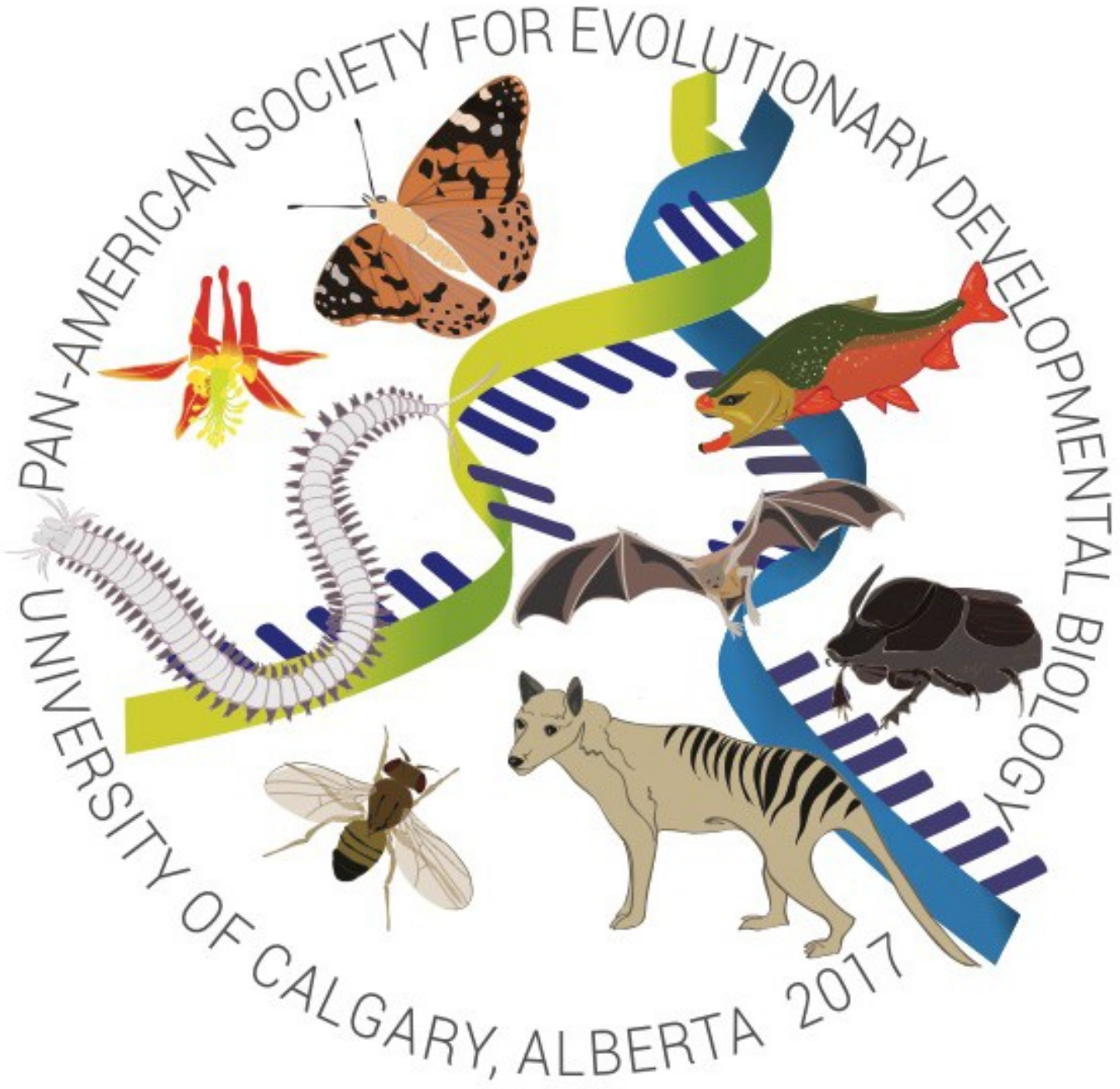

2nd Biennial Meeting

University of Calgary, Calgary, Canada

August 19-23, 2017

Welcome from the President

Dear participants,

Welcome to Calgary and the second meeting of the Pan-American Society for Evolutionary Developmental Biology! This year's meeting features over 60 talks and 100 posters by researchers at all career stages who work on diverse topics and organisms. Calgary is a beautiful setting for our meeting, and I would like to thank our local organizers for their hard work in pulling together a fantastic meeting. I would also like to thank the Scientific Committee for volunteering significant time and effort to making this meeting great. Lastly, I want to extend a huge thank you to NSF, NIH, Journal of Experimental Zoology Part B: Molecular and Developmental Evolution (JEZB), Annals of Botany, University of Alberta, University of Calgary Faculty of Veterinary Medicine, and the Society for Developmental Biology for providing financial support. This meeting wouldn't be happening without your support!

In the two years since our inaugural meeting in Berkeley, our society has matured and flourished. We have initiated new educational efforts, established relationships with other societies, funding agencies, and journals, and continued to support scientists from Latin America. I have been honored to be a part of these efforts, and am looking forward to seeing our society continue to grow under the strong leadership of our next President, Billie Swalla.

We are excited to be presenting three awards at this meeting to celebrate the researchers of evo-devo. The first is the Young Investigator Award to Stacey Smith (sponsored by JEZB), the second is the Pioneers Award to Sean Carroll, and the third is the Kowalevsky Medal of the St. Petersburg Society of Naturalists which will be presented to Günter Wagner by Linda Holland. We will also be hosting workshops and discussions on a number of important topics throughout the meeting, including People of Color in Science, Women in Science, LGBTQ in Science, Evo-devo and Education, Evo-devo in Latin America, and How to Publish in JEZB. Please feel free to attend any of these that are of interest to you - no advance sign-up is necessary.

Our vision for this meeting, and for the society as a whole, is to provide an inclusive forum where researchers with interests that span all aspects of evolutionary and developmental biology can meet and exchange ideas. From plants to vertebrates, theory to field research, and Canada to Chile, you are welcome here.

Wishing you all a great meeting!

Karen Sears
President of the Pan-Am American Society for Evolutionary Developmental Biology

PAN-AMERICAN SOCIETY
FOR EVOLUTIONARY
DEVELOPMENTAL BIOLOGY

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Thanks to the Sponsors of the 2nd Biennial Meeting of the Pan-American Society for Evolutionary Developmental Biology

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Society for Developmental Biology
Advancing the field of developmental biology

General Information

Safety

Emergency: 911
Campus Security: 403-220-5333
campus.security@ucalgary.ca
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Ask someone to contact Safewalk for you at the Registration Desk
For more information call the Safewalk office at 403-220-4750 or email safewalk@ucalgary.ca.

Address

The 2nd biennial meeting of the Pan-American Society for Evolutionary Developmental Biology will take place in the **MacEwan Conference and Event Center (MCEC)** at the University of Calgary (Map at the back of this booklet). MCEC is located minutes by local transportation from downtown Calgary, with easy access to Calgary International Airport (see Transportation, below). There is a food court adjoining the center which offers many quick meal choices and coffee shops, bars. Other amenities nearby include a pharmacy, medical/dental center, gym/pool (see Accommodation for access details). MCEC is a fully wheelchair-accessible facility.

Transportation

When traveling around Calgary, you will hear reference to the four quadrants of the city (NE, NW, SE, SW). The University of Calgary is located in the NW quadrant. The airport is in the NE quadrant. Many city roads are named by number and quadrant (e.g. 24th Ave. NW), with numbers starting low near the city centre and increasing as you move away in any direction. Roads traveling in the North-South direction are generally named Streets, while roads traveling in the East-West direction are generally named Avenues. The main University campus is bounded by 24th Ave NW on its southern edge, 32nd Ave NW on its northern edge, Crowchild Trail on its eastern edge (approximately 24th St NW) and Collegiate Blvd on its western edge (approximately 37th St NW). Zoom in on the map above to navigate.

Travel around the city is by auto, taxi, bus and light rail (C-train). The **'University' C-train (light rail) station** is located at the east edge of campus, near the Biological Sciences building on Crowchild Trail. From there you can travel north-west (Tuscany train) to several shopping malls (Brentwood, Dalhousie, Crowfoot stations), or south-east toward the downtown (Bridlewood-Somerset train). Adult fares are $3.25 flat rate anywhere the public transit system goes in the city. You can transfer between the train and buses with no additional fee within 90 minutes of purchasing your ticket (keep your train ticket as a transfer pass, and ask for a transfer if you first board a bus). You can purchase tickets at any of the train stations before you board the train (cash, debit, credit) or by cash on the bus. You cannot purchase tickets on the train itself, so be sure to purchase tickets before you board. It's an honour system to board the train (purchase your ticket first and walk on when the train arrives), but keep your ticket with you in case an inspector asks to see it. Use of the train is free to shuttle along 7th Avenue downtown. However, if you are catching a train on 7th Ave with the intention of traveling elsewhere (e.g. back to the University), or are elsewhere and intend to travel to 7th Ave (leaving the University), then you must purchase a ticket before you board the train.

Taxis:
Associated Cabs 403-299-1111
Checker Yellow Cabs 403-299-9999
Mayfair Taxi 403-255-6555
Delta Cab 403-278-9999
Calgary United Cabs 403-777-1111

Meals

The campus food court (6:00 am – 11:00 pm), located in the MacEwan Student Centre, provides many quick meal choices and coffee shops. The Den and the Last Defense Lounge are student run pubs located adjacent to the meeting rooms and offer pub meals and refreshments to suit your tastes. For those that want to wander off campus to eat, a 10 minute walk gets you to the Brentwood Village Mall just across Crowchild Trail (Kilkenny's Irish Pub, Jamesons Irish Pub, Wendy's, Harvey's, Starbucks), or the Stadium Shopping Centre a few blocks south from the residences (Moose McGuire's, The Keg, Redwater Rustic Grille). The Bistro Alma, located on campus in the Hotel Alma, offers breakfast, lunch and dinner menus, to stay or to go http://www.hotelalma.ca/bistro_alma.

Breaks during the meeting will include coffee, tea and light snacks, and will take place in MacEwan Ballroom in the mornings, and in MacEwan Hall B/Foyer during the afternoon poster sessions.

WiFi Access

WiFi will be available free of charge throughout the University of Calgary campus. A guest account is required and can be created on first login.

Lectures

All lectures will take place in MacEwan Ballroom or MacEwan Hall A.

Posters

The poster sessions will be held in MacEwan Hall B/Foyer. Presenters should place their posters on their assigned board as of **7:00 am on Sunday August 20**, and should remove them between **5:00 – 6:00 pm on Tuesday August 22**.

Information for plenary speakers

Please come to the room no later than 30 minutes prior to your session to set up your laptop or load your talk. Volunteers and session chairs will be on hand to assist you. Plenary speakers will have 25 minutes for their talk, followed by 5 minutes for questions. Keynote and Award speakers will have 35 minutes for their talk, followed by 10 minutes for questions.

Information for concurrent session speakers

Please come to the room no later than 30 minutes prior to your session to set up your laptop or load your talk. Volunteers and session chairs will be on hand to assist you. Concurrent session speakers will have 12 minutes for their talk, followed by 3 minutes for questions. Timing will be closely monitored to allow movement between lecture venues.

Meeting At-a-Glance

	Saturday Aug 19	Sunday Aug 20	Monday Aug 21	Tuesday Aug 22
8:00 -8:30		Invited Talk -Ehab Abouheif	Concurrent Sessions	Invited Talk -Jukka Jernvall
8:30 -9:00		Abstract Talk -Mihaela Pavlicev		Abstract Talk -Cliff Tabin
9:00 -9:30		Abstract Talk -Isaac Salazar		Abstract Talk -Verónica Di Stilio
9:30 -10:00		Invited Talk -Annalise Paaby	Coffee Break	Abstract Talk -Cheng Ming Chuong
10:00 -10:30		Coffee Break	Concurrent Sessions	Coffee Break
10:30 -11:00		Abstract Talk -Clare Baker		Invited Talk -Thomas Williams
11:00 -11:30		Abstract Talk -Ian Dworkin	Lunch Break	Abstract Talk -Catherine Boisvert
11:30 -12:00		Abstract Talk -Paul François	Solar Eclipse max. 11:33 am	Invited Talk -Tiana Kohlsdorf
12:00 -12:30		Lunch Break	Concurrent Sessions	Lunch Break
12:30 -1:00				
1:00 -1:30			Break	
1:30 -2:00		Posters / Break	Abstract talk -Hans Larsson	Invited Talk -Nadia Fröbisch
2:00 -2:30	Check-in/Registration		Invited talk -Craig Albertson	Abstract Talk -Heather Hines
2:30 -3:00			Abstract talk -Chelsea Specht	Posters / Break
3:00 -3:30			Posters / Break	
3:30 -4:00			How to publish in JEZB	Invited Talk -Mariana Benitez
4:00 -4:30	Executive Council Meeting			Invited Talk -Vincent Lynch
4:30 -5:00		Invited Talk -Cassandra Extavour	Invited Talk -Antonia Monteiro	Poster and Talk Awards
5: 00 -5:30		Invited Talk -Federico Brown	Invited Talk -Andreas Heyland	Dinner Break /
5:30 -6:00		Dinner Break /	Dinner Break /	EvoDevo in Latin America Discussion
6:00 -6:30	Introduction Invited Talk -Dominique Bergmann (6:15)	People of Color in Science Discussion	LGBTQ in Science Discussion	
6:30 -7:00				Kowalevski Medal
	Pioneers Award (6:45)			Günter Wagner
7:00 -7:30	Sean Carroll			Young Investigator Award
7:30 -8:00		Invited Talk -Michael Shapiro	Invited Talk –Pelin Volkan	Stacey Smith
8:00 -8:30	Opening Reception	Abstract Talk -Arnaud Martin	Abstract Talk -Kara Powder	Closing Reception
8:30 -9:00		Women in Science Workshop	EvoDevo Education Workshop	
9:00 -9:30				
9:30 -10:00				

Invited/Abstract Talks | Award Talks | Concurrent Talks | Posters | Receptions | Breaks | Discussions/Workshops

Concurrent Session Schedule

	MacEwan Ballroom			MacEwan Hall A	
	Plasticity			**Cells/Tissues, Vertebrate Skeleton**	
8:00 – 8:15	Rebecca	Green		Kenneth	McKenna
8:15 – 8:30	Maryna	Lesoway		Seth	Donoghue
8:30 – 8:45	Linh	Bui		Christina	Zakas
8:45 – 9:00	Maria	Pesevski		Katerina	Ragkousi
9:00 – 9:15	Dina	Navon		Lachezar	Nikolov
9:15 – 9:30	Sofia	Casasa		Luok Wen	Yong
			BREAK		
10:00 – 10:15	Kirsty	McWhinnie		Tetsuto	Miyashita
10:15 – 10:30	Arjuna	Rajakumar		Sophie	Archambeault
	GRN, gene families			**Macroevolution, Vertebrate Structure**	
10:30 – 10:45	Cheng-Chiang	Wu		Alexa	Sadier
10:45 – 11:00	Ya	Min		Lorna	Cohen
			BREAK		
12:00 – 12:15	Nicolas	Cumplido		Javiera	Chinga
12:15 – 12:30	Charles	Feigin		Rory	Cooper
12:30 – 12:45	Dorit	Hockman		Shuo	Wang
12:45 – 1:00	Stephen	Green		Evan	Kingsley

Detailed Program – Saturday August 19, 2017

2:00 – 6:00 pm	**Hotel Alma and MacEwan Hall B/Foyer**	**Check in and Registration**

4:00 – 5:00 pm	**Boardroom**	**Executive Council Meeting**

6:00 – 6:45 pm	**MacEwan Hall A**	**Welcome and Invited Talk**
6:00 – 6:15 pm	Karen Sears University of California Los Angeles	Welcome and Introduction
6:15 – 6:45 pm	Dominique Bergmann Stanford University	Adjusting the valves: plant stomatal development in space and time

6:45 – 7:45 pm	**MacEwan Hall A**	**Pioneers Award Presentation and Lecture**
6:45 – 7:00 pm	Karen Sears University of California Los Angeles	Introduction and Presentation
7:00 – 7:45 pm	Sean Carroll University of Wisconsin - Madison	The Thrill of Discoveries: Pioneer Stories

8:00 – 10:00 pm	**MacEwan Hall B**	**Opening Reception**

Detailed Program – Sunday August 20, 2017

8:00 – 10:00 am	**MacEwan Ballroom**	**Abstract and Invited Talks**
8:00 – 8:30 am	Ehab Abouheif McGill University	Obligate endosymbiosis in ants reveals evolutionary developmental steps to conflict resolution and integration
8:30 – 9:00 am	Mihaela Pavlicev Cincinnati Children's Hospital	Genomic Red Queen and the evolution of limb genetic architecture
9:00 – 9:30 am	Isaac Salazar-Ciudad University of Helsinki	How can complex gene networks build complex bodies in development and evolution
9:30 – 10:00 am	Annalise Paaby Georgia Institute of Technology	Genetic complexity and the evolution of quantitative traits

10:00 – 10:30 am MacEwan Ballroom Coffee Break

10:30 – 12:00 pm	**MacEwan Ballroom**	**Abstract and Invited Talks**
10:30 – 11:00 am	Clare Baker University of Cambridge	Evolution of the hypoxia-sensitive cells involved in amniote respiratory reflexes
11:00 – 11:30 am	Ian Dworkin McMaster University	Are sex differences in size and shape the same? Insights with the *Drosophila* wing
11:30 – 12:00 pm	Paul François McGill University	Using Machine Learning to reconstruct evolution of segmentation

12:00 – 1:00 pm Lunch Break

Detailed Program – Sunday August 20, 2017, continued

1:00 – 4:30 pm	**MacEwan Hall B/Foyer**	**Posters/Break**

4:30 – 5:30 pm	**MacEwan Ballroom**	**Invited Talks**
4:30 – 5:00 pm	Cassandra Extavour Harvard University	Title TBA
5:00 – 5:30 pm	Federico Brown Universidade de São Paulo	Coloniality in marine chordates: eco-evo-devo approaches to understand different levels of biological organization

5:30 – 7:30 pm	**Dinner Break**

5:30 – 7:30 pm	**Escalus**	**People of Colour in Science Discussion & Dinner**

Discussion Leader: Cassandra Extavour
Join us for the people of colour workshop and discussion. The goal is to meet informally over the dinner hour to share experiences, discuss any areas of concern, and encourage collegiality and support among self-identified people of colour in the Evo-Devo field. There is no need to pre-register, just drop by and join us!

7:30 – 8:30 pm	**MacEwan Ballroom**	**Abstract and Invited Talks**
7:30 – 8:00 pm	Michael Shapiro University of Utah	On the wings of love: pigment patterning and the checkered past of rock pigeons
8:00 – 8:30 pm	Arnaud Martin George Washington University	The Wnt beneath my wings: exploring butterfly pattern formation in the CRISPR era

8:30 – 10:00 pm	**Escalus**	**Women in Science Workshop**

Discussion Leader: Tamara Franz-Odendaal
Join us for the women in science workshop! The aim of this event is to encourage networking among female scientist at all levels of their career. This is a great opportunity to discuss strategies for developing your career and join forces to overcome the difficulties women face in academia. There is no need to pre-register, just drop by and join us!

Detailed Program – Monday August 21, 2017

8:00 – 10:00 am	**MacEwan Ballroom**	**Concurrent Sessions: Plasticity**
8:00 – 8:15 am	Rebecca Green University of Calgary	Developmental Nonlinearity Drives Phenotypic Robustness
8:15 – 8:30 am	Marina Lesoway University of Illinois Urbana Champaign	Sex Change in Slipper Limpets
8:30 – 8:45 am	Linh Bui Indiana University	Functional and molecular evolutionary analysis of polyphenism-specific transcription in the nematode model *Pristionchus pacificus*
8:45 – 9:00 am	Maria Pesevski McMaster University	Genetic and environmental decanalization are not correlated among altitudinal varying populations of *Drosophila melanogaster*
9:00 – 9:15 am	Dina Navon University of Massachusetts Amherst	Evaluating the molecular basis for diet-induced phenotypic plasticity in teleosts
9:15 – 9:30 am	Sofia Casasa Indiana University	Regulation of nutrition-responsive growth and scaling in the horned beetle *Onthophagus taurus*

9:30 – 10:00 am	**MacEwan Ballroom**	**Coffee Break**

Detailed Program – Monday August 21, 2017

8:00 – 10:00 am	**MacEwan Hall A**	**Concurrent Sessions: Cells/Tissues, Vertebrate Skeleton**
8:00 – 8:15 am	Kenneth McKenna Duke University	The development and evolution of morphological scaling relationships
8:15 – 8:30 am	Seth Donoughe Harvard University	The evolution of insect eggs and cellular development across eight orders of magnitude
8:30 – 8:45 am	Christina Zakas New York University	The genetic basis of evolutionary transitions in early development using a polychaete model
8:45 – 9:00 am	Katerina Ragkousi Stowers Institute for Medical Research	Cell-Cycle-Coupled Oscillations in Apical Polarity and Intercellular Contact Maintain Order in Embryonic Epithelia
9:00 – 9:15 am	Lachezar Nikolov Max Planck Institute for Plant Breeding Research	Interspecies gene transfer reveals a distribution of size effects underlying the morphological divergence between species
9:15 – 9:30 am	Luok Wen Yong Academia Sinica	Somite Compartmentalization in Amphioxus: on the Evolutionary Origin of Vertebrate Skeletons

9:30 – 10:00	**MacEwan Ballroom**	**Coffee Break**

Detailed Program – Monday August 21, 2017, continued

10:00 – 11:00 am	**MacEwan Ballroom**	**Concurrent Sessions: GRN, Gene Families 1**
10:00 – 10:15 am	Kirsty McWhinnie University of Glasgow	Exploring Ecological Sexual Dimorphism through Morphometrics, Genetics and Biomechanics
10:15 – 10:30 am	Arjuna Rajakumar McGill University	Reproductive constraint: a developmental mechanism regulating social cohesion in ant societies
10:30 – 10:45 am	Cheng-Chiang Wu Harvard University	Origin and evolution of the WUSCHEL-RELATED (WOX) homeobox transcription factors in plants
10:45 – 11:00 am	Ya Min Harvard University	Sweet genes are made of STYLISH – Members of the STYLISH gene family control both style and nectary development in *Aquilegia* (Ranunculaceae)

11:00 – 12:00 pm Eclipse Viewing/Lunch Break

10:00 – 11:00 am	**MacEwan Hall A**	**Concurrent Sessions: Macroevolution, Vertebrate Structure 1**
10:00 – 10:15 am	Tetsuto Miyashita University of Alberta	Evolutionary origins of the endoskeletal joint in vertebrates
10:15 – 10:30 am	Sophie Archambeault University of Washington/University of Bern	The architecture of adaptation: a master mutation or a mass of mutations?
10:30 – 10:45 am	Alexa Sadier University of California Los Angeles	Deciphering genomic and developmental mechanisms that underlie vision adaptations in noctilionoid bats
10:45 – 11:00 am	Lorna Cohen University of Illinois Chicago	Dissecting the genetic basis of morphology and evolution in *Nasonia*

11:00 – 12:00 pm Eclipse Viewing/Lunch Break

Detailed Program – Monday August 21, 2017, continued

12:00 – 1:00 pm	**MacEwan Ballroom**	**Concurrent Sessions: GRN, Gene Families 2**
12:00 – 12:15 pm	Nicolas Cumplido Universidad de Chile	The Hox code and the identity of the teleostean fish caudal fin
12:15 – 12:30 pm	Charles Feigin University of Melbourne	Genome of the Tasmanian Tiger Provides Insights into the Genetic Basis of Convergent Evolution
12:30 – 12:45 pm	Dorit Hockman University of Oxford	A genome-wide assessment of the ancestral neural crest gene regulatory network
12:45 – 1:00 pm	Stephen Green California Institute of Technology	Evolution of enteric neural crest cells in the vertebrates

1:00 – 1:30 pm Break (own time)

12:00– 1:00 pm	**MacEwan Hall A**	**Concurrent Sessions: Macroevolution, Vertebrate Structure 2**
12:00 – 12:15 pm	Javiera Chinga Pontificia Universidad Católica de Chile	What does ontogenetic integration tell us about how integration patterns arise in *Schizanthus*?
12:15 – 12:30 pm	Rory Cooper University of Sheffield	Developing an ancient epithelial appendage: FGF signalling regulates shark denticle formation
12:30 – 12:45 pm	Shuo Wang Capital Normal University	Ontogenetic tooth reduction in theropod dinosaurs and the macroevolution of avian beaks
12:45 – 1:00 pm	Evan Kingsley Harvard Medical School	Embryonic patterning of airway cartilage and the avian vocal organ

1:00 – 1:30 pm Break (own time)

Detailed Program – Monday August 21, 2017, continued

1:30 – 3:00 pm	MacEwan Ballroom	Abstract and Invited Talks
1:30 – 2:00 pm	Hans Larsson McGill University	Use of experimental atavisms to estimate soft tissue reconstructions of the earliest tetrapod limbs
2:00 – 2:30 pm	Craig Albertson University of Massachusetts Amherst	The genetic basis of jaw shape and plasticity in cichlid fishes
2:30 – 3:00 pm	Chelsea Specht Cornell University	Homology of process: petals and petaloidy in the tropical gingers (Zingiberales)

3:00 – 4:30 pm	MacEwan Hall B/Foyer	Posters/Break

3:00 – 4:30 pm	Escalus	How to Publish in JEZB

During this workshop, the Editor-in-Chief of the *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution*, Günter Wagner at Yale University, and the Associate Editor, Ehab Abouheif at McGill University, will provide advices, tips and pointers on how to get published in their journal.

4:30 – 5:30 pm	MacEwan Ballroom	Invited Talks
4:30 – 5:00 pm	Antonia Monteiro National University of Singapore	The gradual molecular and developmental evolution of butterfly wing eyespots
5:00 – 5:30 pm	Andreas Heyland University of Guelph	Non-genomic actions of thyroid hormones as a potential regulator of larval skeletogenesis in sea urchins

5:30 – 7:30 pm	Dinner Break

Detailed Program – Monday August 21, 2017, continued

5:30 – 7:30 pm	Escalus	LGBTQ in Science Discussion & Dinner

Discussion Leader: Cassandra Extavour
Join us for the LGBTQ workshop and discussion. The goal is to meet informally over the dinner hour to share experiences, discuss any areas of concern, and encourage collegiality and support among self-identified LGBT/queer people in the Evo-Devo field. There is no need to pre-register, just drop by and join us!

7:30 – 8:30 pm	MacEwan Ballroom	Abstract and Invited Talks
7:30 – 8:00 pm	Pelin Volkan Duke University	Patterns of transcriptional parallelism and variation in the developing olfactory system of Drosophila species
8:00 – 8:30 pm	Kara Powder Clemson University	Identifying cis-regulatory enhancers associated with cichlid craniofacial evolution

8:30 – 10:00 pm	Escalus	EvoDevo Education Workshop

Discussion Leader: Neelima Sinha
Join us for the evodevo education workshop! The aim of this event is to encourage discussion of evodevo educational efforts, and ways to improve current efforts. This is a great opportunity to discuss strategies for enhancing evodevo educational efforts, and scientific education efforts in general. There is no need to pre-register, just drop by and join us!

Detailed Program – Tuesday August 22, 2017

8:00 – 10:00 am	MacEwan Ballroom	Abstract and Invited Talks
8:00 – 8:30 am	Jukka Jernvall University of Helsinki	Evo-Devo: Crossing genomes and phenomes
8:30 – 9:00 am	Cliff Tabin Harvard Medical School	Metabolic evolution in cave fish
9:00 – 9:30 am	Verónica Di Stilio University of Washington	The ABC model of flower development in non-core eudicots: a functional synthesis in a ranunculid
9:30 – 10:00 am	Cheng Ming Chuong University of Southern California	Evo-Devo of feathers

10:00 – 10:30 am	MacEwan Ballroom	Coffee Break

10:30 – 12:00 pm	MacEwan Ballroom	Abstract and Invited Talks
10:30 – 11:00 am	Thomas Williams University of Dayton	Endless Networks Most Beautiful: connecting diversity to alterations in a gene regulatory network
11:00 – 11:30 am	Catherine Boisvert Curtin University	Sharks did it first: where our muscle development comes from
11:30 – 12:00 pm	Tiana Kohlsdorf University of São Paulo	Why snakes are so unique? Some insights from developmental pathways

12:00 – 1:00 pm	Lunch Break

Detailed Program – Tuesday August 22, 2017, continued

1:30 – 2:30 pm	**MacEwan Ballroom**	**Abstract and Invited Talks**
1:30 – 2:00 pm	Nadia Fröbisch Humboldt University	Limb development and regeneration in fossil amphibians and extant salamanders
2:00 – 2:30 pm	Heather Hines Pennsylvania State University	The genetic basis of mimetic color diversity in bumble bees

2:30 – 3:30 pm MacEwan Hall B/Foyer Posters/Break

3:30 – 4:30 pm	**MacEwan Ballroom**	**Invited Talks**
3:30 – 4:00 pm	Mariana Benitez Universidad Nacional Autónoma de México	Cell differentiation and pattern formation in the transition to aggregative multicellularity
4:00 – 4:30 pm	Vincent Lynch University of Chicago	Why don't elephants get cancer? Developmental constraints and evolutionary tradeoffs in the resolution of Peto's paradox

4:30 – 5:00 pm MacEwan Ballroom Poster and Talk Awards

5:00 – 6:30 pm Dinner Break

5:00 – 6:30 pm Escalus EvoDevo in Latin America Discussion & Dinner

Discussion Leader: Rodrigo Nunes da Fonseca
Join us for the evodevo in Latin America discussion! The aim of this event is to encourage networking among scientists from Latin America at all levels of their career, and bring together diverse scientists to discuss ways to advance the field of evodevo in Latin America. There is no need to pre-register, or be from Latin America to attend, just drop by and join us!

Detailed Program – Tuesday August 22, 2017, continued

6:30 – 7:10 pm	**MacEwan Hall A**	**Kowalevsky Medal Presentation and Lecture**
6:30 – 6:40 pm	Linda Holland University of California San Diego	Introduction and Presentation
6:40 – 7:10 pm	Günter Wagner Yale University	The Evolutionary Biology of Cell Types: the next frontier of Devo-Evo
7:10 – 8:00 pm	**MacEwan Hall A**	**Young Investigator Award and Lecture** Sponsored by the Journal of Experimental Zoology Part B: Molecular and Developmental Evolution
7:10 – 7:20 pm	Karen Sears University of California Los Angeles	Introduction and Presentation
7:20 – 8:00 pm	Stacey Smith University of Colorado Boulder	Genetic mechanisms and macroevolution of flower color in the tomato family

8:00 – 10:00 pm	**MacEwan Ballroom**	**Closing Reception**

Detailed Program – Wednesday August 23, 2017

All day Departure

Talk Abstracts

Obligate endosymbiosis in ants reveals evolutionary developmental steps to conflict resolution and integration

B. Matteen Rafiqi, Arjuna Rajakumar, and Ehab Abouheif*
McGill University

Major transitions in evolution, including the transition from single cell protists into multicellular animals with differentiated cell types or from solitary individuals into eusocial colonies with differentiated castes, represents a complexity increase through integration of independently replicating lower-level units into a single replicating higher-level unit. However, integration cannot evolve until conflicts between lower-level units are resolved. Yet, the evolutionary developmental steps towards conflict resolution and integration remain unknown. Here we reveal the steps towards a major transition to obligate endosymbiosis in ants, where the bacteria *Blochmannia* live inside the cells of the hyperdiverse genus *Camponotus* (carpenter ants). We show this transition evolved in two steps that drove radical alterations in the early *Camponotus* embryo: a first duplication and divergence of ancestral germplasm that resolved the conflict over the embryonic posterior, followed by a second duplication and divergence of ancestral germplasm leading to *Blochmannia*'s full developmental integration into *Camponotus*. Furthermore, we show that both steps were facilitated by re-wiring of *Hox* genes *ultrabithorax* and *abdominal-A* within the highly-conserved segmentation hierarchy. Environmentally-induced duplication of pre-existing cells, organs, or individuals, coupled with divergence of ancient genes may be a general pathway towards major evolutionary transitions

The genetic basis of jaw shape and plasticity in cichlid fishes

R. Craig Albertson
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A major pursuit in evolutionary biology is to characterize the proximate molecular mechanisms that underlie adaptive phenotypic variation in the wild. While significant progress has been made toward this goal, most of our knowledge comes from studying relatively "simple" shifts in morphology. Much less is known about "complex" adaptive phenotypes, including the craniofacial apparatus, where developmental outcomes are due to a combination of genetic and environmental factors. Cichlids have undergone extensive evolutionary modifications of their skull and jaws, providing a rich array of phenotypic variation for genetics/genomic analyses. Further, the cichlid jaw represents an important model for developmental plasticity (e.g., a morphological flexible stem). Combining these two contributes, I will highlight recent work in the lab that sheds light into three specific questions: (1) what is the molecular nature of evolutionary change? (2) what role does the environment play in the manifestation of adaptive phenotypic variation? (3) what is the genetic basis of phenotypic plasticity? In all I hope to underscore the utility of the cichlid system to address longstanding questions about the nature of phenotypic evolvability.

