhesion-like structures form on both basal and apical surfaces, and compositionally unique cell junctions form at the interface between cells with spicules (siliceous skeletal elements) and between cells and environmental bacteria. These results clarify the diversity, distribution and molecular composition of cell junctions in tissues of *E. muelleri*, but raise new questions about their function and homology to cell junctions in other animals.

P.73 (Marine & Freshwater Invertebrate EvoDevo)

The role of myocardin-related transcription factor in the development of contractile tissue of the freshwater sponge, *Ephydatia muelleri*

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Distinct cell types are a fundamental part of animal multicellularity. However, the evolutionary origin of key animal cell types, such as myocytes, remains poorly resolved. Sponges (Porifera) lack muscles, but their epithelia are able to undergo coordinated, organism-wide contractions in order to help clear debris from their canal system. Understanding the mechanisms by which this tissue contracts and the factors involved in contractile cell fate determination may provide valuable insight into how muscles evolved. Sponges, as well as other non-bilaterians, lack traditional myogenic factors such as MyoD, but have conserved homologs of myocardin related transcription factor (MRTF), and the MADS-box transcription factors; serum response factor (SRF), and myocyte enhancer factor-2 (Mef-2). In bilaterians, nuclear translocation of MRTF and interaction with SRF or Mef-2 drives expression of contractile genes and can result in myocyte differentiation. We explored the function of the sponge homolog of MRTF in the development of contractile tissue in the freshwater sponge, *Ephydatia muelleri*. Immunostaining and pharmacological perturbation of EmMRTF suggest a conserved mechanism of action in sponge cells. We have also find evidence that EmMRTF activity is essential for the development of tissue-level organized contractile actomyosin bundles in the endopinacocytes of the apical pinacoderm (sponge surface epithelium). Activation of EmMRTF in archeocytes (sponge pluripotent stem cells) appears sufficient to drive differentiation into the cell type containing these bundles. These results provide evidence of homology between sponge contractile epithelia and muscles of other animals and point to regulation of the MRTF-MADS-box transcription factor interaction as an important event in myocyte evolution.

P.75 (Marine & Freshwater Invertebrate EvoDevo)

Variations in systems drift within the tunicate heart development gene network

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Mutations in gene regulatory networks often lead to evolutionary divergence without impacting gene expression or developmental patterning. The rules governing this process of developmental systems drift, including the variable impact of selective constraints on different nodes in a gene regulatory network, remain poorly delineated. Here we examine developmental systems drift within the cardiopharyngeal gene regulatory networks of two tunicate species, *Corella inflata* and *Ciona robusta.* Cross-species analysis of regulatory elements suggests that *trans*-regulatory architecture is largely conserved between these highly divergent species. In contrast, *cis*-regulatory elements within this network exhibit distinct levels of conservation. In particular, while most of the regulatory elements we analyzed showed extensive rearrangements of functional binding sites, the enhancer for the cardiopharyngeal transcription factor *FoxF* is remarkably well-conserved. Even minor alterations in spacing between binding sites lead to loss of *FoxF* enhancer function, suggesting that bound trans factors form position-dependent complexes. Additionally, mutational analyses of presumptive FoxF binding sites in other early cardiopharyngeal transcription factors indicate that FoxF plays a critical regulatory role upstream of these factors. Thus, the exceptional conservation of the *FoxF* enhancer may reflect unique structural and functional constraints. More generally, these results suggest that levels of *cis*-regulatory drift are governed by distinct structural constraints that will be difficult to predict based on network architecture.

P.77 (Marine & Freshwater Invertebrate EvoDevo)

Kill your idols! Incongruence in nomenclature vs developmental identity in spiral-cleaving embryos highlights the need for a new developmental framework

Eric Edsinger

Unaffiliated

Spiral cleavage is characterized by a 45-degree inclination of the cleavage spindle across the animal-vegetal axis and by a 90-degree alternation of spindle orientation with each cell cycle. A now classic nomenclature to name each cell based on spatial and cell lineage patterns of division was developed by Wilson (1892) in the annelid *Nereis* and Conklin (1897) in the mollusc *Crepidula*. The nomenclature describes the embryo in terms of quadrants that are established at the 4-cell stage and extend along the animal-vegetal axis and quartets that cut perpendicular to the axis and represent the formation of equivalent sister cells arising within equivalent cell lineages of each quadrant. Thus, based on spatial position and lineage, stereotypic patterns of division in spiral cleavage enable the identification of individual cells in the embryo and the identification of identical cells across species. Today, the system is typically implicit, if not explicit, to interpretations and comparisons of cell lineage, gene expression, and gene editing data in spiral-cleaving lophotrochozoans, often in the context of how the embryonic architecture is translated by gastrulation into the adult body plan. Undoubtedly, the nomenclature of spiral cleavage has been essential in studies of spiral-cleaving embryos for over 125 years. Critically, application of the nomenclature in later developmental stages largely fails to take into account embryonic patterning by the D-quadrant organizer, with its transformation of the developmental architecture and establishment of bilateral symmetry. Based on published organizer-dependent spatial position and cell lineage contributions to the larval prototroch in the gastropod mollusc *Patella vulgata*, combined with newly inferred morphogenetic movements, I highlight here the incongruence of nomenclature identities vs developmental states in the embryo and trochophore, and suggest that long-standing conflation of the two hinders a clear understanding of development in spiral-cleaving embryos.

P.79 (Marine & Freshwater Invertebrate EvoDevo)

Determining the role of oral-aboral patterning on neurogenesis in the sea anemone, *Nematostella vectensis*

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Cnidarian nerve nets are believed to represent the ancestral nervous system that gave rise centralized nervous systems characteristic of bilaterian species. Determining how neural patterning occurs in the net-like ancestral nervous system will allow us to understand the origin and evolution of patterning mechanisms that gave rise to complex centralized nervous systems. Previous work suggested that the oral-aboral (O-A) axis of *Nematostella* is potentially homologous to the anterior-posterior (A-P) axis of bilaterian animals, and that molecularly defined spatial domains established along the oral-aboral axis resemble those that pattern bilaterian centralized nervous systems along the A-P axis. In bilaterians, each molecular domain is established by graded Wnt activity. The molecular domains are described by transcription factors that act directly to pattern distinct neuronal fates. We hypothesized that spatial domains established by axial patterning cues contribute to neuronal patterning the *Nematostella* nerve-net. To address this we identified domain markers, regionally restricted neurogenic genes, and mapped neuronal subtypes to specific domains. Functional disruption of the aboral domain marker *Nvsix3/6* (homologous to the forebrain regulator *six3*) results in the loss of aboral neural genes and expansion of more orally restricted neuronal fates into the aboral domain. Conversely, misexpression of *Nvsix3/6* expanded the aboral neural gene territory more orally at the expense of oral neuronal genes. The molecular domains along the O-A axis are established by graded Wnt activity. *Nvsix3/6* is known to suppress Wnt activity in the aboral domain suggesting that changes to neuronal fates following *Nvsix3/6* disruption are either due to alterations in Wnt activity or are the direct result of disrupted *Nvsix3/6.* To distinguish between these hypotheses we globally misexpressed *Nvsix3/6* in animals treated with the Wnt agonist azenkenpaullone. *Nvsix3/6* was not sufficient to rescue aboral fates indicating that levels of Wnt activity not molecular domain specifiers pattern neuronal subtypes along the oral-aboral axis. To date our data demonstrate a link between Wnt patterning and neuronal specification in cnidarians and bilaterians, but suggest that the significant role for molecular domain specifiers in neuronal fate specification evolved in bilaterians.

P.81 (Marine & Freshwater Invertebrate EvoDevo)

The role of BMP signaling in early development of the spiralian *Capitella teleta*

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BMP (bone morphogenic protein) signaling has long been thought to have a key role in specification of neural ectoderm. In vertebrates (deuterostomes) and insects (ecdysozoans), delimitation of neural fate to one side of the animal relies on inhibition of BMP signaling during dorsal-ventral axis formation. Studies outside model organisms have shown a greater diversity in the extent and role of BMP signaling during these processes, raising the question of how homologous centralized nervous systems are across Bilateria. A major bilaterian clade that has so far been understudied but has shown preliminary evidence that BMP signaling is not necessary for neural fate specification is Spiralia (e.g. molluscs and annelids). Here we investigated the role of BMP signaling in the development of the annelid *Capitella teleta* by injecting mRNA encoding a dominant negative BMP receptor (dnBMPr1) to knock down BMP signaling. In situ hybridizations and antibody staining suggest that suppression of the BMP pathway during development results in drastic reduction of multiple tissues, including trunk mesoderm, foregut, and brain. However, injected animals still form a ventral nerve cord and a dorsal-ventral axis in the trunk. These initial results align with previous experiments that soaked *C. teleta* embryos in recombinant BMP protein. Although preliminary, our works raises some interesting questions about how neural specification in controlled in *C. teleta*, and what this will tell us about the origin of centralized nervous systems in general.

P.83 (Marine & Freshwater Invertebrate EvoDevo)

Molgulid ascidians share a unique Manx/DDX5/17 gene complex not shared with other ascidians: evolution and development of tailless ascidian larvae

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Typical chordate features found in ascidian tadpole larvae have been evolutionarily lost several times independently within the Molgulidae family. Urodele (tailed) Molgulids retain a tail with muscle cells, a notochord, and a dorsal neural tube, whereas the notochord and muscle cells do not differentiate correctly within the tailless species. A locus containing an unusual gene arrangement of the *Bobcat*/*DDX5*/*17 (p68)* gene within the first intron of the *Manx* gene has been shown to be essential for the development of chordate features in molgulid tadpole larvae. Sequencing and closer examination of ascidian genomes show that there is a unique gene arrangement of *SSNA1* upstream and adjacent to *Manx* and *Bobcat* within the Molgulidae which is not found in other ascidians; however, a similar arrangement of *SSNA1* directly upstream of *Bobcat* was found in *Oikopleura dioica* supporting Appendicularia as a sister group to the Molgulidae. *SSNA1* is expressed in tailed *Molgula oculata* gonads and not in tailless *M. occulta* gonads, suggesting *SSNA1* could have a role in the development of tailed larvae. Bobcat/DDX5/17 has been shown to be necessary for chromatin remodeling in vertebrates and acts with transcription factors to allow cells to differentiate. Expression of these key genes may be affected by one another's close proximity, disturbing normal gene expression and thereby larval development of chordate features. We propose that the rearrangement that took place in the molgulid ancestor may be contributing to the numerous instances of the evolution of taillessness found in the Molgulidae.

