2026

Euro Evo Devo

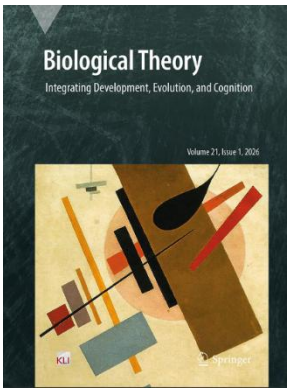
EURO EVO DEVO

Glasgow, Scotland, UK

Programme Book

#EED2026
https://www.evodevoconference26.com/

SPONSORS



BMC
Part of Springer Nature

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The Company of Biologists

Development

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ORGANISERS AND CONFERENCE VENUE

EXECUTIVE COMMITTEE
Annette Becker (Chair)
Carlos Guerrero Bosagna
Daniel Čapek
Rainer Melzer
Gerd Müller
Sylvie Retaux
Tom Van Dooren

PROGRAMME COMMITTEE
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Sandy Hetherington
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Tom Van Dooren

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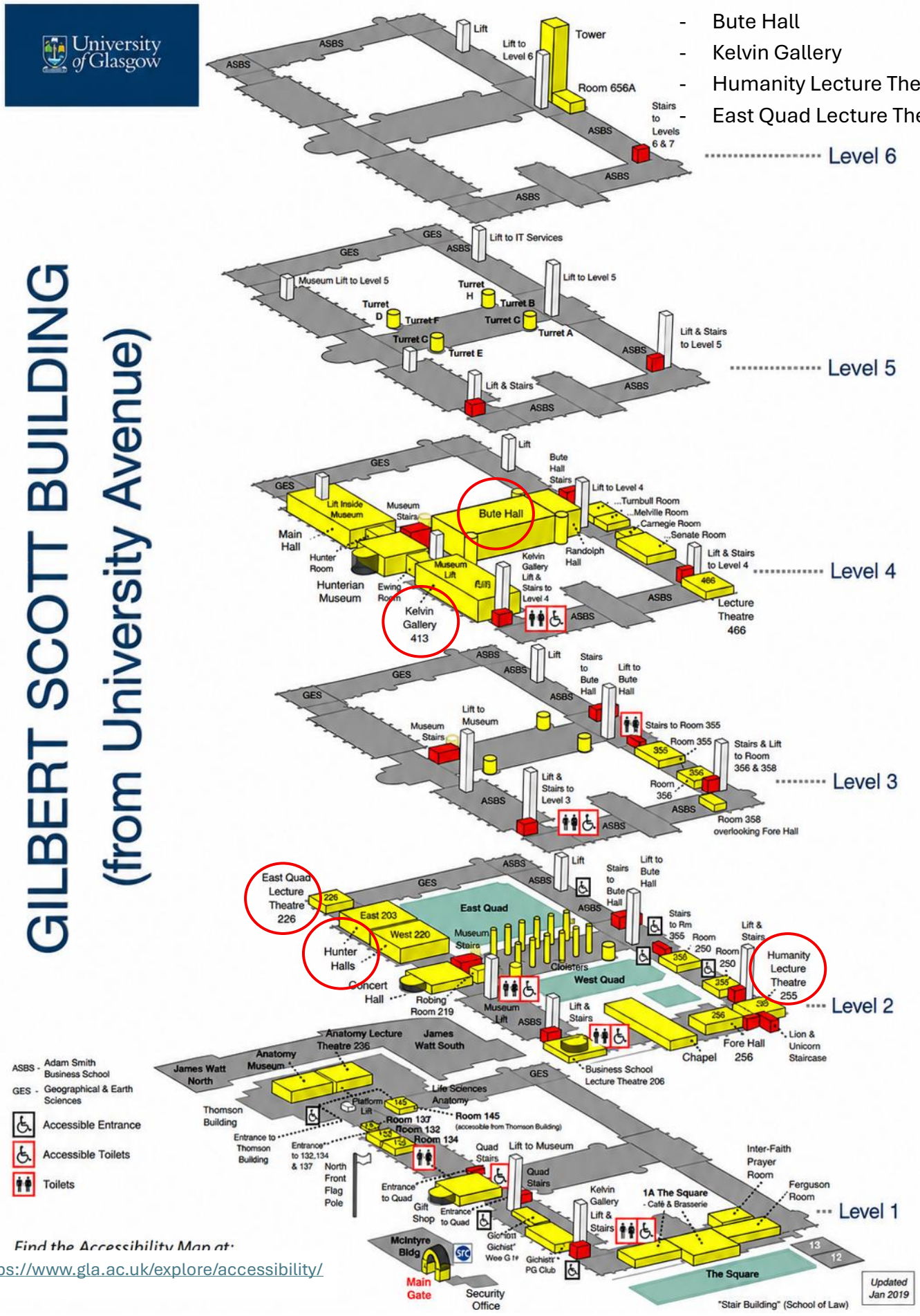
Arne Jacobs
Carmel McDougall
Kevin Parsons
Annis Richardson