Evolution of the hypoxia-sensitive cells involved in amniote respiratory reflexes
Clare V. H. Baker, Dorit Hockman, Alan J. Burns, Gerhard Schlosser, Keith P. Gates, Alessandro Mongera, Shannon Fisher, Gokhan Unlu, Ela W. Knapik, Charles K. Kaufman, Christian Mosimann, Leonard I. Zon, Joseph J. Lancman, P. Duc S. Dong, Heiko Lickert, Abigail S. Tucker"
University of Cambridge

The evolutionary origins of the hypoxia-sensitive cells that trigger amniote respiratory reflexes – carotid body glomus cells, and 'pulmonary neuroendocrine cells' (PNECs) - are obscure. Homology has been proposed between glomus cells, which are neural crest-derived, and the hypoxia-sensitive 'neuroepithelial cells' (NECs) of fish gills, whose embryonic origin is unknown. NECs have also been likened to PNECs, which differentiate in situ within lung airway epithelia. Using genetic lineage-tracing and neural crest-deficient mutants in zebrafish, and physical fate-mapping in frog and lamprey, we find that NECs are not neural crest-derived, but endoderm-derived, like PNECs, whose endodermal origin we confirm. We discover neural crest-derived catecholaminergic cells associated with zebrafish pharyngeal arch blood vessels, and propose a new model for amniote hypoxia-sensitive cell evolution: endoderm-derived NECs were retained as PNECs, while the carotid body evolved via the aggregation of neural crest-derived catecholaminergic (chromaffin) cells already associated with blood vessels in anamniote pharyngeal arches.

Cell differentiation and pattern formation in the transition to aggregative multicellularity
Mariana Benitez Keinrad
Universidad Nacional Autónoma de México

One of the major evolutionary innovations is the appearance of multicellularity, which has occurred in different lineages and has involved not only the aggregation or incomplete separation of cells, but also cell differentiation, metabolic integration and the appearance of new systemic properties. The generation of these features is now being studied with a diversity of causes in consideration, including genetic, ecological, as well as generic physicochemical processes. We use diverse theoretical and experimental tools to integrate some of these multiple causes in the study of aggregative multicellularity. We present new insights on how the mechanical and chemical coupling of intracellular networks may lead to the formation of recurrent patterns in aggregates of hypothetical unicellular entities. Then, we present specific models for cell-fate determination and patterning during the formation of fruiting bodies in *Myxococcus xanthus* and some novel predictions and insights for this particular organism. Finally, we discuss our findings and ongoing experimental work in the broader context of fruiting body formation in Myxobacteria, a group that includes *M. xanthus* and other species with diverse multicellular phenotypes.

Adjusting the valves: plant stomatal development in space and time
Dominique Bergmann, Michael Raissig, Cora MacAlister, Emily Abrash
Stanford University

Stomatal valves that regulate carbon dioxide and water vapor exchange between plants and the atmosphere. Collectively, the activities of stomata on thousands of plant species have major effects on global water and carbon cycles, yet our current mechanistic understanding of how stomata are made is based nearly exclusively on studies in a single species, *Arabidopsis thaliana*. In *Arabidopsis*, five bHLH transcription factors form the core regulatory module for stomatal initiation, proliferation and terminal differentiation. Sequence information from across the plant kingdom suggests parallels between expansion of stomatal lineage complexity and expansion of the stomatal bHLH gene repertoire. Through forward and reverse genetic approaches in *Physcomitrella* and *Brachypodium* and we found that, despite major differences in stomatal lineage initiation and stomatal structure among mosses, grasses and *Arabidopsis*, homologous bHLH transcription factors are indeed recruited. Using fluorescent reporters of gene activity, overexpression and alteration of function (protein and cis-regulatory swaps) tools, we dissected how the same transcription factors could yield different outcomes and found clade-specific differences in gene expression and post-translational modification of protein stability and activity. We are particularly interested in novelty, and the grasses made key innovations to stomatal form and function. Surprisingly, underlying this innovation is not changes in gene expression pattern due to cis-regulatory changes, but rather due to changes in cell-cell mobility of one of the bHLHs. I will discuss our progress using the "natural laboratory" of stomatal development to analyze structure/function of individual proteins and evolution of their regulatory networks.

Sharks did it first: where our muscle development comes from

Catherine A. Boisvert, Anne Rios, Daniel Sieiro Mosti, Frank Tulenko, Marcus Davis, Benjamin Uy, Christophe Marcelle, Peter D. Currie
Curtin University

In all vertebrates, trunk musculature develops from somites. In teleosts, the first muscle fibres develop directly, followed by the establishment of a single cell layer, the External Cell Layer (ECL), which contributes myoblasts for further muscle formation. In contrast, amniotes lack early myofibers and initiate myogenesis through a transient dorso-lateral somitic compartment termed the dermomyotome (DM), a multicellular epithelial structure expressing Pax7. While the ECL and the DM have been hypothesized to be homologues based on gene expression, we argue that the DM is a temporally, morphologically, and mechanistically different structure than the ECL. We immunostained growth series of lampreys, elephant sharks, and paddlefish to characterize the morphological and molecular changes associated with myotome development and compared these data with amniotes and teleosts. Remarkably, elephant shark muscle formation is amniote-like because it is a two-phase process involving an epithelial dermomyotome with four lips, whereas lampreys and paddlefish both exhibit an ECL similar to that of teleosts. These new data show that an ECL-mediated myogenesis is primitive for vertebrates. DM-mediated mode evolved in stem gnathostomes and reverted to ECL-mediated myogenesis secondarily in ray-finned fishes. The DM mediated mode is likely linked to long development time which is primitive for jawed vertebrates.

Coloniality in marine chordates: eco-evo-devo approaches to understand different levels of biological organization

Federico Brown
Universidade de São Paulo

We have undertaken a comparative and multidisciplinary approach to link cellular and molecular understanding of development to the evolution of a major evolutionary transition, i.e. the evolution of coloniality. The Tunicata, sister group of vertebrates, contains clades with solitary species that reproduce only sexually, or colonial species that reproduce both asexually and sexually. These clades have evolved multiple times independently. Whereas solitary forms have evolved tougher tunics and individuals are generally larger to withstand predators, colonial forms can grow rapidly and directional growth may allow them to grow into spatial refuges free of predators. First, we conducted a three-month-long manipulative field experiment under different ecological treatments to determine how biotic factors influence the development and growth of solitary or colonial species. In these studies, competition with other organisms did not show consistent effects between life forms, however predation showe d a significantly higher effect on the growth of colonial species. Second, we hypothesized that the ability of colonial tunicates to reproduce clonally by budding may be related to the remarkable potential of regeneration and a high evolvability in the mechanisms that regulate adult stem cell development. We compared budding mechanisms in three colonial (i.e. *Botryllus schlosseri*, *Symplegma brakenhielmi*, *Polyandrocarpa zorritensis*) and one solitary species (i.e. *Styela plicata*) of the same tunicate clade (i.e. Styelidae). All colonial species differed in budding modes, blood cell types, and in the degree of integration and/or synchronization of developmental modules, whereas *S. plicata* presented a high regenerative potential mediated by circulatory progenitor cells. Similarities and differences in progenitor cells and tissues involved in regeneration or developmental processes of budding among the three colonial species have allowed us to identify potential developmental mechanisms responsible for the evolution of coloniality in this group. Third, we sequenced and continue to assemble the genomes of several colonial species of tunicates to understand genomic changes during the transitions to asexual reproduction, clonality, and coloniality. We are specifically interested in how ncRNAs may have played a role in the regulation of progenitor cells involved in budding and asexual reproduction. We have already found a pool of candidate miRNAs and other ncRNAs that are overrepresented in the genomes of colonial species that need additional validation and further experimentation in developmental studies. We have just begun to unravel some of the evolutionary forces and developmental changes that may have been responsible for the evolution of coloniality in our own phylum.

Functional and molecular evolutionary analysis of polyphenism-specific transcription in the nematode model *Pristionchus pacificus*

Linh T. Bui, R. Taylor Raborn, Erik J. Ragsdale
Indiana University

Polyphenism is the extreme form of developmental plasticity, whereby a developmental switch enables a threshold response to the environment. Polyphenism-specific genes, due to their

conditional expression, may be under the control of specific regulators and evolve under different selective pressures than constitutively expressed genes. Here, we investigate the relationship between polyphenism and molecular evolution in the nematode *Pristionchus pacificus*, which shows two alternative adult morphs (predatory, microbivorous) whose development depends in part on resource availability. By controlling for environmental and genetic backgrounds in this system, using morph-defective and morph-constitutive lines, we precisely identified sets of morph-specific genes. We identified several cis-regulatory elements specific for each set and used yeast-one-hybrid assays to confirm binding of a unique motif by the polyphenism-controlling nuclear receptor NHR-40. We also tested whether morph-specific genes show evolutionary signatures distinct from non-polyphenism genes using >60 fully re-sequenced *P. pacificus* wild isolates, and we studied the evolutionary history of these genes and their homologs in a sister species and in non-polyphenic outgroups. Together, our results provide the first insight into the function of a transcriptional polyphenism switch and reveal the potential of its target genes to evolve with adaptive plastic responses in micro- and macro-evolutionary time.

The Thrill of Discovery: Pioneer Stories
Sean B. Carroll
Howard Hughes Medical Institute

Regulation of nutrition-responsive growth and scaling in the horned beetle *Onthophagus taurus*
Sofia Casasa, Armin P. Moczek
Indiana University

Scaling relationships are critical in enabling the wide diversity of organismal shapes observed within and across taxa. Nutritional conditions are well known to influence scaling relationships, but often do so in a highly trait-dependent manner. Yet how organisms regulate exaggerated growth of some tissues while shielding others from environmental fluctuations and maintaining functionality of the organism as a whole remains poorly understood. In this study, we use the polyphenic horned beetle *Onthophagus taurus* in which high nutrition results in fully horned fighter males, whereas low nutrition development results in hornless sneaker males. While horns exhibit greatly exaggerated growth, other structures, such as genitalia, are almost nutrition insensitive. We investigated the role of the insulin/insulin-like growth factor signaling (IIS) and the Target of Rapamycin (TOR) pathways, known to link nutrition to growth in a wide range of organisms, in the development and evolution of horns and genitalia. Further, we examined possible interactions between IIS and other pathways known to mediate nutrition-responsive horn development in *Onthophagus* beetles. While TOR signaling manipulations are ongoing, our results suggest that IIS by itself, as well as through interactions with doublesex and Hedgehog pathways, regulates nutrition-dependent growth in *Onthophagus taurus* on a trait by trait basis.

What does ontogenetic integration tell us about how integration patterns arise in *Schizanthus*?

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Pontificia Universidad Católica de Chile

To explore how integration level and patterns changes during ontogeny and when adult patterns arise, we propose a new methodology using overlapping ontogenetic windows to calculate integration level during ontogeny without defining any previous ontogenetic stages. We measure flower ontogenetic integration in 6 species of *Schizanthus* representing evolutionary changes from bee-pollinated to hummingbird/moth –pollinated and selfing species. Previous studies shown that the pattern of morphological integration in adult flowers is related with the functionality of the petals in the pollination process. Different ontogenetic window size gave different information: at n=20, general shared tendencies are show, and at n=10, changes in the pattern of integration are clearer. Overall, we see a shared initial stage of low integration, where the final integration pattern is already clear, followed by a rapid increase of the level of integration for bee/hummingbird pollinated flowers and selfing species. The moth pollinated flower in the only one in which early stages present high integration values and the decoupling of structures appear later in ontogeny. However, in all species, important changes in integration patterns always occurs when integration level is low, suggesting that new patterns arise mostly in a low integration context.

Evo-Devo of feathers

Cheng Ming Chuong, Ang Li, Nie Qing, Seth Figueroa
University of Southern California

Adaptation of feathered dinosaurs and Mesozoic birds to new ecological niches was driven by rapid diversification of feather shapes. The paleontology findings identify key evolving feather types (Xu et al., 2014, Science). On the developmental side, we BMP signaling involved in barb branching, and anterior–posterior wnt3a polarization and stem cell ring topology control radial and bilateral feather symmetry (Chen et al., 2015, Annual Rev Animal Sci). For aero-engineer flight, asymmetric feather vane formation is essential, but molecular mechanism remains unclear. Here we use RNA seq to compare the transcriptome of wide versus narrow vanes and found mesenchyme-derived GDF10 and GREM1 are major controllers for the topologies of rachidial and barb generative zones. Their interactions with the anterior-posterior WNT gradient establish the bilateral-symmetric vane configuration. Additionally, retinoic acid (RA) signaling, patterned by differential distributions of CYP26B1, CRABP1 and RALDH3, modulates GREM1 expression and feather epithelial cell shapes. Switch-like modulation of RA signaling enables swift variations in vane morphology. We further develop a mathematical model based on these morphogen molecules. We show a fundamental core branching signaling module which can be modulated by evolutionary novel anisotropic signaling modules dynamically expressed temporal-spatially, introducing new dimensions of feather shape diversification.

Dissecting the genetic basis of morphology and evolution in *Nasonia*

Lorna B. Cohen, Jeremy A. Lynch
University of Illinois Chicago

Proper morphological development of complex organs such as the metazoan head depends on intricate interactions among several genes acting within multiple tissue types. Understanding how multifaceted gene interactions give rise to specific morphology and how changes in gene networks lead to a change in form is a general goal of evolutionary developmental biology. Gene interactions can be exposed by the phenomenon of epistasis, where two alleles produce a phenotype significantly different from the expected sum of their individual effects. Detecting epistasis between loci becomes rapidly intractable in diploid organisms as the number of genes involved increases. This difficulty is significantly reduced using a naturally haploid model system like *Nasonia*. The *Nasonia* genus is fitting for studies in developmental genetics and molecular evolution due to their haplodiploid genetics and the ability for interspecies crosses that result in fertile hybrid offspring. Crosses between two closely related species reveal novel hybrid phenotypes, likely due to negative epistatic interactions. We have begun to study these interactions by generating wasps with targeted loci from one species introgressed into another. Our method of 3D imaging allows for high-throughput phenotyping and quantitative analysis of morphometric differences between introgression strains. Combined, we can use these data to identify developmental incompatibilities and the nature of epistatic interactions among involved loci. Our preliminary results confirm that *Nasonia* is a uniquely powerful system with which to probe the role of complex gene interactions in the evolution of form.

Developing an ancient epithelial appendage: FGF signalling regulates shark denticle formation
Rory L. Cooper, Kyle J. Martin, Alex P. Thiery, Gareth J. Fraser
University of Sheffield

Skin appendages are a diverse group of structures including hair, feathers, and scales. Recent research has provided evidence for their homology throughout amniotes, as they all arise from conserved epithelial placodes. Elasmobranchs are found within the ancient sister lineage of bony vertebrates: Chondrichthyans. They possess tooth-like skin appendages called denticles, which predate the origin of gnathostomes. Using the emerging evo-devo model, the shark (*Scyliorhinus canicula*), we tested the hypothesis that denticles are homologous to other placode-derived amniote epithelial organs. To examine the conservation of putative gene regulatory network (GRN) member function, we undertook chemical inhibition of fibroblast growth factor (FGF) signalling during denticle formation. During early denticle morphogenesis, the shark expresses conserved molecular markers of epithelial placode development and columnar epithelial cells with a reduced rate of proliferation, as observed throughout amniotes. Inhibition of FGF signalling revealed placode development is FGF dependent and resulted in downregulation of associated putative GRN members, as expected from the amniote placode GRN. We provide evidence for the continuous, historical homology of epithelial appendage placodes throughout jawed vertebrates. These findings suggest the core GRN for building vertebrate skin appendages has been highly conserved over 450 million years, from sharks to mammals.

The Hox code and the identity of the teleostean fish caudal fin
Nicolas Cumplido, Salomé Muñoz-Sánchez, Gloria Arratia, Miguel L. Allende
Universidad de Chile

The discovery of Hox genes during the late twentieth century changed the way we approached metazoan development and evolution. This highly conserved family of transcription factors is organized in genomic clusters and is expressed during development in a segmental arrangement along the antero-posterior body axis, conferring distinct regional identities to the body. During evolution, vertebrates acquired at least four Hox clusters (HoxA, HoxB, HoxC and HoxD), required not only for the body's segmental identity, but also for the establishment of median and paired fins. In this context, the teleostean caudal fin stands as a unique structure, as it originates at the posterior body region attached directly to the axial skeleton, linking its segmental position on the body with its fin identity. By using gene editing techniques and gene expression patterns in zebrafish, we found that the cluster HoxB and HoxC are required for the development of the adult caudal fin. This result contrasts with previous works on the other clusters, HoxA and HoxD, and their requirement for the establishment of dorsal, anal and paired fins of zebrafish. Our findings strongly suggest that the teleostean caudal fin represents a separated module in development, opening new possible interpretations over vertebrate fin evolution.

The ABC model of flower development in non-core eudicots: a functional synthesis in a ranunculid

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University of Washington

The elucidation of the “ABC model” of flower development in the 1990’s promised a mechanistic basis for J.W. Goethe’s intuitive idea that floral organs evolved from a leaf bauplan. While the ABC model appeared mostly conserved, much remained to be elucidated considering mounting evidence for the importance of gene duplications in the evolution of development. Multiple duplication events affecting the MADS-box genes and flower morphologies diverging drastically from the prevailing model systems required modifications to the original model. As part of this evolutionary journey into the tinkering of the ABC model, functional studies in a variety of plant groups began to emerge. In the past few years, we have functionally characterized B-, C- and E-class genes in *Thalictrum thalictroides*, a ranunculid representing the sister lineage to the rest of the eudicots. To fill the gap in functional knowledge outside of the core eudicots and to better understand the evolution of floral MADS-box genes, we used virus induced gene silencing, gene expression, protein-protein interactions and mutant analyses. Here, I will synthesize these efforts in the context of data emerging from other ranunculids, highlight cases of sub-, neo-functionalization and redundancy

The evolution of insect eggs and cellular development across eight orders of magnitude

Seth Donoughe, Samuel H. Church, Jordan Hoffmann, Bruno A. S. de Medeiros, Chris H. Rycroft, Cassandra G. Extavour
Harvard University

A core of aim of Evo-Devo is to synthesize diverse genotypic and phenotypic data. Although genotypic data is increasingly accessible, acquiring phenotypic data is still comparatively expensive and time-consuming. The published scientific literature contains a wealth of phenotypic data that has remained inaccessible to large-scale statistical analyses because it is not machine readable. We developed a computational pipeline that empowers the user to quickly and precisely extract quantitative, qualitative, and image-based data from published sources. We used it to compile data on the development, size, and shape of insect eggs across insects. We processed 2,500+ digitized publications that comprise 100,000+ pages, creating a phenotype dataset of 6,500+ species. We found that insect egg volume spans eight orders of magnitude, and that there have been hundreds of times when eggs independently converged on the same size and shape. We then used phylogenetic comparative methods and computational models of early embryonic development to test long-standing hypotheses about how cell size, body size, and ecological factors shape the course of developmental and morphological evolution. We discovered that egg developmental evolution follows predictable patterns across the insect tree. We argue that this approach is a promising avenue for fruitful work in Evo-Devo.

Are sex differences in size and shape the same? Insights with the *Drosophila* wing
Ian Dworkin, Nicholas D. Testa, Maria Pesevski, Lianna Wat, Denise Rebello"
McMaster University

Sexual dimorphism is widespread throughout the animal kingdom. While some examples are exaggerated like the mandibles of stag beetles, most is subtle like differences in human facial shape or height. One striking commonality for sexually dimorphic traits is that they are often highly condition dependent. In particular, a reduction of nutritional resources during development leads to a marked decrease in the degree of sexual size dimorphism. However, it remains unclear to what degree condition influences dimorphism for organismal shape, and how this relates both to overall size and size dimorphism. The *Drosophila* wing is an excellent model to address these questions, as it is a target of sexual selection, has extensive natural variation within and between species for size, shape as well as for sexual dimorphism. I will highlight our research demonstrating that sexual size and shape dimorphism are only partially coupled using variation within and among species and through the use of mutational perturbations in *Drosophila melanogaster*. Furthermore, while nutritional limitation experiments reduce both sexual shape and size dimorphism, it does so in a genotype dependent manner. These results will be discussed in the context of the evolutionary potential of wing shape and size in sexual and non-sexual contexts.

Title TBA
Cassandra G. Extavour
Harvard University

Genome of the Tasmanian Tiger Provides Insights into the Genetic Basis of Convergent Evolution

Charles Y. Feigin, Axel H. Newton, Andrew J. Pask
University of Melbourne

The extinct thylacine (*Thylacinus cynocephalus*) was the largest carnivorous marsupial to exist into the modern era. Together with eutherian canines, it represents an iconic example of convergent evolution. Marsupials and eutherians shared a last common ancestor ~160 million years ago and display conspicuous differences in their development. Yet, the thylacine and canines have independently evolved nearly indistinguishable morphologies. While their phenotypic resemblance is thought to be adaptative, the genetic basis of their exceptional convergence is unknown. We sequenced the genome of a thylacine pouch young and used comparative genomic approaches to clarify the contributions of protein-coding and cis-regulatory evolution in driving its convergence with canines. We show that selection on the thylacine and canine exomes differs markedly and that observed amino acid homoplasies are consistent with neutral evolution. In contrast, we find considerable evidence for adaptive convergence in cis-regulatory elements. Hundreds of vertebrate-conserved, non-coding regions show convergently accelerated evolution. Many elements lie near key osteogenic and craniofacial patterning genes and show convergence in transcription factor binding motifs. Many elements are homologous to functionally validated mouse and human enhancers. Our findings suggest that cis-regulatory elements, rather than protein-coding genes, are likely to be important targets of selection driving convergent morphological evolution.

Using Machine Learning to reconstruct evolution of segmentation

Paul François, Jeremy B. Rothschild, Panagiotis Tsimiklis, Eric D. Siggia
McGill University

In silico evolution is a machine learning technique that can be used to reconstruct the evolution of gene regulatory networks. The expression pattern of gap genes has evolved between Drosophila (fly) and Anopheles (mosquito), yet one of their targets, eve, has remained invariant. Our simulations predict that eve stripe modules 3+7 and 4+6 in fly are homologous to 3+6 and 4+5 in mosquito. As a validation, we show that gap genes and eve patterns in Clogmia are consistent with this predicted evolutionary pathway, and share the mosquito homologies. To account for the evolution of the other pair-rule genes in the posterior, we have to assume in our simulations that the ancestral Dipterian utilized a dynamic method more similar to short germ insects. We propose that the ancestral pair-rule gene network "decodes" the shift of eve stripes towards the anterior for proper patterning.

Limb development and regeneration in fossil amphibians and extant salamanders

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Humboldt University

Modern amphibians are represented by three characteristic groups, frogs, salamanders, and caecilians, which show an amazing diversity with respect to species numbers, ecologies and life

history strategies. This modern diversity in combination with the long evolutionary history and a comparatively good fossil record offer great opportunities for studying aspects of amphibian evolution using both, fossil as well as extant developmental data. Salamanders are a particularly interesting model for this approach, because on the one hand their bauplan arguably is the most plesiomorphic compared to the highly derived baupläne of frogs and caecilians. On the other hand, salamander show unique features in their development, which are of boarder evolutionary and biomedical interest. These include a unique pattern of limb development with a reversed, so called preaxial patterning of skeletal limb elements compared to other tetrapods. Moreover, salamanders with free-swimming larvae have a unique way of forming their digits. Tetrapods normally undergo a paddle stage in which the anlagen of the digits are formed. Larval salamander, on the contrary, bud their digits one by one. The highly derived direct developing plethodontid salamanders, however, show a paddle with a gross morphology comparable to amniotes and frogs while showing preaxial polarity like larval developers. Our studies of gene expression patterns in larval and direct developing salamanders show canonical expression patterns of genes in the limb bud, but some striking differences from other tetrapods in late phases of limb development when the autopod is established. In addition to the marked differences in limb development of salamanders, they also show extremely high regenerative capacities that include full limb and tail regeneration that surpass any other tetrapod. These developmental features are unique among modern tetrapods and hence have classically been interpreted as derived for salamanders. However, data from the fossil record show, that preaxial polarity in limb development and the capacity to regenerate limbs are ancient features among tetrapods that are likely plesiomorphic for Tetrapoda. Moreover, salamander-like tail regeneration is likewise found in Paleozoic anamniotes and evidence for tail autotomy suggests that it had an adaptive significance for these animals. This suggests that salamanders are the only living tetrapods that retained these developmental features, offering a new evolutionary framework for investigating the molecular pathways involved in regeneration and preaxial polarity.

Developmental Nonlinearity Drives Phenotypic Robustness

Rebecca M. Green, Jennifer L. Fish, Nathan M. Young, Francis J. Smith, James M. Cheverud, Charles C. Roseman, Trevor Williams, Ralph S. Marcucio, Benedikt Hallgrimsson
University of Calgary

Robustness to perturbation, or canalization, is a fundamental feature of complex organisms. Mutations are the raw material for evolution, but robustness to their effects is required for populations to tolerate mutational loads. Explanations for this robustness range from the role of specific processes such as heat shock proteins to more general embedded features of developmental systems. Nonlinearities are a ubiquitous feature of development that may link variation in development to phenotypic robustness. Here we manipulate gene dosage of a signalling molecule, Fgf8, which is a critical regulator of vertebrate development. We demonstrate that variation in Fgf8 expression across this series has a non-linear relationship to phenotypic variation, predicting robustness among genotypes. Differences in robustness are not due to gene expression variance or dysregulation, but rather emerge from the nonlinearity of the genotype-phenotype curve for Fgf8 expression, presumably impacting robustness to

environmental variation. This shows emergent features of development rather than specific canalizing mechanisms explain robustness. How such features vary among individuals in natural populations and relate to genetic variation more generally are key questions for unravelling the origin and evolvability of this fundamental feature of organismal development.

Evolution of enteric neural crest cells in the vertebrates

Stephen A. Green, Benjamin R. Uy, Marianne E. Bronner
California Institute of Technology

Evolution of vertebrates is linked to the advent of neural crest cells, a migratory and multipotent cell population that gives rise to many vertebrate characters. Cyclostomes (including the Sea Lamprey *Petromyzon marinus)* are a sister group to jawed vertebrates that have neural crest cells but lack some neural crest derivatives. Here we explore the origin of the lamprey enteric nervous system (ENS). The gnathostome ENS is composed of thousands of interconnected ganglia within the gut wall that autonomously regulate muscle contraction, secretion, and water balance. Our data suggest that lampreys may lack a discrete "vagal" neural crest population akin to those forming the ENS of jawed vertebrates. Rather, we find that late-migrating trunk neural crest cells, originating from the DiI-labeled trunk neural tube and closely associated with nerve fibers, can differentiate into serotonergic neurons within the gut wall. These trunk neural crest cells may be homologous to Schwann cell precursors of mammals, recently shown to populate post-embryonic enteric ganglia of the colon. Thus, we propose that enteric precursors arising from trunk axial levels may represent a plesiomorphic trait of vertebrates, and that vagal neural crest–derived enteric precursors might have arisen in stem gnathostomes.

Non-genomic actions of thyroid hormones as a potential regulator of larval skeletogenesis in sea urchins

Andreas Heyland, Elias Taylor
University of Guelph

Biomineralization provides the rigid material for many adaptations across diverse phyla. Skeletogenesis is the process by which biomineralized structures are formed during embryogenesis. In the sea urchin *S. purpuratus*, it is a well understood process of development and the gene regulatory network (GRN) controlling skeletogenesis in the larva has been described. Previous research shows that TH accelerates the development of the juvenile rudiment in Dendraster excentricus and other irregular urchins while slowing the growth of larval skeleton. Here we tested the hypothesis that thyroid hormones (specifically T4, T3, and Triac) are regulators of skeletogenesis in *S. purpuratus*, upstream of the skeletogenic GRN. Treatment with T4 and T3 accelerated initiation of larval skeletogenesis in gastrulae, plutei, and the juvenile rudiments of late stage larvae, while Triac inhibited skeletogenesis. The use of fluorescently labeled THs suggested that THs bind to skeletogenic primary and secondary mesenchyme cells. Quantitative real-time PCR after TH exposure revealed upregulation of a key transcriptional initiator of skeletogenesis, Ets, as well as various skeletogenic proteins. Some evidence suggests there is a conserved mechanism of developmental regulation of

mesenchyme cells between vertebrates and echinoderms, mediated in part by TH binding to a membrane receptor.

The genetic basis of mimetic color diversity in bumble bees
Heather M. Hines, Li Tian, Sarthok Rahman
Pennsylvania State University

Bumble bees have undergone an extensive diversification in coloration, largely driven by local convergence as a result of Müllerian mimicry. These bees exhibit modular coloration in their setal pile across body sclerites, with myriad orange, yellow, white, and black color patterns across the genus. We are studying how this mimetic color diversity has been attained by targeting the genetic and developmental mechanisms underlying color variation in North American mimicry complexes. Using genomic sequencing of natural variants, we have identified a narrow locus driving a mimetic red/black abdominal color polymorphism in the bumble bee *Bombus melanopygus* to a regulatory region of the abdominal Hox genes, implicating changing expression of these conserved developmental genes in driving late-induced pigment phenotypes. Using developmental genetic tools, we are investigating how this mutation generates such color changes. Examination of this locus in comimicking species revealed that the mimics have acquired the same color patterns as *B. melanopygus* convergently, suggesting rampant mutations as opposed to sorting of ancestral polymorphisms in this mimetic variation. This research provides new insights into how the genome is modified under adaptive diversification, while also revealing processes involved in pigment and Hox gene regulation in a diverse emerging system.

A genome-wide assessment of the ancestral neural crest gene regulatory network
Dorit Hockman, Daria Gavriouchkina, Jeremiah J. Smith, Marianne E. Bronner, Tatjana Sauka-Spengler
University of Oxford

The neural crest (NC) is a vertebrate-specific embryonic cell population that contributes to key adult features including the cranio-facial skeleton and peripheral nervous system. Understanding how NC cells are programmed in the embryo will help determine the causes of NC defects, and assist in harnessing the therapeutic potential of this tissue. Many genes that are required for NC development have been assembled into a gene regulatory network (GRN) describing the hierarchical order and interactions between gene products. We have used an evolutionary approach to elucidate the central players in the NC-GRN by examining NC in lampreys, representatives of an ancient lineage. Analysis of transcriptome datasets for pre-migratory and migratory NC at different stages, as well as control populations, has revealed co-expression modules containing genes co-regulated during NC specification, including known bona fide NC-specifiers, and many loci not previously implicated in NC development. Using lamprey NC ATAC-seq datasets we have identified putative NC enhancers genome-wide, and tested the activity of elements controlling NC-specification genes, Sox10 and TFAP2b, in vivo. By comparing the GRN in lamprey NC to that in jawed vertebrates, our research will reveal the

components of the NC-GRN that are under evolutionary constraint and therefore essential for NC development.

Evo-Devo: Crossing genomes and phenomes
Jukka Jernvall
University of Helsinki

Genomic data from many taxa and individuals are increasingly used to examine links between the genotype and the phenotype. Mechanistically, the 'link' between the genotype and the phenotype is development, and I will discuss, focusing on a collaborative project on species hybridization, how developmental biology can be instructive for investigations building on genome-centric data. Especially in the case of mammals, species hybridization has attracted renewed interest due to paleogenomic evidence that has implicated interbreeding among human taxa. We have investigated using phenomic, genomic, and developmental insights whether hybridization between morphologically disparate taxa can produce developmentally stable, intermediate morphologies. We analyzed teeth of two species of pinnipeds the Grey seal and the Ringed seal, together with a unique hybrid specimen between these two species. Grey seals have fang-shaped post-canine teeth with a prominent central cusp and, when present, small accessory cusps. In contrast, ringed seal post-canines have multiple slender cusps. We contrast these phenotypic differences with genome wide comparisons of genetic differences between the two seal species, between humans and Neanderthals, and between other mammalian species known to hybridize. Finally, I will show how a developmental biology perspective can be provided through a computational model simulating tooth development.

Embryonic patterning of airway cartilage and the avian vocal organ
Evan P. Kingsley, Tom W. Hiscock, Clifford J. Tabin
Harvard Medical School

Most amniotes vocalize with vibratory tissues near the mouth, in the larynx. But avians—and only avians—vocalize with a morphologically distinct vocal organ, the syrinx, at the junction of the trachea and bronchi, nearer the lungs than the mouth. We investigate the development of the syrinx in the context of airway cartilage patterning. The airway is supported in all amniotes by surrounding cartilaginous or bony elements that comprise a set of periodically spaced ring-like segments. Repeating structures are common at many scales in organismal body plans—e.g. the segments of the vertebrate and arthropod anterior-posterior axes, the digits of the vertebrate limb, the photoreceptors of the insect eye—but for only a handful of patterns do we understand the embryonic mechanisms that determine the size, shape, and number of elements. The modified cartilage elements of the syrinx break up the larger pattern of cartilage in the trachea and bronchi, and thus provide an opportunity to identify the molecules that generate the pattern by studying how periodicity is broken. Here we explore two different roles of Hedgehog signaling in patterning tracheobronchial cartilage and in positioning the syrinx in the airway.