Even-Numbered Posters

Sub-sections

I. Sex Determination, Sexual Dimorphism & Reproductive Traits
II. Hormones, Behavior, Life History & EvoDevo
III. EvoDevo of Sensory Systems
IV. Body Plans & Segmentation
V. Networks, Switches Comparative Analyses & Applications

P.2 (Sex Determination, Sexual Dimorphism & Reproductive Traits)

ISL1 targets conserved appendage enhancers during development of the amniote phallus

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The development of the genital tubercle (GT) – the embryonic precursor to the penis and clitoris – involves expression of many genes that play a role in limb development. Similarities in early genital budding across amniotes suggests a shared evolutionary origin of the external genitalia from their last common ancestor over 300 million years ago. ChIP-seq experiments show that many limb enhancers are active during phallus development, raising the possibility that limb regulatory elements were coopted during the evolution of the phallus. The *Isl1* gene encodes a transcription factor required for initiation of hindlimb outgrowth in mice. Conditional knockouts also demonstrate a crucial requirement of *Isl1* for GT outgrowth. Using a combination of RNA-seq and ChIP-seq, we reveal putative direct targets of ISL1 during mouse GT development. Limb genes are overrepresented among these targets, and many appear to be activated via known limb enhancers. ChIP-seq in chick, lizard, and turtle genitalia also reveals conserved binding at some of these enhancers, possibly highlighting targets of functional importance maintained from their common ancestor. A notable example of a conserved target is *Tbx4* and its enhancer HLEB, which is also an ISL1 target during the initiation of hindlimb budding.

P.4 (Sex Determination, Sexual Dimorphism & Reproductive Traits)

A morphological novelty of the *Drosophila* genitalia originates from a pre-existing organizing center

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Investigating the evolution of novel anatomical structures requires deciphering how the genetic programs underlying their development initially form. Work on morphological novelties has frequently implicated a role for conserved signaling pathways in their development and evolution. However, we generally lack an understanding of how the regulation of these pathways are altered to deploy them to novel contexts, and how downstream responses to signaling events evolve to generate new developmental outcomes. To investigate this, we studied the role of Notch signaling during the evolution of a recently evolved structure, the posterior lobe of *Drosophila*, a cuticular outgrowth on the genitalia of males within the *melanogaster* clade. The ligand for the Notch signaling pathway, Delta, is required for posterior lobe development, and its expression has been spatially expanded in lobe-forming species. We've found that the posterior lobe activity of *Delta* is regulated by two transcriptional enhancers. Analysis of enhancer activity indicates that an early enhancer is active in the genital imaginal disc, and a late enhancer becomes active at early pupal stages. Comparisons of the late enhancer's activity to reporters bearing orthologous regions from non-lobed species suggests that changes have occurred upstream of *Delta* to expand into the lobe-forming region. Thus, in contrast to previous examples of novelty, our results suggest that a pre-existing signaling source was expanded to generate a novel structure. We present experiments to decipher the ancestral role of this pre-existing signaling center, dissect the upstream regulators of *Delta*, and investigate downstream targets of the Notch pathway in this novel tissue. These results highlight the nuanced view of novelty that can be obtained through the comparison of closely related species at the level of gene regulatory elements.

P.6 (Sex Determination, Sexual Dimorphism & Reproductive Traits)

Developmental context and the control of gene regulation: a comparison of TBX5 regulatory interactions in embryonic forelimbs and genitalia

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Although the morphology of the amniote limb and phallus differ dramatically, these appendage types express a similar suite of transcription factors and signaling molecules during development. This observation led to the hypothesis that the amniote phallus may have evolved, in part, through co-option of components of an ancient appendage gene regulatory network. Consistent with this, previous work from our lab has shown that many enhancers active in developing limbs are also active in the genital tubercle (GT). However, it remains unknown whether transcription factors expressed in the limbs and phallus interact with the same enhancers to regulate similar suites of target genes. To address this question, we are investigating the regulatory targets of the TBX5 transcription factor. TBX5 plays a critical role in the growth and development of the vertebrate forelimb and is also known to be expressed in the developing genitalia of several amniote species. Using TBX5 ChIP-seq in mouse embryonic forelimbs and GT, we have identified roughly 10,000 putative binding sites in each of these appendage types. Approximately 25% of these peaks are shared between the forelimb and GT and are significantly enriched near genes involved in limb development. Thus, despite the high overlap of active enhancers in embryonic limbs and genitalia, there are differences in the set of TBX5-bound *cis*-regulatory targets in these tissues. To investigate the degree to which TBX5 binding events are conserved in amniote appendages, we are now performing parallel TBX5 ChIP-seq experiments in embryonic forelimbs and hemipenes of the *Anolis* lizard. In addition, we have conditionally knocked out the *Tbx5* gene during the early development of mouse forelimbs and genitalia and will perform RNA-seq in these tissues. By intersecting our ChIP-seq and RNA-seq datasets we will determine the degree to which functional TBX5 regulatory interactions are shared between the forelimb and GT.

P.8 (Sex Determination, Sexual Dimorphism & Reproductive Traits)

The evolutionary connection between hair and mammary glands: mammary development in *Monodelphis domestica* shows a mosaic of ancestral and derived traits

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The nipple is a synapomorphy of marsupials and eutherians, and is thought to have evolved from a hair-associated organ in the mammalian common ancestor from which the lactogenic patch of monotremes is also derived. One of the key lines of evidence for the homology of the therian nipple and the lactogenic patch is that marsupials have retained a transient hair associated with their developing mammary glands, however these structures have not been well documented since the early 20th century. We report results from a study of the developing mammary organs of *Monodelphis domestica* and where we observed the presence mammary hairs in 12 week old females, as well as their absence after 18 weeks of age. Histochemical staining for cystine confirmed the structures as keratinized hairs. Division of myoepithelium and luminal epithelium in *M. domestica* milk ducts was observed by visualization of keratin 14 and 18 in juvenile and adult mammary lobules, and alpha smooth muscle actin was detected in the myoepithelium. These patterns match those in eutherians and suggest a conserved ductal morphology and mechanism of milk expulsion. These results reveal shared characteristics of the *M. domestica* nipple with both the eutherian nipple and the monotreme lactogenic patch, and provide support for the evolutionary derivation of the mammary gland from the ancestral hair organ.

P.10 (Sex Determination, Sexual Dimorphism & Reproductive Traits)

Structure-specific genetic requirements for sexual dimorphism in the Large Milkweed Bug, *Oncopeltus fasciatus*

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Sex determination pathways are fundamental to the development of sexually dimorphic animals and vary greatly despite the conservation of biological sex itself. To date, little is known about the molecular and genetic details of the sex determination pathways of many animals outside a few model species. Here, we studied the following sex determination candidate genes in the hemimetabolous insect, *Oncopeltus fasciatus* (Heteroptera: Lygaeidae): *intersex, fruitless*, and the three paralogs of *doublesex.* We present data on the conservation, expression, and function of these genes. *doublesex* appears to have had a complex evolutionary history of duplication and divergence compared to the relative conservation of the *intersex* and *fruitless* sequences. The expression of all five genes is upregulated during juvenile stages as sexual dimorphism develops in both sexes of *O. fasciatus*. We show that each sex's different dimorphic somatic structures require unique combinations of *intersex, fruitless,* and *doublesex-c* for their specification. Altogether, our results indicate novel developmental functions of *intersex, fruitless,* and the *doublesex* paralogs in *O. fasciatus* compared to other insects. Moreover, our data support recently growing evidence that sexual dimorphisms are specified in a structure-specific manner. Collectively, our results support the theme of diverse mechanisms of sex determination within the insect lineage.

P.12 (Sex Determination, Sexual Dimorphism & Reproductive Traits)

Unlocking the evolution of vertebrate sex determination

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In all non-mammalian vertebrates, sex is primarily determined by the presence or absence of estrogen, acting as a switch to mediate ovarian development. However, in mammals we see a complete switch from a hormonally mediated system to one controlled by a Y-linked gene, SRY, which serves to mediate testis development. Interestingly we have shown that in marsupial mammals estrogen exposure to early developing fetus can completely override the male determining signal from SRY and induce ovarian development. In this study we investigated if this same mechanism exists in eutherian mammals, namely the mouse and human. In each case we show that estrogen plays a highly conserved role in regulating one of the key genes for inducing testis development, SOX9. This leads to a cell fate switch in the gonads from male to female. These data indicate a highly conserved role for estrogen in mammals that has persisted for over 160 million years and show that it directly impacts early fate decision in the gonads. These data have broad implications for the evolution of sex determining mechanisms and our susceptibility to environmental contaminants that mimic estrogen.

P.14 (Sex Determination, Sexual Dimorphism & Reproductive Traits)

Inspecting the role for the trans-regulatory landscape to the origin, diversification, and loss of a sexually dimorphic fruit fly pigmentation trait

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A major goal for evolutionary-developmental biology research is to identify the genetic changes underlying the origins, diversification, and loss of morphological traits. Such traits are built by the spatial and temporal regulation of gene expression, and thus the evolution of gene expression is often involved in their evolutionary histories. Gene expression is under the control of a network of transcription factors (trans-landscape) that ultimately impinge on the cis-regulatory elements (CREs) of differentiation genes whose encoded proteins produce particular traits. Transcription factor genes are often highly pleiotropic, as they can regulate the expression of multiple genes for multiple traits. Thus, it seems reasonable to expect that evolutionary changes in gene expression more frequently occurred by mutations altering the CREs for differentiation genes than changes to the trans-landscape. Our research aims to test whether this expectation for a conserved trans-landscape applies to the origin, diversification, and loss of a well-studied fruit fly pigmentation trait in the Sophophora subgenus. The origin of a male-specific pattern of abdominal tergite pigmentation involved the gain of CREs controlling the expressions of pigmentation enzyme genes responsive to the prevailing trans-landscape of body plan patterning and sexual dimorphism transcription factors. Here, we share our results from tests of these CREs in transgenic hosts that represent the ancestral sexually monomorphic trait, diverse forms of the derived dimorphic trait, and a secondary loss of the dimorphic trait. The outcomes from these tests will reveal the extent to which this particular trans-landscape has remained conserved while the pigmentation phenotype has widely evolved.