UNIVERSITY OF GLASGOW, GLASGOW, UK
Gilbert Scott Building
University of Glasgow
Glasgow
G12 8QQ

The main conference spaces are circled in red on the below map:

- Hunter Halls
- Bute Hall
- Kelvin Gallery
- Humanity Lecture Theatre
- East Quad Lecture Theatre



Find the Accessibility Map at:
https://www.gla.ac.uk/explore/accessibility/

WELCOME FROM THE PRESIDENT

On behalf of the Executive Committee, it is my great pleasure to welcome you to the 10th biannual Conference of the European Society for Evolutionary Developmental Biology. Here in the historic city of Glasgow we are not only celebrating two decades of science, collaboration and community, but also enjoying the opportunity to host such a diverse and vibrant group of scientists—635 participants from 40 countries, of whom 154 are students. It is heartening to see our Society grow from its European roots into a truly global network, and I hope that many of our young members will continue to contribute to EuroEvoDevo's future initiatives with fresh ideas and enthusiasm.

The programme is rich and wide-ranging. It includes 212 oral presentations, four keynote talks, and a record-breaking 312 posters. This breadth of topics highlights the depth and diversity of research in evolutionary developmental biology and offers each of us ample opportunity to explore new approaches and ideas. We are proud to have Bute Hall—University of Glasgow's iconic venue—host all joint sessions; the hall's historic, "Harry Potter-ish" ambience provides a fitting backdrop for the lively scientific exchange that will take place. Our opening-night reception, hosted in the city's most prestigious building—the City Chambers with its impressive marble staircase—offers a perfect start to a Scottish evening. A short walk away, we will gather for a conference dinner at the science museum, where traditional Scottish cuisine and locally produced drinks will create a warm, convivial atmosphere.

I would be remiss not to thank the many people who made this conference possible. Kevin Parsons has been our devoted point person on the ground in Glasgow, while David Ferrier and Chema Martin Duran, who helped scout and secure the venues, have been invaluable. I also gratefully acknowledge Nicola Hardaker and Lauren Duffy from Hg3 Conference Services, who handled the logistical details with professionalism and care. The scientific programme itself owes much to Sylvie Retaux, Chair of the Programme Committee, who, together with our session chairs and the Scientific Committee, had to make difficult decisions to convert several oral talks to poster presentations after receiving far more applications than we could accommodate.

In this era of climate change, the importance of face-to-face collaboration has never been greater. Although we each must consider the environmental impact of our travel, the opportunity to meet in person—to knit our scientific networks, discuss ideas over a drink, and lay the foundations for future projects—remains priceless. Students are especially encouraged to seize this chance to connect with colleagues and to make yourself heard even if you feel new to the field.

Finally, let us preserve this conference as a safe intellectual space where scientists can freely share ideas and unpublished data without fear of being "snitched" on. If you need a specific slide from a presentation, feel free to ask the speaker; you needn't resort to taking photos of the entire talk. I urge you to wander through the poster sessions, engage patiently with presenters, and take advantage of the unique opportunity to learn from each other in an environment of trust and openness.

I look forward to meeting each of you over the coming days and to the exciting scientific conversations that will certainly emerge from our shared passion for evolutionary developmental biology.

Warm regards,

Annette Becker
President, EuroEvoDevo Society

INFORMATION FOR ATTENDEES

The Venue

Address Gilbert Scott Building**:**
The University of Glasgow
Glasgow
G12 8QQ

Website: https://www.gla.ac.uk/

Email UoG Events: uog-events@glasgow.ac.uk

Conference Secretariat

Address: Hg3 Conferences Ltd
4 Dragon Road
Harrogate
HG1 5DF

Tel: +44 (0)1423 529333

Email: events@hg3.co.uk

Website: www.hg3.co.uk

Conference Website
The conference website can be accessed via the following link:
https://www.evodevoconference26.com/

Satellite Meetings
Before the main conference begins, 4 Satellite meetings will be running from the **8th – 9th June** in the Gilbert Scott Building at the University of Glasgow. If you are booked to attend one of these meetings, please arrive at the venue and register at the following times.

The registration desk is located in the Concert Hall in the Gilbert Scott building. Please click here to view the map of the venue. There will be signage to help you when on site.