Why snakes are so unique? Some insights from developmental pathways

Tiana Kohlsdorf
University of São Paulo

Snakelike morphologies consist of elongated trunks and reduced or absent limbs. This type of morphology evolved multiple times in Tetrapoda, being a classical example of snakelike animals the snakes. The axial morphology of this lineage is characterized by increased number of vertebrae, which in the trunk are all associated to ribs. Although such snakelike characteristics evolved independently in different tetrapod lineages, including Gymnophiona, Amphisbaenia and Serpentes, some remarkable changes in developmental pathways seem unique to snakes. In this talk I will first present data from regulatory regions of genes expressed during embryo development, and then focus on results obtained from coding regions of Hox genes sequenced in snakelike lineages. Analyses of coding regions compared models of molecular evolution among lineages using PAML, and provided evidence for positive selection in Hox genes in the Serpentes clade. Functional assays suggest that molecular signatures unique to this lineage may affect developmental pathways involved in axial elongation.

Use of experimental atavisms to estimate soft tissue reconstructions of the earliest tetrapod limbs

Hans C.E. Larsson, Yacine Kherdjemil, Marie Kmita
McGill University

The fish-tetrapod transition 400 million years ago involved extensive anatomical and physiological transformations. Most evidence of evolutionary transformations about this transition are based on the preserved skeletal fossil record. Soft tissue and physiological reconstructions are limited to comparisons of relevant extant taxa about this transition. These include extant coelacanths, lungfish, and amphibians. Although some musculoskeletal comparisons have been made, they are hindered by the extreme disparity between these taxa. One potential solution to this problem is to examine experimental atavisms. Although there are many caveats to this approach, it does present an intriguing avenue of evidence by altering developmental processes to yield seemingly plesiomorphic morphologies. We use a recently engineered Hoxa11 antisense regulation mouse mutant. These mutants express varying degrees of polydactyly with remarkable similarity to some digit morphologies present in the earliest tetrapods. Using soft tissue enhanced staining and a nano-focus X-ray microscope, the entire soft tissue patterns are reconstructed for these mutant mice limbs. Reconstructions of the vasculature, nerves, muscles, tendons, ligaments, cartilage, and ossified skeleton are compared to controls and to the fossil record. The soft tissue patterns are remarkably consistent among the experimental limbs, suggesting a conserved patterning mechanism for these tissues. Using this conserved architecture as a guide, these soft tissues are reconstructed for fossils about the fish-tetrapod transition. The reconstructed morphologies suggest a complex muscular organization in early tetrapods and yet maintaining a relatively simple neurovascular pattern. Muscular patterning in subsequent tetrapods with the reduced pentadactyl pattern are hypothesized to have reduced only the pre- and post-axial muscles. Although the use of natural and experimental atavisms have a long history in evolutionary

biology, current sophisticated genetic engineering offers novel methods to explore the relationship between developmental processes and macroevolutionary transitions.

Sex Change in Slipper Limpets
Maryna P. Lesoway, Jonathan J. Henry
University of Illinois Urbana Champaign

Sex is a central problem of biology. However, current understanding of the evolution of discrete sexes and development of sexual characters focuses on organisms with separate sexes. Existing hermaphroditic models either do not change sex, or lack tractability as developmental models. Slipper limpets (*Crepidula*) are protandric hermaphrodites, transitioning from male to female. *Crepidula* snails are easily maintained, have a well-supported phylogeny, and well-studied development. To study sexual transitions, we have established the direct developing, fast-growing *Crepidula atrasolea* as a model. We confirm that the endocrine disrupter, tributyltin (TBT), causes ectopic male genital formation in female *C. atrasolea* (imposex), and show that it accelerates penis development in juveniles. Several developmental pathways implicated in sexual transitions are potentially disrupted by TBT, including steroid metabolism, neuroendocrine secretion, retinoic acid, and PPAR pathways, but their precise roles and relationship to normal sexual development are poorly characterized. Ongoing work compares ectopic and normal male development using pharmacological manipulation, and examination of gene expression patterns using candidate gene and unbiased approaches. Comparison to sexual development in other *Crepidula* could be of practical use in controlling invasive *Crepidula* species.

Why don't elephants get cancer? Developmental constraints and evolutionary tradeoffs in the resolution of Peto's paradox
Vincent Lynch
University of Chicago

A major developmental constraint on the evolution of large body sizes in animals is an increased risk of developing cancer. If all cells have a similar risk of malignant transformation and equivalent cancer suppression mechanisms, organism with many cells should have a higher risk of developing cancer than organisms with fewer cells. Similarly organisms with long lifespans have more time to accumulate cancer-causing mutations than organisms with shorter lifespans and therefore should be at an increased risk of developing cancer, a risk that is compounded in large bodied, long-lived organisms. There are no correlations, however, between body size and cancer risk or lifespan and cancer risk across species, this lack of correlation is often referred to as 'Peto's Paradox'. In this talk I will discuss some of the genetic mechanisms by which elephants evolved enhance cancer suppression mechanisms and the price they paid for reducing their risk of developing cancer.

The Wnt beneath my wings: exploring butterfly pattern formation in the CRISPR era
Arnaud Martin, Anyi Mazo-Vargas, Carolina Concha, Richard Wallbank, Luca Livraghi, Linlin Zhang, Robert Reed, Nipam Patel, Owen McMillan, Virginie Courtier-Orgogozo

George Washington University

Understanding the generative mechanisms of morphological diversification requires the routine manipulation of genomes in a comparative context. Here, I present how current work using CRISPR mutagenesis has allowed to decipher developmental mechanisms that may have driven the diversification of a spectacular of morphological radiation: the color wing patterns of butterflies. Indeed, mosaic Knock-Outs induce wing both pattern and color modifications at high-efficiency in any butterfly suitable for laboratory rearing. I describe the multiple phenotypic effects of the Wnt ligand WntA in seven species, and illustrate how this signaling molecule has been essential for both pattern formation and exploration for the morphospace on the butterfly wing. A total of eighteen cis-regulatory alleles of WntA (all of adaptive relevance linked to mimicry) have been formally mapped to date in at least three radiations of butterflies. Thus, CRISPR offers an opportunity to validate genetic function in non-traditional model organisms is of tremendous importance for further understanding the genome-to-phenome relationship at different taxonomic nodes, from population levels to more macro-evolutionary scales. I will discuss this principle in the broader context of GepheBase (www.gephebase.org), a database of known genotype-phenotype that compiles from the literature more than 1600 allele pairs across all Eukaryotes.

The development and evolution of morphological scaling relationships

Kenneth Z. McKenna, H. Frederik Nijhout
Duke University

Arguably one of the great unanswered questions in biology is that of scaling. Taking a glance at variation in any group of organism, some natural questions arise: how do body parts scale with body size when there is intra- and inter-specific variation in body size? And how do these scaling relationships evolve? Experimental studies have demonstrated that there is genetic variation for allometries and that scaling relationships can evolve under artificial selection. Insects provide excellent examples of evolutionary changes in allometry, displaying some of the most exaggerated traits in the animal world, e.g. eyes on the ends of long stalks, forelegs longer than twice the body length, and horns that emerge from the head and thorax. We will discuss how changes in nonlinear growth kinetics can give rise to novel allometric relationships. Using wing-body scaling in the tobacco hornworm, *Manduca sexta*, we show that the size attained by wings depends on parameters that influence both body growth and wing growth. We describe how the scaling relationships and underlying developmental parameters change under directional selection on body size and relative wing size. Finally, we demonstrate how novel morphological scaling relationships can arise by evolutionary change in growth kinetics.

Exploring Ecological Sexual Dimorphism through Morphometrics, Genetics and Biomechanics

Kirstie C. McWhinnie, Kevin J. Parsons
University of Glasgow

Understanding connections between adaptive phenotypes and the mechanisms underlying them provides a central focus for evo-devo. The changes in these connections can occur

through adaptive divergence, a phenomenon usually studied between species. Ecological sexual dimorphism (ESD) represents another form of adaptive divergence that evolves between sexes due to alternate ecological conditions and can result in differences in trophic morphology, a key feature of many adaptive radiations. Here, I explore adaptive variation in trophic morphology using the African cichlid mandible. Sexual dimorphism in colour and body size is prevalent amongst cichlids, suggesting sexual selection, but potential adaptive differences between sexes are rarely considered. Therefore, I combine techniques from evo-devo and engineering to test for evidence of ESD. In this project, I use 3D measures of shape and QTL mapping to determine the genetic basis of differences between species and sexes. In addition, as it has been well established that phenotypic plasticity is itself an evolvable trait, I examine whether developmentally plastic responses to alternate foraging environments are influenced by sex, and test whether these sex-specific developmental responses are adaptive. Taken together, this research addresses ESD from a developmental and genetic perspective to provide a wider understanding of how adaptive divergence proceeds.

Sweet genes are made of STYLISH – Members of the STYLISH gene family control both style and nectary development in *Aquilegia* (Ranunculaceae)

Ya Min, J. Imani Bunn, Elena M. Kramer
Harvard University

The nectar spurs of *Aquilegia* flowers have long been regarded as a textbook example of key innovation that facilitated the adaptive radiation of the New World *Aquilegia* species. In an effort to understand the genetic basis of nectar spur development in *Aquilegia*, we conducted functional studies of genes from the STYLISH (STY) family in *A. coerulea*. All three members, AqSTY1, AqSTY2, and AqLRP, were shown to be enriched in young petal cups in previous RNA-sequencing data, while AqSTY1 showed the strongest differential expression between petal cups and blades among all candidate genes. In situ hybridization revealed similar expression patterns of these three genes during all stages of carpel development and also the earliest stages of flower development. However, their expression differed when the nascent petal spurs start to emerge, during which AqLRP is not detectable in the petal, AqSTY2 showed diffused expression in the growing spur cup, but AqSTY1 exhibits concentrated expression in a highly restricted area of the inner surface of the growing spur tip. Single and triple gene knock down via RNAi-based method revealed that three genes function redundantly in style and nectary development, and silencing all three genes led to the absence of nectar. Since members of the STY family are known to redundantly control auxin synthesis both early-diverging land plants and core-eudicots, this functional conservation may explain the defects in stigmatic tissue in *Aquilegia* flower. However, no previous study among the core eudicot has reported STY genes function in nectary development, suggesting the genes have been co-opted to this role in *Aquilegia*. Moreover, our preliminary data suggests that this co-option event is likely to have occurred before the diversification of the family Ranunculaceae.

Evolutionary origins of the endoskeletal joint in vertebrates

Tetsuto Miyashita, Stephen A. Green, A. Phil Oel, A. Richard Palmer, W. Ted Allison, Marianne E. Bronner

University of Alberta

A diarthrosis is a functional prerequisite to the mineralized endoskeletons of vertebrates, but its evolutionary origin remains a puzzle. The elastic cartilaginous skeletons of cyclostomes have no proper diarthrosis. Nor do stem cyclostomes possess any correlates of a joint in the areas of the chondrocranium that are highly elastic as in living cyclostomes. The distribution of characters in stem gnathostomes suggests jaw and pectoral joints as the earliest diarthrosis consisting of a fibrous capsule and articular cartilages. While the morphology of pectoral joints varies markedly and non-parsimoniously from one lineage to another, the jaw joint is conservative and tractable through comparison of distantly related living vertebrate models. We tested three structures in cyclostomes as potential homologues of the jaw joint: (1) muscular articulation in the lingual apparatus; (2) mucocartilage (fibrous cartilage-like structures in larval lampreys); and (3) an intercartilaginous blood sinus. Expression profiles of jaw- or general-joint marker genes (Bapx, Barx, Gdf5/6/7, Prg4, etc.) in the lamprey *Petromyzon marinus* rule out (1) muscular articulations but cannot discriminate between (2) mucocartilage and (3) blood sinus. The expression domains overlap significantly in the anlagen for (2) and (3), but are not specific or exclusive to either. CRISPR/Cas9-mediated partial knockouts of Bapx and Barx in lampreys resulted in equivocal phenotypes (CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats). Thus, we formulated two possible scenarios that may have occurred at the origin of the jaw: (a) mucocartilage or blood sinus acquired expression of the transcription factors (Bapx, Barx) to specify the presumptive jaw joint; or (b) the transcription factors acquired functions to target effector genes such as Col2a to modify mucocartilage or blood sinus into a jaw joint. To test these hypotheses, we used CRISPR/Cas9 to generate homozygous bapx mutant zebrafish in which the jaw joint is predicted to fuse. These mutants might partially phenocopy the states before and after the origin of the jaw, through comparison with the oropharyngeal morphology in osteostracans and placoderms. Comparison of gene expression patterns between the knockout lampreys and the mutant zebrafish may provide insight into whether transcription factors or effectors are key for endoskeletal joint evolution in vertebrates.

The gradual molecular and developmental evolution of butterfly wing eyespots

Antonia Monteiro, Heidi Connahs, Nesibe Oszu, Sham Tlili, Yuji Matsuoka, Tirtha Banerjee, Jelle van Creij, Sarah Monroe, Mainak das Gupta, Jeffrey Oliver, Timothy Saunders
National University of Singapore

An important and still largely unanswered question in the field of evo-devo concerns the molecular changes that lead to the origin of novel complex traits. One such trait is the eyespot color patterns on the wings of nymphalid butterflies. My lab is combining a series of evo-devo approaches to identify the molecular and developmental changes underlying eyespot evolution. A multi-species comparative phylogenetic approach led to the identification of the ancestral lineage where eyespots likely evolved, and also where four genes started becoming expressed in eyespot centers. A transcriptomics approach, applied to a single species, identified a much larger number of genes (186) expressed in eyespot centers that are differently expressed in homologous wing tissue without eyespots. From this pool of genes, a CRISPR/Cas9 gene editing

approach identified three genes required for eyespot development that when mutated lead to wings without eyespots. Two of the genes evolved their new expression pattern concurrently with eyespot origins whereas the third gene evolved its expression at a later date in a subset of butterfly lineages. This means that genes that become associated with the development of a novel trait at a late stage in their evolution can become essential developmental genes. In addition, a combination of in situ gene expression with modeling work, led to reconstructions of mosaic mutant wing patterns and to the identity of two potential ligands involved in eyespot center differentiation via simple reaction-diffusion mechanisms. I'll put all this data together to offer a possible molecular model underlying the origin and gradual evolution of butterfly eyespots on the wings of nymphalid butterflies.

The architecture of adaptation: a master mutation or a mass of mutations?
Sophie L. Archambeault, Catherine L. Peichel
University of Washington/University of Bern

Adaptation to divergent environments often requires changes in many different phenotypes. Adaptation can therefore be facilitated when the genetic loci that underlie different phenotypes are clustered in the genome. Although genetic analyses in many systems is starting to reveal that such "phenotypic hotspots" exist, it is almost completely unknown how these hotspots are built during evolution and whether they are due to linkage of multiple mutations or to pleiotropic effects of a single mutation. Several "phenotypic hotspots" have been identified in the threespine stickleback (Gasterosteus aculeatus), an excellent model for studying the genetic basis of adaptive evolution. We are focusing on a hotspot on chromosome IV that overlaps with a region of high genomic divergence between marine and freshwater ecotypes, suggestive of repeated selection on one (or more) of the phenotypes that map to the hotspot. To determine whether linkage or pleiotropy is responsible for this hotspot, we are performing association mapping of multiple traits in a fully interbreeding, polymorphic, freshwater population of threespine stickleback. Our preliminary data suggest that the clustering of traits in this region is due at least in part to pleiotropic effects of a single genetic change.

Evaluating the molecular basis for diet-induced phenotypic plasticity in teleosts
Dina Navon, Ira Male, Nathan Olearcyzk, Rolf O. Karlstrom, R. Craig Albertson
University of Massachusetts Amherst

Plasticity allows species to respond rapidly to environmental changes and may guide future evolution (via the flexible stem model). While it's thought that plasticity can evolve, little is known about its genetic underpinnings. Many teleost lineages have diverged along a benthopelagic axis encompassing coordinated shifts among behavior, morphology, and ecological niche. Importantly, some species exhibit significant plasticity along this axis while others do not. Previous work in our lab has identified candidate genes that underlie this phenomenon, including ptch1, a member of the Hedgehog (Hh) pathway. In order to test the hypothesis that Hh signaling mediates benthopelagic plasticity, we quantified rates of bone deposition in transgenic zebrafish in which Hh levels were manipulated via heatshock as well as

in three species of cichlids with different ptch1 genotypes. Calcium-binding fluorochromes labeled bone at the beginning and end of the experiment, and fish were split into diet treatments mimicking benthic and pelagic feeding modes. We focused on bones under high mechanical load during feeding, but also examined bones not engaged in foraging efforts as internal controls. In all, these data will serve to functionally test the relevance of candidate genes in establishing a plastic response, filling an important gap in the field.

Interspecies gene transfer reveals a distribution of size effects underlying the morphological divergence between species

Lachezar A. Nikolov, Michiel Kwantes, Miguel Anton Perez, Stefan Strütt, Xiangchao Gan, Miltos Tsiantis
Max Planck Institute for Plant Breeding Research

High-throughput sequencing technologies have revolutionized evolutionary biology but the identification of causal genetic changes underpinning the diversity of biological forms remains a significant challenge. This is particularly relevant at the macroevolutionary scale, where classical approaches based on interspecific crosses are difficult to use to link genotypic and phenotypic variation. Leaves of seed plants offer attractive prospects to understand the molecular basis of morphological change because they exhibit substantial heritable variation at different evolutionary scales. Comparisons between the reference plant *Arabidopsis thaliana*, which bears simple leaves and its complex-leaved relative *Cardamine hirsuta* have led to considerable insight on how leaf form develops and diversifies. To gain further insight into the mechanisms that gave rise to different leaf shapes in these two species, we compared their gene expression profiles in developing leaves. We identified several transcription factors expressed at higher levels in *Cardamine* than in *Arabidopsis*, and expressed all of them in *Arabidopsis* to test whether they are sufficient to generate leaf complexity in simple leaves. We also reduced their expression in *Cardamine* to assess whether they are necessary for compound leaf development. Our results reveal a skewed distribution of effect sizes, with a handful of differentially expressed transcription factors showing a disproportionate ability to alter leaf shape in the interspecies gene transfer assays. Our findings suggest that change in leaf morphology occurs via a limited number of evolutionary paths, influenced by a defined set of major-effect molecular players.

Genetic complexity and the evolution of quantitative traits

Annalise Paaby
Georgia Institute of Technology

The evolution of complex processes, like embryogenesis, require the interaction of many genetic elements. These interactions may allow the accumulation of conditionally functional mutations, alleles that are normally silent but which can be revealed when the functions of other genes are perturbed. In this talk I describe the existence of a vast reserve of such natural genetic variation, in the gene networks of *C. elegans* embryogenesis. We see that these modifier alleles are ubiquitous and segregate at intermediate frequencies in the wild, and that they demonstrate low developmental pleiotropy such that specific, rather than general,

perturbations are required to reveal them. These findings underscore the importance of genetic background in characterizing gene function and provide a model for the expression of conditionally functional effects that may be fundamental in mechanisms of trait evolution. However, mapping these alleles is monstrous and potentially impossible. So how can we probe these interactions in order to elucidate functional relationships among genes, proteins, pathways, cell-biological events and other mechanisms at the sub-organismal level? I will describe the approaches my new lab is taking to measure and understand variation in one complex process, early embryogenesis, in order to understand how it evolves over both micro and macro scales.

Genomic Red Queen and the evolution of limb genetic architecture
Mihaela Pavlicev, Fernando Andrade, Günter Wagner
Cincinnati Children's Hospital

Genes and developmental pathways are re-used in complex organisms in different traits. Consequently, genes become pleiotropic and mutations in such genes cause covariation of traits. Depending on how much of the trait`s genetic basis is based on pleiotropic genes, such entanglement can substantially constrain the trait's response to selection. We and others have suggested that decoupling of genetic covariation due to pleiotropy may involve local epistatic modification of deleterious pleiotropic effects (selection-pleiotropy-compensation), causing accumulation of genetic changes in traits under stabilizing selection, to compensate for the side effects of selection on other traits. This genomic red queen principle (evolving genetic basis in order to maintain the phenotype) also predicts that a greater number of genes may be involved in genetic basis of traits under stabilizing, than those under directional selection. To test this hypothesis, we focus on serially homologous traits, which share a great deal of pleiotropic genes but have undergone substantial divergence. We chose limbs of four vertebrates, which differ in specialization of either fore-, or hind limbs: rabbit, mouse, chick and bat, and performed RNAseq analysis on limb buds at multiple developmental stages. We will present an analysis of divergences between fore and hind limbs across these species.

Genetic and environmental decanalization are not correlated among altitudinal varying populations of *Drosophila melanogaster*
Maria Pesevski, Will Pitchers, Justin Lack, John Pool, Ian Dworkin
McMaster University

Organisms are exposed to environmental and mutational effects that influence the mean and variance of phenotypes. These potentially deleterious effects can be ameliorated by the evolution of buffering (canalizing) mechanisms which reduce phenotypic variation within and among individuals. Theory predicts that canalizing mechanisms for environmental and mutational stresses should co-evolve, and thus will be correlated. Yet empirical evidence has not supported this prediction. In a recent study, Lack et al. (2016) observed that African populations of *Drosophila melanogaster* adapted to high altitudes via an increase in body and wing size. The high-altitude populations had more qualitative mutational defects, relative to ancestral populations from lower altitudes. Lack et al. (2016) demonstrated that this was due to

higher sensitivity to genetic perturbation. They concluded that the high-altitude populations have reduced genetic canalization. This system provides a unique opportunity to test the prediction of a correlated response between environmental and genetic canalization. We used the same populations to test if the high-altitude populations show a decrease in micro-environmental canalization. We quantified the size and shape of wings, measured the density of cells across 16 regions of the wing, and quantified the frequencies of mutational defects in lines derived from these populations. We observed the expected differences in size, shape, cell density and mutational defects between the two populations. However, we observed little evidence for a relationship between measures of micro-environmental canalization for wing size and shape with population or defects frequency. Our results do not support the theoretical prediction of an association between genetic and environmental canalization.

Identifying cis-regulatory enhancers associated with cichlid craniofacial evolution
Kara E. Powder, R. Craig Albertson
Clemson University

Evo-devo theory posits that variation in cis-regulatory enhancers, which regulate the spatiotemporal pattern of gene expression, is a primary mechanism for morphological evolution. However, identifying relevant enhancers and characterizing their in vivo function remains a challenge. Recent studies in model organisms have experimentally identified >75000 enhancers active during facial, cartilage, and/or bone development. In order to identify which of these enhancers may mediate the unparalleled craniofacial variation that is a hallmark of the adaptive radiation of cichlid fishes, we utilized a bioinformatic approach. Specifically, we mapped enhancers experimentally identified in mammals to the tilapia genome, and cross reference these to both craniofacial QTL and genomic regions that exhibit high levels of genotypic divergence (i.e. high FST) among phenotypically divergent cichlid species. This approach prioritizes enhancers in an efficient and unbiased way from ten of thousands to dozens for functional assays. Using this method, we prioritized a putative sox9b enhancer that lies within QTLs for both lower jaw width and length and has fixed mutations in species that vary in jaw phenotypes. Using the CRISPR system in zebrafish, we show that genetic variation in the sox9b enhancer results in the highly specific loss of ceratobranchial cartilages as well as phenotypic variation in the lower jaw that mimics natural variation in jaw shape between cichlid species. We suggest that the bioinformatic integration of QTL and population genomic data from evolutionary models like cichlids with enhancer data from model organisms offers a powerful approach to both prioritize and functionally evaluate enhancers that may mediate morphological evolution.

Cell-Cycle-Coupled Oscillations in Apical Polarity and Intercellular Contact Maintain Order in Embryonic Epithelia
Katerina Ragkousi, Kendra Marr, Sean McKinney, Lacey Ellington, Matthew C. Gibson
Stowers Institute for Medical Research

Throughout animals, embryonic cells must ultimately organize into polarized epithelial layers that provide the structural basis for gastrulation or subsequent developmental events.

Precisely how this primary epithelium maintains continuous integrity during rapid and repeated cell divisions has never been directly addressed, particularly in cases where early cleavages are driven in synchrony. Representing the early-branching non-bilaterian phylum Cnidaria, embryos of the sea anemone *Nematostella vectensis* undergo rapid synchronous cell divisions and ultimately give rise to a diploblastic epithelial body plan after gastrulation. Using live imaging of apical polarity proteins in *Nematostella* embryos, we demonstrate that cell polarity is established by the four-cell stage and then reiteratively lost during subsequent mitoses, correlating with transient adhesion disengagement and dramatic deformations of embryonic morphology. Intriguingly, the re-establishment of polarity and adhesion during each interphase is associated with a process of whole-embryo compaction analogous to that observed in mammals. Because similar protein dynamics are observed in dividing epithelial cells in *Drosophila melanogaster*, we propose that cell-cycle-coupled oscillations in apical polarity may be conserved throughout Metazoa.

Reproductive constraint: a developmental mechanism regulating social cohesion in ant societies

Arjuna Rajakumar, Ehab Abouheif
McGill University

A key feature of ant societies is their reproductive division of labor within the colony, where queens reproduce and workers perform most other tasks. This cooperation carries with it a potential conflict between queens and workers – in the majority of ant genera workers have retained their ovaries and are capable of laying eggs, creating a potential conflict. This conflict is reduced by 'Reproductive Constraint', which has evolved in advanced ant societies. Reproductive Constraint is an adaptive mechanism that causes the mislocalization of germplasm components, like oskar (maternal determinants that regulate germline and axis specification), in worker ovaries and as a consequence, workers produce 'failed' and 'trophic' eggs used for colony nutrition, limiting production of 'viable' eggs. In *Camponotus floridanus*, we discovered inter-individual variation in Reproductive Constraint. We found that soldiers have greater reproductive potential (ovariole and oocyte number) and realized reproduction (brood) than minor workers. However, soldiers also have a higher degree of Reproductive Constraint. Furthermore, the developmental stage a worker is orphaned can influence its ability to oviposit. Our results suggest the evolution of higher degrees of Reproductive Constraint in soldiers to deter their greater reproductive potential and that queens can socially-regulate worker oviposition to limit worker reproduction.

Deciphering genomic and developmental mechanisms that underlie vision adaptations in noctilionoid bats

Alexa A. Sadier, Liliana Davalos, Elizabeth Dumont, Stephen Rossiter, Kalina Davies, Karen Sears
University of California Los Angeles

Key innovations, novel traits that promote diversification, often involve sensory adaptations. Among these adaptations, the ability to see new wavelengths has been linked to advantages in foraging and hunting in multiple groups. We studied vision evolution in noctilionoid bats that

exhibits high diet variability, placing selective pressures on vision evolution. uMRI scans of adults from 20 diverse species demonstrated an association of eye phenotype and diet. Immunohistochemistry and RNAseq data for S (blue/UV) and L (red/green) cones in adults of 85 species also show an association between photoreceptor composition and diet. Insectivores have only L cones, while S cones have likely re-evolved in frugivores and nectarivores, such that also possess L cones. Interestingly, the photoreceptor density varies a lot between species, indicating fine scale differences in the capabilities to see wavelength among species. Immunohistochemistry on developing bats from 6 species is in progress but our first results suggest that some early factors act like a switch to regulate both the density and the balance between S and L cones. Together with existing behavioral data, our results suggest a complex mosaic evolution of S cones and UV vision through separate mechanisms in multiple noctilionoid lineages in association with diet selective pressures.

How can complex gene networks build complex bodies in development and evolution
Isaac Salazar-Ciudad, Pascal Felix Hagolani, Roland Zimm
University of Helsinki

Image you could build any gene regulatory network (GRN) and embedded it in any species developmental stage (e.g. the zygote of a fly). The question would then be: how should we wire the GRN in order to lead to pattern formation and morphogenesis producing the complex morphologies we observe in animals. We have done exactly that but in a general computational model of animal development, the Embryomaker. This model implements any possible gene network, all cell behaviours known to animal cells (signaling, adhesion, etc...), the genetic regulation of those and realistic biophysical interactions between cells and tissues in 3D. Using this model we explore the range of morphologies theoretically possible in animal development for a massive number of GRNs. Counter-intuitively, the complexity of the GRN has, in most cases, a limited effect on the complexity of the resulting morphology but a strong effect on how stable a morphology would be to external perturbations. We explain why and why this should apply to all animal development. We also discuss some properties of this space of possible developments that are relevant for evo-devo; most notably local and global degeneracy, or the fact that the same morphologies can be produced by completely different GRNs.

Homology of process: petals and petaloidy in the tropical gingers (Zingiberales)
Chelsea Specht
Cornell University
One of the most striking elements of angiosperm evolution is the diversity of floral forms represented. Flowers can modify existing structures or evolve novel elements that are often linked with functions, such as mechanisms for pollination or effective seed dispersal. Yet despite the diversity of floral forms, certain flower structures are highly conserved, as are many of the genes that underlie their basic form. In addition, transitions between certain floral forms are more common than others, indicating a certain level of canalization that may occur during the elaboration of floral development. We first discuss some of the patterns and processes underlying conserved floral forms, providing an idea of the bauplan that drives basic floral morphologies. We then extend to discuss two particular processes, polarity and fusion, that

drive novelties in floral organ development and are responsible for some of the most impressive diversity in flowers. The Zingiberales (tropical gingers) provide a model system for investigating the role of these two developmental processes in the evolution of floral form.

On the wings of love: pigment patterning and the checkered past of rock pigeons
Michael Shapiro
University of Utah

The rock pigeon (*Columba livia*) is among the most phenotypically diverse and widely distributed avian species in the world. We are combining traditional genetics, whole-genome sequencing, and developmental analyses to understand the evolution of diversity among the hundreds of breeds within this species. In doing so, we are addressing fundamental questions about the genetic architecture of phenotypic change. In particular, our combined approach is revealing a spectrum of mutations that control the orientation, color, and placement of plumage within and among breeds. Patterns of pigment deposition on feathers are especially variable in this species, and while we understand the molecular origins of switches in pigment type, we know considerably less about how patterns are controlled. Classical genetic studies determined that four major pigment patterns represent an allelic series at a single locus, and we found that different molecular changes in one gene are associated with much of this variation. Increased melanism is advantageous for pigeons in urban environments, and these adaptive melanic alleles originate from a surprising source.

Genetic mechanisms and macroevolution of flower color in the tomato family
Stacey D. Smith
University of Colorado Boulder

Genetic mechanisms have the potential to shape both the trajectories of trait evolution and the consequences of trait evolution for subsequent diversification. In this talk, I will explore relationship between the structure and regulation of floral pigment pathways and the macroevolution of flower color. Using the tomato family, Solanaceae, as a model clade, I will focus on two types of evolutionary color transitions: gains and losses of floral anthocyanin pigmentation and shifts between types of anthocyanin pigments. Ancestral state reconstructions suggest that floral anthocyanins have been lost and regained many times during the ca. 50 million year history of the family, and this extreme lability may be explained by the interplay of rapidly evolving transcriptional activators and repressors. Transitions among types of anthocyanins appear to be rare, by contrast, and may require mutations at multiple loci. Looking to the future, a grand challenge will be to move beyond individual case studies to build an integrative and predictive understanding of how genetic systems relate to phenotypic variation on short and deep time scales. Floral pigmentation may serve as an ideal trait for such efforts given the relatively well understood underlying pathways and the tremendous variation in flower color across angiosperms.

Metabolic evolution in cave fish

Cliff Tabin, Nick Rohner, Ariel Aspiras, Misty Riddle, Julius Tabin, Brian Martineau, Karin Zueckert-Gaudenz, Robert Peuss, Andrew Box, Michaella Levy, Suzanne McGaugh, Richard Borowsky
Harvard Medical School

Periodic food shortage is one of the biggest challenges organisms face in many natural habitats. Caves represent an extreme setting where animals have had to adapt to continuously nutrient-poor conditions, as most cave environments lack a primary energy source. We find that cave-adapted populations of the Mexican tetra *Astyanax mexicanus* have significantly altered sugar and fat metabolism, including changes that would be characteristic of pathological conditions such as diabetes in most other animals. Yet we are not able to detect any negative health effects of these changes in the cavefish. Genes responsible for these metabolic changes, and for the compensatory protective changes that accompany them, are being addressed through a combination of genomic analysis and QTL studies.