P.16 (Sex Determination, Sexual Dimorphism & Reproductive Traits)

Resolving the evolution and diversification of a Hox-regulated pigmentation trait

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Morphological diversity appears to evolve primarily from functional changes within cis-regulatory elements of developmental genes. In animals, the identity of each body segment is determined by Hox transcription factors. Due to their prominent developmental role, changes in the regulation of Hox genes have been implicated in the evolution of animal diversity. In fly species from the melanogaster group the abdominal pigmentation is sexually dimorphic, with the A5 and A6 abdominal segments covered with melanic pigmentation only in males. This trait represents a novelty that evolved within a monomorphically pigmented lineage 45 million years ago. The formation of adult pigments depends on the activity of enzymatic genes that are expressed during late pupal development under the control of the Hox gene Abdominal-B. In this study we aim to test whether regulatory changes in Abdominal-B were crucial for the origin of the dimorphic pigmentation pattern within the melanogaster group. Specifically, we hypothesize that in species from monomorphic lineages – that resemble the ancestral state – Abdominal-B expression is arrested during pupal development; in dimorphic species, however, regulatory changes resulted in an extended temporal expression of Abdominal-B followed by the activation of the downstream pigmentation genes. By taking advantage of the extensive knowledge of the abdominal pigmentation gene regulatory network and the available molecular tools in the fruit fly we hope to provide a thorough example of how a novel morphological trait can be originated by regulatory changes in a Hox gene.

P.18 (Hormones, Behavior, Life History & EvoDevo)

The role of maternal genetic effects in brain development and behavior in the cavefish *Astyanax mexicanus*

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A. mexicanus is a single species of fish that consists of two forms, a river-dwelling surface form and a cave form adapted to survive and thrive in multiple caves in Mexico. Cavefish have evolved a number of morphological, physiological and behavioral traits. Cavefish and surface fish are interfertile, allowing for examination of the genetic basis of cavefish trait evolution. Previous studies have demonstrated that parental genetic effects play a role in two cavefish-evolved traits, eye degeneration and vibration attraction behavior. However, it is currently unknown if parental genetic effects play a role in the evolution of the brain or other behaviors that have evolved in cavefish. We have leveraged the ability to hybridize cave and surface fish to examine the role of parental genetic effects on the evolution of brain development and sleep loss in cavefish. We have generated reciprocal hybrid fish by crossing male surface fish with female cavefish and male cavefish with female surface fish. By examining brain morphology, brain activity, and behavior in these reciprocal hybrid fish, we have determined the role that parental genetic effects play in the evolution of these potentially adaptive traits.

P.20 (Hormones, Behavior, Life History & EvoDevo)

Ontogeny of schooling and shoaling behavior in *Astyanax mexicanus*

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Groups of animals have complex social dynamics that are influenced by both internal and external factors. Fish form social groups called schools and shoals. Schooling is a group behavior in which the movements of the group members are coordinated in speed and direction, while shoaling refers to the tendency to aggregate regardless of speed and direction. Both environmental factors, such as predator presence and food availability, and genetic factors influence the likelihood of schooling. *Astyanax mexicanus* is a species of fish that exists in two extant morphs: cavefish and river-dwelling surface fish. Cavefish populations have evolved eye and pigment loss, as well as a variety of behavioral phenotypes. The genetic similarities between populations, paired with robust differences in behavior, provide a unique opportunity to investigate the molecular and cellular bases of these complex behaviors. Previous studies indicate that surface fish exhibit schooling and shoaling, while no social cohesiveness has been identified in cavefish. However, it is currently unknown when during development these behavioral differences arise. To examine the ontogeny of schooling and shoaling in *A. mexicanus,* we recorded and analyzed the movement of surface fish and cavefish alone and in groups at various points in development. Through analysis of nearest neighbor distances, velocity, and relative orientation, we were able to identify when during development surface fish begin to school, and to determine when social behavior in surface and cavefish diverges.

P.22 (Hormones, Behavior, Life History & EvoDevo)

Establishing the Mexican cavefish as a model for studying the evolution of a decision-making circuit

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Anti-predator adaptations are essential for the survival of most organisms. Effective predator evasion involves careful evaluation of sensory input followed by the execution of an appropriate behavioral output. The Mauthner neurons, or M cells, are a pair of reticulospinal neurons conserved across fish and amphibians that are responsible for integrating sensory information and initiating the c-bend escape response. The variety in sensory input and highly stereotyped behavioral output make the M cells a useful model for investigating sensory integration and decision making. Although extensive work has been done characterizing the morphology and firing properties of the M cells in various species, little is known about how they evolve. The teleost *Astyanax mexicanus* consists of ancestral surface-dwelling populations, as well as multiple independently evolved cave populations. While the surface populations experience predation, no predators have been identified for the cave populations. Thus, the Mexican cavefish presents an ideal opportunity to understand how this decision-making circuit may evolve in response to environmental differences. Here, we elicit c-bend startle responses using vibrational stimuli to assess responsiveness of cave and surface fish larvae to various stimuli and conduct kinematic analyses to assess differences in latency, angular speed, and bend angle. Kinematic analyses reveal that Pachón cavefish larvae respond to stimuli with diminished angular speed and increased latency compared to surface fish. These findings suggest that surface fish may be better adapted at predator evasion. Pachón were more likely to perform c-bend responses than their surface counterparts. These contradictory results may be related to the involvement of the M cells in prey-capture techniques. Taken together, these data suggest that the Mexican cavefish may serve as a novel model for studying the evolution of the Mauthner neurons in sensory integration and decision making.

P.24 (Hormones, Behavior, Life History & EvoDevo)

Examining the role of albinism in the evolution of cave populations of *Astyanax mexicanus*

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Understanding the role of pleiotropy in the evolution of adaptive traits is a central question in evolutionary genetics. Key to this is evaluating the phenotypic consequences of naturally occurring mutations. The fish *Astyanax mexicanus* exists in two interfertile eco-morphotypes: a river-dwelling surface form, and multiple populations of a cave form. Some cave populations have independently evolved two striking regressive traits: loss of eyes, and albinism. While the genetic and developmental bases of these traits have been studied extensively, the evolutionary forces that drive regressive trait evolution are still unclear. The identification of the causal mutations for albinism in the gene *oculocutaneous albinism type 2 (oca2)* in two independently evolved cave populations provides the opportunity to investigate the repeated evolution of this trait. Recent evidence suggests that *oca2* plays a role in both pigmentation and the catecholamine synthesis pathway. The catecholamines dopamine and norepinephrine have been implicated in cave-evolved behaviors including sleep, schooling and feeding. Thus, albinism may have evolved due to indirect selection for catecholamine-mediated behavioral changes. To examine the role that *oca2* plays in behavior, we used CRISPR/Cas9 gene editing to generate mutant alleles of *oca2* in surface fish, thereby rendering them albino. We compared behaviors in albino fish and their pigmented, wild-type siblings focusing on sleep and foraging behavior. Together, this work will improve our understanding of the role that pleiotropy plays in adaptation to a novel environment.

P.26 (Hormones, Behavior, Life History & EvoDevo)

How to quantify complex behavioral phenotypes across vertebrates: leveraging network analysis to study the evolution of sociality

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Modern 'omics approaches provide us with powerful, quantitative “big data” to ask questions about the molecular and developmental origins and evolution of complex phenotypes. Such comparative studies of phenotypic evolution often suggest homoplasy, or the evolution of similar phenotypes in unrelated species. In fact, recent studies have identified shared neuromolecular underpinnings of behavioral phenotypes across vast phylogenetic distances – for example learned vocalizations, aggression, and monogamous mating systems. However, comparing complex phenotypes fairly across distantly related species requires the development of rigorous, quantitative metrics (e.g., geometric morphometrics), which have been elusive for the study of social evolution because behavioral phenotypes represent emergent properties of the organism and integrate multiple organismal systems (e.g., sensory and motor systems). Here, we present and apply a novel quantitative approach to characterize diverse forms of sociality across vertebrates to compare independent evolutionary transitions to social dominance. Social dominance systems – where some individuals are dominant over subordinate group members, defend resources, and have increased access to reproductive opportunities – have evolved repeatedly across vertebrates and beyond. In such groups, individuals may assume a specific set of behavioral characteristics – such as social polymorphisms or reproductive tactics (or “types”) – because of genotype, developmental events, individual condition, and/or social or ecological opportunity. Using a quantitative social network modeling approach, we compare attributes of social status and network position across “types” in independent evolutionary transitions to social dominance systems. We ask how components of social dominance vary, whether similar social and reproductive “types” emerge, and how “types” vary in social status attributes and network properties across vertebrates. Finally, we discuss the implication of our approach for identifying the evolutionary origins and underlying neuromolecular mechanisms of social dominance systems.

P.28 (Hormones, Behavior, Life History & EvoDevo)

Evolution of acoustic communication in the blind cavefish *Astyanax mexicanus*.

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Acoustic communication is an essential feature to exchange information related to social cohesion and coordination. We recently found that *Astyanax mexicanus* is a highly sonic species, in the laboratory and in the wild, and cave and surface morphotypes share a repertoire of at least six sounds. Based on major differences concerning eye loss and reduced aggressiveness in cavefish we hypothesised that one sound of the repertoire, the "Sharp click", would be differentially used and have a different meaning in the two *Astyanax* morphs. We demonstrated that Sharp click is a visually-triggered sound produced during agonistic behaviour in surface fish and a chemosensory-triggered sound produced by cavefish during foraging behaviour. Resident-intruder assays were performed in the two morphotypes to investigate relationships between Sharp clicks production and agonistic events. In addition, groups of surface fish and cavefish submitted to starvation, overfeeding or normal diets for several months were recorded during chemosensory-triggered foraging stimulation. Finally, the functional value of Sharp clicks was assessed on social groups by playing back either surface fish or cavefish Sharp clicks, or white noise. The results demonstrate that acoustic communication does exist and has evolved in blind cavefish, accompanying the evolution of its behaviours. Thus, *A. mexicanus* appears like a powerful model to address the question of the role of acoustic communication in evolution, and possibly speciation.