The registration desk will be open at the following times:

Monday 8th June (Satellite meetings)	08.00 – 18:00
Tuesday 9th June (Satellite meeting and start of the main meeting)	08.00 – 18:00
Wednesday 10th June	08.00 – 18:00
Thursday 11th June	08.00 – 18:00
Friday 12th June	08.00 – 18:00

Merchandise
For those who have purchased merchandise, this can be collected by the registration desk at the refreshment breaks on Wednesday and Thursday. Our student helpers will be available to issue your purchased items.

Getting to the University of Glasgow, Glasgow

How to reach the venue
By car- Motorway links provide access to major UK cities.

From Carlisle, Newcastle and the south, exit M74 on to the M73, joining the M8 at the Old Monkland junction. M8 exits for the University are J18 if travelling from the south, Edinburgh and the north and J19 if coming from Glasgow Airport and the west. Other main routes serving the University include Great Western Road (A82, north of campus), Clydeside Expressway (A814, south) and Byres Road (B808, west).

By bus- For useful information on travelling on buses in Glasgow, please see the link below:
Visitors Guide to Glasgow by bus

By rail- Glasgow is well connected by train from across the UK by 2 main stations:

- Glasgow Central Station links Glasgow to the south
- Glasgow Queen Street Station operates routes mainly to Edinburgh and the north
- Avanti West Coast runs several daily services to London with a journey time of approximately 4 hours 30 minutes

The Caledonian Sleeper connects London to Glasgow with overnight services.
Eurostar international high-speed railway service connects European mainland destinations to Glasgow via London (St Pancras station)

By air- Glasgow is served by 3 international airports, with connections across the UK and the world: Glasgow Airport is only 15 minutes from the city centre with an express bus service from outside the terminal. Glasgow Prestwick Airport is very accessible with a 45-minute train or bus ride to the city centre. Edinburgh Airport has a direct bus service from the airport to Glasgow's Buchanan Bus Station which takes under an hour.

Taxis- Here are a couple of taxi suggestions:

Glasgow Taxis	0141 429 7070 Download the app: https://www.glasgowtaxis.co.uk/passenger-services/smartphone-apps/
Saltire Private Hire	0141 319 5344 E: contact@saltireprivatehire.co.uk
GlasGo Cabs (private hire)	0141 332 5050 / 0141 774 3000 Download the app: https://glasgocabs.co.uk/app/

Parking- On campus parking is limited to permit holders. Car parking in the areas around the campus is extremely difficult. There are various pay & display parking spaces around the local Byres Road and Kelvin Way areas but spaces are limited. You may want to consider using the city's Subway Park & Ride scheme. This service costs around £5 and Kelvinbridge subway station has parking which is only 5 minutes walk away. If you wish to book car parking then please feel free to email: carparking@glasgow.ac.uk

Exhibition and Posters

The Exhibition and Posters will take place in Hunter Hall, please take time to visit all of the exciting exhibition stands and posters. The exhibition will run from Wednesday through to Friday during the main meeting days.

Should you have any queries relating to your poster at the conference, please go to the conference registration desk. Velcro for posters will be provided by the organisers. No drawing pins, staples, sticky pads or any other form of adhesive should be used.

Catering
Refreshment breaks throughout the conference will be provided in Hunter Halls. **PLEASE NOTE that there will be limited bottled water available but there are 2 water refilling stations at either end of Hunter Halls. Please bring your own water bottles so that these can be refilled during the conference.**

Dietary requirements
Please inform waiting staff if you have a specific food allergy or dietary requirements and you will be offered/served an appropriate meal.

Civic Reception – Tuesday 9th June from 18.00 hours
There will be a Civic Reception on Tuesday 9th June 2026 held at Glasgow City Chambers at 18:00. This unique event is hosted by Glasgow City Council and is held in the prestigious surroundings of the City Chambers, which were opened in 1888.

Address: 82 George Square, Glasgow G2 1DU
Walking: 45 minute walk from Bute Hall
Drive: 19 minute drive
Public transport: 22 minutes on the subway, 23 minutes on the Number 15 bus. For more details please click here.

Conference Dinner – Friday 12th June, Glasgow Science Centre – 19.15 – 01.00
This is a ticketed event, and attendees will have to have prebooked their attendance. It is recommended to wear appropriate footwear for the Ceilidh.
There are several ways to reach the Science Centre, please see details below:

Address: 50 Pacific Quay, Glasgow G51 1EA
Walking: 32 minute walk from Bute Hall
Drive: 13 minute drive from Bute Hall
Public transport: 30 minutes on the subway, for details on how to plan your journey please click here.

Internet Access
Attendees can connect to the **UofG visitor** wireless network using your device, no registration or password is required. For more information, please see the link here. If you're visiting from another institution that uses eduroam, you can connect here too.