Patterns of transcriptional parallelism and variation in the developing olfactory system of Drosophila species

Pelin C. Volkan, Jia Pan, Qingyun Li, Scott Barish, Sumie Okuwa, Songhui Zhao, Charlie Soeder, Matthew Kanke, Corbin Jones
Duke University

Organisms have evolved strikingly parallel phenotypes to similar selection pressures suggesting shared constraints limiting the possible evolutionary trajectories. For example, behavioral adaptation of specialist Drosophila species to specific host plants exhibit parallel changes in their adult olfactory neuroanatomy. We investigated the genetic basis of these parallel changes by comparing gene expression during the development of the olfactory system of these two specialist *Drosophila* species to that of four other generalists. Our results show that the parallelism in adult olfactory neuroanatomy of specialists, unpredicted from their phylogenetic relationship, extends broader to expression profile and patterns of many transcription factor combinations specifying olfactory receptor neuron (ORN) fates during development. We also found that a subset of olfactory receptor genes expressed, particularly Or22a expressed by ORNs housed in basiconic ab3 sensilla, along with a subset of the transcription factors governing their development, are disproportionally variable across all six *Drosophila* species relative to other olfactory receptor neuronal lineages. These patterns imply the presence of non-random levels of interspecies variation within the components of both the adult *Drosophila* olfactory system, and its developmental programs. Further examination of these developmental components may be able to inform a deeper understanding of how traits evolve.

The Evolutionary Biology of Cell Types: the next frontier of Devo-Evo

Günter P. Wagner
Yale University

Cells and cell types are the building blocks of animals, plants and other multicellular forms of life. The number of cell types is also a major determinant of body plan complexity, with about 6 cell types in the simplest free-living metazoan, *Trichoplax adhaerens*, and in mammals at least 500 cell types, but probably many more. Hence the origin of novel cell types is a key event in the evolution of complex organisms. To investigate the origin of novel cell types, however, requires an, at least preliminary, model of what cell types are and how they are realized mechanistically. Recently an evolutionary cell type concept has been proposed (2016, Nature Reviews Genetics 17:744ff) building upon the intellectual framework of the evolutionary homology concept: a cell type is a group of developmentally individualized cells that are able to execute a cell differentiation program different from that of other cells in the body. A corollary of this characterization is that cell types are also able to evolve cell type specific phenotypes and functions, i.e. are units of phenotypic evolution. At the mechanistic level, it is proposed that the cell type specific gene expression is enabled through the formation of a cell type specific "core regulatory complex" (CoRC), that is a physical complex of transcription factor proteins that cooperatively regulate the expression of target genes. For Devo-Evo an evolutionary approach to cell types opens a vast new field of study, the evolutionary history of all cell types, a project even larger than that of the tree of life. It will involve developing methods to identify homologous cell types across large phylogenetic distances, the reconstruction of the evolutionary history of all cell types and investigating the mechanisms underlying the origin of novel cell types. The benefits of this project will go far beyond the confines of Devo-Evo and even evolutionary biology in general. As phylogenetic information is essential to reap the full benefits of comparative genomics, the evolutionary history of cell types will enhance the study of cell biology, even the comparative study of cells within one species, like the human."

Ontogenetic tooth reduction in theropod dinosaurs and the macroevolution of avian beaks
Shuo Wang, Josef Stiegler, Ping Wu, Cheng-Ming Chuong, Dongyu Hu, Amy Balanoff, Yachun Zhou, Xing Xu
Capital Normal University

Beaks are innovative structures characterizing several theropod lineages including modern birds, but little is known about how developmental processes influenced the macroevolution of these important structures. Here we show that vestigial alveoli are present in several beaked lineages of theropod dinosaurs, including caenagnathid oviraptorosaurs and Early Cretaceous bird *Sapeornis*, suggesting the previously reported ontogenetic tooth reduction phenomenon is also present in these theropod lineages. Based on new morphological data and study of macroevolutionary patterns of tooth reduction in extant vertebrates, we propose that ontogenetic truncation of tooth development is a mechanism contributing to tooth reduction in various theropod lineages that eventually reach edentulism. Dental reduction in theropods appears to have passed through at least four common steps: (I) normal tooth development and tooth replacement with an apomorphic keratinized rhamphotheca covering only the rostral-most portion of the jaws; (II) tooth replacement is impeded by external closure and/or constriction of alveoli, perhaps in association with adjacent growth of the rhamphotheca—

regional tooth reduction occurs; (III) as the keratinized rhamphotheca enlarges, the remaining teeth are either functionally reduced or redundant; and (IV) alveolar remodeling is complete or nearly complete, the edentulous beak is completely covered by the rhamphotheca.

Endless Networks Most Beautiful: connecting diversity to alterations in a gene regulatory network

Thomas M. Williams
University of Dayton

The origination and diversification of morphology represents a key problem in understanding the evolution of development. Morphological traits result from gene regulatory networks (GRNs) that form a web of transcription factors, which regulate *cis*-regulatory element (CRE) sequences to control the expression of differentiation genes. The formation and modification of GRNs must ultimately be understood at the level of individual regulatory linkages (transcription factor binding sites within CREs) that constitute the network. Our research studies the pigmentation patterns adorning the segmentally repeated abdominal tergites of fruit flies. In the *Sophophora* subgenus, it is thought that dimorphic pigmentation of the male tergites evolved from a monomorphic ancestral state in the *Drosophila melanogaster* lineage. Pigmentation is made by temporally-, spatially-, and sex-specific expression of differentiation genes encoding pigment metabolism enzymes. CREs for these genes respond to the landscape of abdominal *trans*-regulators. I will share how dimorphic pigmentation and its diversification resulted from the GRN's recruitment of ancient duplicated transcription factors through changes to pigment enzyme CREs, and how diversity has been driven by changes elsewhere to the landscape of *trans*-regulators. We suggest that these observations typify how evolutionary changes in GRNs individualize segmentally repeated traits to generate more elaborate morphologies.

Origin and evolution of the WUSCHEL-RELATED (WOX) homeobox transcription factors in plants

Cheng-Chiang Wu, Elena M. Kramer
Harvard University

The WUSCHEL-RELATED (WOX) transcription factor family encodes homeobox-containing proteins that regulate the maintenance of stem-cell niche and organ patterning in plants. The radiation of the WOX gene family has been postulated as a factor in the morphological complexity of land plants. Previous phylogenetic studies classified the WOX genes into three superclades (the ancient, intermediate and modern clades). However, the relationship among WOX clades remains unclear due to excessive sequence divergence outside the short and highly conserved homeodomain, and limited sampling across plant lineages. In order to decipher the origin and evolution of the WOX family, we curated WOX protein sequences from 36 genomes and 351 transcriptomes spanning Plantae and Rhodophyta for phylogenetic reconstruction. The WOX phylogeny inferred from 1019 WOX proteins of 254 species implies that the WOX protein family may have originated in Plantae with three ancient superclades. Analyses of synteny, protein motifs, and promoter sequences, reveal lineage-specific gene duplications, protein

diversification, and deep conservation of the WOX-involved gene regulatory networks (GRNs). This study provides the phylogenetic context for functional studies of the GRNs that orchestrate morphological complexity in land plants.

Somite Compartmentalization in Amphioxus: on the Evolutionary Origin of Vertebrate Skeletons

Luok Wen Yong, Tsai-Ming Lu, Song-Wei Huang, Che-Huang Tung, Rui-Jen Chiu, Kun-Lung Li, Jr-Kai Yu
Academia Sinica

Mineralized skeletal tissues are widely considered as a vertebrate innovation. However, the evolutionary origin of the vertebrate skeletal tissue has not been investigated thoroughly in invertebrates. Our observations, amongst others, show that amphioxus possess cartilaginous components in the oral cirri, notochordal sheath, and in a cell layer underneath the epidermis. The development of these structures has been shown to be closely associated with the mesothelial cells, derived from the non-myotome part of the somite. In this study, we characterized extracellular matrix genes crucial for skeletal tissue development and known somitic marker genes. We show that the amphioxus somite is compartmentalized into four regions, including a central region (myotome) and three lateral sub-regions, possibly corresponding to the dermatome, sclerotome, and lateral plate mesoderm. Additionally, we confirmed the role of Bone morphogenetic protein (BMP) signaling pathway on medio-lateral patterning of the somite. Since the amphioxus possess rudimentary skeletal elements derived from the somite which share various features of the vertebrate somites, we postulate that mesoderm-derived skeletogenic tissues arose in the common ancestor of all chordates. The expansion of tissue mineralization genes would act upon the foundation of such rudimentary skeletogenic elements to give rise to the hypermineralized skeleton of the vertebrates.

The genetic basis of evolutionary transitions in early development using a polychaete model

Christina Zakas, Matthew Rockman
New York University

Phenotypic evolution in animals is constrained by the mechanics of early development. How do major transitions in development occur? Historically, efforts to address this question have been limited to comparative methods. The polychaete annelid *Streblospio benedicti* provides a unique opportunity to use forward genetics to experimentally dissect a major transition in animal development. *S. benedicti* is ideal because it produces two distinct offspring types that differ in egg size, early development, and larval morphology. *S. benedicti* is thus a genetic model for the evolutionarily common transition between indirect and direct development. Using genetic crosses between these types, we constructed the first annelid genetic map, which reveals the distribution of genetic factors affecting a suite of genetically separable developmental phenotypes. Because early development is strongly influenced by maternal effects, our cross design disentangles maternal and zygotic genetic effects and shows that a transition from indirect to direct development requires contributions from both the zygotic and maternal genome; an increase in egg size alone is not sufficient to change development mode.

By identifying the loci responsible for regulating early development, we uncover how the dimorphic developmental program is determined on a whole-genome level.

Poster Abstracts

1. Does the Developing Eye Affect Assembly of the Upper Jaw in Reptiles?
John Abramyan
University of Michigan, Dearborn

Reptile embryos (including those of birds) develop disproportionately large eyes when compared to mammals. The developing eyes span the lateral sides of the head and directly abut against the embryonic craniofacial prominences, which consist of the frontonasal mass (including medial nasal processes), lateral nasal processes and maxillary prominences. The facial prominences eventually fuse to form an intact upper jaw, encompassing the nasal cavities, upper lip and anterior palate (together termed the primary palate). Perturbation of this developmental process may result in deformation of the upper jaw, including the formation of a cleft; a potentially life-threatening condition in vertebrates. Due to the position and substantial size of the eyes, we and others hypothesized a role for these large eyes in influencing fusion of the prominences during primary palatogenesis by pushing the prominences forward during the fusion process. To test the aforementioned hypothesis, we performed unilateral ablation of the eye primordium in chicken embryos and allowed them to develop past the point of primary palate fusion. Contrary to our hypothesis, the eye did not seem to substantially affect fusion of the primary palate. Fusion still occurred almost simultaneously on treated and untreated sides, although we did observe minor changes in size, shape and position of the component structures in the primary palate.

2. *CANCELLED

3. Nervous system organization: A molecular characterization of neuronal cell types in Saccoglossus
Jose M. Andrade Lopez, Ariel Pani, Paul Minor, Christopher Lowe
Stanford University

Vertebrates and other bilaterians are characterized by a central nervous system (CNS) with a brain and nerve cord, and their neural organization has been well-characterized. Hemichordate enteropneusts have elements of both central and diffusely organized nervous systems because they have both a diffuse epithelial plexus and ventral and dorsal nerve cords. However, we have a poor understanding of the composition and organization of this nervous system. Interestingly, the gene regulatory network (GRN) for anterior-posterior patterning is well conserved between vertebrates and hemichordates even though the nervous system they specify are anatomically different. This makes direct comparisons challenging. It remains a possibility that there are neural cell type homologies under conserved positional regulation between vertebrates and hemichordates. To test this hypothesis, a comprehensive molecular characterization of the hemichordate nervous system is required. With a sequenced genome and extensive EST resources, we performed molecular comparative studies to determine whether there is any

conservation between AP patterning and neural cell type specification. We use immunohistochemistry, in situ hybridization, and transgenic approaches to characterize neural sub-type regionalization and neural morphology. This will facilitate the identification of regions of integration, and a more sophisticated understanding of nervous system organization for more informative comparisons with chordates.

4. Functional genomics of host-associated differences in an insect wing polyphenism
David R. Angelini, Meghan M. Fawcett, Mary C. Parks, Alice E. Tibbetts, Elizabeth M. Richards, Juan Camilo Vanegas, Jane S. Swart, Wenzhen Stacey Hou, William R. Simmons, Laura Crowley, Joshua Steele
Colby College
Phenotypic plasticity is thought to evolve through changes in development altering the integration of environmental cues. Our lab studies the evolution of plasticity and its underlying developmental regulation in populations of the red-shouldered soapberry bug, *Jadera haematoloma* (Hemiptera: Rhopalidae). These insects exhibit polyphenism in which a non-linear response to juvenile nutrition produces distinct morphs that specialize in dispersal versus fecundity. The response of soapberry bug morphs to their nutritional environment has evolved in approximately 80 years as they have adapted to an introduced host plant. Differences in morphs and host-associated ecotypes are associated with differences in insulin signaling, and the plastic response of ancestral-state bugs can be shifted to resemble the low plasticity response of derived bugs by RNA interference targeting insulin-signaling components. These results suggest that evolution of this polyphenic reaction norm has occurred by changes in insulin signaling during adaptation to a novel nutritional environment. We characterize this system using a combination of geometric morphometrics, transcriptome comparisons, cross rearing, functional genetic tests, as well as studies of fecundity.

5. The architecture of adaptation: a master mutation or a mass of mutations?
Sophie L. Archambeault, Catherine L. Peichel
University of Washington; University of Bern

Adaptation to divergent environments often requires changes in many different phenotypes. Adaptation can therefore be facilitated when the genetic loci that underlie different phenotypes are clustered in the genome. Although genetic analyses in many systems is starting to reveal that such “phenotypic hotspots” exist, it is almost completely unknown how these hotspots are built during evolution and whether they are due to linkage of multiple mutations or to pleiotropic effects of a single mutation. Several “phenotypic hotspots” have been identified in the threespine stickleback (*Gasterosteus aculeatus*), an excellent model for studying the genetic basis of adaptive evolution. We are focusing on a hotspot on chromosome IV that overlaps with a region of high genomic divergence between marine and freshwater ecotypes, suggestive of repeated selection on one (or more) of the phenotypes that map to the hotspot. To determine whether linkage or pleiotropy is responsible for this hotspot, we are performing association mapping of multiple traits in a fully interbreeding, polymorphic, freshwater population of threespine stickleback. Our preliminary data suggest that the

clustering of traits in this region is due at least in part to pleiotropic effects of a single genetic change.

6. A bone-like tissue in the vertebral skeleton of Skates
Oghenevwogaga J. Atake, David Cooper, Brian Frank Eames
University of Saskatchewan

Bone is a feature of the vertebrate skeleton, but cartilaginous fishes (sharks, skates, chimaeras and relatives) are believed to have evolved without the ability to make bone. Despite reports of bone and bone-like tissue in the vertebrae of sharks, the presence or absence of “true” bone in cartilaginous fishes remains a debate. How widespread this feature might be in other lineages of cartilaginous fishes remains unknown. Using micro-CT 3D imaging and histology, we tested the hypothesis that a bone-like tissue is present in the vertebrae of cartilaginous fishes. By analysing the patterns and staining properties of mineralized skeletal tissues in skate vertebrae, we show that an extensively mineralized bone-like tissue is present in the neural arches. This bone-like tissue surrounded a core of mineralized cartilage. We also show evidence of trabecular tesserae – a mineralized tissue with a trabecular microarchitecture but exhibiting histological features of classic tesserae – in the neural spine of the vertebrae. Quantitative analysis using CTan further showed that trabecular tesserae has a thickness like trabecular bone. The presence of a bone-like tissue and trabecular tesserae provide a novel illustration of the reinforcement of the vertebrae of skates by methods analogous to cortical thickening and trabeculation in vertebrate bone

7. Reduction of the braincase in the Dissorophoidea clade
Jade Atkins, Robert R. Reisz, Hillary C. Maddin
Carleton University

Dissorophoideans are temnospondyls that first appear in the Late Carboniferous. Dissorophoidea has two clades, the Olsoniformes and Amphibamidae, the latter of which likely includes lissamphibians. The lissamphibian braincase has long been thought to be representative of the ancestral tetrapod form. Older research suggested that early tetrapods, including lissamphibians, had fewer elements in their braincases and amniote evolution involved the recruitment of additional elements. However, recent research has shown that instead the lissamphibian braincase may be the product of a series of transformations. Hindering research into this question has been the lack of phylogenetic analyses for Dissorophoidea that includes taxonomically dense sampling and characters to document braincase changes. Thus, our goal is twofold: (1) produce a high-resolution Dissorophoidean phylogeny and (2) use it to visualize braincase evolution. Our analysis includes 117 characters and 47 taxa. Our matrix allows us to study braincase evolution and shows a clear trend towards reduction leading to lissamphibians, characterized by one absence and three losses. Dissorophoidea lacks a supraoccipital. Olsoniforms have a basioccipital, which amphibamids have lost. Lissamphibia share the additional losses of the basisphenoid and hypoglossal nerve foramina. Together, these losses suggest the lissamphibian braincase is highly derived and not representative of tetrapod ancestral morphology.

8. Evolution of the hypoxia-sensitive cells involved in amniote respiratory reflexes
Clare V.H. Baker, Dorit Hockman, Alan J. Burns, Gerhard Schlosser, Keith P. Gates, Alessandro Mongera, Shannon Fisher, Gokhan Unlu, Ela W. Knapik, Charles K. Kaufman, Christian Mosimann, Leonard I. Zon, Joseph J. Lancman, P. Duc S. Dong, Heiko Lickert, Abigail S. Tucker
University of Cambridge

*NOW A TALK. See abstract above.

9. Putative molecular mechanisms of light-dependent magnetoreception in zebrafish
Spencer D. Balay, W. Ted Allison
University of Alberta

Earth's magnetic field acts as a navigational cue for many organisms. Although a large amount of behavioral evidence exists, the underlying mechanisms of magnetoreception remain unknown. Cryptochrome (Cry), a highly-conserved protein involved in the circadian clock, is a likely molecular candidate. Cry is co-localized with UV cone (sws1) photoreceptors in birds and the homologous blue cones in some mammals. Due to the molecular inaccessibility of the favoured avian model, cones requirement for Cry expression and ultimately magnetoreception has seldom been tested. This project uses zebrafish (*Danio reiro*) to study UV cones role in *Cry* expression. Zebrafish have six isoforms of *cry*. While most participate in circadian clock, two genes (*cry2* and *cry4*) appear functionally divergent. We hypothesized that non-circadian *cry* expression would be dependent on sws1 expression. We predicted that non-circadian *cry* expression would decrease if *sws1* expression decreased. Using fluorescent in situ hybridization we found that *cry4* is expressed in zebrafish UV cones. Using nitroreductase (NTR) mediated cell ablation and qRT-PCR, we found that *cry4* expression dramatically decreased when UV cones (*sws1*) were ablated, but not when blue cones (*sws2*) were ablated. This study serves as the first evidence that *cry4* is co-localized in zebrafish UV cones and suggests a potential role in magnetoreception.

10. *POPOVICH* encodes a C2H2 transcription factor essential for the development of a key innovation, floral nectar spurs, in the genus *Aquilegia*
Evangeline S. Ballerini, Scott A Hodges
University of California, Santa Barbara

The evolution of novel features that allow organisms to explore and exploit their environment in new ways, such as eyes or wings, can lead to increased diversification rates. Therefore, understanding the genetic and developmental mechanisms involved in the evolution of these key innovations has long been of interest to evolutionary biologists. In flowering plants, floral nectar spurs represent such a key innovation, with the independent evolution of spurs leading to increased diversification rates in multiple lineages through their ability to influence pollinator shifts. As none of the traditional model taxa have nectar spurs, little is known about their genetic or developmental basis. Nectar spurs are a defining feature of the genus *Aquilegia* (columbine), a lineage that has experienced a relatively recent radiation and for which a

number of genetic tools have been developed. We used a combination of genetic mapping, gene expression analyses, and functional assays to identify a gene crucial for nectar spur development, *POPOVICH* (*POP*), which encodes a C2H2 transcription factor. We further examine the molecular evolution and expression patterns of POP between *Aquilegia* and its closest spurless relatives, the genera *Semiaquilegia* and *Urophysa*, to begin to understand the role that POP may have played in the evolution of nectar spurs in *Aquilegia*.

11. Lab Versus Wild: Phenotypic Covariation in Threespine Stickleback
Tegan Barry, Heather A. Jamniczky, Sean Rogers

The integrated nature of form and function dictates that suites of phenotypic traits often vary together in response to selective pressures. This correlation between traits, or phenotypic covariation, along with its underlying genetic architecture, allows for the investigation of how organisms respond and adapt to novel environments and challenges. The Threespine Stickleback (*Gasterosteus aculeatus;* stickleback) are an ideal model to characterize phenotypic covariation due to their repeated parallel colonizations of freshwater lakes from the marine environment. Examination of this radiation along with its characteristic phenotypic changes allows for the investigation of the changes in phenotype and phenotypic covariation between the putative ancestral, marine form and derived freshwater form; however, whether these differences are caused by plastic phenotypic changes induced by varied environments or genetic mechanisms remains a question of great interest. Using micro-computed tomography and three dimensional (3D) geometric morphometrics, skeletal phenotypes of both wild-caught parental and lab-reared F1 stickleback were quantified and compared to determine if the phenotypic covariation patterns seen in wild populations persist in a common-garden environment. Investigation into the phenotypic covariation patterns seen in wild-caught fish have served as a starting point for comparison of both pure and marine-freshwater hybrid F1s to examine if these patterns are conserved between parent and offspring, and to determine the extent to which these patterns are altered in hybrid fish. The results from this study will help us understand the influence of both environmental and genetic factors on the nature of phenotypic covariation.

12. *CANCELLED

13. Egg size mediated maternal effects and early developmental changes in gene expression in Arctic char
Samantha Victoria Beck, Katja Räsanen, Zophonías O. Jónsson, Bjarni K. Kristjánsson, Skúli Skúlason, Camille A. Leblanc
University of Iceland

Understanding how developmental processes interact with the environment, affecting gene expression, is key to furthering our understanding of the determinants of phenotypic variation and diversification of natural populations. Maternal effects are putative but rarely investigated, facilitators of adaptive diversification. In polymorphic Arctic charr (*Salvelinus alpinus*), the differential distribution of maternal resources (i.e. egg size) has been shown to influence

offspring morphology and feeding behaviour, suggesting that egg size mediated maternal effects, jointly with developmental plasticity of offspring, may play a key role in the evolution of resource polymorphism. We used a wild population with large variation in egg size to test the hypothesis that smaller embryos have higher expression of genes related to growth and skeletal development. In partial accordance with our hypothesis, smaller embryos had higher skeletal-related gene expression at eye stage and hatching, whilst larger embryos had higher expression of growth-related genes, but at hatching stage only. We propose that smaller individuals may invest in developing important craniofacial structures to enable earlier feeding, whilst larger individuals continue to grow larger likely because of increased survival benefits.
Our results provide novel insight into the potential role of maternal effects in influencing divergence processes in natural populations of Arctic charr.

14. Clarity on skeleton evolution

Joao F. Botelho, Bhart-Anjan Bhullar
Yale University

The questions about the mechanisms which control skeletal differentiation and their evolutionary consequences can be addressed by making comparisons between different species. Nevertheless, the study of initial stages of endochondral ossification faces some technical hindrances regarding sectioning, orientation of samples and low cell density (which reduces signal in experimental assays). In order overcome most of these obstacles, we adapted recently developed whole-mount techniques based on hydrogel matrix embedding (Clarity). The technique allows detailed temporal comparisons of immunolabeled embryonic skeletons and associate tissues in model and non-model tetrapods (chicken, quails, ducks, anoles, alligators, turtles, and opossums). We have shown that the protocol is also compatible with fluorescent in situ hybridization using hairpin amplification. Combined with optical section using structured illumination, the technique permits characterizing precisely histological and molecular changes during the development of the skeleton of whole mount embryos, facilitating the integration of molecular data to changes in the growth and shape of the skeletal system.

15. A morphological and histological investigation of imperfect appendage regeneration of the salamander Ambystoma mexicanum and the West African lungfish *Protopterus annectens*

Vivien Bothe, Nadia B. Fröbisch
Museum für Naturkunde Berlin, Leibniz Institute for Research on Evolution and Biodiversity

Axolotl display a high degree of tissue regeneration which is unparalleled in its ability to completely replace body parts. Outside Tetrapoda, lungfish show regenerative capacities that are comparable to those of salamanders. Although major studies made great progress in understanding the cellular mechanisms on a molecular level during regeneration, much is still unclear regarding the histology and gross morphology of regenerated appendages beyond the blastemal forming phases. The presented study provides a morphological and histological investigation of regenerated axolotl limbs and lungfish fins to assess whether regenerated body parts are exact replica of the original. Data from 55 axolotl larvae, 29 with bitten limbs and 26

with amputations, were analyzed. Amputations were executed in several larval stages and at different limb positions (middle of the humerus, above the mesopod). In addition, two lungfish with regenerated fins after bite injuries were examined. For all samples histological serial sections, 3D reconstructions, and x-ray microtomography scans were generated. After regeneration, both axolotl limbs and lungfish fins show an abundance of abnormalities which pointed to imperfect regeneration. The severity of pathologies in axolotl varies in correlation with amputation vs. bite injuries, amputation along the proximodistal limb axis, and with ontogenetic age of the axolotl.

16. Can anatomical network analysis help explain anatomical evolution in marsupials and placentals?

Julia C. Boughner, Janine M Ziermann, Rui Diogo, Borja Esteve-Altava
University of Saskatchewan

Animal body parts evolve with variable degrees of integration but result nonetheless in functional adult phenotypes. But how do parts evolve without compromising function and survival? Building on recent insights that physical contacts among body parts influence evo-devo integration and modularity, we studied here the musculoskeletal modularity and integration of adult heads, and upper and lower limbs, of mouse, opossum and human using Anatomical Network Analysis (AnNA). Using tools from network theory, AnNA quantifies the arrangement of contacts among body parts with even very different shapes, sizes and tissue types. We hypothesized significant differences between the networks of marsupials and placentals, particularly for cranial musculoskeletal networks. The topology of contacts between bone:bone, bone:muscle, and muscle:muscle showed that, among all three species, skeletal networks were more similar than musculoskeletal networks, and human and mouse networks were more similar to each other than to opossum. Also, differences were greatest among musculoskeletal networks of heads, and then, of forelimbs, which showed more variation than hindlimbs. We discuss our results in light of evo-devo variation in mammalian life histories including development, and functional adaptations including locomotion and suckling, and explore ways to include further systems biology and anatomical network analyses into evo-devo research.

17. Ancient fishes illuminate the genomic basis of vertebrate Evo-Devo

Ingo Braasch, Spotted Gar Genome Consortium, John H. Postlethwait
Michigan State University

Teleost fishes like zebrafish are commonly used to investigate vertebrate developmental and genomic evolution. However, teleosts are derived from a teleost-specific genome duplication (TGD) that had major impact on their genome and gene function evolution. Together with the earlier vertebrate genome duplications (VGD1/2), this complicates vertebrate macroevolutionary comparisons: 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. We show that spotted gar, an ‘ancient’ fish that diverged before the TGD, provides connectivity among vertebrate genomes and informs the

ancestry of vertebrate development. The gar genome is representative of the bony vertebrate ancestor and retained many VGD1/2 paralogs differentially lost in other lineages. Gar facilitates the identification of cis-regulatory elements, revealing hidden orthology of regulatory elements across distant vertebrates. Genomic alignments of gar to other vertebrates allow genome-wide identification of regulatory element gains and losses during vertebrate evolution and reveal the role of paralog sub-/neofunctionalization for vertebrate development. We rear gars in the laboratory to developmentally test hypotheses about the evolutionary origins of vertebrate gene functions. Gar is thus a powerful new model to study the genomic foundation of vertebrate Evo-Devo.

18. Embryonic odontogenesis in the leopard gecko reveals contributions to tooth size disparity

Kirstin S. Brink, Theresa M Grieco, Joy M Richman
University of British Columbia

The leopard gecko (*Eublepharis macularius*) offers an opportunity to study the developmental controls over tooth size as it has egg teeth at hatching that are five times larger than the contemporaneous marginal teeth and yet form from the same maxillary dental lamina. We examined embryonic odontogenesis to determine whether larger cell size, an increased rate of cell proliferation, or larger cell numbers within tooth germs can explain differences in tooth size. PCNA immunofluorescence on 28 embryonic specimens reveals that at all stages, the size of epithelial cells in the tooth germs and the amount of proliferation are equivalent between tooth types. However, at the earliest stage, the egg teeth are at cap stage and have up to three times as many cells as the bud stage maxillary teeth. Therefore, the large size of the egg teeth is created by earlier initiation and allocation of more cells to tooth germs, but not increased proliferation. The developmental origins of tooth size disparity in this model suggest that changes to placode patterning and developmental timing may be stronger variables than differential growth in creating fixed size differences in heterodont dentitions.

19. Stranger than finches: molecular mechanisms of adaptive skull shapes in neotropical leaf-nosed bats

Jasmin Camacho, Rachel Moon, Samantha Smith, Cliff Tabin, Arhat Abzhanov
Harvard University

The New World leaf-nosed bats (phyllostomids), the most ecologically diverse clade of mammals, display exemplary morphological adaptations associated with specialized modes of feeding. Phyllostomid bat skulls underwent significant alteration, most notably in the cranial base, cranial vault, and the length of the face. These changes, among others, occurred over a very short evolutionary interval, which brings into focus underlying developmental mechanisms behind those changes. Despite the wealth of studies into bat evolution virtually nothing is understood about the developmental events responsible for the emergence of their cranial diversity. In this study, we describe, quantify and compare BMP signaling molecules required for cartilage and bone development in the evolution and development of phyllostomid bats. Preliminary data on proliferation, apoptosis, and cranial neural crest cell distribution are

highlighted during craniofacial development between bat species. This study provides the foundation for testing hypothesis about the link between ontogeny and phylogeny. Specifically, gene expression may be experimentally replicated in the mouse embryo and subsequent phenotype examined to evaluate if evolutionary patterns are mimicked. This will reveal causative connection between morphological and molecular changes.

20. Temperature impacts evolvability through multiple forms of phenotypic plasticity
Calum S. Campbell, Colin Adams, Colin Bean, Kevin Parsons
University of Glasgow

Temperature can alter development and in turn the phenotypic variation available to selection (i.e. evolvability). Therefore, understanding interactions between development and temperature will inform mitigation strategies in the face of climate change. Adaptive divergence in the form of resource polymorphisms is common in Arctic charr (Salvelinus alpinus) usually occurring between benthic and limnetic habitats. While these habitats provide differences in foraging ecology it is less appreciated that they also include differences in temperature, and thus developmental conditions. We focused on skeletal development in charr and hypothesized that temperature would modify 1) ossification and bone metabolism, 2) morphological plasticity, and 3) performance. Embryos were incubated at two temperatures (5°C and 9°C to mimic a predicted arctic temperature increase due to climate change) and were sampled at two developmental stages. Ossification and bone metabolism differed at equivalent stages between temperatures, while foraging trials indicated that morphological responses to temperature conferred adaptively relevant effects. Notably, phenotypic plasticity was increased under higher temperatures. Given these results we argue a developmental perspective can greatly inform fundamental questions surrounding evolvability which can also be relevant to imminent conservation issues.

21. The evolution of organ developmental programs across mammals
Margarida Cardoso-Moreira, Henrik Kaessman
University of Heidelberg

The evolution of novel phenotypes requires changes in pre-existing developmental programs. One approach to identify the molecular changes that underlie new mammalian phenotypes is, therefore, to compare developmental gene expression programs across mammals. To this end, we generated gene expression profiles covering development in 7 species (human, macaque, mouse, rat, rabbit, opossum and chicken) for 7 major organs (brain, cerebellum, heart, kidney, liver, ovary and testis). Our developmental time courses cover most of organogenesis and later tissue development (both prenatal and postnatal, for a total of ~ 2,000 RNA-seq libraries). In agreement with the observed increase in morphological divergence between species with developmental time, as first described by von Baer, we find that the conservation of developmental programs decreases over time. One facet of this decrease in conservation is the increased deployment of lineage-specific genes with developmental time, thus supporting an important role for new genes in driving morphological/physiological change. After matching developmental stages across species, we identified the genes that have significantly changed

their developmental profiles between species. Overall, these genes are strong candidates for underlying lineage- and species-specific morphological and physiological phenotypes and further help elucidate the molecular changes responsible for developmental change and thus new phenotypes.