P.30 (Hormones, Behavior, Life History & EvoDevo)

Evolution of an endocrine gland underlies the evolution of distinct life history strategies of moths and flies

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The fruit fly *Drosophila melanogaster* has served as an outstanding model system for developmental biologists although embryonic development has undergone considerable modification to allow for its rapid life cycle. Recent studies using fruitflies have demonstrated that a steroidogenic gland called prothoracic gland is a critical regulator that determines the timing of metamorphosis. To determine the generality of this model, we compared the functions of prothoracic glands between the fruitflies and the tobacco hornworm, *Manduca sexta*. The endocrine regulation and nutrient-dependency of this gland was found to differ between the two species: In fruitflies, the gland plays key roles in dictating the timing of metamorphosis in flies, whereas in tobacco hornworms, they merely serve as mediators of a distinct process that regulates the timing of metamorphosis. The two species differ considerably in their life history strategies, and our study highlights how post-embryonic development of fruitflies have also undergone unique evolutionary changes to adapt to its rapid life cycle. Our findings demonstrate the selection acting on an endocrine gland underlies the evolution of life history strategies.

P.32 (Hormones, Behavior, Life History & EvoDevo)

Genomic mechanisms of developmental delay and environmentally-cued hatching in annual killifishes, an emerging Eco-Evo-Devo model

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Annual killifishes are emerging Eco-Evo-Devo models due to their unique embryonic dormant stages. They are being used to study development, metabolism, cell proliferation, and stress tolerance in vertebrates. They inhabit seasonal pools that desiccate, resulting in the death of the adult population. Unique adaptations including specialized egg structures, desiccation resistance, and up to three ontogenetic diapause stages slowing developmental and metabolic rates enable the embryonic population to survive annual dry seasons. When the habitat floods, annual killifish terminate their third and final diapause (DIII), hatch, and begin a new lifecycle. Here we explore the genomics of embryonic DIII in annual killifishes, the only vertebrate diapause known to occur after completion of organogenesis. We use scanning electron microscopy, comparative developmental transcriptomics, phylogenomics, genome sequencing and model rates of gene evolution to investigate the genetic basis of killifish annualism, diapause, and environmentally-cued hatching. We discover hundreds of candidate genes involved in diapause and delayed hatching, comparing annual and non-annual killifish species. Specifically, we find numerous differentially expressed killifish transcripts with homologs also differentially expressed in the same direction during dormancy stages in other animals. These transcripts illustrate conserved roles of these homologs during delayed development in metazoans from insects to killifish to mammals. Additionally, tight linkage of diapause and hatching with the expression of a complex family of hatching enzymes leads us to analyze regulatory mechanisms associated with environmentally-cued hatching in comparison to other aquatic vertebrates. Lastly, we show that diapause has up to 7 origins in killifishes and detect over 160 genes that have increased rates of molecular evolution in annual compared to non-annual killifishes. Our integrative framework combining development, genomics, evolution, and ecology provides important insights into the mechanisms of diapause and the diversity of vertebrate hatching strategies.

P.34 (Hormones, Behavior, Life History & EvoDevo)

Monarch butterflies use an environmentally sensitive, internal timer to control overwintering dynamics

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The monarch butterfly (*Danaus plexippus*) complements its iconic migration with diapause, a hormonally controlled developmental program that contributes to winter survival at overwintering sites. Although timing is a critical adaptive feature of diapause, how environmental cues are integrated with genetically-determined physiological mechanisms to time diapause development, particularly termination, is not well understood. In a design that subjected western North American monarchs to different environmental chamber conditions over time, we modularized constituent components of an environmentally-controlled, internal diapause termination timer. Using comparative transcriptomics, we identified molecular controllers of these specific diapause termination components. Calcium signaling mediated environmental sensitivity of the diapause timer, and we speculate that it is a key integrator of environmental condition (cold temperature) with downstream hormonal control of diapause. Juvenile hormone (JH) signaling changed spontaneously in diapause-inducing conditions, capacitating response to future environmental condition. Although JH is a major target of the internal timer, it is not itself the timer. Epigenetic mechanisms are implicated to be the proximate timing mechanism. Ecdysteroid, JH, and insulin/insulin-like peptide (IIS) signaling are major targets of the diapause program used to control response to permissive environmental conditions. Understanding the environmental and physiological mechanisms of diapause termination sheds light on fundamental properties of biological timing, and also helps inform expectations for how monarch populations may respond to future climate change.

P.36 (Hormones, Behavior, Life History & EvoDevo)

Competitive strategies of the predatory nematode *P. pacificus*: "boom and bust" dynamics and cross-generational pheromone signaling

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Population size is a major ecological constraint on resources, which can affect behavioral, physiological, and morphological traits of future generations through density-dependent selection. While many species are capable of dynamically responding to population density through phenotypic plasticity, the underlaying mechanisms facilitating these changes are often poorly understood. Therefore, to study presumed age-dependent influences on phenotypic plasticity we performed developmental pheromone profiling in the necromenic nematode *Pristionchus pacificus,* which responds to high population densities by inducing a predatory mouth form or an arrested developmental stage called dauer. Surprisingly, we observed adult-specific production of small signaling molecules that induce the predatory morph, even though adult phenotypes are no longer plastic. We investigated the potential influence of adults on younger generations by introducing a novel dye-based method to differentiate populations in mixed cultures. Indeed, adults, but not juvenile peers, influence juvenile mouth forms, revealing an age-dependent signaling mechanism acting through pheromone secretions influencing both density-dependent selection and phenotypic plasticity. Furthermore, due to the necromenic association between *P. pacificus* and scarab beetles we explored population density and dispersal within this ecological context by performing experiments in the wild. We found that there are two major dispersal events of the worms from the host. While food is abundant, the worms reproduce and the population grows rapidly; as resources are diminished, *P. pacificus* enters dauer and disperses. Thus, *P. pacificus* exhibits a "boom and bust" dispersal cycle with the evolution of the predatory morph serving as a competition strategy to both broaden dietary range and consume nematode competitors on decaying host carcasses.

P.38 (Hormones, Behavior, Life History & EvoDevo)

Dramatic evolution of body length due to postembryonic changes in cell size in a close relative of *Caenorhabditis elegans*

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Understanding morphological diversity—and morphological constraint—has been a central question in evolutionary biology since its inception. Nematodes of the genus *Caenorhabditis*, which contains the well-studied model organism *C. elegans*, display remarkable morphological consistency in the face of extensive genetic divergence. Here, we provide a description of the broad developmental patterns of a newly discovered species, *C. inopinata*, which was isolated from fresh figs in Okinawa and which is among the closest known relatives of *C. elegans*. *C. inopinata* displays an extremely large body size; it can grow to be nearly twice as long as *C. elegans* and all other known members of the genus. Observations of the timing of developmental milestones reveal that *C. inopinata* develops about twice as slowly as *C. elegans*. Measurements of embryonic and larval size show that the size difference between *C. inopinata* and *C. elegans* is largely due to post-embryonic events, particularly during the transition from larval to adult stages. This difference in size is not attributable to differences in germ line chromosome number or the number of somatic cells. The overall difference in body size is therefore largely attributable to changes in cell size via increased cytoplasmic volume. Because of its close relationship to *C. elegans*, the distinctness of *C. inopinata* provides an ideal system for the detailed analysis of evolutionary diversification. Ongoing comparative genomic and evo-devo studies are generating and testing hypotheses regarding the causes and consequences of ecological specialization and morphological divergence. The context of over forty years of *C. elegans* developmental genetics also reveals clues into how natural selection and developmental constraint act jointly to promote patterns of morphological stasis and divergence in this group.

P.40 (Hormones, Behavior, Life History & EvoDevo)

Hormonal regulation of cardiomyocyte regeneration across phylogeny

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Cardiomyocyte proliferative and regenerative potential displays striking divergence across phylogeny and ontogeny, but the underlying mechanisms remain enigmatic. Loss of mammalian cardiac regenerative potential correlates with cardiomyocyte cell-cycle arrest and polyploidization as well as the development of postnatal endothermy. We reveal that diploid cardiomyocyte abundance across 41 species conforms to Kleiber's law—the ¾-power law scaling of metabolism with bodyweight—and inversely correlates with standard metabolic rate and body temperature. We analyzed 3 hormonal pathways that may contribute to CM proliferative and regenerative potential: thyroid, glucocorticoid, and vitamin D hormones. Inactivation of thyroid hormone signaling reduces mouse cardiomyocyte polyploidization, delays cell-cycle exit, and retains cardiac regenerative potential in adults. Conversely, exogenous thyroid hormones inhibit zebrafish heart regeneration. Moreover, thyroid hormone levels inversely correlate with cardiomyocyte ploidy across vertebrates and parallel the ectothermy-endothermy transition. Thus, our findings suggest that loss of heart regenerative capacity in adult mammals is triggered by increasing thyroid hormones and may be a trade-off for the acquisition of endothermy.