Social media
Please use this hashtag when posting about the conference: #EED2026

Mobile phones and photography
Please ensure that your phone is switched off or on silent mode when you are in a session.

Delegate Discounts and Offers
Please use the link here to access several discounts and offers open to attendees.

Smoking
Smoking is not permitted in any rooms or buildings. This also includes vaping and e-cigarettes.

Fire Policy
The University provides fire signs within all its buildings. This includes fire directional signs, and signs indicating fire doors, manual call points and portable fire-fighting equipment.

On fire alarm activation, all building occupants should immediately leave the building by the nearest evacuation route or, if physical assistance is needed, proceed to the nearest temporary waiting area and alert Security by a phone call to 0141 330 4444. Fire assembly points are detailed in the rooms.

Cloak room
Coat racks will be located next to the registration desk.

Code of conduct
The organisers of the EED 2026 meeting want to ensure that everyone enjoys the event. Therefore, by registering for and attending EED 2026, we all agree to the following code of conduct: We will respect everyone regardless of age, race, religion, ethnicity, sexual orientation, gender identity, gender expression, marital status, nationality, political affiliation, health, care responsibilities, physical appearance, disability, or educational background. We will also avoid language and behaviour that reinforces discrimination or oppression based on any of these characteristics. We will not engage in any form of discrimination, harassment, or retaliation. This includes intimidating behaviour or language, inappropriate jokes or comments, unwanted attention, stalking, or displaying offensive images. If you are unhappy or uncomfortable with the behaviour of others at EED 2026, please speak to one of the organisers or email us (events@hg3.co.uk). Your comments will be taken seriously and treated confidentially. If you do not treat others with the respect and tolerance we all deserve, you may be asked to leave. Please speak to one of the organisers or email us (events@hg3.co.uk). Your comments will be taken seriously and treated confidentially. If you do not treat others with the respect and tolerance we all deserve, you may be asked to leave.

Emergency Contact Details
In the event of an emergency, please alert the registration desk and or team immediately. Ensure that you note all emergency exits and instructions provided. If you require medical assistance while resident in your hotel/accommodation, you are advised to contact your reception. Fire/Ambulance Emergency Number in Scotland is 999.

Lost and Found
During the conference, any lost property found should be brought to the registration desk.

Information about Scotland
Language: In Glasgow, the primary language spoken is English. You may also hear Scots and Scottish Gaelic, though English is used in everyday communication and all public services. The local accent can be strong, but people are generally friendly and happy to repeat or clarify if needed.

Some useful basics:
Hello: Hello / Hi
Bye: Bye / See you
Thank you: Thank you / Cheers (informal)
Excuse me: Excuse me / Sorry

Electricity: In United Kingdom, type G power plugs and sockets are used. The standard voltage is 230 V with a frequency of 50 Hz. This is different from most European countries, so you may need an adapter if you're travelling from abroad.

Time zone: Glasgow operates on Greenwich Mean Time (GMT, UTC+0) during winter and British Summer Time (BST, UTC+1) during daylight saving months. During BST, Glasgow is one hour behind Central European Summer Time (CEST).

Smoking:
Smoking is prohibited inside public buildings, including restaurants, bars, and public transport. Many venues may provide designated outdoor smoking areas.

Opening Hours
Typical opening hours around the University of Glasgow and the wider Glasgow area are:

- Banks: Weekdays: 9:00 AM to 4:30 PM; Weekends: Closed
- Bars & Pubs: Weekdays: 12:00 PM to 11:00 PM; Weekends: 12:00 PM to 1:00 AM (Friday–Saturday); 12:00 PM to 11:00 PM (Sunday)
- Restaurants: Weekdays: 12:00 PM to 10:00 PM; Weekends: 12:00 PM to 10:30 PM (Friday–Saturday); 12:00 PM to 9:00 PM (Sunday)
- Cafés: Weekdays: 7:00 AM to 6:00 PM; Weekends: 8:00 AM to 5:00 PM
- Museums: Usually 10:00 AM to 5:00 PM; some may have late openings on certain days
- Shopping Centres: Weekdays: 9:00 AM to 8:00 PM; Weekends: 9:00 AM to 6:00 PM (Saturday); 10:00 AM to 6:00 PM (Sunday)
- Supermarkets: Often 7:00 AM to 10:00 PM daily; larger stores may have reduced hours on Sundays
- Shops (High Street): Weekdays: 9:00/10:00 AM to 6:00 PM; Weekends: similar Saturday hours; Sunday typically 10:00 AM to 6:00 PM

Note: Opening hours can vary on public holidays (e.g., Christmas Day, Boxing Day, Easter Sunday). It's always best to check in advance.