22. Investigating the evolution and genetic basis of jointed fruits in the Brassiceae (Brassicaceae)

Shane F. Carey, Jocelyn C. Hall, Kerrin Mendler, Navjot Singh
University of Alberta

Fruits of Brassicaceae represent an ideal system to investigate the evolution of novel, ecologically important traits. The remarkable morphological variation seen likely impacts seed dispersal. Members of the tribe Brassiceae have a unique fruit type in the family, where the fruits are laterally segmented by a novel structure referred to as the joint. This segmentation, known as heteroarthrocarpy, is accompanied by variable dehiscence in the proximal segments. Further, indehiscence of proximal segment is often correlated with two segments abscising (separating) at maturity. Here we continue our investigations of *Erucaria erucarioides* and *Cakile lanceolata*, closely-related species which vary both in proximal dehiscence and joint abscission. Thus, we can make both within fruit and between species comparisons. We build on previous comparative gene expression studies by comparing RNA-seq data obtained from three regions of young fruits: proximal, joint, and distal. These data are complemented by qRT-PCR expression profiles at multiple developmental stages. Analyses suggest that the joint has a significantly different gene expression profile, especially when compared to the proximal segment. These data shed light on how differential expression of fruit patterning genes in these taxa lead to variable morphologies and dispersal capabilities.

23. Determining the influence of environment on quantitative size generation through epigenetic regulation

Travis W.L. Chen, Ehab Abouheif
McGill University

Quantitative traits vary continuously within a population and are a pervasive feature in all organisms. Body size, reproductive ability, and disease susceptibility are all examples of traits that vary quantitatively in nature. Although there is a wealth of knowledge on the genetic basis of quantitative traits, the role of the environment and how it interacts with genes to produce quantitative phenotypes in nature is poorly understood. DNA methylation is an epigenetic mechanism known to mediate this interaction by chemically modifying DNA in response to variation in the environment, and consequently, can alter gene expression and phenotype. We have previously shown that quantitative DNA methylation of several nucleotides on the gene epidermal growth factor receptor (*Egfr*) generates continuous size distribution between workers of the ant *Camponotus floridanus*. Quantitative methylation of major effect genes, like *EGFR*, may be a general mechanism by which DNA methylation mediates environmental factors to generate quantitative phenotypic variation in natural populations. However, the link between environmental factors and quantitative DNA methylation of Egfr remains unknown.

The goal of this research is to understand the complex interactions between the quantitative DNA methylation of genes and nutrition, hormones, socially-regulated pheromones, quantitative size variation and allometry in ants.

24. The Developmental Transcriptome Atlas of the Echiuran Worm Urechis unicinctus Sung-Jin Cho, Yong-Hee Han, Sung-Gwon Lee, Kyoung-Bin Ryu, Elizabeth M. A. Kern, Jong-Seok Park, Joong-Ki Park, Chungoo Park
Chungbuk National University

Urechis unicinctus, an echiuran worm inhabiting the U-shaped burrows in the intertidal and subtidal mudflats, is an important commercial and ecological invertebrate in China, Korea, Russia, and Japan, which has potential applications in the study of lophotrochozoan evolution and marine drug development. However, knowledge is limited on the developmental molecular mechanism of *U. unicinctus*. The goal of this research was to enhance our understanding of gene expression during embryogenic development. Here we report the transcriptome profiling analysis of *U. unicinctus* early developing embryos using RNA-Seq as an attempt to gain insight into the molecular and cellular events associated with radial cleavage and spiral cleavage stage. It is an invaluable model species for research in the fields of evolution and phylogeny. This transcriptome sequencing effort has dramatically increased the number of known genes for this echiurans, and provides an invaluable resource for the discovery of potential roles of genes involved in various embryological and larval development processes in the *U. unicinctus*.

25. Finding wings in a non-winged arthropod
Courtney M. Clark-Hachtel, Nipam H. Patel, Yoshinori Tomoyasu
Miami University

The origin of insect wings is a biological mystery that has fascinated scientists for centuries. Through extensive investigations performed across various fields, two possible wing origin tissues have been identified; a lateral outgrowth of the dorsal body wall (tergum) and ancestral proximal leg structures. With each idea offering its strengths and weaknesses, these two schools of thought have been in an intellectual battle for decades without reaching a consensus. Identification of tissues homologous to insect wings from lineages related to insects will provide crucial information to resolve this conundrum. Here, through expression analyses and CRISPR/Cas9-based genome-editing in the crustacean, Parhyale hawaiensis, we show that both crustacean tergum and proximal leg segments share a gene regulatory network with the insect wing, and thus are likely crustacean wing homologs. These results parallel previous findings in some wingless segments of insects, where wing serial homologs are maintained as two separate tissues, suggesting that the situation in these segments reflects an ancestral state for the tissues that gave rise to the insect wing. These outcomes point toward a dual origin of insect wings, and thus provide a crucial opportunity to unify the two historically competing hypotheses on the origin of this evolutionarily monumental structure.

26. Genetics of an extra-cusp in insular domestic mice
Julien Claude, Arthur Weyna, Sabrina Renaud, Institut

des Sciences de l'Evolution de Montpellier

Understanding how cusps appear and evolve in mammalian teeth has motivated a long research in developmental and comparative biology. Although developmental and evolutionary models have been produced, we know little about the influence of genetic and environmental variation at the intraspecific level. Variation in cusp pattern is known within and between populations in several rodent species but the way that this patterning transmits from generations to generations has not been yet studied. We investigated here the environmental and genetic determinisms behind the formation of an anterior extra-cusp in domestic mice from the Orkney islands, North of Scotland. Based on large pedigree and Genotyping By Sequencing data, we investigated the relationship between the morphology of the tooth and genomic, genetic and environmental information. In order to understand how genomic and/or genetic divergences could explain the observed patterns, we incorporated either the social pedigree or the genomic distance in a linear mixed models. Our results suggest that the extra- cusp is heritable but that a large part of this heritability is non genomic; maternal effects likely play an important role, but other epigenetic effects are expected and could explain the gap of predictions between genetic and genomic data

27. Evolutionary sequence conservation in the identification of enhancers that regulate short-stature homeobox (Shox) gene expression during limb development in humans and mice
John Cobb, Isabella Skuplik, Anja Ljubojevic, Edgardo Rodriguez-Carballo, Samuel Abassah-Oppong
University of Calgary

Léri-Weill dyschondrosteosis (LWD) is characterized by a specific shortening of the zeugopodal elements of the limbs. A large number of LWD cases are caused by deletions of noncoding sequences clustered downstream of SHOX within the pseudoautosomal region 1 (PAR1) of the X and Y chromosomes. Presumably these deletions remove an enhancer or enhancers necessary for SHOX expression in developing limbs. We searched for this active sequence using a transgenic mouse assay and identified a 563 bp zeugopodal enhancer that is removed in almost all pathogenic deletions known to cause LWD. This sequence has previously escaped notice because of its poor evolutionary conservation, although it does contain an approximately 100 bp core sequence that is largely conserved in mammals. In parallel experiments, we sought to determine the cis-regulatory sequences controlling the expression of the mouse Shox2 gene in developing limb buds. Shox2 has a similar function as SHOX during limb development, although its function is required to form the more proximal styolopodal elements. We predict that an overlapping set of proteins regulate human SHOX and mouse Shox2 expression. We have generated a map of the Shox2 regulatory landscape using chromosome conformation capture techniques (4C-Seq) complimented by a transgenic mouse assay.

28. Using Developmental Simulations To Understand Odontode Patterning In Basal Gnathostomes
Aidan M. Couzens, Martin Rücklin
Naturalis Biodiversity Centre

Gene regulatory networks (GRN) controlling the development of homologous structures in phylogenetically disparate organisms are often highly conserved. But how can a fundamentally conserved GRN permit the generation of morphological diversity? One possibility is that modulation of signaling pathway interactions enables phenotypic disparity to emerge even when the GRN is invariant. During vertebrate evolution there has been diversification of mineralized epithelial organs (odontodes) such as denticles and teeth. Gene expression studies suggest that chondrichthyans (cat-shark) and osteichthyans (cichlids) share a similar odontode GRN but it is poorly understood how components of this GRN interact to pattern odontodes. Here, we outline a 'phenotype-back' approach which uses simulations of odontode patterning to examine oral odontode complexity. We aim to: (1) quantify the complexity of oral odontodes in basal crown-group gnathostomes; and (2) use the ToothMaker platform to replicate observed patterns of oral odontode complexity. We will use micro-computed tomography to scan oral odontodes from fossil and modern gnathostomes spanning the split between chondrichthyans and osteichthyans and then measure surface complexity with orientated patch count (OPC). We hypothesise that varying the synergism between signaling pathways and their interactions with tissue growth will provide mechanisms to generate different types of odontode complexity in silico.

29. Evolution of the pea aphid photoperiod response

Gregory K. Davis, Erin R. Bonner, Emily L. Spiegel, Grisilda Bakiasi
Bryn Mawr College

The pea aphid, *Acyrthosiphon pisum*, exhibits a remarkable adaptive response to seasonal changes in photoperiod. In spring and summer, aphids reproduce asexually, yielding large numbers of genetically identical female offspring. The longer nights accompanying the fall induce these asexual aphids to produce sexual males and females, which mate to lay frost-resistant eggs. These eggs diapause through the cold winter months, hatch into asexually reproducing females in the spring that found new clonal populations. Pea aphid populations have been shown to exhibit latitudinal variation in this photoperiod response, presumably reflecting local adaptation to variation in the timing of the first frost. Populations from the southern United States have been reported to exhibit attenuated photoperiod responses or to have lost the ability to produce sexuals altogether. Here we describe a previously detected difference in the photoperiod response between strains from the Northern and Southern United States. With an eye toward understanding what underlies this difference, we also describe differences in how these strains respond to juvenile hormone, which has been implicated in the induction of asexual fate, as well as strain differences in the expression of members of the juvenile hormone pathway.

30. A voxel-based morphometry study of intrauterine growth restriction

Jay P. Devine, Malcolm Eaton, James C. Cross, Benedikt Hallgrimsson
University of Calgary

The intrauterine growth restriction (IUGR) phenotype is generally described as differential growth restriction of various feto-placental tissues. "Brain sparing" (BS) is one hypothesis that is invoked to explain such asymmetric growth. BS suggests that during periods of intense

environmental stress, growth of other vital fetal organs (e.g., heart, liver and kidney) are sacrificed to preserve brain weight and head size, especially during late-gestation when the brain is rapidly growing and particularly vulnerable to growth retardation. IUGR organ growth trade-offs nevertheless remain relatively unexplored, largely due to poor imaging. Aside from chromosomal disorders, maternal malnutrition and placental insufficiency are the most frequent causes of IUGR. Using a mouse model of maternal protein malnutrition and placental insufficiency via placental prolactin-related gene knockouts, we examine variation in total fetal brain volume and shape, as well as region-based brain volumes to test the BS hypothesis and identify regional growth trajectories in the brain. Heart, liver, and kidney volumes are calculated in a subsample of embryos. To do so, we employ a combination of contrast-enhanced μCT scanning, image processing techniques (e.g. linear/non-linear registration and automated segmentation), and morphometric analyses.

31. A Model System for Studying Epistasis between Maternal and Zygotic Genomes

Margaret C. Dickson, Dan Pers, Jeremy A. Lynch
University of Illinois at Chicago

In *Nasonia*, a genus of parasitoid wasps, all species are able to produce viable F1 hybrids after being cured of *Wolbachia* infections. Recently, a laboratory strain of *N. longicornis* was found to have developed asymmetric F1 hybrid inviability with *N. vitripennis*, caused by an incompatibility between a maternal genomic factor from *N. longicornis* epistatically interacting with a zygotic factor from *N. vitripennis*. Examination of hybrid embryos has shown a failure of dorsal closure is the likely cause of the lethality. We propose that this is caused by disruption in extraembryonic development. I will take multiple complementary approaches to understand the evolutionary and developmental origins of this embryonic incompatibility. I will characterize the patterning and morphogenesis of extraembryonic membranes in the two parental species, and in both cross directions, to understand which types of developmental variation are and are not compatible with successful development. I will also take advantage of the convenient genetic tools in Nasonia to identify the loci that cause the incompatibility in order to gain insight into the molecular basis of the failure in development. These results will provide unique insights into the interactions of maternal and zygotic effects in evolution and development.

32. *CANCELLED

33. The evolutionary history of vertebrate vision through the eyes of the Pacific hagfish (*Eptatretus stoutii*)

Emily M. Dong, W. Ted Allison
University of Alberta

Cyclostomes occupy an important role in understanding the evolutionary history of vertebrate eyes. This highly contested group includes the only living representatives of jawless fish: lamprey and hagfish. Though lamprey exhibit a camera-style eye like that of jawed vertebrates, hagfish differ in that their eye is hidden beneath a layer of epidermis and lacks the characteristic 3-layered organization of a vertebrate retina. Due to this, the “simple” hagfish

eye is more closely compared to a pineal than to a vertebrate eye. Its primitive appearance paired with the phylogenetic positioning of hagfish allow for a unique perspective in the study of the evolution of the vertebrate eye. Preliminary evidence of rhodopsin, a visual pigment, expressed in the photoreceptors suggests that hagfish may share more diagnostic "vertebrate" features than previously observed. Using RNAseq, protein and gene expression assays, this project aims to explore the hagfish eye, with particular attention paid to photoreceptor sub-types and synaptic layers in the retina. Our goal is to capture any conserved retinal characters that might aid us in revealing the ancestral state that allowed for the evolution of the vertebrate eye.

34. Comparison of linear and nonlinear dimensionality reduction methods on morphometric data

Trina Y. Du
McGill University

Principal component analysis is the most widely used dimensionality reduction method in the biological sciences, and is commonly used to construct empirical morphospaces based on geometric morphometric data. However, inferences about evolution and development based on the distribution of morphologies in morphospace may be misled by PCA's assumption of linear dependency between variables. The performance of PCA was compared to nonlinear dimensionality methods on the task of 2D visualization of morphological variation. Visualizations generated by nonlinear methods were more trustworthy than those generated by PCA for many artificial and natural morphometric datasets. Choice of dimensionality reduction method has implications for the interpretation of morphospace analyses, particularly in the context of the prevalence of non-linearity in morphogenesis and the genotype-to-phenotype map.

35. Altered covariance structure reveals relaxation of developmental constraints in an asexual hybrid vertebrate

Kevin K. Duclos, Richard Cloutier, Bernard Angers, Heather A Jamniczky
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Changes in developmental constraints affect phenotypic variability and, subsequently, ecology. In this regard, hybrid organisms are interesting models as interspecific hybridization disrupts genomic coadaptation which may change developmental constraints in hybrids with regards to parental species. While the effects of hybridization are observable in first generation hybrids, second generation hybrids display additional changes following sexual recombination. An ideal model system would thus be a group of asexually reproducing hybrids. The *Chrosomus eos-neogaeus* hybridization complex harbors such asexual hybrids known to display genotype-dependent differences in phenotypes and ecology, and impressive phenotypic plasticity. This study aims to investigate how differences in developmental constraints affect phenotypic variability in *C. eos-neogaeus* hybrids. Morphological variation and covariation in the craniofacial skeleton were assessed and compared across two hybrid genotypes and their parental species using μCT imaging and three dimensional geometric morphometric analyses. Preliminary results reveal differences in morphology and in the structure of phenotypic

covariation, between both lineages and between hybrids and parental species. Hybrids seem to benefit from relaxed constraints, allowing phenotypic transgression. It remains unclear whether changes in developmental constraints in hybrids lead to an increase in variability and if this affects the ecology of a given hybrid genotype.

36. Development of curved petal nectar spurs in bee-pollinated *Aquilegia* (columbine)
Molly B. Edwards, Evangeline S Ballerini, Gary Choi, Elena M Kramer
Harvard University

Plant organ sculpting requires fine control of the timing and patterning of cell proliferation and expansion. The petals of *Aquilegia* (columbine) flowers are an extreme example of organ sculpting: they form elongated, three-dimensional nectar spurs that vary in shape and length depending on their pollinators (bee, hummingbird, or hawkmoth). The development of petals with the latter two syndromes has been well-characterized: early in petal development, differential cell division creates an out-pocketing at the site of the nascent spur; then, highly anisotropic cell elongation generates 90% of the final size of the long, straight spurs. The nectar spurs of bee-pollinated species, however, are short and curved. What cellular processes during development are responsible for creating curvature in the spur, and how do they differ from those in straight spurs? Given that the bee syndrome is the ancestral state, is the same developmental strategy deployed across bee-pollinated species to generate spur curvature? Answering these fundamental questions will inform our investigation into the genetic basis of nectar spur curvature.

37. The systematic-taxonomic hierarchy as an explanandum for Evo-Devo
Alejandro Fábregas-Tejeda, Francisco Vergara-Silva
Universidad Nacional Autónoma de México

In 1998, Gellon and McGinnis proposed a model in which differences in Hox gene number, changes in their expression domains, and modifications in the regulation of downstream genes are correlated with morphological divergences associated to three higher-level ranks –namely, phylum, class and order– of the systematic-taxonomic hierarchy (STH). Independently, Davidson and Erwin (2006) suggested another model where changes in hierarchically arranged modules of gene regulatory networks (GRNs) also have morphological consequences at specific ranks of the STH (e.g. kernels/phyla, plug-ins/classes). Davidson and Peters (2011) further elaborated this proposal, claiming that the hierarchical structure of GRNs in fact 'explains' the correspondingly nested structure of the STH. From a philosophy of biology standpoint, here we describe the epistemic similarities and discrepancies of the Gellon-McGinnis and Davidson-Erwin-Peters models, in order to analyze how Evo-Devo accounts for the hierarchically-arranged aspects of morphology associated to the STH. Our analysis, which rests on a historiographic reconstruction of the separate research traditions represented by our case studies, also involves an examination of the support that recent animal Evo-Devo empirical data provides for the models under consideration. We conclude addressing the implications of our historical-epistemological evaluation of Evo-Devo approaches to 'explanation' for the 'evo-devo versus devo-evo' distinction.

38. Kryptolebias marmoratus, a developing genetic model for vertebrate sexual plasticity

John A. Ficklin, Troy Anlage, Lena Boyer, Brianna Pierce, Helena Boldt, Matthew P. Harris, Ryan L. Earley, Eric S. Haag
University of Maryland

The mangrove killifish, *Kryptolebias marmoratus*, is an emerging model organism that shows great potential for use in genetic studies and in the study of developmental plasticity. The primary mode of reproduction for these fish is self-fertile hermaphroditism, mediated by small testis nodes in the outwardly female sex. *K. marmoratus* also have the potential to switch from hermaphrodite to male at low frequency as they age. The males court hermaphrodites and can fertilize a fraction of their eggs. This combination of selfing and facultative outcrossing is reminiscent of *Caenorhabditis elegans* and *Arabidopsis thaliana*, two of the most powerful genetic systems for multicellular eukaryotes. Here we present progress on several fronts that collectively set the stage for genetic analysis and manipulation, focusing on sexual plasticity. We have identified mutants from a mutagenesis screen in the Honduran strain Hon11, including a class which exhibit maleness at Mendelian frequencies. We also describe efforts to genetically map both induced and natural genomic variants related to sequential hermaphroditism. Finally, we describe experiments suggesting a simple way to increase fecundity of *K. marmoratus* hermaphrodites from all strains: frequent removal of eggs. These steps set the stage for positional cloning and genome modifications in *K. marmoratus*.

39. Comparative transcriptomics support the wing gene co-option hypothesis for the origin of the novel treehopper helmet

Cera Ruth-Marie Fisher, Elizabeth L. Jockusch
University of Connecticut

Understanding the origins of novel morphology is crucial to explaining the diversity of life. In the insects, new body parts often arise as projections of the body wall that are then sculpted by natural selection, e.g. beetles' horns and leaf insects' lobes. A stunning example of novelty is found in the hemipteran family Membracidae (treehoppers), sap-sucking insects with enlarged, sometimes elaborate projections of the pronotum (dorsal body wall) termed "helmets". Treehopper helmets have been molded by natural selection in an array of structures aiding predator defense via crypsis, mimicry, and aposematism. Members of their sister group, the leafhoppers (Cicadellidae), exhibit the plesiomorphic condition, a flat, shield-like pronotum. We tested three hypotheses for the developmental origin of the treehopper helmet using comparative transcriptomics in four tissues of nymphal *Entylia carinata* (a treehopper) and Homalodisca vitripennis (a leafhopper). Differential gene expression analysis indicates that in the leafhopper, the pronotum and mesonotum (both dorsal body wall) are most similar, as would be predicted of serial homologs. In treehoppers, however, the gene expression in the developing pronotum/helmet is most similar to that of wings. Many transcripts upregulated in both wings and helmet of the treehopper are known for their roles in *Drosophila* wing development, including nub, vg, and wg. This preliminary evidence supports a wing-co-option scenario for the origin of the treehopper helmet, wherein elements of the wing-patterning network are redeployed in the novel context of developing pronotal tissue. We are now evaluating the functional significance of these expression similarities with comparative RNAi in

E. carinata and *Oncopeltus* milkweed bugs.

40. Evolution And Multiple Roles Of The Pancrustacea Specific Transcription Factor zelda In Insects

Rodrigo Nunes da Fonseca, Lupis Ribeiro, Vitoria Tobias-Santos, Danielle Santos, Felipe Antunes, Georgia Feltran, Jackson de Souza Menezes, L. Aravind, Thiago M. Venancio
Federal University of Rio de Janeiro

Gene regulatory networks (GRN) evolve as a result of the coevolutionary process acting on transcription factors and the cis-regulatory modules (CRMs) they bind. The zinc-finger transcription factor (TF) zelda (zld) is essential for maternal zygotic transition (MZT) in *Drosophila melanogaster*, where it directly binds over thousand CRMs to regulate chromatin accessibility. *D. melanogaster* displays a long germ type of embryonic development, where all segments are simultaneously generated along the whole egg. However, it remains unclear if zld is also involved in MZT of short-germ insects (including those from basal lineages) or in other biological processes. Here we show that zld is an innovation of the Pancrustacea lineage, being absent in more distant arthropods (e.g. chelicerates) and other organisms. To better understand zld's ancestral function, we thoroughly investigated its roles in a short-germ beetle, *Tribolium castaneum*, using molecular biology and computational approaches. Our results demonstrate roles for zld not only during the MZT, but also in posterior segmentation and patterning of imaginal disc derived structures. Further, we also demonstrate that zld is critical for posterior segmentation in the hemipteran *Rhodnius prolixus*, indicating this function predates the origin of holometabolous insects and was subsequently lost in long-germ insects. Our results unveil new roles of zld in maintaining pluripotent state of progenitor cells at the posterior region and suggest that changes in expression of zld (and probably other pioneer TFs) are critical in the evolution of insect GRNs.

41. Using Machine Learning to reconstruct evolution of segmentation networks

Paul François, Jeremy B. Rothschild, Panagiotis Tsimiklis, Eric D. Siggia
McGill University

*NOW A TALK. See abstract above.

42. Presence and Absence of the Ocular Skeleton

Tamara A. Franz-Odendaal
Mount Saint Vincent University

The ocular skeleton of vertebrates was first studied by Tilley Edinger, a German scientist and daughter of a famous palaeontologist. Her work together with that of Walls two decades later laid the foundation for an assessment of the presence and absence of the ocular skeleton within vertebrates. Additional research has investigated the synapsids, mammal-like reptiles, as well as various squamates and teleosts. This study will summarize this data and she d light on where the gaps are in our current understanding. The homology of these structures across vertebrate groups will also be discussed.

43. Evolution of the gas bladder, a morphological novelty, in ray-finned fishes
Emily Funk
Cornell University

Understanding the origin of phenotypic novelties, such as jaws and limbs, is an important frontier of evolutionary biology. Because development governs body plan patterning, the evolution of new developmental programs and changes in expression of key regulatory genes must underlie the origin of these novelties. The gas bladder, derived from the lungs of the bony vertebrate common ancestor, originated as a phenotypic novelty in Actinopteri (all ray-finned fishes except bichirs). The distinguishing characteristic between the gas bladder and lungs is the developmental budding site; the gas bladder buds dorsally and the lungs ventrally from the foregut. Comparing gene expression patterns during development of ray-finned fish taxa that phylogenetically bracket the gas bladder origin will reveal spatial and temporal expression changes associated with the morphological shift from ventrally budding lungs to dorsally budding gas bladder. These taxa include bichirs (*Polypterus*), the only ray-finned fish family that still have lungs and bowfin (*Amia calva*), an early diverging lineage that develops a gas bladder. The homology between the gas bladder and lungs as air-filled organs is an ideal morphological transformation to investigate genetic changes underlying evolutionary novelty.

44. Molecular fingerprinting of skeletal tissues provides new insight into the gene regulatory networks (GRNs) driving mature cartilage differentiation
Patsy Gomez Picos, Amir Ashique, Katie Ovens, Ian McQuillan, Brian F Eames
University of Saskatchewan

A molecular fingerprint is the set of genes expressed by a cell type, and it is organized into several GRNs.We believe that the GRNs regulating immature cartilage, mature cartilage and bone are related. We hypothesize that mature cartilage is a combination of the GRNs driving immature cartilage and bone.We used LCM-RNAseq to identify the molecular fingerprints of these skeletal tissues.Several findings support the hypothesis. First,mature cartilage had the least DEGs and “unique” genes meaning that its molecular fingerprint is an overlap between immature cartilage and bone. Second, clustering analyses show that GRNs are acting independently in mature cartilage,whereas other clusters reveal that these GRNs are interacting.One particular cluster showed enrichment for genes in mature cartilage, meaning that a portion of the GRNs in this tissue are acting synergistically.Our data show that besides Sox9 and Runx2, there are other transcription factors that are directly correlated with genes in mature cartilage,suggesting that some of the GRNs driving this tissue have not been revealed. Finally,we identified a number of novel genes in mature cartilage, expression of which were confirmed using RNA-ISH. Future application of this technique to animals of different clades will permit quantitative assessments of the evolution of skeletal cell molecular fingerprints.

45. Investigating the role of gene duplication and divergence during arachnid evolution
Luis M. Baudouin-Gonzalez, Daniel Leite, Prashant Sharma, Alistair McGregor
Oxford Brookes University

Gene duplication underlies the origin and evolution of novel genes with new functions that can contribute to organismal divergence, as it allows for the accumulation of mutations that,

modifying coding and/or regulatory sequences of duplicated genes, can lead to subfunctionalization or neofunctionalization. Recent studies of spiders and scorpions have revealed a high prevalence of duplicated genes, including two Hox clusters and approximately 40% of other homeodomain containing genes. This supports the finding that there was a whole genome duplication event in the common ancestor of these animals (arachnopulmonates) that is not found in outgroups like ticks, mites and harvestmen. To better understand the impact of this WGD event during arachnid evolution, we have analysed the expression patterns of duplicated homeodomain genes in spiders and their single copy orthologues in harvestmen. Our results reveal striking patterns of sub and neofuncationalisation among ohnologs of homeodomain genes during embryogenesis in spiders when compared to harvestmen. This suggests that the retention of duplicated homeodomain genes after WGD in the ancestor of arachnopulmonates has made an important contribution to the diversification of these animals.

46. Deciphering the role of developmental constraints in the evolution of conodonts

Nicolas Goudemand, Louise Souquet, Pauline Guenser
ENS Lyon, Institute of Functional Genomics

We are interested in deciphering the relative roles of 'external' (environmental) and 'internal' (developmental) constraints on the course of morphological evolution in deep time. Conodonts were small, eel-shaped, marine vertebrates whose abundant fossils consist mostly of their tiny, phosphatic feeding elements. The set of possible conodont element morphologies is obviously constrained by the way these morphologies can be generated during ontogeny, which may have driven conodonts' evolution into preferential directions. Owing to their 300-Myr-long and very rich record, conodonts have a great potential for analyzing the relative roles of environmental vs. developmental factors in evolution. Yet, hitherto, conodonts have been used mostly as biochronological or geochemical tools but patterns of morphological variation have been documented only qualitatively and at the species level (e.g. within taxonomic diagnoses), and no generalized pattern of variation or covariation has been described for conodonts. In order to constrain our interpretations of presumed adaptation to diets and environments, it is mandatory to better understand if and which traits might be adaptive. Here we have 3d scanned hundreds of conodont elements and investigated quantitatively patterns of phenotypic integration in two distinct and evolutionary distant lineages, one from the Late Devonian, and one from the Late Triassic.

47. Single cell developmental transcriptomics in *Saccoglossus kowalevskii*

Jessica Gray, James A. Briggs, Leonid Peshkin, John Gerhart, Allon M. Klein, Marc W. Kirschner
Harvard Medical School

Diverse cell types are generated over the course of both developmental and evolutionary timescales. While the developmental mechanisms for generating particular cell fates through the expression of distinct sets of genes are increasingly well understood, it is unclear how novel cell types are generated or how different animals produce similar cell types through different developmental paths. To investigate the diversity and origins of cell types in deuterostomes we have used a droplet-microfluidic barcoding method to determine single-cell gene expression profiles across development in the hemichordate *Saccoglossus*. More than 15,000 single cell

transcriptomes were sequenced from 5 stages, from blastula to 1 gill slit, and clustered to characterize cell types. We can track lineage segregation and identify increasingly diverse populations of cells through development. This comprehensive dataset is being used to assess the numbers and diversity of cell types in *Saccoglossus* and gain insight into the developmental evolution of cell type diversity. We are focusing on developmental cell fate choices as well as neural diversity and organization in the very differently organized nervous system of *Saccoglossus*. This developmental atlas of cell types by gene expression will prove a useful resource for future comparative developmental studies and investigations of cell type evolution.

48. The developmental basis of forelimb reduction in the emu (*Dromaius novaehollandiae*)

Phil D. Grayson, John J. Young, Clifford J. Tabin, Scott V. Edwards
Harvard University

Flight has been lost numerous times across the avian tree in diverse taxa including parrots, rails, ducks, and ratites (flightless Palaeognaths). One of the striking convergent phenotypes in flightless birds is forelimb reduction. Within the ratites, the emu, kiwi, and cassowaries possess vestigial forelimbs with digit reductions, whereas the extinct moa had no forelimbs whatsoever. Developmentally, emu forelimbs are delayed and reduced compared to its hindlimbs, and to fore- and hindlimbs of flight-capable (volant) species, but the mechanism for this delay is unknown. Prior to limb outgrowth, lateral plate mesoderm (LPM) undergoes an epithelial-mesenchymal transition (EMT). We have determined that, as in chickens, EMT is complete in the emu forelimb region by HH16, and precursor muscle cells are present by HH18 despite low proliferation. HH18 emu forelimbs display restricted or reduced expression of *Tbx5* and *Fgf10*, with no appreciable *Fgf8* expression. *Fgf8* bead insertion, chicken LPM transplantation, or *Fgf10* overexpression all induce early emu forelimb proliferation, suggesting that emu LPM expresses *Fgf10* at insufficient levels to induce *Fgf8*, resulting in forelimb heterochrony. Experiments are underway using RNA-seq and ATAC-seq in chicken and emu to identify additional candidate genes and specific regulatory changes associated with the delayed and reduced emu forelimb.

49. Identifying requirements for continuous tooth replacement through dental surgery in leopard geckos

Theresa M. Grieco, Kirstin S. Brink, Joy M. Richman
University of British Columbia

Many non-mammalian vertebrates continuously generate and replace teeth throughout life, whereas mammals including humans have reduced this ability. We use the adult leopard gecko (*Eublepharis macularius*) to investigate the requirement for different dental tissues in sustaining tooth cycling. In our model, lingually situated dental tissues are exposed rather than encased in bone. We developed survival surgery techniques for localized replacement tooth extraction and disruption of dental soft tissues. These permit us to study multiple treatment conditions in a single animal and characterize short- and long-term phenotypes. Thus far, animals have been sacrificed 1, 3, and 7 days after surgery, and were microCT scanned, decalcified, and sectioned for histology and immunofluorescence. MicroCT and histological data confirm the extraction of all mineralizing replacement teeth from surgical sites. After three

days, mesenchymal cell proliferation is elevated after both extraction and ablation. By seven days, the truncated dental lamina extends aborally and encloses nearby blood clots within cysts. Cell proliferation is evident in the new, much thicker epithelial tip of the dental lamina. These results suggest that the removal of a developing tooth triggers a robust response in the dental tissues, and the dental lamina has capacity to repair significant damage into adulthood.

50. The role and evolution of the *Dopa decarboxylase* gene in the origin of a derived dimorphic fruit fly pigmentation trait

Sumant Grover, Melissa E.Williams, Thomas M. Williams
University of Dayton

Understanding the genetic and molecular underpinnings for trait diversity remains a central goal of evo-devo research. Traits arise by the orchestrated expression of numerous genes in a gene regulatory network. Remaining poorly understood is how these networks and their expression patterns are initially assembled and subsequently diversify. Gene expression is controlled by DNA sequences known as cis-regulatory elements (CREs) that possess binding sites for transcription factors whose binding drives a specific pattern of expression. It is anticipated that gene expression evolution often occurs through the formation, modification, and destruction of CREs, presumably by changes that create or destroy binding sites for transcription factors. However, the binding site level of CRE evolution has been worked out in few cases. The fruit fly species *Drosophila melanogaster* has a male-specific pattern of abdominal pigmentation for which the enzyme encoding genes and several of their expression-regulating transcription factors are known. However, the details of how these regulators interact with CREs remain largely uncharacterized, including the *Dopa decaboxylase* (*Ddc*) pigmentation enzyme gene. Here we share the results of our efforts to uncover the CRE-basis of this gene's expression pattern, and how this regulation and pattern of expression evolved during the origin of this male-specific trait.