P.42 (Hormones, Behavior, Life History & EvoDevo)

A rare direct test of selection: phosphorus limitation does not drive bony plate loss in freshwater stickleback

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A central goal of biology is to understand the selective forces that drive evolution. Connecting these forces to phenotypes and their underlying genotypes is key to understanding the constraints and abilities of organisms to adapt to changing environments. However, there are few cases in which the selective force acting on an adaptive allele is known. Stickleback are a powerful model for adaptive evolution for several reasons. First, marine stickleback have repeatedly adapted to freshwater environments across the northern hemisphere through a suite of largely parallel phenotypic changes. Second, for many of the changes, quantitative trait loci (QTL) studies have revealed the genomic regions responsible. The *Ectodysplasin (Eda)* locus is highly divergent between marine and freshwater sticklebacks, and a regulatory mutation in the *Eda* gene causes a reduction in the number of bony armor plates covering the fish. These plates are protective against predatory attacks, however the cause of repeated loss of the plates in freshwater is unknown. We empirically tested one hypothesis for the adaptive loss of these plates: loss of plates provides a growth advantage during development due to the dietary limitation of phosphorus for freshwater sticklebacks. We made crosses of marine fish that were heterozygous for the freshwater *Eda* allele and measured the growth of offspring under different dietary and salinity conditions. We found no difference in growth between siblings carrying the marine and freshwater *Eda* alleles. Given these results, we have further tested a subset of fish for differences in phosphorus excretion rate and levels of bone mineralization-mechanisms which could mediate the impacts of phosphorus limitation and explain the lack of interaction between diet and genotype. This study highlights the importance of rigorous experimental testing to identify the selective forces acting on genotypes and phenotypes in the wild.

P.44 (Hormones, Behavior, Life History & EvoDevo)

Mitochondrial function in the terrestrialized *Polypterus senegalus*

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Through the study of the phenotypic plasticity of extant amphibious fish, it is possible to gain insight into how plastic responses that increase performance on land may have facilitated the evolution of tetrapods. *Polypterus senegalus* is a basal ray-finned fish that is capable of emersion out of water, breathing air using rudimentary lungs, and terrestrial locomotion using its strong pectoral fins. The move from water to land involves coping with changes in gravity, desiccation, O_2 and CO_2 levels, and NH_3 solubility and amphibious fish must have the energy capacity to meet these increased demands. Mitochondria are the powerhouse of the cell and so we investigated mitochondrial phenotypic plasticity in the body muscle of terrestrialized *Polypterus*. To determine whether mitochondrial traits are modified to increase terrestrial performance, we assigned *Polypterus* to the following conditions: control aquatic (n=7), gravel terrestrial (n=5), or ramp terrestrial (n=6). After one week of acclimation, we exercised the terrestrial fish daily (i.e., gently encouraged them to walk). After 5 weeks, we sacrificed the fish, excised body muscle and placed the tissue into a mitochondrial-friendly respiration buffer (miR05). We added diluted body muscle tissue homogenate to a 26°C Clark-type electrode chamber (HansaTech) and measured the respiratory function of the mitochondria electron transport chain (ETC) through the addition of ETC complex-specific substrates and inhibitors. Compared to aquatic controls, we observed decreased locomotive activity and body mass over 5-weeks in the terrestrialized *Polypterus*, plus a statistically significant ($p=0.0013$) increase in liver mass normalized to body weight (indicating metabolic stress). Comparing tissue performance, we observed no significant difference between basal tissue respiration, ETC-driven ADP phosphorylation, maximal ETC respiration, or ETC complex IV respiration in the body muscle of either young or older fish; however, we observed a trend towards an increase for all four mitochondrial traits in terrestrial fish, with high inter-individual variability. To determine differences in mitochondrial function independent of mitochondrial density, we will be presenting mitochondrial respiratory function data normalized to the mitochondrial marker, citrate synthase. Amphibious fish that are active out of water typically have the same or an increased metabolic rate and our *Polypterus* mitochondrial respiration data support this conclusion. Further study is needed to determine if there is mitochondrial remodelling (e.g., mitobiogenesis) during emersion to maintain threshold metabolic function.

P.46 (Hormones, Behavior, Life History & EvoDevo)

Developmental and evolutionary aspects of multicellularity in Myxobacteria and Dicytostelids: insights from the interplay between generic and agent-like properties

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Myxobacteria and Dictyostelids are prokaryotic and eukaryotic multicellular lineages, respectively, that after nutrient depletion, aggregate and develop into structures called fruiting bodies. The developmental processes and the resulting morphological diversity resemble one another to a remarkable extent despite their independent origins, the evolutionary distance between them and the lack of tractable levels of homology in the molecular mechanisms in each group. We hypothesize that the morphological outcomes, and thus the parallelism between the two lineages, arise as the consequence of the interplay between generic (i.e., physically predictable) processes acting upon the multicellular materials and agent-like behaviors characteristic of the constituent cells. In this context, we analyze the relative contribution of the generic and agent-like properties in the main features observed during Myxobacteria and Dictyostelid development and how they explain the emergence of their shared traits. We suggest that as a consequence of aggregation, the nascent multicellular mass becomes subject to new sets of patterning and morphogenetic processes whereby collective cell-cell contacts mediate the emergence of a fluid-like properties. In both lineages, this leads to developmental processes, e.g., streaming, rippling, similar to behaviors observed in non-living fluids. We attribute the deviations of the dynamics and morphological outcomes of the multicellular mass from the generic predictions to the contribution of agent-like behaviors, e.g., directed migration, synchronization, of the cells themselves. This occurs in response to external cues through lineage-specific regulatory and signaling mechanisms that reflect the evolutionary history of the respective organisms. We conclude that the similar developmental programs of Myxobacteria and Dictyostelids are more likely due to shared generic physical processes in coordination with analogous agent-type behaviors than to convergent evolution under parallel natural selection regimes.

P.48 (EvoDevo of Sensory Systems)

Chitin is found endogenously within the electrosensory organs of cartilaginous fish

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Chitin is one of earth's most abundant polysaccharides and a major component of rigid biological structures such as the outer cuticle of arthropods. The molecule is produced by a myriad of organisms using enzymes called chitin synthases. For decades, it had been widely assumed that vertebrates don't produce chitin, but recently our lab discovered it in the gut lumen of zebrafish and the epidermis of both fishes and salamanders. In our ongoing investigations into vertebrate chitin, we unexpectedly found chitin within the electrosensory organs (known as ampullae of Lorenzini or AoL) of cartilaginous fish such as sharks, skates, and rays (class Chondrichthyes). All living organisms emit weak electric fields and chondrichthyans use their AoL to orient towards potential prey or mates. A single AoL consists of a gel-filled tubular canal that opens to the environment *via* a pore in the epidermis and terminates in a lobular structure made up of specialized electrosensory cells that are responsible for signal detection. Using histochemical reagents and chemical analyses, we found that chitin is a component of the viscous hydrogel which fills the whole tubular AoL. We subsequently identified chitin synthase genes in the genomes of several cartilaginous fish species. Using *in situ* hybridization with a chitin synthase sequence from the little skate (*Leucoraja erinacea*), we observed the localized expression of chitin synthase to the AoL of both embryos and adults, indicating that they are indeed synthesizing chitin endogenously. We have observed the presence of AoL chitin in representatives from several chondrichthyan clades belonging to both subclasses, Elasmobranchii and Holocephali, suggesting that this character evolved ancestrally to all cartilaginous fish over 450 million years ago. We are now investigating the evolution of chitinous hydrogel in non-chondrichthyan fish and also how chitin contributes to the development and/or function of the electrosensory system.

P.50 (EvoDevo of Sensory Systems)

Elucidating the role of eye loss in the evolution of adaptive traits in the cavefish *Astyanax mexicanus*

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Regressive evolution, the loss of traits over time, has occurred across taxa. However, the evolutionary forces that drive regressive evolution remain unclear. One striking example of regressive evolution is the reduction or loss of eyes that has evolved repeatedly in cave-dwelling organisms. The freshwater fish *Astyanax mexicanus* exists as a surface morph and multiple populations of a cave morph, some of which have independently evolved. Cavefish from multiple cave populations have repeatedly evolved eye degeneration. Eye loss could have evolved through neutral mutations in the absence of positive selection for eyes in the darkness of caves. Alternatively, eye loss could have evolved due to selection, either directly for eye degeneration or indirectly due to selection for another trait influenced by the same gene(s). We know from previous studies that cavefish have an increase in number of neuromasts, the sensory organs of the lateral line, and an enhanced Vibration Attraction Behavior (VAB), an attraction to vibrating objects in the water, relative to surface fish. Either or both of these traits could be advantageous in the cave, contributing to more effective foraging in the dark. Further, crosses and mapping studies suggest that eye size, neuromast number, and VAB could have evolved through the same genes. In this study, we use eyeless surface fish generated by two different methods: lensectomy, and CRISPR/Cas9 mediated knockout of the eye gene, *rx3*. By examining neuromasts and VAB in these eyeless surface fish we aim to understand the role that eye loss plays in other, potentially adaptive, traits.

P.52 (EvoDevo of Sensory Systems)

Molecular and functional role of bitter taste receptors in Mexican cavefish (*Astyanax mexicanus)*

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A sensory paucity both at the individual and species level can be counterbalanced by enhanced other chemosensory perception. In fish, the chemosensory system is highly developed and very important for a wide range of activities like navigation, mate recognition, and food searching. The taste receptors family show a dynamic evolution in land-living vertebrates, with as little as 2–3 functional genes in some avian species and more than 50 genes in an amphibian species. In contrast, teleost fish appeared to have a small and rather constant repertoire of 3–6 bitter taste receptor (T2Rs) genes. The small teleost fish *Astyanax mexicanus* (AM) consists of interfertile river-dwelling and cave-dwelling populations, referred to as 'surface fish' and 'cavefish', respectively. So far a small number or T2Rs are reported in AM, therefore our objective was to investigate the expression and functional role of T2Rs in AM. Genome database of AM (ensemble and NCBI) showed 7 T2Rs in AM viz. Tas2R1, Tas2R3 (2isoforms), Tas3R4, Tas2R8, Tas2R60, and Tas2R114. enabled the exploration of taste receptor genes in fish species. We performed quantitative PCR and in situ hybridization to the expression of Tas2R1, Tas2R3, Tas3R4, and Tas2R144 in 2 and 30 days post fertilization juvenile fish. These genes have been already characterized in human. We found Tas2R1 expression was maximum and localized throughout the embryo. Tas2Rs expression was identified in different organs (liver, fins, jaws, and gills) in adult cavefish. To see the functionality of T2Rs, we transiently transfected all 4 selected T2Rs in HEK293T heterologous expression system and treated with quinine and dextromethorphan (DXM). Quinine and DXM both are well-known ligands for T2Rs in Human. We observed a high response of T2R4 with Quinine and DXM in heterogenous system. This study will provide novel insights into the role and importance of the chemosensory system in fishes and will identify the crosstalk between the different sensory systems in vertebrates.