Shopping: There are several shopping destinations near the University of Glasgow. Popular options include:

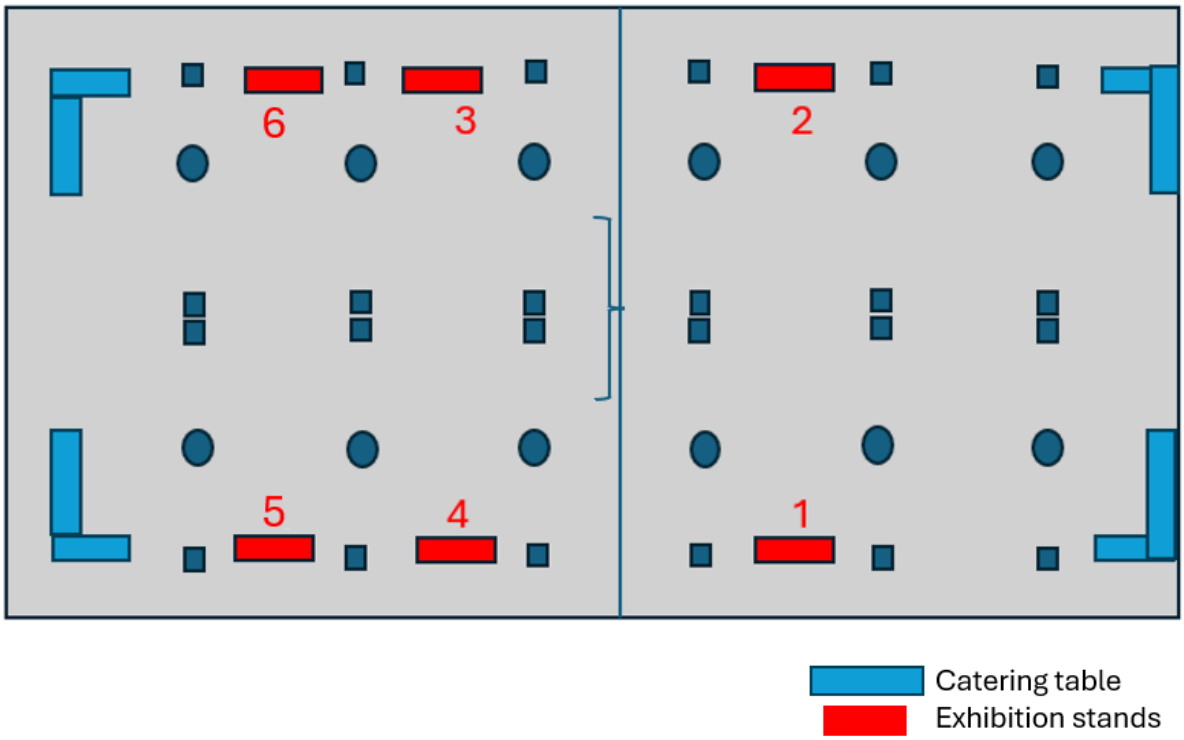
- Buchanan Galleries – a major city-centre shopping centre
- St. Enoch Centre – another large indoor shopping centre
- Princes Square – known for boutique stores and dining

You'll also find many independent shops, cafés, and boutiques in the nearby West End area.

Payment and Tipping: In Glasgow and across the UK, cashless payments are widely used and accepted. A 10–12.5% service charge may be included at restaurants. If not, tipping around 10% for good service is customary.

What to do in Glasgow: for information on activities in Glasgow, please follow the link here.

EXHIBITION FLOORPLAN



Stand No.	Company Name
1	Novogene
2	BMKGene
3	BGI
4	Developmental Biology Advances (Springer)
5	Royal Society
6	Molecular Instruments

PROGRAMME OVERVIEW

MONDAY 8 June				
08:00	Registration in the Concert Hall			
09:00 – 10:30	Fish Satellite Kelvin Gallery (413)	Arthropod Satellite Humanities Lecture Theatre (255)	Amphioxus Satellite Lecture Theatre (466)	Platynereis and other annelids Satellite East Quad Lecture Theatre (226)
10:30 – 11:00	Coffee Break, Kelvin Gallery			
11:00 – 12:30	Fish Satellite Kelvin Gallery (413)	Arthropod Satellite Humanities Lecture Theatre (255)	Amphioxus Satellite Lecture Theatre (466)	Platynereis and other annelids Satellite East Quad Lecture Theatre (226)
12:30 – 13:30	Packed Lunch, Kelvin Gallery			
13:30 – 15:30 (maximum)	Fish Satellite Kelvin Gallery (413)	Arthropod Satellite Humanities Lecture Theatre (255)	Amphioxus Satellite Lecture Theatre (466)	Platynereis and other annelids Satellite East Quad Lecture Theatre (226)
15:30 – 16:00	Coffee Break, Kelvin Gallery			
16:00 onwards	Fish Satellite Kelvin Gallery (413)	Arthropod Satellite Humanities Lecture Theatre (255)	Amphioxus Satellite Lecture Theatre (466)	Platynereis and other annelids Satellite East Quad Lecture Theatre (226)