51. REDfly and SCRMshaw: powerful tools for insect evo-devo studies

Marc S. Halfon
University at Buffalo-State University of New York

We have developed two powerful resources to facilitate evo-devo studies and regulatory genomics in insects. REDfly (Regulatory Element Database) is a comprehensive database of *Drosophila melanogaster* gene regulatory sequences containing records for empirically validated cis-regulatory modules (CRMs) and transcription factor binding sites from the published literature. REDfly currently contains more than 22,000 records of reporter constructs regulating over 630 genes. A major feature of this resource is detailed information about the spatio-temporal patterns of gene expression regulated by each CRM. Plans are in place to add additional insect species in the near future. CRM data from REDfly serve as input for SCRMshaw, a computational method for CRM discovery. SCRMshaw, which is highly effective with a low false-positive rate, uses the wealth of *Drosophila* CRM data to enable CRM discovery in diverse insect species including mosquitoes, beetles, and bees. Because the same training data are used by SCRMshaw to search all species, chances of discovering homologous (or functionally similar) CRMs for orthologous genes are strong, a decided advantage for evo-devo studies. Several compelling examples have already been identified. Together, REDfly and

SCRMshaw will allow for rapid, cost-efficient insect genome annotation and provide a rich source of regulatory elements for evo-devo studies.

52. Gaining Focus: Using RNAi to Understand How *T. marmoratus* Larval Eyes Maintain Focus

Jenni C. Hassert, Elke K Buschbeck, Aaron L Stahl
University of Cincinnati

Visual systems are complex and require that all pieces work together to form clear images. The refractive power of the lens is fundamentally important for any eye to maintain correct focusing. During growth all parts of the eye need to coordinate to maintain focus. Previous studies have thoroughly examined how vertebrates can preserve this property during growth, but there are few studies which attempt to answer the question in invertebrates. An excellent model for eye development are *Thermonectus marmoratus* larvae which have exceptional eyes that use a bifocal lens to focus images on two retinas. These larvae undergo rapid growth between their 2nd and 3rd larval stages and substantially reform their lenses to accommodate this growth. The cuticular protein Lens3 is a major contributor to the lens. In this project we use RNAi to knock down *Lens3* expression and to investigate if reduction of this major lens protein leads to refractive errors, or if *T. marmoratus* eye development contains compensatory mechanisms that allow correct focus to be maintained. Knockdowns can be measured using a customized ophthalmoscope to determine focusing abilities. This study will provide insights towards the question of whether invertebrates use active or passive regulation to maintain focus.

53. Developmental bias primes toads for convergent middle ear losses

Kim L. Hoke, Molly C Womack, Jennifer L Stynoski
Colorado State University

Middle ear structures have disappeared among true toads (Bufonidae) an estimated 12-17 times, even though toads primarily communicate with sound and species without ears have reduced sensitivity to sound in mid- and high-frequency ranges. Moreover, ancestral reconstructions suggest two likely regains of middle ears within toads. To better understand the developmental biases that promote middle ear transitions in toads, we have investigated ear development in different lineages within the bufonid clade. We examined morphology of skull structures across development in captive bred species from Ecuador and Peru. All species initiate the earliest stages of middle ear development at similar developmental times, but this process is interrupted in two species that lack middle ears as adults. Middle ear development is particularly late in bufonids compared to other frogs, and current evidence suggests that expanded genome sizes may have altered developmental timing and contributed to ear loss. Ongoing analyses are comparing gene sequences and gene expression patterns to further characterize developmental mechanisms involved in parallel losses and regains of middle ears.

54. Hybrids between the two most phylogenetically distant genera of cephalochordates give Insights into the evolution of pharyngeal development

Linda Z. Holland, Hiroki Ono
University of California San Diego

In the cephalochordate *Branchiostoma*, larval gill slits are on the right and mouth on the left. At metamorphosis, the mouth moves anteriorly, a second row of gill slits appears on the right, the first row moves left and gill bars divide each slit. Pharyngeal development in *Asymmetron*, which split from *Branchiostoma* ~150 mya, is similar, but gill slits are ventral. The emerging picture of gill slit formation involves first specifying the territory for larval gill slits, then positioning them and dividing the territory into gill slit primordia. Surprisingly, the two genera hybridize. Both crosses (*Branchiostoma* female x *Asymmetron* male and vice versa) give larvae with gill slits in intermediate positions. Some hybrids metamorphose, albeit with an abnormal pharynx. How does pharyngeal patterning differ between purebreds and hybrids? Is the mechanism patterning the second row of gill slits conserved with that for the first? Why is the second row of gill slits in hybrids abnormal?

55. Revealing when, how, and how often a pigmentation gene network evolved to be seuxally dimorphic in a fruit fly subgenus

Jesse T. Hughes, Abigail M. Groszkiewicz, Thomas M. Williams
University of Dayton

Since the origin of the 36 recognized animal phyla, evolution can be largely summarized as the diversification of characteristics among these original body plans. As animal characteristics are the products of development, a key challenge for contemporary research is to reveal the ways in which development evolves through changes in the use of genes within a gene regulatory network. One ideal trait for deep mechanistic study is the coloration patterns observed on the abdominal tergites of fruit fly species from the *Sophophora* subgenus. Prior research has supported a scenario where elaborate melanic pigmentation limited to the male abdomen evolved once within this clade through the evolution of a sexually dimorphic pattern of expression for the *bric-à- brac* transcription factor genes. My research seeks to confirm or revise this scenario by bringing attention to the distribution of species with elaborate male pigmentation among the diverse *Sophophora* species groups and interrogating the patterns of *bric-à- brac* expression during the development and coloration of abdominal tergites.

56. Comparative transcriptome of early development in basal deuterostomes

Keita Ikegami, Mayuko Hamada, Noriyuki Satoh
Okinawa Institute of Science and Technology Graduate School

In metazoans, comparison of developmental transcriptome has been successful in studying conservation of gene expression patterns between species in inter- and intra-phylum. Interestingly previous study showed an overlap between developmental period where embryos resembled the most and the period where gene expression conserved the most. The overlapped period is considered to be a source of body plan. In this study, we attempted to compare stage association between developmental transcriptome of basal deuterostomes from four different phyla. Namely, the urochordate, *Ciona intestinalis,* the cephalochordate, Branchiostoma floridae, the hemichordate Ptychodera flava, and the echinoderm, *Acanthaster planci*. For each target organisms, 1-cell stage to larva stage were sampled. Special attention was paid to stage associations between different developmental stages of different organisms

based on gene expression profile. To this end, dynamic programming technique was applied to compare similarity of gene expression profiles. We are particularly interested in how developmental modes of basal deuterostomes and corresponding stages are related to each other.

57. Evolution of collective action despite genetic conflict and free riding in a bacterial biofilm
Neal Jahren, Jessica Dewey, Sarah M Hong, Michael Travisano
University of Minnesota

The emergence of multicellular life histories and developmental programs during evolution must be consistent with the overall conditions pertaining to collective action. The holobiont concept has motivated hypotheses that multicellular organisms evolved from multispecies communities despite the apparent contradiction that genetic heterogeneity among cells undermines the collective program. Here we show the evolution of bacterial collective action resistant to undermining conflicts. We induced physical clustering of *Pseudomonas fluorescens* cells by subjecting populations to settling selection. Clustering emerges through the production of a costly extra-cellular matrix (ECM), and the populations evolve long-term coexistence of strains with distinct adaptations. In particular, some strains evolve the capability to survive settling selection by free riding. Thus, these clusters provide a system to investigate how collective action can emerge and survive inter-cellular conflicts. Previous research into the collective action of *P. fluorescens* has focused on the pellicle that grows on the surface of a static culture. A subset of ECM-producing strains isolated from our experiments also demonstrate adaptation to static culture, suggesting that during evolution in shaking culture they avoided a trade-off that constrains pellicle-forming strains. These results provide evidence for the existence of a new mode of collective action in bacterial biofilms.

58. Genetic Interactions among Hox genes shape appendage diversity in the amphipod crustacean *Parhyale hawaiensis*
Erin Jarvis, Nipam H. Patel
University of California, Berkeley

Striking homeotic shifts in limb identity demonstrate the significance of individual Hox genes in specifying regional identities of segments and their associated limbs in arthropods. To expand our understanding of the developmental role of Hox genes and how they might have contributed to the evolution of animal body plans, we have undertaken a comprehensive analysis of Hox gene function and cross-regulatory interactions in the crustacean *Parhyale hawaiensis*, focusing on the genes equivalent to the Bithorax complex of *Drosophila*---*Ultrabithorax* (*Ph-Ubx*), *abdominal-A* (*Ph-abd-A*), and *Abdominal-B* (*Ph-Abd-B*). Initially motivated by the non-linear transformation of the abdominal limbs of *Abd-B* knockouts and the transformation of limbs to both more anterior and posterior fates in *abd-A* knockouts (Martin et al. 2016), we here present our characterization of double mutants for all combinations of the Hox genes *Ph-Ubx, abd-A,* and *Abd-B*, as well as the simultaneous knockout of all three. We examine how the Hox genes interact with one another by observing the induced patterns of gene expression for these and neighboring Hox genes in the various mutants, which allows us to significantly advance our understanding of the Hox "code" that specifies limb identity and

how this is dependent upon specific Hox gene interactions. Most strikingly, we find that the model of posterior prevalence, which dominates our interpretation of the Bithorax-complex in *Drosophila*, does not strictly apply in *Parhyale*, most notably in the manner in which *abd-A* interacts with *Ubx* and *Abd-B*.

59. What controls the timing of seed maturation in Arabidopsis thaliana (Brassicaceae)?
Pablo D. Jenik, John P. O'Neill, Kristen T. Colon
Franklin & Marshall College

The angiosperm seed is a structure that is resistant to environmental insults, dormant and full of nutrients. All those attributes, which contributed significantly to the evolutionary success of the group, are the outcome of a developmental program known as “seed maturation”. The primary positive regulators of this process have been identified, a group of transcription factors (the AFL genes) that appear to be broadly conserved. However, what controls the timing of initiation of maturation is less well understood. In the Brassicaceae embryos develop synchronously inside the fruit (silique), which means that all seeds mature at the same time. It is unclear whether each seed decides to start maturation autonomously or is influenced by a silique-wide signal, and whether the process is triggered by time after pollination or stage of development. A signal caused by the cellularization of the endosperm (reserve tissue) has also been postulated. We address these questions by monitoring the timing of maturation-related traits in siliques segregating mutants that are developmentally slow but morphologically almost normal, and in mutants with altered endosperm cellularization. Preliminary data suggest that maturation is seed-autonomous, that it starts at a specific developmental stage, and that it is uncoupled from endosperm cellularization.

60. Parallel developmental processes in the repeated evolution of hummingbird-adapted flowers
Amanda M. Katzer, Carolyn A Wessinger, Lena C Hileman
University of Kansas

Similar phenotypes may arise multiple times independently through parallel evolution. When parallel phenotypes exhibit similarity in developmental or genetic patterning, it suggests constraints on the underlying processes. The flowering plant genus *Penstemon* is ideally suited to leverage parallel evolution of a complex phenotype to investigate potential constraints on trait evolution. During *Penstemon* diversification there have been multiple pollination syndrome shifts from ancestral blue/purple, short and wide, bee-pollinated flowers to red, long and narrow, hummingbird-pollinated flowers. To investigate potential developmental parallelism in this system, we used cell size analyses to test whether the repeated evolution of *Penstemon* flowers with long, narrow corolla tubes results from predictable differences in cell expansion and/or cell proliferation processes. Our findings demonstrate that transitions to longer, narrow corolla tubes occur through predictable processes of cell proliferation and cell expansion. Interspecific variation in petal tube length across multiple species pairs representing independent transitions to hummingbird adaptation results from increased cell proliferation. However, across the same species pairs, variation in corolla tube width is strongly associated with anisotropic (asymmetric) cell expansion processes. Our results demonstrate parallel

developmental processes underlying repeated evolution of a complex phenotype and suggest the potential for parallel genetic mechanisms in floral shape evolution.

61. Rates of Variation in Vertebral Counts in Populations of Chickens and Mice: A Test of Constraint

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University of Massachusetts, Dartmouth

Population variation is the raw material of evolution. Theory demonstrates that limited variants available for selection can result in developmental constraint on evolution. Some vertebral regions seem especially constrained in mammals, e.g. 7 cervical vertebrae. Vertebral regionalization is specified very early in development, defining cervical, thoracic, lumbar, sacral and caudal regions with species-specific counts. Here, we examined vertebral count variation in 1) a population of outbred chickens from a research farm, and 2) a population of *Peromyscus* mice whose recent ancestors were wild caught and descendents had been kept in laboratory colonies for a few generations. Approximately 200 cleared-and-stained chicken embryos were scored, since skeletons were unfused and variations could be more easily determined. Radiographs of 400 adult mice were scored. The most common phenotype was determined after scoring all individuals, and other variants were then tallied. We found that cervical ribs were rare in both populations (~1%) and no significant differences in percentages of cervical ribs were found between the chicken and mouse populations. We found significant differences in percentages of variants in thoracic and lumbar counts. Variations in rib segments, connections, and uncinate processes also differed between species. This information adds to the debate on the origin of proposed constraints in these traits

62. Genetic basis of the evolution of polyphenism in horned beetle

Teiya Kijimoto
West Virginia University

Understanding the genetic and developmental mechanisms underlying the origin and diversification of novel, complex traits is a fundamental objective of evolutionary biology. The recruitment of modular developmental genetic components into novel developmental contexts (co-option) has been proposed as a central mechanism enabling the origin of novel traits without necessitating the invention of novel genes or developmental pathways. At the same time, co-option of such “old” genes and pathways into novel developmental contexts has the potential to create new opportunities for morphological diversification. One particularly significant axis of diversification concerns environment-responsive trait formation. Developmental plasticity is ubiquitous across trait types and taxa, and creates especially significant evolutionary degrees of freedom for novel, complex traits. In this conference, by using the beetle horn as an example, I will present the significance of two “old” genetic pathways, Hedgehog (Hh) signaling pathway, a deeply conserved cellular transduction pathway in the axis patterning, and *doublesex* (*dsx*), a master regulator of somatic sex determination, in the evolution of polyphenism - an extreme case of developmental plasticity. Then I would like

to further discuss how the regulation of such newly-deployed pathways might be contributing to the maintenance of horn polyphenism in beetles.

63. *CANCELLED

64. Mechanisms regulating soldier development in a hyperdiverse genus of ants
Sophie M. Koch, Ehab Abouheif
McGill University

The hyperdiverse ant genus *Pheidole* is ecologically dominant, evolutionarily successful and a striking example of polyphenism – a winged queen and a wingless worker caste are determined by ecological and nutritional cues during development. A characteristic feature shared by all Pheidole species is subdivision of the worker caste into two morphologically discrete subcastes: big-headed soldiers and small-headed minor workers. Although they are completely wingless, soldier larvae develop a pair of vestigial or rudimentary forewing imaginal discs that degenerate during metamorphosis. Previous work in the Abouheif lab discovered that these rudimentary wing discs have been co-opted during ant evolution to regulate soldier-specific allometry. However, it remains unclear whether these rudimentary discs directly or indirectly regulate head and body size and whether they are sufficient to induce soldier development. We aim to address these questions by using in vitro co-culturing techniques and wing disc transplantations to identify the signaling mechanisms by which the wing discs regulate soldier allometry. Preliminary results show that in soldiers the wing disc proliferates rapidly in the fourth and final instar whereas the wing imaginal cells are never activated in minor workers. These experiments and preliminary results are a first step towards understanding the evolution of social and morphological complexity in ants.

65. Evolution and development of muscle tissues at the head/trunk interface
Rie Kusakabe, Shigeru Kuratani
RIKEN

Vertebrate skeletal and cardiac muscles have their developmental origins in multiple embryonic tissues such as somites, lateral plate mesoderm and unsegmented mesoderm of the head. Near the embryonic region bordering between head and trunk, these tissues generate myogenic precursors that differentiate into a variety of complex and functionally specialized muscles, such as muscles in the shoulders and limbs, tongue muscles, and the chambered heart. In order to clarify the evolutionary mechanisms underlying the formation of complex muscles and associated tissues, we have examined the expression of developmental genes and protein markers in the cyclostome lampreys, the shark, and other gnathostome species. In lamprey embryos, precursors forming a coherent hypobranchial muscle at each lateral side of the pharynx, emerge from the ventral edges of the anterior somites. Lamprey hypobranchial muscles undergo muscle differentiation in the early larval stage and are anatomically equivalent to the tongue muscles of the vertebrates. On the other hand, sharks possess paired fins and compartmented hypobranchial muscles, similarly to other gnathostome species. Comparison of the tissue structure and of the expression of developmental markers have illustrated the

temporal order of differentiation of various muscles in each species. Our analysis has provided new insights for cellular and molecular characteristics of each musculature as well as for their contribution to the complexity of the vertebrate body plan.

66. Evolution and Development of Craniofacial Variation in Bahamian Pupfishes

Ezra Lencer
Cornell University

Understanding the origins of novel phenotypic variation is fundamental to the study of biodiversity. Many studies have investigated the role of selection in driving phenotypic change, however equally important is how phenotypic variation is produced. The overarching goal of my thesis research is to understand the genetic and developmental sources of novel skull variation in a clade of three morphologically and trophically differentiated pupfish species (genus *Cyprinodon*) that co-occur on San Salvador Island, Bahamas. I used RNA-seq to identify modifications to gene expression among species of pupfishes. Genes differentially expressed among species include both pathways known to affect craniofacial development, such as Wnt signaling, as well as novel genes and pathways not previously implicated in craniofacial development. These data are being used along with information about differences in growth rates of jaw elements to identify how modifications to gene expression underlie the origins of ecologically critical phenotypes. Ongoing work is linking changes in gene expression to modifications in cell behavior to understand how growth rates are modified at both a genetic and cellular level.

67. Gene-By-Environment Interactions Drive Responses to Antibiotic Exposure in Threespine Stickleback

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Disruptions to gut microbiota due to antibiotic exposure can have both immediate and long-term consequences on host development, such as increased inflammation and altered behavioral patterns. Much of what is known about host-microbe interactions has been studied using traditional inbred animal models, which limit researchers' abilities to understand the vital role of host genetic background in shaping microbial community composition and its response to disturbance. In our experiments using the genetically variable threespine stickleback fish (*Gasterosteus aculeatus*), we show that gene-by-environment interactions drive differences in morphological, but not behavioral, responses to short-term tetracycline exposure early in development, suggesting population-level variation in developmental instability. Future work will focus on the influence of tetracycline exposure on immune system development and microbial community diversity in fish chronically exposed to antibiotics throughout their lifespan. Results will increase our understanding of how a population's response to microbial disturbance could be indicative of its resilience to environmental perturbations.

68. Glass sponges and the evolution of canonical Wnt signalling

Sally P. Leys, Jasmine Mah
University of Alberta

Components of the canonical Wnt pathway were in place before multicellular animals arose, but the full pathway is only known from metazoans where it is typically involved in aspects of body polarity. In sponges (Porifera) Wnts are expressed at the posterior pole of the larva and at the incurrent and excurrent openings of the water filtration system around which the sponge is polarized; but solid evidence for canonical Wnt signalling is lacking. Glass sponges (Hexactinellida) are one of the earliest branching animal groups. They are syncytial but arise from a cellular embryo; importantly they lack motile cells. We have scoured several transcriptomes of the glass sponge *Aphrocallistes vastus* and while components of the Wnt pathway are present, we find no Wnts. Has Wnt signalling been lost in glass sponges, or did it arise after glass sponges diverged from other Porifera? Alternatively, might glass sponges, because they lack motile cells, use a non-secreted factor to interact with the remainder of the pathway components? Or, perhaps canonical Wnt signalling arose after sponges diverged from other metazoans and what we see in sponges is an alternate use of the classical components used in Wnt signalling? Our findings on these questions will be discussed.

69. On identifying evolutionary lineages and delimiting species: a case study in *Glycine* subgenus *Glycine* (*leguminosae*)

Shujie Li, Sue Sherman-Broyles, Jeffrey Doyle
Cornell University

The concept of species is one of the most fundamental in biology. Definition of the term is highly variable, depending on the field of study and the goals of the research in question. Species definitions emphasizing different characteristics of lineage differentiation lead to differences in species delimitation. Allopolyploid complexes in plants are particularly challenging for species delimitation due to the complex morphological character patterns created by hybridization, coupled with reproductive isolation by ploidy. Such a species complex is *Glycine tomentella*, one of the wild perennial relatives of the cultivated soybean (*G. max*), in *Glycine* subgenus *Glycine*. *G. tomentella* T4 and several of its potential diploid progenitors were studied. Various analytical methods were used to provide separate and general results, including principal component analysis, decision tree, Tukey's test and random forest. Most results showed that within ploidy, the species/subgroups are morphologically different, with tetraploid T4 highly resembling one of its potential diploid progenitors. A dichotomous key of the plants studied was generated, and the most differentiating characters were identified. Combining preliminary results from genotyping-by-sequencing (GBS, Sherman-Broyles et al., unpublished data), all the diploid subgroups in this study were proposed to merit recognition as species, but the tetraploid T4's lineages should remain unclassified due to uncertainty concerning genomic donors. Further analysis of the GBS data may help us compare patterns of morphological and molecular variation for these taxa and study the contributions of diploid progenitors to morphological variation in the allopolyploid.

70. Collembola Ubx on Appendage Repression: Molecular Evolution from Crustacea to Insecta

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Insect Ubx/abdA suppress limb formation via repression on Dll transcription, but crustacean Ubx doesn't, since the phosphorylation sites in C-terminus of crustacean Ubx inhibit the limb repression function of the protein. Collembola, one group of basal hexapods intermediate between Crustacea and Insecta, bears three kinds of appendages on abdominal segments I, III and IV. Previous studies suggest collembolan Ubx/abdA specify abdominal appendages, but don't repress collembolan Dll transcription. Hitherto, the mechanism of collembolan Ubx/abdA on appendage formation remains unclear. Here, by using EMSAs and fly ectopic expression system, we prove the collembolan Ubx could bind the fly regulatory element Dll304 and repress the fly Dll expression, with similar function as insect Ubx. However, the transcriptome analyses indicate collembolan Ubx is involved in the regulation of phosphorylation/dephosphorylation. Notably, C-terminus of collembolan Ubx contains potential phosphorylation sites, and it can confer de-repression function on fly Ubx in the dual luciferase reporter assays, which is similar to crustacean Ubx. Our data suggest collembolan Ubx has both de-repression and repression function on Dll expression, which could interpret why collembolans possess appendages only on three abdominal segments. In addition, collembolan Ubx represents an evolutionary transition in sequence and function from Crustacea to Insecta.

71. A Conserved Gene Regulatory Module Specifies Lateral Neural Borders Across Bilaterians

Xiao Liu, Yongbin Li, Di Zhao, Takeo Horie, Geng Chen, Hongcun Bao, Siyu Chen, Weihong Liu, Ryoko Horie, Qinghua Tao
Tsinghua University

The lateral neural plate border (NPB) of vertebrates is the precursor of substantial evolutionary innovation, including the neural crest, a major contributor of PNS. In invertebrates, PNS progenitors are also juxtaposed to the lateral boundary of the CNS. Whether there are conserved molecular mechanisms determining vertebrate and invertebrate lateral neural border remains unclear. Using single-cell resolution gene-expression profiling and genetic analysis, we present evidence that orthologues of the NPB specification module specify invertebrate lateral neural border. First, like in vertebrates, the conserved neuroectoderm lateral border specifier Msx/vab-15 specifies lateral neuroblasts in *Caenorhabditis elegans*. Second, orthologues of vertebrate NPB specification module (*Msx/vab-15, Pax3/7/pax-3* and *Zic/ref-2*) are significantly enriched in worm lateral neuroblasts. And like in other bilaterians, the expression domain of *Msx/vab-15* is more lateral than those of *Pax3/7/pax-3* and *Zic/ref-2* in *C. elegans*. Thirdly, we showed that *Msx/vab-15* regulates the development of mechanosensory neurons derived from lateral neural progenitors in multiple invertebrate species, including *C. elegans, Drosophila melanogaster,* and *Ciona inestinalis*. In addition, we identified a novel lateral neural border specifier *ZNF703/tlp-1*, which functions synergistically with *Msx/vab-15* in both *C. elegans* and *Xenopus laevis*. These data suggest a common origin of the molecular mechanism specifying lateral neural borders across bilaterians.

72. What is a conserved mechanism?
Alan C. Love University
of Minnesota

The “conservation” of molecular genetic mechanisms is central to the reasoning practices of contemporary developmental biology, such as deriving explanatory generalizations from model organisms, and is a major source of its recent success in elucidating how animals and plants develop. Conserved mechanisms also play a key role in evo-devo, such as in appeals to deep homology in order to explain the origins of evolutionary novelty. However, conserved molecular genetic mechanisms are not identical and therefore a question arises about how deep the similarities must be to license these inferences. Additionally, mechanisms are individuated by the outcomes they produce. Since the claim of conservation is a judgment of homology, which is typically based on structure rather than function, what constitutes the individuation conditions for a conserved mechanism? I address these questions in the context of philosophical literature on mechanisms with an example from insect segment formation. This analysis identifies a further, neglected issue about the dynamic constitution and organization of molecular genetic mechanisms during ontogeny and its evolution.

73. Genomic studies on dicyemids: phylogenetic position and genomic adaptations for parasitic lifestyle
Tsai-Ming Lu, Noriyuki Satoh, Hidetaka Furuya
Okinawa Institute of Science and Technology Graduate University

To obtain the data from enigmatic taxa is essential to better comprehend animal evolution. Dicyemids are microscopic endoparasites inhabiting the renal sacs of some cephalopods, and have long fascinated biologists because of their highly simplified body organization and poorly known life-cycles. The phylogenetic position of dicyemids remains controversial, leading to hypothetical scenarios of spiralian evolution remain contentious. Their simple body organization, consisting of approximately 40 cells, may be the adaptation to a parasitic lifestyle. However, previously available data have not clarified their phylogenic affinity, and the genomic changes for adapting to parasitic lifestyle are not well studied. In the present study, we first decoded the transcriptome and genome of *Dicyema japonicum*, and performed phylogenomic and comparative genomic analyses. Our results indicated that dicyemids are a group of morphologically reduced spiralian, having a close affinity to the Orthonectida, and they diverged early as a sister group to the Rouphozoa. Second, the genome size of *D. japonicum* is compact with extraordinarily shortened introns. Besides, many regulatory genes and pathways were not found in the predicted gene models, and only two Hox genes that control the body plan along the anterior-posterior axis were obtained, perhaps corresponding to their parasitic life style.

74. Cis-regulatory changes at two loci interact in cell type evolution of *Drosophila prolongata*
David M. Luecke, Artyom Kopp
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Gene expression evolution is a major contributor to phenotype diversification, yet the complexity of transcriptional networks often leaves the molecular mechanisms of expression change unclear. Instances of phenotypic evolution with candidates for causal gene expression evolution provide powerful leverage on this question. A species-specific increase in the number of chemosensory bristles on forelegs of male *Drosophila prolongata* male forelegs provides such a model. Male-specific chemosensory bristle expansion suggests two candidate transcription factors, *doublesex (dsx)* and *Pox neuro (Poxn)*, which are responsible for sexually dimorphic development and chemosensory bristle fate respectively. Antibody staining shows expression of both these genes has expanded into the novel chemosensory bristles. *D. prolongata* cis-regulatory elements (CREs) from both loci, driving reporter assays in transgenic *D. melanogaster* flies, show expanded expression into bristle cells relative to homologous CREs from sister species *D. rhopaloa*. The expansion from the dsx CRE faithfully replicates the expression seen in male *D. prolongata*, but the *Poxn* CRE only drives into a subset of relevant bristles. This suggests CRE evolution produces dsx expansion, which in turn acts as a trans factor that interacts with *Poxn* CRE changes to expand *Poxn* expression, and thus chemosensory bristle development.

75. Dorsal–ventral patterning in brachiopods and the evolution of BMP gradients in neural induction

Yi-Jyun Luo, Noriyuki Satoh
Okinawa Institute of Science and Technology Graduate University

Bone morphogenetic protein (BMP) signaling regulates axial patterning and cell fate determination during early embryogenesis in animals. Interactions between BMP ligands and their antagonists, such as chordin, establish the BMP gradients, which subsequently subdivide the embryos into distinct territories and organize body axes. The molecular control and evolutionary origin of dorsal–ventral (DV) patterning in spiralians have been obscure. Although belonged to spiralians, the cleavage pattern of the brachiopod Lingula anatina exhibits deuterostome-like development, allowing direct topological comparison between spiralian and deuterostome embryos. Here we provide a functional analysis of DV patterning under the control of BMP signaling in the Lingula embryos. We find that BMP signaling is activated at the animal pole, which subsequently becomes the dorsal side of the larva, opposite to the blastopore. Using small-molecule drugs and recombinant proteins together with deep RNA sequencing, we demonstrate that BMP signaling is required for the folding of mantle lobes along the larval anterior–posterior axis. We also show that BMP signals inhibit the expression of neuronal markers in the lophophore. Our findings reveal a conserved function of BMP gradients in brachiopods comparable to that of basal deuterostomes, such as sea urchins.

76. Distinct properties of wasp germ plasm correlate with its divergent complement of localized RNA

Jeremy A. Lynch, Honghu Quan
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Many animals set aside primordial germ cells in the earliest stages of embryogenesis, using maternally provisioned and localized germ plasm. In holometabolous insects, germ plasm is assembled at the posterior of the oocyte. Typically, syncytial nuclei of the embryo that enter the germ plasm cellularize precociously (becoming pole cells), take on germ cell traits, and later migrate to the gonad. Surprisingly, germ plasm and pole cell features are quite diverse among the Holometabola. For example, the germ plasm in the wasp *Nasonia* (aka oosome) is spheroid, moves freely in the posterior half of the egg, and then buds in a single mass at the pole. This contrasts with the small, stationary polar granules and individual germ cell buds in *Drosophila*. Later, the *Nasonia* pole cells take a distinct migratory path to the gonads. To understand these differences, we have sequenced RNAs of anterior and posterior fragments of *Nasonia* embryos, and have identified more than 30 posteriorly localized mRNAs potentially involved in germ cell determination. Only a handful of the fly orthologs of these transcripts are localized or have a described germ cell role. Functional analysis has confirmed that several of the wasp specific transcripts are important for the unique properties of the *Nasonia* oosome.

77. Rho GTPases and morphogenesis during early development in the cnidarian, *Nematostella vectensis*

Craig R. Magie, Spencer Hess, John O. Wheeler, Colby Ledoux, Alexa McWhinnie, Setareh Khalili
Quinnipiac University

Understanding the control of morphogenesis in early-branching metazoans, coupled with comparisons across the Metazoa, will help clarify the evolution of morphogenetic mechanisms. To this end we are examining the cell biology underlying gastrulation in the cnidarian *Nematostella vectensis*. Gastrulation in *Nematostella* occurs via invagination of the endoderm, though the molecular details underlying this process remain to be elucidated. One group of likely candidates for regulating cell shape changes in the endoderm is the Rho family of small GTPases. Phylogenetic analyses have identified *Nematostella* orthologs of Rho, Rac, and Cdc42, and all are expressed ubiquitously during the gastrula stage. We are currently utilizing a variety of approaches to perturb function of Rho GTPases and their downstream effectors. Treatment of embryos with a pharmacological inhibitor of Rho-kinase (ROCK), a downstream target of Rho, affects cell morphology and the ability of treated embryos to complete gastrulation. Pharmacological inhibition of Rac also results in morphogenetic defects, possibly indicating a role in cell division. Additionally, microinjection of morpholinos or mutant mRNA targeting Rac, Rho and Cdc42 have shown similar effects. Our data suggest that the molecular mechanisms underlying Rho GTPase function in *Nematostella* may be distinct from those in bilaterian taxa.

78. Neural genes and the sponge osculum

Jasmine L. Mah, Sally P. Leys
University of Alberta

The proposal that ctenophores are the most basal animal conflicts with the apparent simplicity of sponges. Yet sponges have a surprising degree of genetic complexity, including the presence of neural-marker genes. Given that ctenophores possess a nervous system, do neural-marker genes suggest a ‘proto-nervous’ system or nervous system loss in sponges? In non-bilaterian

animals bilaterian gene function is not necessarily conserved; genetic and functional context is needed to interpret the presence of neural-marker genes. We performed a meta-analysis of sponge in situ hybridization studies to examine all current gene expression data. It revealed that while sensory structures do express neural-marker genes, so do cell types with no known sensory function. We also performed an RNA-seq experiment investigating the osculum, a sponge sensory organ. Oscular development was examined by comparing the pre-oscular and juvenile stages of *Spongilla lacustris*, while the osculum itself was investigated by comparing the oscular and body tissue of *Aphrocallistes vastus*. While a distinct cohort of genes was upregulated during oscular development and in the osculum itself, with few exceptions neural-marker genes were not. Thus, the osculum is a specialized structure at the molecular level but neural-marker genes may hold cryptic non-sensory functions in sponges.