P.54 (EvoDevo of Sensory Systems)

The genetic and molecular mechanisms of environmental perception during mouth-form plasticity regulation in the nematode *Pristionchus pacificus*

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Polyphenism, a discrete case of phenotypic plasticity, is widely present in nature. Nevertheless, the genetic mechanisms regulating polyphenisms are not well understood, as genetic and molecular tools are limited in many of these organisms. One of the most well-studied developmental switch networks regulating a polyphenism in animals is the stomatal dimorphism in the hermaphroditic nematode *Pristionchus pacificus,* which is responsible for the formation of either a predatory or bacterivorous morph. A switch network involving the *eud-1*/sulfatase has been identified and characterized in great detail. However, the upstream pathways that perceive environmental information into the switch network are currently not known. Temperature is a general developmental cue for phenotypic plasticity. Therefore, we are investigating the role of temperature cues in the mouth-form decision in *P. pacificus*. Phenotyping mouth-form ratios in 10 wild isolates on temperatures between 12° and 28°C revealed a conserved concave reaction norm, but also substantial natural variation. We conducted a forward mutagenesis screen using the strain RSA635 at 27° C and isolated 12 mutants, which were defective for the high temperature response. Identification of the genetic lesion in one of these mutants revealed the *P. pacificus daf-11/guanylate cyclase*. In *C. elegans*, *daf-11* is expressed in the nervous system and is inhibiting high temperature mediated induction of dauer larvae, another polyphenic trait. However, we do not observe constitutive dauer larvae in *Ppa-daf-11* mutants on high temperatures. Therefore, our results show co-option of the high-temperature dauer forming pathway into mouth-form plasticity, and suggest developmental drift of the high-temperature dauer induction between these two related nematodes. We will describe our ongoing studies to identify the molecular mechanisms of temperature perception during mouth-form plasticity in *P. pacificus*.

P.56 (EvoDevo of Sensory Systems)

Morphological variations in eye size: does rx3 play a role?

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The retinal homeobox transcription factor RX has an evolutionarily conserved role during vertebrate eye development. Loss of function mutations in Rx in mammals (*rx3* in fish) result in loss of optic vesicle evagination and a complete lack of eyes. In humans, RX has been associated with structural eye defects such as anophthalmia (absence of eyes) or microphthalmia (small eyes), but little is known about how genetic variation in RX results in these morphological variations in eye size. *Astyanax mexicanus* is a single species consisting of eyed surface fish and multiple, independently-evolved blind cavefish morphs. Cavefish initiate eye formation, but developmental alterations lead to differences in eye size and ultimately eye degeneration. These distinct morphological differences that arise during development make *Astyanax* a powerful system for studying the genetic regulation of eye size. Previous studies demonstrated that *rx3* is located under a QTL for eye size in *A. mexicanus*, suggesting that this gene may play a role in the evolution of eye size reduction in cavefish. We have found that both the *rx3* expression domain size and total *rx3* expression levels are reduced in early Pachón cavefish development compared to surface fish. Interestingly, this reduction in *rx3* expression was not observed in Molino cavefish, suggesting that different molecular and developmental mechanisms underlie reduced eye size in these two cave populations. Further, we have used CRISPR/Cas9 gene-editing to generate surface fish with mutations in *rx3*. Injected surface fish have eyes that are greatly reduced in size or entirely absent, suggesting a conserved role for this gene in early eye development in *A. mexicanus*. Together, these results will allow us to unravel the role that *rx3* plays during eye development and how differences in *rx3* expression regulate differences in eye morphogenesis, critical to understanding the mechanisms that lead to different congenital eye disorders.

P.58 (EvoDevo of Sensory Systems)

Gene duplication and regulatory network cooption in the developing cephalopod lens and the evolution of visual system complexity

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The evolutionary origin of new and unique morphologies in the natural world remains a difficult and unresolved question. Coleoid cephalopods, a group of molluscs that include squid, cuttlefish, and octopus, are exceptional animals for the study of morphological novelty in the Metazoa as they have evolved an incredible array of complex adaptations. One of these includes a highly acute visual system. The cephalopod visual organ has a single lens in the anterior, capturing and focusing light on the cup-shaped retina in the posterior. The lens is essential to acuity and image formation in complex visual systems. The camera-type morphology of the cephalopod eye resembles the vertebrate visual system but this complexity is convergently evolved. The development of the vertebrate lens has been extensively studied, yet little is known about lens development in the cephalopod. We have begun to describe lens development and dissect the gene regulatory network underlying this process in the squid Doryteuthis pealeii. Our work shows a gene duplication of Sp6-9 and cooption of the canonical Sp6-9/Dlx limb regulatory network in the developing cephalopod lens and anterior segment. We have further identified that lens-generating cell differentiation is regulated by Wnt signaling. This work is the first functional characterization of lens development outside of traditional models and supports the importance of gene duplication in the evolution of novel phenotypes.

P.60 (Body Plans & Segmentation)

The developmental underpinnings of the miniaturized body plan of Tardigrada

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Tardigrada is a phylum of microscopic animals that are characterized by a highly compact body plan. Our investigations of Hox genes and other anteroposterior axis patterning genes revealed that the compact body plan of tardigrades evolved by the loss of a mid-trunk region. Here we present results of our more recent studies, which provide clues to how the mid-trunk region was lost, and reveal additional insights into the evolution of the compact tardigrade body plan. First, in most arthropods and probably onychophorans, Wnt genes and *caudal* (*cad*) are co-expressed in a posterior domain where they regulate segment addition by posterior growth. As predicted, *cad* expression is restricted to a posterior domain during development in *Hypsibius exemplaris*. However, Wnt genes are not restricted to a posterior domain in this species. Furthermore, the specific Wnt genes that have been implicated in regulating posterior growth in arthropods are missing in the genomes of two tardigrade species that we analyzed. Therefore, the loss of these genes may explain the loss of posterior growth in Tardigrada, and the loss of the corresponding mid-trunk segments that develop by posterior growth in other panarthropods. Second, *Distal-less* (*Dll*), *dachshund* (*dac*), and *homothorax* (*hth*) + *extradenticle* (*exd*) specify distal, intermediate, and proximal appendage domains, respectively, in arthropods and onychophorans. In *H. exemplaris*, *Dll* is expressed across the entire appendage bud, but *hth/exd* do not specify an anteroposterior appendage domain. Additionally, *dac* is missing in tardigrade genomes. These results suggest that tardigrades have lost proximal and intermediate appendage domains. Together, our results reveal modifications to ancestral developmental mechanisms that may explain the evolution of the miniaturized and simplified body plan of Tardigrada.

P.62 (Body Plans & Segmentation)

The miniaturization of tardigrade legs is correlated with the loss of proximal and intermediate appendage domains

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Tardigrades are microscopic invertebrates with a body plan that is small and compact. They evolved their small body plan through the loss of a significant portion of their anteroposterior axis. The legs of tardigrades are also remarkably small when compared to the legs of arthropods and onychophorans—their closest relatives. In both arthropods and onychophorans, the leg gap genes *Distal-less* (*Dll*), *dachshund* (*dac*), *homothorax* (*hth*), and *extradenticle* (*exd*) show regionalized expression patterns during leg development. *Dll* patterns the distal region of the leg, *dac* patterns the intermediate region of the leg, and *hth* and *exd* work in combination to pattern the proximal region of the leg. We investigated the leg gap genes to determine whether the mechanism that patterns the very disparate leg morphologies of onychophorans and arthropods also patterns the tiny legs of tardigrades. We identified single orthologs of *Dll* and *hth* in the genomes of two tardigrade species—*Hypsibius exemplaris* and *Ramazzottius varieornatus*, as well as three orthologs of *exd* in these species. Using *in-situ* hybridization, we detected strong *Dll* signal across all four tardigrade developing leg pairs. We detected little to no expression of *exd1* or *exd2* in developing legs. *Hth* and *exd3* show variable expression between leg pairs, suggesting they are not playing a role in specifying proximal identity of developing legs. We could not identify a *dac* ortholog, even though it is present in out-groups, suggesting that this gene was lost in the tardigrade lineage. Of the leg gap genes, only *Dll* appears to be required for development of all tardigrade legs. This suggests that tardigrade legs may possess only distal identity, and that they have lost a significant portion of the proximodistal axis. This lends further support to the miniaturization of Tardigrada through the loss of regions of body axes.

P.64 (Body Plans & Segmentation)

Isolation of *Drosophila* pair-rule gene orthologs from the harlequin bug *Murgantia histrionica* (Hemiptera: Pentatomidae)

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Studies of the genes controlling embryonic development in the model insect *Drosophila melanogaster* during the 1980s led to the discovery of genes that act sequentially to establish the basic body plan of the fly. A set of nine pair-rule genes (PRGs) is responsible for segment formation in *Drosophila* with most PRGs expressed in stripes in the primordia of the alternate segmental units missing in corresponding mutants. While roles of individual PRGs are often conserved within holometabolous insects, work from our lab and others suggests extensive variation in these genes in non-holometabolous insects. For example, in *Oncopeltus fasciatus*, *Drosophila* PRG-orthologs are not expressed in *Drosophila*-like pair-rule patterns and RNAi knockdown does not produce pair-rule defects. Interestingly the only reported PRG in *O. fasciatus* is *E75A*, a nuclear receptor that regulates larval development in *D. melanogaster.* In order to understand these evolutionary changes in critical regulatory genes, additional model organisms are needed. Here, we present the harlequin bug, *Murgantia histrionica*, another hemipteran, as a model system. Segmentation in the harlequin bug occurs in a sequential mode, with segments added sequentially from the segment addition zone. We established lab cultures of *M. histrionica* and have maintained this colony for multiple generations. To assess similarities and differences in the PRG-orthologs between *O. fasciatus* and *M. histrionica*, we isolated all nine PRG-orthologs as well as *E75A*, using degenerate PCR and Rapid amplification of cDNA ends (RACE). We modified protocols for in situ hybridization from *O. fasciatus* and verified this technique using *M. histrionica engrailed*, which is expressed segmentally, as expected. Interestingly, *E75A* showed pair-rule gene expression, similar to *O. fasciatus*. Preliminary analysis of the expression patterns of the Drosophila PRG-orthologs suggests both similarities and differences compared to *O. fasciatus*. Future goals are to establish RNAi in this species to assess gene function.