TUESDAY 9 June				
08:00	Registration in the Concert Hall			
09:00 – 10:30	Fish Satellite Kelvin Gallery (413)	Arthropod Satellite Humanities Lecture Theatre (255)	Amphioxus Satellite Lecture Theatre (466)	Platynereis and other annelids Satellite East Quad Lecture Theatre (226)
10:30 – 11:00	Coffee Break, Hunter Halls			
11:00 – 12:30	Fish Satellite Kelvin Gallery (413)	Arthropod Satellite Humanities Lecture Theatre (255)	Amphioxus Satellite Lecture Theatre (466)	Platynereis and other annelids Satellite East Quad Lecture Theatre
12:30 – 13:30	Packed Lunch, Hunter Halls			
13:30 – 15:30 (maximum)	Fish Satellite Kelvin Gallery (413)	Arthropod Satellite Humanities Lecture Theatre (255)	Amphioxus Satellite Lecture Theatre (466)	Platynereis and other annelids Satellite East Quad Lecture Theatre
15:30 – 16:00	Coffee Break, Hunter Halls			
	Start of the Main Meeting			
16:00 - 17:00	Opening Keynote – Bute Hall Kevin Lala, *University of St Andrews* Evolution Evolving: The Developmental Origins of Adaptation and Biodiversity			
18:00 onwards	Civic Reception Glasgow City Chambers			

WEDNESDAY 10 June				
08:00	Registration in the Concert Hall			
	Symposia in 4 Parallel Sessions			
	"Theory" in Humanities Lecture Theatre (255)	**"Molecular" in Bute Hall**	**"Physiology" In Kelvin Gallery (413)**	**"Specific Systems" in East Quad Lecture Theatre (226)**
09:00 - 10:40	**S06- Evolution through Simplification and Loss**	**S02- From Cell Type- to Tissue-Evolution**	**S04- Mechanical Forces in EvoDevo**	**S08- Integrative Perspectives on Craniofacial Evo-Devo**
10:40 - 11:05	Coffee Break, in Hunter Halls			
11:05 - 12:30	**C06- Evolution through Simplification and Loss** **6 talks**	**C02- From Cell Type- to Tissue-Evolution** **6 talks**	**C04- Mechanical Forces in EvoDevo** **6 talks**	**C08- Integrative Perspectives on Craniofacial Evo-Devo** **6 talks**
12:30 - 13:30	Lunch Break in Hunter Halls WO1 – Publishing workshops, in East Quad Lecture Theatre (226)			
13:30 - 15:10	**S13- Leveraging Evo-Devo for Conservation Biology and Agroecology**	**S14- The Role of Soma-to-Germline Inheritance in Evolutionary Change**	**S22- Forty Years of Molecular Paleontology and Beyond**	**S01- Evo-Devo Principles of Plant Specialized Metabolism**
15:10 - 15:30	Coffee Break in Hunter Halls			

15:30 - 16:40	**C20- Open Symposium (Patterning) 5 talks**	**C14- The Role of Soma-to-Germline Inheritance in Evolutionary Change 5 Talks**	**C22-Forty Years of Molecular Paleontology and Beyond 5 Talks**	**C20- Open Symposium (Neuro) 5 talks**
16:40 - 18:15	Poster Session: Odd numbers, in Hunter Halls // H01- Historical artifacts, in Concert Hall			
18:15 - 19:15	Keynote – Bute Hall Beverley Glover, *University of Cambridge* How to build a (fake) fly: the convergent evolution of sexually deceptive pollination			

Thursday 11 June				
08:00	Registration in the Concert Hall			
	"Theory" in Humanities Lecture Theatre (255)	**"Molecular" in Bute Hall**	**"Physiology" In Kelvin Gallery (413)**	**"Specific Systems" in East Quad Lecture Theatre (226)**
09:00 - 10:40	**S16- Modularity, Robustness and Evolvability**	**S19- Integrating Gene Regulatory Networks into Evo-Devo: Linking Molecular Mechanisms to Trait Evolution**	**S05- The Impacts of Microbiota on Animal and Plant Development**	**S11-Pixels, Patterns, and Perception: Integrative Eco–Evo–Devo Perspectives on Biological Colour**
10:40 - 11:05	Coffee Break, in Hunter Halls			
11:05 - 12:30	**C16- Modularity, Robustness and**	**C19- Integrating Gene Regulatory**	**C05- The Impacts of Microbiota on Animal**	**C11- Pixels, Patterns, and Perception:**