79. Characterization & Culture of Spermatogonial Stem Cells of the Gray Short-Tailed Opossum

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Monodelphis domestica, (the gray short-tailed opossum), is a model system in biomedical and evo-devo research. Its biology gives it advantages in some contexts over mice. Opossums are born prematurely with several organ systems rudimentarily developed. The forelimbs are well-formed at birth while the hind limbs remain paddle-like. This disparity has been exploited in marsupial limb studies. Opossums are also used to study many other biomedical questions. A drawback to the opossum system is a lack of transgenic or knockout techniques, complicating functional testing. Spermatogonial stem cell (SSC) transplantation has been used to generate transgenic rodents; we will adapt it to opossums. Briefly, SSCs are isolated, cultured, modified, and transplanted into a recipient male who is bred for modified offspring. Little is known of marsupial SSCs, therefore we characterized markers of SSC formation known from rodents in opossums using RT-qPCR and immunofluorescence. We also purified SSCs from testes by differential culture to remove somatic cells. SSC identity was confirmed and methods to expand, freeze, and transfect opossum SSCs are being developed. Based on these results, we hope to be able to generate transgenic opossums in the near future, increasing the utility of opossum as a model for evo-devo and biomedical research.

80. The Elkhorn coral *Acropora palmata* as a model for EvoDevo research in corals

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Corals from the genus *Acropora* are distributed in seas around the world, they can be found in the Pacific and the Atlantic, either in the Australia great reef barrier or at the Caribbean Mesoamerican reef barrier. Once abundant they are now a species with special protection from several environmental agencies worldwide. These are reef forming stony corals, located in shallow waters, and little is known about their development, since they only reproduce once a year, during summertime massive spawning events. There is genomic information available

from Acroporidae corals, therefore it is considered an emerging model, along other cnidarians, to study early events of animal evolution. We have built a development staging table for *Acropora palmata* also known as the "Elkhorn coral" that is one of the *Acropora* corals in the Caribbean Sea and we will be looking at the expression of several marker genes for cnidarian development that will be useful for future studies and to compare development among other cnidarian species, like sea anemones or jellyfishes. At the same time, we setup a protocol for culturing cells from *A. palmata*, in order to also study gene expression in somatic and germ cells.

81. Trichome and Root Hair Development in *Arabis alpina*

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Evolutionary developmental approach is used to compare homologous processes in closely related species. In this study, we use *Arabis alpina*, as a second model system in Brassicacea to study trichome and root hair development and compare it to Arabidopsis thaliana. It has been shown previously that trichome and root hair pattern in *A. alpina* is different from *A. thaliana* (Chopra et al. 2014). To understand the genetic reason for this difference, we screened two EMS populations of *A. alpina* and selected mutants with defects in trichome and root hair patterning and morphology and found a similar range of phenotypes as known in *A. thaliana*. We therefore sequenced selected candidate genes as judged by the mutant phenotype to identify the mutated genes in *A. alpina*. We found that mutations in *Arabis TTG1* and *TRY* lead to same phenotypes. However, *gl3* mutants in *A. alpina* showed a glabrous phenotype similar as the *gl3 egl3* double mutant in *A. thaliana*; indicating an evolutionary change of the *GL3* and *EGL3* functions. Similarly, *Arabis GL2* function appears to be changed as it functions only in a subset of trichomes. We also identified the branching mutant *Aastichel* and several mutants in the *DISTORTED* genes, namely *AaCROOKED*, *AaGNARLED* and *Aaspirrig*. Interestingly, many mutants in *Arabis alpina* carry no mutations in any of the known *Arabidopsis thaliana'*s genes with the respective phenotypes. We will also present the identification of patterning and morphogenesis mutants affecting root hair development.

82. A new A-P compartment boundary and organizer in holometabolous insect wings

Jeffrey M. Marcus, Roohollah Abbasi
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Decades of research on the highly modified wings of *Drosophila melanogaster* has suggested that insect wings are divided into two Anterior-Posterior (A-P) compartments separated by an axis of symmetry. Butterflies possess more typical insect wings and butterfly wing colour patterns provide many landmarks for studies of wing structure and development. Using eyespot colour pattern variation in *Vanessa* butterflies, here we show an additional A-P axis of symmetry running between wing sectors 3 and 4. Boundaries of *Drosophila* mitotic clones suggest the existence of a previously undetected Far-Posterior (F-P) compartment boundary that coincides with this additional A-P axis. A similar compartment boundary is evident in

butterfly mosaic gynandromorphs. We suggest that this compartment boundary and its associated developmental organizer create an axis of wing colour pattern symmetry and a gene expression-based combinatorial code, permitting each insect wing compartment to acquire a unique identity, allowing for the individuation of butterfly eyespots. This code both explains the basis of patterns of eyespot correlation observed in butterflies and provides a mechanism for how these correlations can be broken to produce the observed pattern diversity.

83. Cryptic genetic variation in natural populations of *Drosophila melanogaster*

Sarah Marzec, Ian Dworkin
McMaster University

Cryptic genetic variation is standing genetic variation that has little influence on organismal phenotypes under normal circumstances, but can lead to heritable variation under rare or novel conditions. Under consistent selection in these novel environments, the initially plastic traits can become genetically fixed, a process known as genetic assimilation. Little is known about the underlying genetic architecture enabling cryptic genetic variation and genetic assimilation, and why such variation segregates in natural populations. Using environmental stress-induced crossveinless wings in *Drosophila melanogaster*, I will address how cryptic genetic variation is maintained in natural populations and by what mechanisms genetic assimilation operates.

84. Thyroid Hormone Coordinates Craniofacial Osteogenesis

Catherine M. May, Stephanie Keer, L. Patricia Hernandez, Sarah McMenamin
Boston College

Thyroid hormone plays a vital role in skeletogenesis during post-embryonic development. Cranial bones in larval zebrafish undergo substantial growth and remodeling during the post-embryonic metamorphosis from larva to juvenile. Using a transgenic thyroid ablation system, we found that thyroid hormone plays a crucial role in coordinating the ossification sequence of the bones in the skull. Hypothyroid fish show a lack of ossification in many cranial bones even at late stages of development. We are investigating in greater detail the roles of thyroid hormone in regulating the timing and coordination of skeletal changes during the larval-to-juvenile transition. Comparing pre-metamorphic, mid-metamorphic and post-metamorphic stages we characterize the changes in skull shape and ossification that are controlled by thyroid hormone.

85. Evolvability and Robustness in Models of Pattern Formation

Christine Mayer, Thomas F Hansen
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Evolvability is critical to understand the origination of complex organisms. By investigating how genetic variation translates into phenotypic variation, we can enhance our knowledge about evolvability and how it shapes evolutionary processes. It is assumed that properties of embryological development are playing an important role in determining how genetic variation translates into phenotypic variation. Less is known about how population variation and

selection over time influence the relationship between evolvability and robustness. By combining the concept of the genotype-phenotype map with models of development on a population level, we can explore different aspects that affect the evolution of evolvability. We use reaction-diffusion models of pattern formation to simulate evolutionary change of populations under natural selection and systems drift to study the evolution of the relationship between evolvability and robustness. We are exploring the ability of the system to produce and maintain genetic variation over long-term evolutionary change. Thereby, we can identify processes of pattern formation that facilitate evolvability and can give insight in the origination of novel patterns over time.

86. Evolution of the vertebrate neural crest from an ancestral pan-neural cell population

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University of Oklahoma

A major challenge in evolutionary developmental biology is to identify the molecular-genetic features that facilitated the origin of neural crest cells from ancestral precursors. Here, we show that the transcription factor Snail—a key neural crest regulator in jawed vertebrates—is expressed throughout the developing central nervous system (CNS) in a primitively jawless vertebrate, the sea lamprey. Using CRISPR-Cas9-mediated mutagenesis, we show that lamprey *Snail* is required for both CNS and neural crest specification. We find that an invertebrate chordate orthologue of lamprey *Snail*, *AmphiSnail*, drives precocious neuronal differentiation in the lamprey CNS. Taken together with analysis of invertebrate *Snail* genes, our results suggest a pan-bilaterian mechanism for regulating CNS development involving the *Snail* family of transcription factors. We propose that ancestral vertebrates deployed pan-neural *Snail* expression, coupling CNS and neural crest specification. Following their divergence from jawless vertebrates, we suggest jawed vertebrates retained *Snail* for neural crest specification. Instead of being co-opted exclusively to the dorsal neural tube, our results raise the possibility that neural crest cells may have originated from an ancestral *Snail*-dependent neural-neural crest axis deployed throughout the ancestral vertebrate CNS that was subsequently partitioned to the neural folds and dorsal neural tube in jawed vertebrates.

87. The genetic basis of genital evolution among *Drosophila* species

Cláudia C. Mendes, Joanna F. Hagen, Kentaro M. Tanaka, Maria D. S. Nunes, Alistair P. McGregor
Oxford Brookes University

External male genitalia can exhibit remarkable morphological diversity to the extent that even closely related *Drosophila* species show striking variation in genital structures. Here, we focused on two species of the *D. simulans* clade, *D. mauritiana* and *D. simulans*, which show considerable differences in the morphology of two external genital structures: the claspers and the posterior lobes. Using QTL and fine-scale introgression mapping, we identified several regions on chromosome arms 3L and 3R that contribute to interspecific variation in these two structures. However, regions involved in clasper size differences are mutually exclusive from those with effects on posterior lobe morphology, suggesting that different genes underlie the

evolution of these genital traits. We then performed an RNAi screen in *D. melanogaster*, which allowed us to identify compelling positional and developmental candidate genes. While *fear-of-intimacy (foi)* and *grunge (gug)* may largely contribute to the differences in clasper size between *D. mauritiana* and *D. simulans*, *cuticular protein 67B (cpr67B)* and *male-specific lethal 3 (msl3)* might underlie posterior lobe interspecific variation. We are now applying population genetic tools to explore the evolution of these genes. Our study will significantly enhance our understanding of how sexual selection leads to the rapid divergence in morphological traits.

88. Evolution of centralized nervous systems: insights from the annelid *Capitella teleta*

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Clark University

A key question concerning animal evolution is how centralized nervous systems (CNSs) evolved and contributed to organismal diversity. In many animals with a CNS, a region of ectoderm receives extrinsic signals instructing it to become neural during the process of dorsal-ventral axis specification. In vertebrates and insects, both processes rely in part on inhibition of BMP signaling, leading some to infer that neural induction is homologous within Bilateria. Using a combination of blastomere isolations and incubation in recombinant BMP4 protein in the annelid *Capitella teleta*, we studied to what extent extrinsic versus intrinsic signals are involved in neural specification in a spiralian. BMP4 protein did not block CNS formation or affect dorsal-ventral axis formation. Instead, BMP4 dramatically affected brain formation, causing a third brain lobe and eye to form. To assess the role of extrinsic versus intrinsic signaling in neural induction, we isolated blastomeres from 2- to 16-cell *C. teleta* embryos and assayed for neural fate in partial larvae after 6 days. Daughters of isolated 1st-quartet micromeres expressed the pan-neuronal gene *Ct-elav1*, indicating a possible role for neural determinants during brain formation in *C. teleta*. These results suggest something more complicated than a single origin of CNSs.

89. Developmental Biology meets Quantitative Genetics

Lisandro Milocco, Isaac Salazar-Ciudad
University of Helsinki

Quantitative genetics has given a mathematical framework to the study of evolution of quantitative traits in a population under selection. From the evo-devo perspective, we recognize the importance of development in the determination of evolutionary trajectories, largely neglected by this framework. This omission, we argue, produces several limitations in the applications of its results. In this study, we explore what type of errors arise from the simplification of developmental interactions in the Quantitative Genetics framework. Specifically, we test the predictive performance of the multivariate breeder's equation. Its best-known form was proposed by Russell Lande in the 80s, and allows to predict a population's response to a selective pressure, on several traits. To test its performance, we have developed an evolutionary algorithm in which different genotype-phenotype maps can be tested. We study the performance under a linear genotype-phenotype map and under a map that emerges from a realistic representation of development, given by a previously published model for tooth

development. We conclude that the framework works under the linear map but can give considerable errors otherwise. Furthermore, we have found that part of these errors are systematic, i.e. arising from aspects of the system that the framework fails to consider.

90. Molecular and cellular mechanisms of mesoderm formation during gastrulation and segmentation in the cricket *Gryllus bimaculatus*

Taro Nakamura, Jordan Hoffmann, Seth Donoughe, Chris Rycroft, Cassandra Extavour
Harvard University

Gastrulation is a fundamental process required for the formation of various complex organs during animal embryogenesis. In insects, the molecular mechanisms and cellular dynamics of gastrulation have been extensively studied in *Drosophila melanogaster*. In *Drosophila*, the first step in gastrulation is the formation of a ventral furrow, in which invagination accompanied by cell shape changes gives rise to the presumptive mesodermal tissues. However, classical histological studies of insects that branch basally to Holometabola (the Hemimetabola), have reported that a ventral furrow is either absent or weak during the gastrulation process. Furthermore, in contrast with *Drosophila* embryogenesis, which is of the "long-germ" segmentation type, basally branching insects undergo short-germ or intermediate-germ segmentation, in which only anterior segments are formed during gastrulation, and the remaining segments are added subsequently in a secondary growth process. Therefore, in the context of these segmentation modes, the mesoderm in the newly arising abdominal segments may be formed by different mechanisms than those operative in the initial gastrulation process. However, modern studies elucidating the morphogenetic movements and molecular machinery underlying mesoderm formation in these insects are extremely scarce. I seek to address the ancestral insect mechanisms of mesoderm formation during gastrulation and segmentation in the cricket *Gryllus bimaculatus*, which is an emerging model Hemimetabolous insect, by using live-imaging and CRISPR/Cas9-mediated gene modification techniques.

91. Empirical measurement of the prevalence of cis-trans regulatory interactions across phyla

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A large number of embryonic cis-regulatory modules (CRMs) can be a useful resource to elucidate and validate gene regulatory programs for early embryonic development. Despite their importance, only a minute fraction of embryonic CRMs has been discovered due to limited accessibility to embryos and/or lack of efficient tools. Sea urchin embryos have the potential as an ideal surrogate system for high-throughput discovery of embryonic CRMs thanks to the virtually unlimited number of synchronized embryos and well-established high-throughput reporter assays. As a pilot study, we tested the utility of sea urchin embryos to discover human CRMs. Considering the vast evolutionary distance between human and sea urchin, an obvious concern is that there may not be many human CRMs that are active in sea urchin embryo. Based on the empirical discovery rate of 0.017 (26 out of ~1500 random fragments) at the mesenchyme blastula stage, we estimate ≥68,000 fragments in the entire human genome to be active in sea urchin embryos. In addition, the 26 human fragments displayed diverse spatial

patterns in sea urchin embryos. Together, these results foretell the utility of sea urchin embryos to discover and examine an unprecedentedly large number of embryonic CRMs even across phyla.

92. The Inhibitory Cascade Model of Phenotypic Integration in the Molar Row

Amanda B. Neves, Benedikt Hallgrimsson, Charles Roseman, Rebecca Green, Christopher Percival
University of Calgary

Understanding the ways in which development converts genetic and environmental influences into phenotypic variation is a central question of EvoDevo biology. We test variational predictions the of Inhibitory Cascade (IC) model that predicts relative tooth size differences along the molar row as a function of the activating and inhibiting signals during molar growth. The IC model is supported by developmental experiments on the tooth row and in comparative studies of macroevolutionary trends in dental evolution. Less well understood, however, is whether the IC model can describe the genotype-phenotype map that channels genetic variation on which evolutionary processes can act to permit evolutionary change. We used high resolution micro-CT imaging and three-dimensional computational visualization to measure the occlusal surface area of each molar of individuals from the F34 of the WUSTL: LG, SM advanced intercross population. We found that the observed size of M3 was smaller than expected under the IC model. Additionally, we found that the variance of M3 was also much lower than expected under the model. These results suggest that the IC is not the exclusive determinant of covariation in the mammalian molar row.

93. Investigating the mechanisms that contribute to convergent craniofacial evolution between the thylacine and canids

Axel H. Newton, Charles Y Feigin, Christy Hipsley, Andrew J Pask
University of Melbourne

Convergent evolution occurs when unrelated species evolve similar characteristics to exploit similar ecological niches. A remarkable example of convergence exists in the skeletal and craniofacial morphology of the marsupial thylacine and placental canids. Despite their similarities, the thylacine and canids last shared a common ancestor ~160 MYA. We have quantified craniofacial convergence between these species using geometric morphometric analyses of skull shape across marsupial and placental mammals. We then explored the role of RUNX2, a master regulator of osteoblast differentiation and craniofacial evolution in the canids, as a regulator of craniofacial shape in marsupials. Repeat variation in RUNX2 has been correlated with facial length variation within several orders of placental mammals. Remarkably we found little-to-no variation in repeat length across, or within, orders of marsupials, despite a large degree of facial length diversity. Our data suggests that facial length variation in marsupials, and convergent craniofacial morphology between the canids and the thylacine, cannot be explained by RUNX2 repeat variation and must be under the control of other genes or alterations to their regulation.

94. A conserved role for transcription factor nrl in the evolution of rod development across vertebrates

A. Phillip Oel, Keon Collett, W. Ted Allison
University of Alberta

Rod and cone photoreceptors mediate vertebrate vision; rods work in dim light, while cones enable high-acuity and colour vision in bright light. The ancestral jawed vertebrate retina was likely cone-dominant, but nocturnal animals commonly enrich for rods. One way to build a rod-enriched retina is to convert cones to rods, which we recently showed happened in early mammals. We hypothesize that the transcription factor NRL, a rod development gene, played a central role in this cone-to-rod conversion. To pursue whether ancestral NRL had this capacity, we used zebrafish as a cone-dominant vertebrate outgroup to mammals. We characterized the role of zebrafish nrl in rod development, and then tested the hypothesis that ectopic non-mammalian nrl can convert cones to rods. Knockout/knockdown (via CRISPR and morpholino) showed that loss of nrl blocks rod generation, and that lack of rods is concurrent with increases in abundance of at least two cone subtypes. Transgenic nrl expression in UV cones caused the UV cones to cease expressing their hallmark opsin, instead expressing rod markers. This work establishes a conserved role for NRL in rod development. Such conservation of important genetic architecture will enable us to further explore the origins of derived mammalian traits using zebrafish.

95. What is the key developmental change for insect wing evolution?

Takahiro Ohde, Taro Mito, Teruyuki Niimi
Kyoto University

Despite the long history of discussion, it remains unclear what the key innovation in the developmental program to evolve novel insect wing is. To understand key change in development for wing evolution, we first investigated ancestral type of wing development in the two-spotted cricket *Gryllus bimaculatus*, then examine the conservation of the developmental program between *Gryllus* and an apterygote (non-winged) insect *Thermobia domestica*. In Gryllus, we examined spatiotemporal gene expression pattern of wing formation gene orthologs in late embryonic stage, and found that vestigial, a wing field selector gene in *Drosophila*, specifically express in tergal margin cells. Following gene functional analysis revealed that wing gene orthologs are required to form larval tergal tissue that differentiates into wing in later stage. *Thermobia* is considered to represent a body plan before the first insect wing evolved. We found that *Thermobia* wing gene orthologs indicate similar spatial expression pattern to those in *Gryllus*, suggesting that tergal margins are homologous between ancestral apterygote and pterygote insects, and that evolution of the mechanism to differentiate wing from tergal margin in late development was a key innovation to acquire flight apparatus in insects.

96. A deeply conserved Turing mechanism in the fin and limb development

Koh Onimaru, Luciano Marcon, Marco Musy, Mikiko Tanaka, James Sharpe
RIKEN Center for Life Science Technologies

For the testability and the predictability of hypotheses of morphological evolution, formal mathematical modelings are one of the demanding challenges in the evo-devo field. In this presentation, we propose that the skeletal pattern difference between the fish fin and tetrapod digits can be explained by shared reaction-diffusion equations that represent Bmp-Sox9-Wnt interactions. We examine catshark fin development, and find the distal nodular elements arise from a periodic spot pattern of *Sox9* expression, in contrast to the stripe pattern in mouse digit patterning. However, our computer model shows that reaction-diffusion equations that is proposed to underlie mouse digit formation, with altered spatial modulation can reproduce the Sox9 expression of catshark fin development in silico. Finally, experimental perturbations of Bmp or Wnt signalling in catshark embryos produces skeletal alterations which match in silico predictions. Together, our results suggest that the morphological diversity of the distal fin and limb elements arose from the spatial re-organization of a deeply conserved Turing mechanism.

97. *CANCELLED

98. Horizontally acquired genes contribute to novelty within the DV GRN patterning Chalcid wasp embryos

Daniel Pers, Jeremy A. Lynch
University of Illinois, Chicago

A global comparison of *Drosophila* and *Nasonia* dorsoventral GRNs has shown that a conserved patterning output, such as tissue specification in the embryonic blastoderm, can arise from GRNs that share surprisingly little similarity in terms of molecular composition. A set of genes unique to *Nasonia* is characterized by the presence of PRANC-Ankyrin domains. These genes have no clear orthologs outside of a family of parasitic wasps, and are in fact most similar to genes found in bacteria and viruses, suggesting acquisition via horizontal gene transfer. Furthermore, knockdown of these genes indicates that they have gained crucial functions in regulating expression patterns of other DV genes, morphogenetic movements, and developmental timing. To understand how the incorporation of these genes into the genome resulted in their incorporation into the DV GRN, functional studies on a wider breadth of diversity must be conducted. We have developed tools (in situ hybridization, RNAi, RNAseq) to establish the wasp *Melittobia digitata* as a model system for comparison to *Nasonia*. We are characterizing the expression and function of Melittobia PRANC-Ankyrin type genes. The results of this analysis will provide the first insights into the pattern and process of incorporation of novel genes into the wasp DV GRN.

99. Gain, loss and reshuffling of pair-rule segmentation genes during insect evolution

Leslie Pick, Jie Xiang, Katie Reding, Patricia Graham, Faith Kung, Alys Cheatle Jarvela
University of Maryland

Constraint on changes to key features of animal body plans may lead to re-wiring of gene regulatory networks such that downstream gene expression is more highly constrained than upstream regulators controlling these genes. This appears to apply to the segmentation gene

network in arthropods, where a segmented body plan is universal but the mechanisms regulating segmentation vary. The pair-rule segmentation genes (PRGs), identified in the model insect *Drosophila melanogaster* are the most proximate regulators of segments per se and this cohort of nine genes is largely retained in insect genomes. We are examining the extent to which expression and function of PRGs has varied during insect radiations. Perhaps the most variable PRG is fushi tarazu that arose as a homeotic gene but was coopted into the PRG network as a result of changes in both its expression and its function. Other PRGs show variation in expression pattern in different taxa and a smaller number have been lost from the PRG cohort entirely. We will present an analysis of PRG expression and function in hemi- and holometabolous insects, along with progress on strategies to unravel re-wiring events that maintain segment formation and downstream segmentation gene expression despite reshuffling of upstream regulators.

100. Phylogenomic resolution and the dynamic evolutionary history of the cnidarian photo-senses

David C. Plachetzki, M. Sabrina Pankey, Joseph Ryan, Allen Collins, Ehsan Kayal, Bastian Bentlage
University of New Hampshire

The Cnidaria display a rich sensory capacity, but a lack of comprehensive genome-scale resources and confusion surrounding relationships within the phylum has impeded understanding of cnidarian sensory evolution. Here we describe a well-resolved phylogeny for Cnidaria based on 67 new or publically available genomic and transcriptome datasets that includes representatives of all cnidarian classes. We use this phylogeny to address longstanding questions regarding the evolutionary history of the photo-senses in Cnidaria. We first explore the character history of several cnidarian sensory structures including pigment cup eyes, lens eyes, rhopalia and statocysts. We find that rhopalia are restricted to Scyphozoa and Cubozoa, but the phylogenetic position of Staurozoa, together with transcriptome data, suggests that their unique anchor structures may be, in part, homologous to rhopalia. A more dynamic character history is recovered for the other sensory characters with numerous gains and losses noted. Next, using our dataset, we estimate a comprehensive phylogeny for cnidarian opsins. We identify several clades of opsin that were present at the origin of the Cnidaria and characterize dynamic expansions and contractions of the opsin gene family during cnidarian diversification. Finally, we assess the possibility of functional classes of cnidarian opsins based on their expression across taxa.

101. The radiation of a transcription factor family rewired cell-fate specification in *Caenorhabditis*

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The GATA-type zinc finger transcription factor family expanded prodigiously in the Caenorhabditis genus. The bilaterian ancestor likely had two GATA factors; vertebrates make do with six. Of the eleven GATA factors in the nematode *C. elegans*, six comprise the key gene

network that specifies endoderm cell fate, and four are expressed only in the endoderm. By reconstructing the evolutionary history of this protein family within the *Caenorhabditis* genus, we discovered that this expansion was restricted to a subclade. Since five of the six known endoderm-specifying GATA factors are part of this radiation and since the promoter of the sixth – a regulatory target of the others – was restructured at the same time, evolution substantially rewired the early embryonic developmental genetics establishing endoderm cell identity, all without overt changes in morphology. The phylogenetic history also shows that this radiation involved pattern of repeated gene duplication and loss, a result consistent with developmental genetic experiments that show robustness of gene expression in this network despite fluctuations in gene copy number. We propose that this churning of paralogs represents a new type of developmental systems drift and will discuss the developmental genetic conditions that, we hypothesize, made it possible.

102. Friend or Foe, Cavefish Adaptation to a Low Parasitic Load

Nicolas Rohner, Robert Peuss Stowers
Institute for Medical Research

Host-parasite interactions are one of the major driving forces in evolution and the loss of parasite diversity in modern societies strongly correlates with an increase of autoimmune-diseases caused by immune-regulatory defects in humans, however, no suitable model organisms has been established yet to study adaptation to low parasitic diversity. Here we introduce the cavefish system as a new model system for host-parasite coevolution to study how changes in parasite diversity can alter the evolutionary trajectory of the host immune system. Cave environments typically have a less diverse fauna, including parasites, than the respective surface environments. We therefore hypothesized that cavefish would have acquired differences in their immune system to adapt to the difference in parasite diversity. Indeed, we found drastic changes in the organization of immune cells as well as their cellular function. These changes persist in a laboratory environment and are therefore most likely genetically encoded and part of an adaptive strategy as we find evidence of parallel evolution within specific immunological traits of two independently derived cave populations. Intriguingly, these changes correlate with a lower burden of inflammation in previously studied metabolic disease phenotypes in these fish, potentially explaining some of the increased health aspects we previously observed.

103. Artificial selection as a tool for micro-evo-devo: the case of the Longshanks mouse

Cambpell Rolian
University of Calgary

As a comparative approach, evolutionary developmental biology has provided fundamental insights into the evolution of developmental systems and processes among multicellular organisms. To date, however, evo-devo has focused largely on macroevolutionary patterns and processes at broad phylogenetic scales, e.g., understanding the developmental basis of evolutionary novelties, of major evolutionary transitions, and of extreme morphologies in homologous anatomical structures. In contrast, studies that focus on the relationships between

developmental variation among individuals, and heritable phenotypic variation within species (a.k.a. micro-evo-devo), remain rare, despite the central role of phenotypic variation in adaptive evolution. This is especially true for studies above the genomic level, e.g., at the level of cells and tissues: most recent examples of micro-evo-devo research focus on the identification of polymorphisms in key developmental genes and their outcomes on phenotype, but rarely address how these polymorphisms translate into variation in cell and tissue processes of morphogenesis. Here, I will discuss the benefits and challenges of a micro-evo-devo approach focused on cell, tissue and organ-level tissue determinants of morphogenesis, present some recent examples, including our ongoing work on the genetic and cellular basis of skeletal variation within species, and discuss complementary approaches that can be leveraged to address this overlooked topic.

104. Linking new genes to an odontogenesis gene regulatory network peripheral to jaw morphogenesis

Nasim Rostampour, Julia C Boughner
University of Saskatchewan

Despite that teeth and jawbones can form and evolve independently of each other, the genetic processes that regulate cranial and dental morphogenesis independently remain unclear. Previous microarray screens indicated that a p63 gene regulatory network is integral to odontogenesis but peripheral to jaw morphogenesis. Here, we characterized the expression of four genes flagged by our microarray screens, comparing wildtype and edentate p63-/- mutant mouse mandible prominences. We hypothesized that in dental (but not gnathic) epithelium, p63 down-regulates Frmt1 and Pltp and up-regulates Cbln1 and Krt8. We validated the expression of these genes in p63-/- mutant and wildtype mice using RNA in-situ hybridization on paraffin sections at two stages, E11.5, just after odontogenesis begins, and at E13.5, just after odontogenesis arrests in the mutant. Our in situ results validated our previous microarray screens; for example, confirming in mutant decreased Fermt1 expression and increased Cbln1 expression in the dental epithelium, not the jaw mesenchyme. Also, our in situ results mapped the expression of these genes specifically in the dental epithelium and tooth bud. We propose that these genes help regulate odontogenesis, and enable evolutionary change in the dentition, independently from the lower jaw skeleton.

105. Par proteins in the endomesoderm of basal metazoans: implications for the evolution of mesoderm

Miguel Salinas-Saavedra, Mark Q. Martindale
University of Florida

In bilateral animals, the “Par system” regulates embryonic cell polarity and the integrity of both ectodermal and endodermal epithelial tissues. However, in embryos of the cnidarian Nematostella vectensis, Par components do not display asymmetric localization until epithelial blastula stages and are degraded altogether in the endomesodermal epithelium during gastrulation. Interestingly, Par protein downregulation in N. vectensis temporally correlates with the expression of brachyury and snail, at the site of gastrulation, two genes that affect the

Par system in bilaterian systems. In contrast, ctenophores (comb jellies), which, like N. vectensis also gastrulate at the animal pole, lack snail orthologues and develop under a highly stereotyped cleavage program. Are Par genes involved in cell polarity in this group of animals? By in vivo imaging we characterized the Par protein localization during early embryogenesis of the ctenophore Mnemiopsis leidyi. mRNA expression of the components of the ctenophore Par system shows that these proteins distribute differently compared to what we have described for N. vectensis embryos and Par proteins are expressed in the endodermal epithelium of M. leidyi. These data will provide implications in to the evolution of mesoderm in metazoan embryos.

106. The evolution of vertebrate appendage regeneration

Igor Schneider, Carinne Monteiro, Amanda Cass, Ingo Braasch, Patricia N. Schneider, Nadia B. Fröbisch, Marcus C. Davis, Sylvain Darnet
Universidade Federal do Pará

Salamanders are the only tetrapods capable of regenerating limbs as adults, however the evolutionary origin of this remarkable ability remains unclear. The only other sarcopterygian capable of regenerating its fin endoskeleton are the lungfishes. Regeneration of dermal fin rays is common among teleost fish, yet fin endoskeleton regeneration has only been reported in living representatives of the non-teleost clade Cladistia, family Polypteridae. Therefore, the explanation for the phylogenetic distribution of vertebrate appendage regeneration as a trait remains elusive. Here, we combine experimental studies in non-teleost actinopterygians and comparative RNA-seq analyses in an effort to resolve the evolutionary origin of limb and fin regeneration. First, we demonstrate that among actinopterygians, fin endoskeleton regeneration is not restricted to living representatives of early diverging clade Cladistia, but is also present in species of the other two non-teleost clades: the paddlefish, Polyodon spathula (Chondrostei) and the spotted gar, Lepisosteus oculatus (Holostei). Next, we generated transcriptome assemblies of regenerating and non-regenerating appendages for Polypterus, lungfish, axolotl, zebrafish and mouse digit tips. Our comparative RNA-seq analysis provides compelling evidence for a shared appendage regeneration program between axolotl limbs and Polypterus and lungfish fins. Altogether, our findings provide strong support for an evolutionary scenario in which an appendage endoskeleton regeneration program first arose in osteichthyes and was subsequently lost in amniotes and teleosts.