P.66 (Body Plans & Segmentation)

Combining RNA-seq and a candidate gene approach to probe the segmentation gene network of a sequentially segmenting insect *Oncopeltus fasciatus* (Hemiptera: Lygaeidae)

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Our current understanding of embryonic patterning in insects is derived mostly from the studies of segmentation genes in *Drosophila melanogaster*. A model for a developmental hierarchy has emerged, one level of which is represented by the pair-rule genes (PRGs) which encode transcription factors involved in promoting the formation of body segments. Interestingly, previous work showed that orthologs of the *Drosophila* PRG *eve* and a non-PRG *E75A* have non-PRG and PRG function, respectively, in the sequentially segmenting insect, *Oncopeltus fasciatus* (Liu & Kaufman, 2005; Erezyilmaz et al., 2009). By evaluating the expression of the *Drosophila* PRG orthologs in *O. fasciatus*, we have found that most of these genes are expressed in every segment, not every other segment as in *Drosophila.* Only one ortholog—*runt*—is expressed in a manner suggestive of pair-rule patterning. Parental RNAi was used to better understand the function of each ortholog during *Oncopeltus* embryogenesis. The expression of *invected,* a gene expressed in the posterior of every segment, was analyzed in knockdown embryos to assess the effects of each gene's depletion on segmentation. Severe segmentation defects were observed after *odd, prd,* and *slp* RNAi, however every segment was affected more or less equally. Loss of alternate segments, as seen in *Drosophila* PRG mutants, was not observed. To search for other non-*Drosophila* PRGs like *E75A*, we sequenced RNA to isolate genes with an *E75A*-like transcription profile by differential expression (DE) analysis. Putative transcription factors were then identified in this cluster by functional domain assignment using InterProScan. *E75A* was uncovered through this analysis, validating our DE analysis and transcription factor screening pipeline. An *in situ* screen of these transcription factors will serve as an unbiased approach to reveal novel pair-rule genes in this species as well as provide a useful atlas of transcription factor expression patterns.

P.68 (Body Plans & Segmentation)

Regulation of segmentation during embryonic development of the red flour beetle, *Tribolium castaneum*: an RNA-Seq analysis

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Embryonic development in *Drosophila melanogaster* is extensively-studied and well understood, but its development is highly-derived and distinct from the likely ancestral and more common short-germ type development of many arthropods. *Tribolium castaneum* development is more similar to other insect lineages and has features analogous to other segmented animals, including annelids and vertebrates. Yet, the genetic regulation of early development, particularly segmentation, is much less studied than in *Drosophila*. In order to characterize changes in transcription during key phases of anterior-posterior patterning in *T. castaneum,* we prepared and sequenced cDNA libraries extracted within narrow one-hour time windows during germband condensation, extension and elongation, and the termination of segmentation. We compared transcriptomes at different stages for differential expression and looked for enrichment of particular categories of biological process, molecular functions as well as correlated changes among genes in the same biochemical pathways. We found an enrichment of transcription factors among genes that were differentially expressed. In *Tribolium* a genetic oscillator controls segment addition in the posterior of the embryo, but modulators of the oscillator are not well characterized. Our findings constrain models of the regulation of segmentation by broadly screening for the presence, absence and differential expression of transcription factors at key time points when we have previously shown that the rate of segmentation changes.

P.70 (Body Plans & Segmentation)

A transgenic study reveals evolutionary conservation in regulation of gene Chordin

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Establishment of dorso-ventral body axis occurs before and during gastrulation and it is controlled by a complex program. Protein Chordin plays an important antagonizing role to BMP (Bone Morphogenetic Protein) signaling in the region of dorsal blastopore margin (organizer). Concurrently, the expression pattern of Chordin reliably marks the organizer of gastrulation. However, regulation of Chordin transcription is not clear and evolution of vertebrate gene regulatory network (GRN) for organizer formation remains vague. To understand how vertebrate GRN evolve, it is necessary to obtain knowledge from animal lineages proxy to vertebrates. Cephalochordates (e.g. Florida lancelet, or amphioxus) belongs to phylum Chordata. They possess a simple body plan organization reminding fossil putative chordates. Their genome shares with vertebrates a basic set of chordate genes involved in development or signaling, as well as numerous transcriptional enhancers. For these reasons, lancelets are one of the best model to study the evolution of vertebrates. We created a reporter gene consists of cis-regulatory region from amphioxus Chordin (BfCh) fused with GFP gene and introduced it into a vertebrate genome (zebrafish) to observe its behavior in the context of vertebrate gene regulatory network. Further, we expose this system to chemicals that manipulate the developmental signaling pathways (Nodal, Wnt) in order to find out similarities and differences of Chordin regulation. We use regulation of Chordin transcription as a tool for comparison of GRN between a vertebrate (zebrafish) and an invertebrate (amphioxus) model. By taking advantage of comparison regulatory systems in distinct animals, we can discuss the possible evolutionary scenario of changes in gene network regulating dorso-ventral body axis formation in vertebrates.

P.72 (Body Plans & Segmentation)

Tracing the evolutionary origin of chordate somites in the indirect-developing hemichordate *Ptychodera flava*

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Somites are a novel character of the chordate body plan and their evolutionary origins remain unclear. In vertebrates, paraxial mesoderm of the trunk region becomes segmented somites, which are further subdivided into specialized compartments: the sclerotome gives rise to the vertebral and rib cartilage and the dermomyotome gives rise to skeletal muscle and the dermis of the back. To elucidate the deuterostome origin of somites and the ancestral mechanism of mesoderm patterning, it is useful to study the chordate's closest sister group, Ambulacraria, which includes hemichordates and echinoderms. In this study, we utilized the indirect-developing hemichordate *Ptychodera flava* to investigate the genetic conservation of mesoderm developmental mechanisms within the deuterostome lineage. First, we used a candidate gene approach where we identified homologues of chordate mesoderm and somite markers in *P. flava* and observed their expressions during embryogenesis. Our data show that many of these candidate genes are also expressed in the hemichordate mesoderm, suggesting that these genes may play conserved roles in mesodermal development. Second, we employed an RNA-seq approach to comprehensively screen for genes associated with mesodermal development in the *P. flava* embryo, which is positively regulated by FGF signaling. In the tornaria larva, we found 244 genes to be concurrently upregulated under FGF signaling activation and downregulated under FGF signaling inhibition. Using *in situ* hybridization, we verified most of these genes were indeed expressed in mesodermal tissue. Our results show that some genes identified in this study may be part of an evolutionarily conserved toolkit for mesoderm development in deuterostomes. Moreover, the mesoderm of the hemichordate also displays compartmentalization, suggesting that regionalization of the mesoderm is an ancestral deuterostome trait. Our results set a foundation for further studies tracing the development of mesodermal tissues in the hemichordate and elucidating the deuterostome origin of somites.

P.74 (Networks, Switches, Comparative Analyses & Applications)

Ancestral states, null hypotheses, and phylogenetic comparative methods for developmental genetics

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An unprecedented amount of developmental genetic data is now accessible for investigation, and much of it is from organisms and lineages previously understudied in laboratory conditions. This presents a unique opportunity to compare developmental genetic data across species, gaining insight into the evolutionary history of developmental processes. We suggest that this opportunity comes with the renewed necessity to incorporate theoretical advances in phylogenetic comparative methods into the interpretation of developmental comparisons. In particular, we focus on two analytical efforts: reliably reconstructing ancestral states on an evolutionary tree, and establishing appropriate null hypotheses for interspecific comparisons. In each case, we present a worked example based on data collected across arthropod species. For the first, we discuss the evolution of the function of the gene *oskar*, which in *Drosophila melanogaster* is necessary and sufficient for germ line specification. For the second we compare tissue specific RNAseq datasets across species of Hawaiian *Drosophila*. These examples demonstrate both the potential pitfalls as well as the power of rigorous evolutionary comparisons of developmental genetic data.

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P.76 (Networks, Switches, Comparative Analyses & Applications)

Differential embryonic sub-genome activation in an allotetraploid

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After fertilization, a maternal contribution of factors to the egg activates the previously silent embryonic genome. Newly transcribed RNA replace the maternally contributed RNA, effectively reprogramming the embryo to eventually give rise to all adult cells in the organism. Although this "maternal-to-zygotic transition" occurs across taxa, the mechanisms underlying it are highly divergent. Here, we present our efforts to understand the mechanisms underlying embryonic genome activation and how they change by taking advantage of the allotetraploid genome of *Xenopus laevis*, which is composed of two homeologous subgenomes diverged ~16 million years, prior to hybridization in *X. laevis*. We find that in the context of a shared maternal contribution in the egg, these two subgenomes show differential activation. To investigate how differential gene activation is regulated, we performed low-input chromatin profiling (CUT&RUN) to identify enhancers across the two embryonic subgenomes marked by H3K27ac, a histone modification correlated with transcriptional activity. We find that differentially activated homeologous gene pairs exhibit differential enhancer activity, suggesting differential engagement of maternal factors at these loci. Comparative motif analysis reveals selective enrichment of specific transcription factor binding motifs at active enhancers, including ones corresponding to the mammalian pluripotency-inducing factors OCT4 and SOX2. Inspection of the egg transcriptome confirms high expression of *pou5f3.3* (OCT4 homolog) and *sox3* (SOX2 homolog) in the maternal contribution, implicating these factors in mediating differential subgenome activation. Future loss-of-function analyses will confirm their roles in the early embryo. By taking a comparative approach to understanding the maternal-to-zygotic transition in egg-laying vertebrates, in which homologs of mammalian pluripotency inducing factors orchestrate gene regulation in the early embryo, we will gain further insight into how transcriptomes are reprogrammed and how pluripotency is induced across vertebrates.