	Evolvability **6 talks**	**Networks into Evo-Devo: Linking Molecular Mechanisms to Trait Evolution** **6 talks**	**and Plant Development** **4 talks + Discussion Time**	**Integrative Eco–Evo–Devo Perspectives on Biological Colour** **6 talks**
12:30 - 13:30	Lunch Break, in Hunter Halls RT01 – KLI Round Table, in East Quad Lecture Theatre (226) EED Council Meeting, in Humanities Lecture Theatre (255)			
13:30 - 15:10	**S17- Micro-Evo-Devo: Developmental Origins of Within-Population Variation**	**S18- Evo-Devo of Plant Forms & Patterns**	**S09- Nutritional Regulation of Developmental Processes**	**S15- Evolution and Development of Digestive Systems: from Specialised Cell Types to Complex Ecosystems**
15:10 - 15:30	Coffee Break, in Hunter Halls			
15:30 - 16:40	**C17- Micro-Evo-Devo: Developmental Origins of Within-Population Variation** **5 talks**	**C18- Evo-Devo of Plant Forms & Patterns** **5 talks**	**C19- Integrating Gene Regulatory Networks into Evo-Devo: Linking Molecular Mechanisms to Trait Evolution** **4 talks**	**S20- Open Symposium (Cell-Tissue)** **5 talks**
16:40 - 18:15	Poster Session : Even numbers, in Hunter Halls			
18:15 - 19:15	Keynote – Bute Hall Laura Nuno de la Rosa, *Spanish National Research Council, Institute of Philosophy* Reproduction as a Mediator of Development and Evolution			

Friday 12 June				
08:00	Registration in the Concert Hall			
	"Theory" in Humanities Lecture Theatre (255)	**"Molecular" in Bute Hall**	**"Physiology" In Kelvin Gallery (413)**	**"Specific Systems" in East Quad Lecture Theatre (226)**
09:00 - 10:40	**S21- Advances in Theoretical Evo-Devo**	**S12- Genomics in Evo-Devo - And What Next?**	**S07- Evo-Devo of Complex Life Histories: Agametic Development, Regeneration and Clonality**	**S10- Skin Deep: Common Threads in the Evolution of Bilaterian Ectodermal Appendages**
10:40 - 11:05	Coffee Break, in Hunter Halls			
11:05 - 12:30	**C21- Advances in Theoretical Evo-Devo** **6 talks**	**C12- Genomics in Evo-Devo - And What Next?** **6 talks**	**C07- Evo-Devo of Complex Life Histories: Agametic Development, Regeneration and Clonality** **6 talks**	**C10- Skin Deep: Common Threads in the Evolution of Bilaterian Ectodermal Appendages** **6 talks**
12:30 - 13:30	Lunch Break, Hunter Hall ERC workshop, in East Quad Lecture Theatre (226)			
13:30 - 15:10	**S20- Open Symposium (Theory)** **5 talks**	**C02- From Cell Type-to Tissue-Evolution (Continued)** **5 talks**	**S03- Bioelectric Signals as a new Frontier in Evo-Devo** **4 talks**	**S20- Open Symposium (Artho)** **5 talks**

15:10 - 15:30	Coffee Break, in Hunter Halls
15:30 - 16:30	Poster Session: All numbers, in Hunter Halls
16:30 - 17:25	EED Society Business Meeting – Poster Prizes – EED Society Medal – Bute Hall
17:25 - 18:25	Keynote – Bute Hall Abderrahman Khila, *CNRS* Selection, plasticity and variation: any connection?
18:25 - 18:30	Closing remarks
19:15 - 01:00	**Conference Dinner** Glasgow Science Centre