107. How to build a spider: the regulation of segment addition in *Parasteatoda tepidariorum*

Anna Schoenauer, Christian Bonatto Paese, Alistair McGregor
Oxford Brookes University

It has been shown that Wnt and Delta-Notch signalling regulate posterior segmentation ancestrally in arthropods. However, it remains unclear how these signalling pathways interact and regulate putative downstream segmentation genes. Therefore, we studied the regulatory interactions between Delta, Notch, Wnt8, caudal (cad), even-skipped (eve), runt-1 (run-1) and odd-skipped (odd) during posterior segmentation in Parasteatoda tepidariorum. We showed that Delta initially activates Wnt8 in the posterior SAZ, but conversely inhibits Wnt8 expression

in the anterior SAZ. This shows that the spider SAZ can be divided into at least two regions: a posterior region of uncommitted cells and an anterior region where cells become committed to forming a new segment. Furthermore, the dynamic expression of eve, run-1 and odd in uncommitted cells and forming segments is regulated by the read out of Delta-Notch and Wnt signaling via cad. However, unlike the other pair-rule gene orthologs, it appears that hairy is part of the oscillatory mechanism rather than a read-out from it. Taken together, our results suggest that in the ancestor of short germ arthropods, Delta-Notch, hairy and Wnt8 form an oscillatory mechanism in the SAZ that maintains uncommitted posterior cells and regulates the formation of segments by pair-rule gene orthologs via cad.

108. Developmental basis of the adaptive radiation of bat molars

Karen E. Sears, Alexa Sadier, Sharlene Santana, Jukka Jernvall
University of California, Los Angeles

Teeth are among the most diverse of mammalian organs. Because of this, mammalian teeth have become a model system for study of morphological variation and its underlying developmental basis. Previous research in rodents suggests that first molar development inhibits latter molar development and thus constrains molar proportions (M1>M2>M3). However, recent studies in non-rodents have challenged this inhibitory constraint (IC) model. To test this model, and explore the developmental basis of adaptive diversification, we are investigating tooth evo-devo in Noctilionoid bats, a hyperdiverse group with exceptional molar variability. Using morphometric data from 117 species with diverse diets, we found insectivorous and frugivorous bats (>25% of Noctilionoids) that do not follow the IC model, with larger M2s than M1s or M3s. Our morphometric data further suggest that these model divergences are achieved through two distinct developmental mechanisms, with some species exhibiting larger M3s than expected, and others smaller. uCT analysis of molar development in 10 Noctilionoids also reveal that, unlike rodents, the bat premolar/molar row is likely established by two distinct signaling centers. In sum, our results suggest that the process of tooth development, and the rules governing it, likely differ in bats and rodents, and perhaps in other groups as well.

109. Contributions from a distant cellular source during regeneration in the annelid *Capitella teleta*

Elaine C. Seaver, Danielle M. de Jong
University of Florida

There is great diversity in relative regenerative capabilities across animals. Understanding the complex evolutionary history of regeneration requires detailed knowledge of the cellular and molecular regulatory controls of the regeneration process within a phylogenetic context. Annelids are well known for their substantial regeneration abilities, and the polychaete annelid, *Capitella teleta*, displays robust posterior regeneration. However, the source, behavior and molecular characteristics of cells that form new tissue during regeneration are unknown. We present evidence that cells migrate into the wound site during posterior regeneration in juveniles. To localize a source of migrating stem cells, we examined a cell cluster localized to

segments 4 - 6 that express stem cell markers. We hypothesize that a heterogenous population of multipotent cells contributes to the germ line and serves as a source of somatic stem cells during regeneration. Using CapI-vasa as a marker to examine the characteristics of this cell population, dynamics were tracked following transverse amputation. We also assessed the relative capacity for posterior regeneration in juveniles with and without the putative stem cell population. This is the first study in *C. teleta* to address the cellular source of regenerating segments, and adds to a growing body of work of the origin of the regeneration blastema in annelids.

110. Commissureless regulation of Slit-Robo signalling in insects

Mark A. Seeger, David Glasbrenner
The Ohio State University

Slit-Robo signaling is a key mediator of axon guidance decisions in organisms ranging from planaria to vertebrates. In contrast to this conservation of ligand and receptor, organisms have evolved various mechanisms to regulate Slit-Robo signaling. In *Drosophila*, *Commissureless* is a key post-translational regulator of the Robo receptor that functions to prevent cell surface accumulation of Robo. Two additional *Comm*-family members are found in *Drosophila* and they vary in their ability to regulate Robo receptors. We are investigating the evolution and function of *Comm*-like genes in insects. Bioinformatic studies indicate that *Comm*-like genes are present in most Dipteran genomes, although the number of *Comm*-family members varies. Divergent *Comm*-like genes can be identified in many insects, suggesting it was present early in insect evolution. There is evidence supporting three independent losses of this *Comm*-like gene in insects: 1) the absence from sequenced Lepidopteran genomes, 2) the absence from *Tribolium* but presence in more basal Coleopteran genomes, and 3) the presence in basal Hymenoptera, like the sawfly, and absence in more derived Hymenoptera including ants, bees, and most wasps. We are investigating the functional properties of divergent *Comm*-family members from a variety of insects using multiple approaches.

111. Beggars can't be choosers: developmental integration of a horizontally-acquired gene by co-option

Bogdan Sieriebriennikov, Vladislav Susoy, Christian Roedelsperger, Metta Riebesell, Nermin Akduman, Iuliia Boichenko, Ralf J. Sommer
Max Planck Institute for Developmental Biology

Horizontal gene transfer is the passage of genetic material between phylogenetically distant lineages. Using whole-genome sequencing platforms, horizontally-acquired genes have been identified in numerous organisms including eukaryotes. However, to become functional, new genes must be integrated into gene regulatory networks, and little is known about how this is achieved. We studied developmental integration of a horizontally-acquired cellulase cel-2 in the nematode Pristionchus pacificus. We show that cel-2 is expressed in pharyngeal gland cells and the secretory-excretory system, consistent with the presumptive extracorporeal hydrolytic function of the encoded enzyme. Strikingly, cel-2 expression is tightly linked to the regulation of a dimorphism of feeding structures. We observe that cel-2 is more highly expressed in the

'stenostomatous' microbivorous morph than in the 'eurystomatous' morph that can kill other nematodes. Using mutants in the developmental switch genes eud-1 and nhr-40, which regulate the mouth-form decision, we could elucidate that these genes also regulate the level and pattern of cel-2 expression. In addition, cel-2 expression is influenced by bacteria provided to the worm as food and is increased upon starvation. We conclude that a horizontally-acquired cellulase gene in P. pacificus was integrated into development by co-option of existing regulators of feeding plasticity.

112. Comparative development of the archosaurs pectoral musculature

Daniel Smith-Paredes, Miccaella Vergara Cereghino, Joao F. Botelho, Bhart-Anjan S. Bhullar
Yale University

One of the most impressive features of bird biology is the capacity of powered flight. Although not all modern birds are able to, they all descend from a flying ancestor that, in turn, evolved from flightless non-avian theropod dinosaurs. The presence and arrangement of some muscles involved in flight can be studied by looking at some particular fossils within the dinosaur-bird transition, but a deeper understanding of the ancestral condition, and what changed along bird evolution, comes from the comparative study of the anatomy of their closest living relatives, the other living archosaurs. We compared the development of the muscles and skeletal elements involved in flight in modern bird and alligator embryos, following the origin and fate of individual muscles. The number of separate adult muscles is roughly similar, though their accepted homologies are not always representative of their developmental trajectory. Some muscles also display a high expansion from their origin to their attachment point, which also varies according to flight capability. By comparing their development, we gain understanding of the homologies of the archosaurs pectoral muscles, and more insight into the mechanistic causes behind the differences originating such diverse group of organisms.

113. The evolution and development of symbiosis-associated traits: on the study of mycangial development in ambrosia beetles and spore development of fungal symbionts

Ellie J. Spahr, Matthew Kasson, Teiya Kijimoto
West Virginia University

Symbiotic relationships may facilitate specialized structural development in both host and symbiont. The ability of ambrosia beetles to culture and subsist on fungi has driven their divergence from their bark beetle ancestors. This nutritional symbiosis has routinely coincided with the development of structures, termed 'mycangia', which allow beetles to vector fungal symbionts. Mycangia vary significantly in location and complexity across ambrosia beetle tribes. In our system, the ambrosia beetle Euwallacea validus develops internal, pre-mandibular mycangia to transmit its Fusarium sp. symbionts. Fusarium species, when associated with Euwallacea, develop shortened, bulbous conidia (spores) likely through the selective 'farming' behavior of beetles; i.e. species with no known beetle association sporulate in the wildtype 'fusiform' shape. To understand the genetic and evolutionary underpinnings of symbiosis-associated trait development, we utilize the Euwallacea-Fusarium symbiosis to resolve 1) how genetic co-option in beetles gives rise to mycangia, 2) the genetic underpinnings of modified

spore development. We will present our current data as it pertains to both host and symbiont development.

114. Beyond the jaw joint: endothelins drove neural crest cell differentiation in stem vertebrates

Tyler A. Square, David Jandzik, Haley P. Stein, Andrew W. Hansen, Marek Romášek, Amrita Purkayastha, James L. Massey, Maria V. Cattell, Daniel M. Medeiros
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In model jawed vertebrates (gnathostomes), Endothelin signaling mediates several aspects of neural crest cell (NCC) development, including their differentiation into the jaws. Although lampreys lack jaws, recent work has shown they express a gnathostome-like complement of Endothelin ligands (Edns) and receptors (Ednrs) during early development. This suggests Endothelin signaling had functions in neural crest differentiation and/or patterning that predate jaws. To test this, we disrupted the function of embryonically-expressed Sea Lamprey (*Petromyzon marinus*) *ednrs* and *edns* using CRISPR/Cas9-mediated mutagenesis. To aid in side-by-side comparisons, and add phylogenetic depth within gnathostomes, we also mutagenized *Xenopus laevis* ednrs and edns. We found overall conservation of Endothelin functions in neural crest migration and differentiation, supporting a role for endothelin signaling in early neural crest evolution. Similarly, nested *Dlx*, but not *Hand* expression in the pharynx relies on endothelin signaling in *P. marinus*. This suggests that endothelin signaling acquired some new skeletal patterning functions in gnathostomes (i.e. hand expression, joint placement, and bone development), but importantly Edn-driven *dlx* expression in the pharynx predates the jaw by more than 75 million years.

115. Molgulid Tales

Billie J. Swalla
University of Washington

Transcriptome and genome data offer an exciting new approach to examine the origin and evolution of the chordate body plan. Chordate body plan evolution can be studied with two tunicate species with radically different larval body plans that are found sympatrically off the coast of Roscoff, France - the tailed ascidian Molgula oculata and the tailless M. occulta. Tailed M. oculata embryos have forty notochord cells that are converged and extended in the center of the tadpole tail, as most ascidian larvae. The larvae also have tail muscle cells flanking the notochord in the tail, and in the head is the otolith, a gravity sensory organ. The tailless M. occulta does not form a tail in their larval stage, and have only twenty notochord cells that do not converge and extend during larval development. We have sequenced the genomes of these two species and a third species, M. occidentalis in collaboration with the Christiaen lab, and they are available on Aniseed (Stolfi et al. 2014). We show by transcriptome and in situ hybridization analysis that the notochord gene network is expressed at the right time and place in the tailless M. occulta embryos and larvae, although the notochord collapses into a "notoball" near the posterior. We show by transcriptome analyses that the ascidian metamorphosis program begins much earlier in molgulid ascidians, during early development.

This radical heterochronic shift has been documented in another tailless ascidian, Molgula tectiformis, and is now reported for three additional species: the tailed molgulid species, Molgula oculata, Molgula occidentalis, and the tailless Molgula occulta. Further functional data is necessary to determine if this pronounced heterochrony is the necessary preadaptation for tailless tadpole to evolve in molgulid ascidians. This is an excellent model system to study the evolution of gene networks underlying morphology.

116. Identification of Notch pathway components in the tardigrade *Hypsibius dujardini*

Jennifer Tenlen, Braeden Wiebe
Seattle Pacific University

In many animals, the Notch pathway plays an essential role in stochastic signaling events that establish cell fates. Of particular interest is the role of the Notch pathway in promoting proliferation of the germline in organisms such as *Drosophila melanogaster* and *Caenorhabditis elegans*. Preliminary cell fate analysis in the emerging model organism *Hypsibius dujardini* (Phylum Tardigrada) has suggested that germ cells may arise by an induction event early in embryogenesis. To test the hypothesis that the Notch pathway is necessary for this induction, we have begun to clone and characterize Notch pathway components from *H. dujardini*. We have identified putative homologs of nearly all Notch pathway components in the *H. dujardini* genome, and have isolated nearly full-length sequences for several components. We will describe our initial characterization of these components, including expression analysis and effects of down-regulation of gene expression via RNA interference.

117. The shape of things to come: Developmental System Drift in *Caenorhabditis* nematode embryos

Nicholas D. Testa, Annalise B. Paaby
Georgia Institute of Technology

Developmental system drift (DSD) occurs when selection for a stable phenotype allows for developmental mechanisms to diverge over evolutionary time. Nematodes are an exemplary model of DSD with highly stereotyped and near-invariant early embryonic cell divisions. Only by highly quantitative phenotyping methods have previous studies demonstrated any cellular or developmental variation; however, many of the sequences for genes activated in early development (especially par-2) have diverged significantly across taxa. Here, we investigate DSD by collecting videos of embryogenesis in multiple nematode species within the genus, Caenorhabditis. Videos start in the single-cell stage and progress past 4-cells, allowing us to collect images at precise time points corresponding to both 2-cell and 4-cell embryos. Using geometric morphometrics we have uncovered species-specific differences in shape for 2- and 4-cell embryos at normal and stressful temperatures. If the underlying mechanisms that regulate embryonic shape remained the same across species, we would expect to see all species react similarly to stress during development. Instead, our data demonstrate that developmental shape trajectories diverge in near-lethal temperatures (30˚C), revealing divergence in the genetic mechanisms that mediate the near-identical early cell divisions.

118. Investigating the developmental timing of cyclical tooth regeneration in the catshark (*Scyliorhinus canicula*)

Alex P. Thiery, Kyle Martin, Rory Cooper, Zerina Johansen, Gareth Fraser
University of Sheffield

Research on dental regeneration has focused mostly on identifying dental progenitor cells which underlie the formation of new dental generations, with a specific focus on the regeneration of a tooth from a single tooth family. However, the regulation of regeneration in both a spatial and temporal context is not well understood. Sharks develop their regenerative teeth along a continuous and permanent epithelial dental lamina. A sheet of dental progenitors are housed within compartments of the dental lamina, with adjacent tooth families connected via this epithelial layer. We performed RNAseq analyses on specific cell layers associated with shark tooth regeneration and identified candidate genes involved in the initiation of continuous dental regeneration within the dental lamina. With adjacent tooth families staggered in the timing of their initiation in the catshark, we investigated the expression of the RNAseq candidate genes between tooth families to look for spatial shifts in expression. Our findings shed light on the developmental regulation of both the timing of continued dental initiation and the maintenance of stem progenitors for rapid tooth regeneration in an exciting emerging Evo-Devo model, the shark (Scyliorhinus canicula).

119. Evolution of Annualism in Killifishes: An Eco-Evo-Devo Approach

Andrew W. Thompson, Andrew I. Furness, Corinne Stone, Anais Hayes, Cristina Rade, Ingo Braasch, Jason E. Podrabsky, Guillermo Ortí
Michigan State University

Developmental arrest or diapause is found in many invertebrate lineages but is exceedingly rare in vertebrates. Here we explore the evolution of embryonic diapause in annual killifishes, the only vertebrate case known to occur after completion of organogenesis. Annual killifishes inhabit seasonal pools that desiccate, resulting in the death of the adult population. Unique adaptations including specialized eggs, desiccation resistance, and up to three ontogenetic diapause stages that slow developmental and metabolic rates enable the embryonic population to survive annual dry seasons. When the habitat floods, annual killifish terminate diapause, hatch, and begin a new lifecycle. We use scanning electron microscopy, comparative transcriptomics, and phylogenomics to investigate the evolution of killifish annualism, diapause, and environmentally-cued hatching. We characterize egg ultrastructure illustrating convergent egg development, discover candidate genes involved in diapause and desiccation tolerance, and infer a phylogeny with hundreds of loci that supports convergent origins of diapause within killifishes. 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. Our integrative Eco-Evo-Devo framework provides important insights into the evolution of diapause and the diversity of vertebrate hatching strategies.

120. Exploring insect wing origin through cis analysis of *vestigial* in *Drosophila*

Yoshinori Tomoyasu, Yi-Ting Lai, Kevin D. Deem
Miami University

Insect wings represent a classic example of morphological novelty, but their origin remains a chief conundrum in biology. Throughout the history of the insect wing origin debate, popular opinion has shifted between two contrasting hypotheses, the tergal-origin hypothesis and pleural-origin hypothesis, without reaching any consensus. Through the study of *vestigial* (*vg*) in the *Tribolium* beetle, we have previously obtained functional evidence supporting a "dual origin" of insect wings, which potentially combines the two hypotheses. *vg* is an important gene to trace the developmental and evolutionary history of wing structures. We reasoned that comprehensive cis-analysis for *vg* will provide novel insights into the origin of insect wings. In *Drosophila*, *vg* is expressed in several tissues, including wings and muscles. Our analysis revealed intricate cis-regulatory mechanisms operating at the *vg* locus. Intriguingly, one of the wing enhancers was also active in the larval tergum (i.e these two tissues share a similar transcriptional regulatory landscape), suggesting an evolutionary connection between the two tissues (thus supporting either tergal or dual origin). Unfortunately, we could not evaluate the pleural-origin hypothesis through cis-analysis due to the derived dipteran body plan. Nonetheless, our study provides a framework to investigate the insect wing origin from a cis-perspective.

121. 5'Hoxd genes and Gli3 determine anterior vs posterior dominance of the primary limb axis

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National Cancer Institute, Frederick

In most vertebrates, the primary limb axis runs through the posterior limb; postaxial ulna/fibula and digit4 condense first. However, urodeles display preaxial dominance (radius/tibia/digit2 condense first), which may represent the basal state based on fossil record, and be linked with adult regeneration. How this variant axis formation is controlled, despite otherwise normal limb AP polarity, remains enigmatic. Urodele preaxial dominance is proposed to result from altered late phase 5'Hoxd expression. We find that preaxial elements form first in 5'HoxdDel mice (Hoxd11-d13 deleted), as in urodeles. 5'Hoxd genes regulate replication licensing and cell adhesion. Gli3 repressor, which antagonizes 5'Hoxd function, likewise regulates proliferation and condensation complementarily. Indeed, in compound 5'HoxdDel/Gli3 mutants, postaxial dominance is restored. Changes in key early condensation steps (cell cycle exit, aggregation rate) in anterior versus posterior limb bud may drive axial polarity. We find that 5'HoxdDel anterior limb cell proliferation is reduced (prolonged G1) at stages just before condensations arise. We hypothesize that accelerated anterior cell cycle exit is linked to earlier condensation, and live cell imaging is underway to examine aggregation. These data demonstrate genetically that 5'Hoxd and Gli3 genes regulate primary limb axis appearance polarity, potentially via antagonistic cell cycle and mesenchymal aggregation effects.

122. Origin and Diversification of Novel Structures of the Bat Wing

Daniel J. Urban, Karen E. Sears

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Bats achieved powered flight through specializations in their forelimbs, including longer digits and novel wing membranes. The earliest known fossil bats date back to ~50mya and were already morphologically very similar to modern bats. Thus, understanding how bats attained the morphological novelties required for powered flight requires studies that reach beyond the fossil record. We have been using development as a tool to gain insights into the morphological evolution of key hallmarks of the bat wing. Previous study has provided developmental explanations for the bat wing's elongated digits and retained interdigital tissues. These structures arise through multiple mechanisms, including larger fields of Shh and Fgf8 expression, as well as a novel reactivation of Shh creating a self-sustaining feedback loop with Bmp2. We are currently investigating the developmental origins of the novel membranes of the bat wing (plagiopatagium, uropatagium). We have established that these membranes begin to form ~St. 14-15. By studying a developmental sequence of embryological stages in multiple Noctilionoid bats with varying wing morphologies, we intend to discover when and how these novel wing membranes initially form and eventually diverge among species.

123. Patterns of transcriptional parallelism and variation in the developing olfactory system of *Drosophila* species
Pelin C. Volkan, Jia Pan, Qingyun Li, Scott Barish, Sumie Okuwa, Songhui Zhao, Charlie Soeder, Matthew Kanke, Corbin Jones
Duke University

*NOW A TALK. See abstract above.

124. Bridging pattern and process: How do snails grow shell sculpture?
Nicole B. Webster, A. Richard Palmer
University of Alberta

Mollusc shells are a prime example of "endless forms most beautiful" and exhibit many diverse and complicated patterns, including both colour and sculpture. The shell is secreted by the mantle, a flexible hydrostatic tissue that lines the opening of the shell (aperture). Great strides have been made in recent years to understand the molecular processes involved in calcium carbonate secretion. Furthermore, the patterns of shell shape and sculpture has been modelled extensively, but whether these models reflect the biological reality is unclear. Nothing is known about the how the process of shell secretion can be modified to yield different patterns of shell sculpture. Here we work to answer: What aspect of the mantle changes to produce different shell sculpture? Nucella ostrina (Ocenebrinae: Muricidae) is a small predatory intertidal snail whose shell varies from strong spiral ribs to a smooth shell. We examined the mantle of ribbed and smooth snails using histology, TEM, 3D reconstructions, and histochemistry. Altogether, these observations revealed a relatively simple mechanism: in a rib, the mantle is longer with taller cells. This would increase the volume of shell secreting tissue, producing the thicker shell of a rib when compared to the adjacent, thinner, inter-rib.

125. Dual neural crest-neurogenic role of lamprey Snail links CNS patterning to neural crest evolution

Joshua R. York, Tian Yuan, Kevin Zehnder, David W McCauley
University of Oklahoma

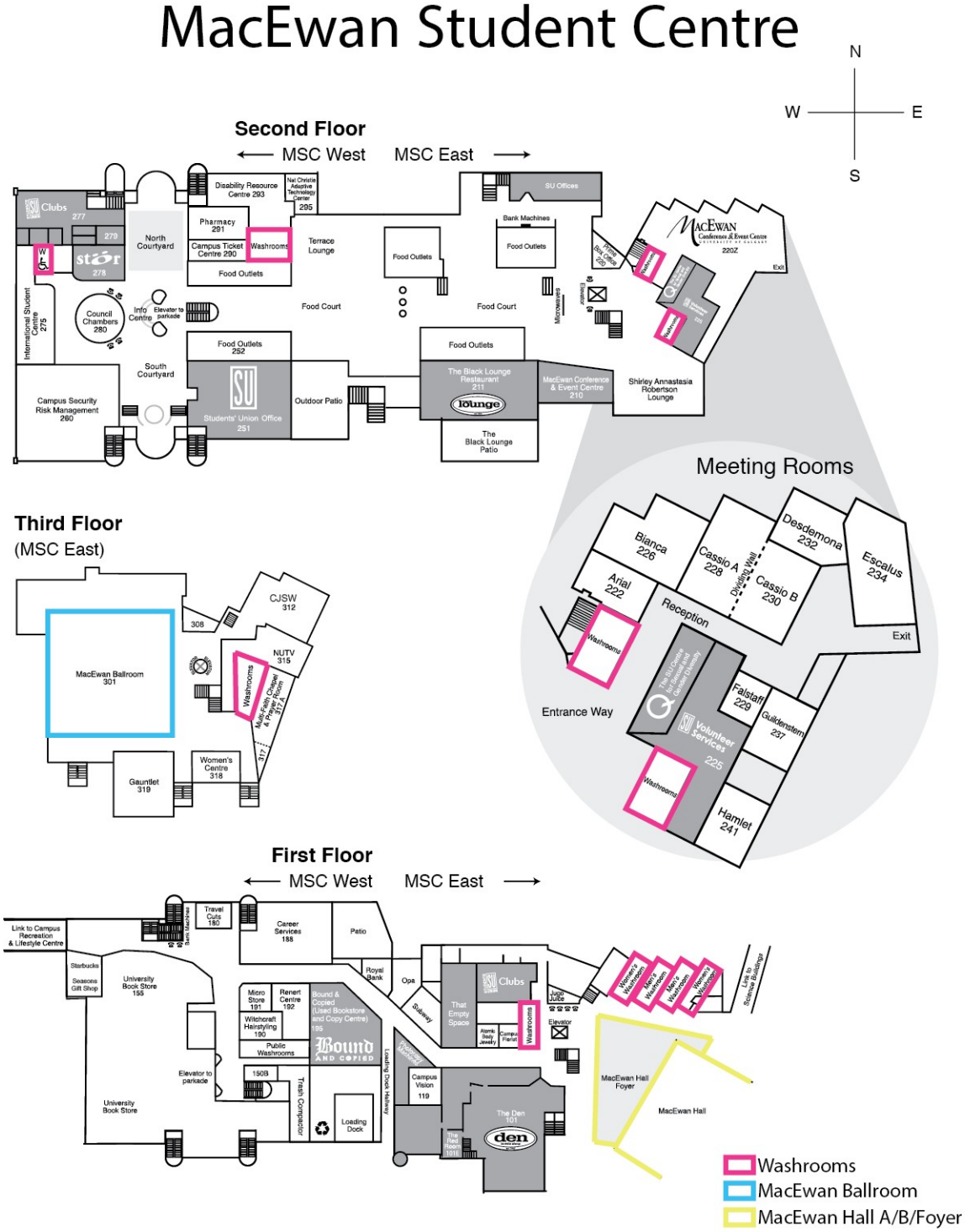
A major challenge in evolutionary developmental biology is to identify the molecular-genetic features that facilitated the origin of vertebrate neural crest cells from ancestral precursors. Here, we show that the transcription factor Snail—a key regulator of neural crest development in jawed vertebrates—is expressed throughout the developing central nervous system (CNS) in a primitively jawless vertebrate, the sea lamprey. Using CRISPR-Cas9-mediated mutagenesis, we show that lamprey Snail is required for both CNS and neural crest specification. We find that an invertebrate chordate orthologue of lamprey Snail, AmphiSnail, drives precocious neuronal differentiation in the lamprey CNS. Taken together with analysis of invertebrate Snail genes, our results suggest a pan-bilaterian mechanism for regulating CNS development controlled by the Snail family of transcription factors. We propose that ancestral vertebrates deployed pan-neural Snail expression, coupling CNS and neural crest specification. Following their divergence from jawless vertebrates, we suggest jawed vertebrates retained Snail for neural crest specification. Instead of being co-opted exclusively to the dorsal neural tube, our results raise the possibility that neural crest cells may have originated from an ancestral Snail-dependent neural-neural crest axis deployed throughout the ancestral vertebrate CNS that was partitioned to the neural folds and dorsal neural tube.

126. Somite Compartmentalization in Amphioxus: on the Evolutionary Origin of Vertebrate Skeletons

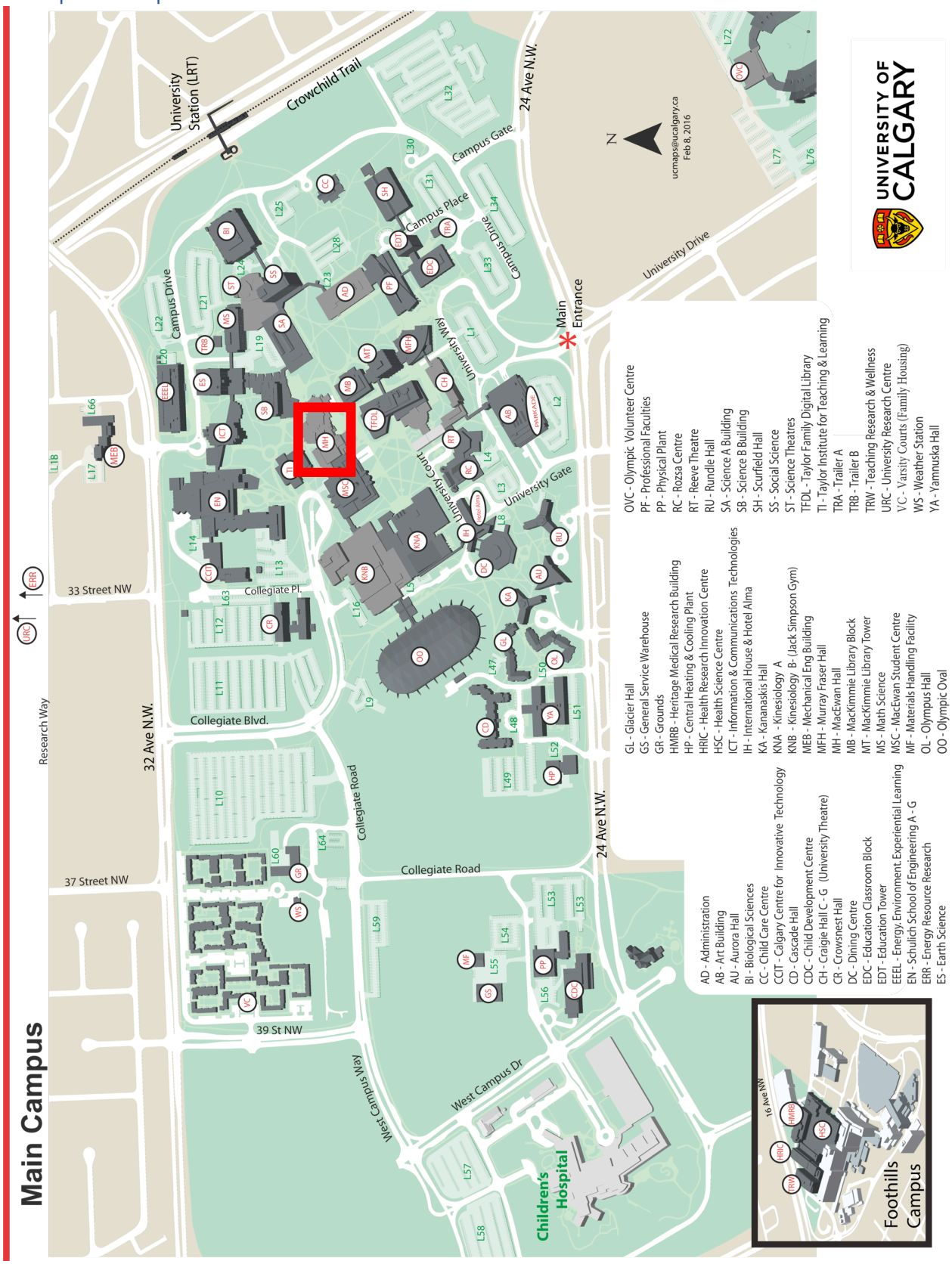
Luok Wen Yong, Tsai-Ming Lu, Song-Wei Huang, Che-Huang Tung, Rui-Jen Chiu, Kun-Lung Li, Jr-Kai Yu
Academia Sinica

*SUPPLEMENTAL TO A TALK. See abstract above.

MacEwan Conference and Event Centre Map



Campus Map


Main Campus
Foothills Campus
University Station (LRT)
Crowchild Trail
24 Ave N.W.
Campus Gate
Campus Place
Campus Drive
University Drive
University Way
University Gate
Main Entrance
Research Way
33 Street NW
32 Ave N.W.
Collegiate Blvd.
Collegiate Pl.
Collegiate Road
37 Street NW
39 St NW
West Campus Dr
West Campus Way
Children's Hospital
16 Ave NW
N
ucmaps@ucalgary.ca
Feb 8, 2016
UNIVERSITY OF CALGARY
AD - Administration
AB - Art Building
AU - Aurora Hall
BI - Biological Sciences
CC - Child Care Centre
CCIT - Calgary Centre for Innovative Technology
CD - Cascade Hall
CDC - Child Development Centre
CH - Craigie Hall C - G (University Theatre)
CR - Crowsnest Hall
DC - Dining Centre
EDC - Education Classroom Block
EDT - Education Tower
EEEL - Energy, Environment, Experiential Learning
EN - Schulich School of Engineering A - G
ERR - Energy Resource Research
ES - Earth Science
GL - Glacier Hall
GS - General Service Warehouse
GR - Grounds
HMRB - Heritage Medical Research Building
HP - Central Heating & Cooling Plant
HRIC - Health Research Innovation Centre
HSC - Health Science Centre
ICT - Information & Communications Technologies
IH - International House & Hotel Alma
KA - Kananaskis Hall
KNA - Kinesiology A
KNB - Kinesiology B- (Jack Simpson Gym)
MEB - Mechanical Eng Building
MFH - Murray Fraser Hall
MH - MacEwan Hall
MB - MacKimmie Library Block
MT - MacKimmie Library Tower
MS - Math Science
MSC - MacEwan Student Centre
MF - Materials Handling Facility
OL - Olympus Hall
OO - Olympic Oval
OVC - Olympic Volunteer Centre
PF - Professional Faculties
PP - Physical Plant
RC - Rozsa Centre
RT - Reeve Theatre
RU - Rundle Hall
SA - Science A Building
SB - Science B Building
SH - Scurfield Hall
SS - Social Science
ST - Science Theatres
TFDL - Taylor Family Digital Library
TI - Taylor Institute for Teaching & Learning
TRA - Trailer A
TRB - Trailer B
TRW - Teaching Research & Wellness
URC - University Research Centre
VC - Varsity Courts (Family Housing)
WS - Weather Station
YA - Yamnuska Hall