P.78 (Networks, Switches, Comparative Analyses & Applications)

An evolutionarily novel essential gene regulatory network determines endoderm identity in *Caenorhabditis elegans*

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Wagner's Genetic Theory of Homology postulates that developmental genetic networks are organized into a hierarchy of three functional groups. The first set of genes establishes positional information in a developing embryo. A second set processes this positional information, establishes character identity, and then activates a third set of genes that builds the part and controls character state. According to the theory, the link between molecular and morphological homology lies in the second set of genes, which is more conserved than the other two. The endoderm specification network in *C. elegans* appears to be a canonical example of a character identity network. At the morphological level, endoderm development within *Caenorhabditis* is highly conserved. The core network is activated by maternally deposited transcription factors that are differentially translated and segregated to spatially divide the embryo. Once the last gene of the network is activated, it stays on throughout the worm's life and regulates hundreds of genes that build and run the worm gut. Within the Elegans supergroup, a subclade within the Caenorhabditis genus, there is some variation in copy numbers, but all Elegans supergroup species sequenced so far have at least one ortholog of each of the genes in the *C. elegans* network. However, contrary to this three-tiered model, this endoderm specification network cannot be responsible for the undeniable homology of endoderm identity even within the Caenorhabditis genus. Except for the last gene, the genes of the *C. elegans* endoderm specification network simply do not exist outside the Elegans supergroup. This essential gene regulatory network that interprets positional information in the early *C. elegans* embryo and establishes endoderm identity is an evolutionary novelty that arose within the *Caenorhabditis* genus and somehow supplanted the ancestral network, whatever it was, all without any obvious change above the cell level in how the endoderm develops.

P.80 (Networks, Switches, Comparative Analyses & Applications)

Commissureless regulation of slit-robo signaling in arthropods

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Slit-Robo signaling is a key mediator of axon guidance decisions at the CNS midline in divergent organisms from planaria to vertebrates. Not surprisingly, Slit and Robo are conserved throughout arthropods. In contrast to this conservation of ligand and receptor, animals have evolved various mechanisms to regulate Slit-Robo signaling. In Drosophila, Commissureless (Comm) is an essential post-translational regulator of the Robo receptor that functions to prevent cell surface accumulation of the Robo receptor. Two additional Comm-family members are found in Drosophila and they vary in their ability to regulate Robo. We are investigating the evolution and function of Comm-like genes in arthropods. Divergent Comm-like genes can be identified in most Insect orders, but not in crustaceans, myriapods, chelicerates (or organisms outside of arthropods). The presence of a Comm-like gene in basal insect orders like Zygentoma and its pervasiveness throughout Insecta suggests an early origin in insect evolution. In some Diptera (the Neodiptera) there is an expansion to three Comm-family members. In contrast, bioinformatic analyses identify at minimum four independent losses of Comm, including 1) absence from Lepidopteran genomes and transcriptomes, 2) presence in basal Hymenoptera (e.g. sawflies) but absence in more derived Hymenoptera including ants, bees, and most wasps, 3), absence from the Dipteran family Chironomidae (non-biting midges), and 4) absence from *Tribolium castaneum* and related Tenebrionoidea despite presence in other Coleopteran genomes Functional studies using a Drosophila S2 cell assay demonstrate that Comm-like proteins from the beetle *Onthophagus taurus* and the Hemipteran *Oncopeltus fasciatus* can regulate their endogenous Robo1 proteins, similar to Drosophila Comm. Thus, the ability of Comm-like proteins to regulate Robo protein distribution is conserved outside of Diptera. These same studies reveal coevolution of these proteins in maintaining this regulatory interaction.

P.82 (Networks, Switches, Comparative Analyses & Applications)

A mechanism-based strategy to assess risks of RNA interference in diverse insects

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Widespread use of RNAi-based pest control strategies is likely in the near future, yet we are poorly prepared to assess risks to non-target organisms (NTOs). We are making use of our expertise in developmental genetics to devise novel strategies to assess effectiveness and risk of RNAi implementation. Most insect bioassays–including those currently in use for tests of RNAi efficacy-are based upon tests of lethality alone; these assays not only fail to detect sublethal effects, they suffer from the high levels of lethality that occur simply from brining field insects into a lab setting. In contrast, our approach will utilize the range of specific defects caused by gene knockdown and will detect even weak effects of RNAi delivery. We are using six indicator species from three insect orders that generally vary in their susceptibility to RNAi: Diptera (*Drosophila suzukii*, Anophlelesstephensi), Coleoptera (*Dermestes maculatus*, Coleomegilla maculata); Hemiptera (*Oncopeltus fasciatus*, *Acyrthosiphon pisum*). We use three well-studied regulatory genes –even-skipped (*eve*), ftz-f1, and Sex combs reduced (*Scr*), for which RNAi-mediated generates a range of highly specific defects. For example, parental RNAi knockdown of *eve* in beetles and true bugs generates offspring with defects ranging from partial segment fusions to 'head-only'. We are using these three developmental regulatory genes to 1) establish a rubric to assess susceptibility; 2) determine whether insects in different orders biosynthesize gene silencing molecules via secondary RNAs; 3) evaluate the severity and extent of hazard to non-target organisms from trophic exposure to gene silencing molecules (for example, does feeding dsRNA to aphids result in silencing in lady beetle predators?). This work will contribute to our long-term goal to identify the mechanism-based parameters that effectively predict species-level RNAi susceptibility and improve risk assessment for insecticidal RNAi. This work is funded by USDA-Biotechnology Risk Assessment Grant #2018-33522-28712.

P.84 (Networks, Switches, Comparative Analyses & Applications)

Finding the switches that activate animal genes through a combined *in silico* and *in vivo* approach

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Genomes encode in DNA sequence the recipes for cellular products, notably proteins, and the switches that determine when during life and in which cell types these products are made. While the genetic code for protein recipes is known, a comparable code for these switches is lacking. This impedes understanding the genetic underpinnings of animals and their evolution, as switches (CREs) outnumber protein-coding genes by over an order of magnitude and switch evolution is thought to be a predominant mechanism of trait evolution. Both *in vivo* and *in silico* approaches exist to study CREs, but the former is low throughput, and the latter lacks validation of predictions. Our research merges these approaches to identify CREs controlling genes for an evolving fruit fly pigmentation trait. We will use sequences of CREs known to activate genes involved in pigmentation, in order to find the unknown CREs with similar activity. We will use the SCRMshaw bioinformatic tool to find putative CREs in the *Drosophila melanogaster* genome that control novel genes involved in pigmentation, based upon the putative CREs possessing DNA motifs similar to those within the known CREs. From this list, we will test twenty for CRE activity *in vivo* as reporter transgenes. As a control, we will test a set of five randomly selected sequences of similar length and deoxyribonucleotide composition for *in vivo* activity. The results will reveal the extent this *in silico* method succeeded in CRE identification. For the validated CREs, we will elucidate the molecular mechanisms by which they similarly control gene expression, and whether they evolved in route to the gain, loss, and modification of male-specific abdomen pigmentation. The encoding of information in CREs is a universal feature of life, so these results bear upon life at every level.

P.86 (Networks, Switches, Comparative Analyses & Applications)

Resolving the molecular mechanisms by which mutations alter the function of an evolving genetic switch

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Animal genomes possesses anywhere from tens of thousands to more than a million mutations that are genetic baggage from mutations that occurred in the past. Each mutation can either improve, reduce, or have no effect on fitness. Moreover, the effects of such mutations can depend on the presence or absence of other mutations, so called epistatic interactions. A goal of evolutionary-developmental biology research is to identify the mutations responsible for the evolution of form and function, and to understand the molecular mechanisms of their effects. This goal remains out of reach, as the effects of mutations and epistatic interactions are difficult to predict without knowing the function of the DNA sequence they reside in. This difficulty is heightened for mutations occurring in cis-regulatory element sequences that act as switches to control gene transcription. We are using a fruit fly model to test hypotheses about the molecular mechanisms by which mutations alter a genetic switch's activity, and whether these function-altering mutations are subjected to the tyranny of epistatic interactions. Specifically, we are investigating the Drosophila melanogaster dimorphic element that is a transcription-regulating switch for the bric-à-brac genes. Three mutations in the dimorphic element were identified that individually alter the level of bric-à-brac transcription. The presence or absence of epistatic interactions will be determined by measuring the activity of dimorphic elements from related species that have been engineered to possess the Drosophila melanogaster mutations. I will also test the hypothesis that these mutations impart their effects by creating or destroying binding sites for transcription factors. The results will provide a needed example where an understanding of molecular mechanisms bridges the gap between a cis-regulatory element's DNA sequence and its functional evolution.

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Bombard and conquer: DNA transposon-driven *de novo* enhancer evolution

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In sharp contrast to their "junk DNA" nickname, repeated genomic sequences are known to fulfill a wide range of functions. In particular, studies performed in distinct mammalian tissues have revealed a direct contribution of retroposon families to developmental enhancers. To tackle this issue from a different phylogenetic and biological perspective, we examined the transcriptome (RNA-seq) and epigenome (ChIP-seq, ATAC-seq) of osteoblasts freshly extracted from the calvaria (skull bone) of the amphibian *Xenopus tropicalis*. We detect thousands of putatively active/poised promoters and enhancers. While promoters are strongly depleted from all retroposon and DNA-transposon types, we identify 6 DNA-transposon families specifically enriched at transcriptional enhancers in *Xenopus tropicalis*. Intra-enhancer repeated elements are under selection because they evolve at a slower rate and maintain a higher GC content and CpG density than extra-enhancer elements from the same DNA transposon family. We also find that mobile element-containing enhancers are *Xenopus tropicalis*-specific and, therefore, evolutionarily recent. By contrast, *Xenopus tropicalis* enhancers devoid of mobile elements are more likely to have evolved before the tetrapod radiation because they are more conserved with mammalian enhancers than expected by chance. Remarkably, intra-enhancer transposon sequences exclude nucleosomes in *in vitro* reconstitution assays, suggesting that they may open chromatin regions to facilitate transcription factor access. We propose that bursts of DNA transposons periodically bombard eukaryotic genomes with nucleosome-depleted CpG islands, henceforth facilitating the emergence of de novo transcriptional enhancers. Acknowledgements: Fondecyt 1190926