DETAILED PROGRAMME

MONDAY 8th JUNE 2026- SATELLITE MEETINGS				
08:00	Registration in the Concert Hall			
09:00 – 10:30	Fish Satellite Kelvin Gallery (413)	Arthropod Satellite Humanities Lecture Theatre (255)	Amphioxus Satellite Lecture Theatre (466)	Platynereis and other annelids Satellite East Quad Lecture Theatre (226)
10:30 – 11:00	Coffee Break, Kelvin Gallery			
11:00 – 12:30	Fish Satellite Kelvin Gallery (413)	Arthropod Satellite Humanities Lecture Theatre (255)	Amphioxus Satellite Lecture Theatre (466)	Platynereis and other annelids Satellite East Quad Lecture Theatre (226)
12:30 – 13:30	Packed Lunch, Kelvin Gallery			
13:30 – 15:30 (maximum)	Fish Satellite Kelvin Gallery (413)	Arthropod Satellite Humanities Lecture Theatre (255)	Amphioxus Satellite Lecture Theatre (466)	Platynereis and other annelids Satellite East Quad Lecture Theatre (226)
15:30 – 16:00	Coffee Break, Kelvin Gallery			
16:00 onwards	Fish Satellite Kelvin Gallery (413)	Arthropod Satellite Humanities Lecture Theatre (255)	Amphioxus Satellite Lecture Theatre (466)	Platynereis and other annelids Satellite East Quad Lecture Theatre (226)

TUESDAY 9TH JUNE 2026- SATELLITE MEETINGS AND START OF MAIN MEETING				
08:00	Registration in the Concert Hall			
09:00 – 10:30	Fish Satellite Kelvin Gallery (413)	Arthropod Satellite Humanities Lecture Theatre (255)	Amphioxus Satellite Lecture Theatre (466)	Platynereis and other annelids Satellite East Quad Lecture Theatre (226)
10:30 – 11:00	Coffee Break, Hunter Halls			
11:00 – 12:30	Fish Satellite Kelvin Gallery (413)	Arthropod Satellite Humanities Lecture Theatre (255)	Amphioxus Satellite Lecture Theatre (466)	Platynereis and other annelids Satellite East Quad Lecture Theatre
12:30 – 13:30	Packed Lunch, Hunter Halls			
13:30 – 15:30 (maximum)	Fish Satellite Kelvin Gallery (413)	Arthropod Satellite Humanities Lecture Theatre (255)	Amphioxus Satellite Lecture Theatre (466)	Platynereis and other annelids Satellite East Quad Lecture Theatre
15:30 – 16:00	Coffee Break, Hunter Halls			
	Start of the Main Meeting			
16:00 - 17:00	Opening Keynote – Bute Hall Kevin Lala, *University of St Andrews* Evolution Evolving: The Developmental Origins of Adaptation and Biodiversity			
18:00 onwards	Civic Reception Glasgow City Chambers			

WEDNESDAY 10TH JUNE 2026 – SECOND DAY		
08:00	Registration, The Concert Hall	
09:00 - 10:40 "Theory" in Humanities Lecture Theatre (255)		
S06- Evolution through simplification and loss		
09:00 - 09:25 S06.1	Stepwise loss of recombination preceeded the origin of asexuality in *Schmidtea mediterranea*	Jeremias N. Brand
09:25 - 09:50 S06.2	Evolution through simplification and loss – A panarthropod perspective	Ralf Janssen
09:50 - 10:15 S06.3	Small but mighty: How miniaturization shapes metazoan evolution	Katrine Worsaae
10:15 - 10:40 S06.4	Loss-driven genomic remodeling enables a freshwater–marine transition in a teleost	Filipe Castro
09:00 - 10:40 “Molecular” in Bute Hall		
S02- From cell type- to tissue-evolution		
09:00 - 09:25 S02.1	Principles of cell type and tissue evolution: Origin and centralisation of the nervous system	Detlev Arendt
09:25 - 09:50 S02.2	Evolutionary assembly of vertebrate digestive organs	Arun Chavan
09:50 - 10:15 S02.3	Metabolic complementation between cells drives the evolution of tissues and organs	James DiFrisco
10:15 - 10:40 S02.4	Whole genome duplication drove cell-type evolution in the vertebrate brain	Yuanzhen Zhu

WEDNESDAY 10TH JUNE 2026 – SECOND DAY		
09:00 - 10:40 “Physiology” in Kelvin Gallery		
S04- Mechanical Forces in EvoDevo		
09:00 - 09:25 S04.1	Mechanics of the cleavage pattern of the brown alga *Fucus* embryo	Bénédicte Charrier
09:25 - 09:50 S04.2	The mechanics of crocodile and tortoise head scale patterning	Rory Cooper
09:50 - 10:15 S04.3	The origin and evolution of animal morphogenesis: insights from choanoflagellates	Thibaut Brunet
10:15 - 10:40 S04.4	Evolution of gastrulation as a mechanically constrained process	Steffen Lemke
09:00 - 10:40 “Specific Systems” in East Quad Lecture Theatre		
S08- Integrative Perspectives on Craniofacial Evo-Devo		
09:00 - 09:25 S08.1	Evolutionary transition of the vertebrate pituitary from a pharyngeal gland revealed in embryos of basal fishes	Agata Horckova
09:25 - 09:50 S08.2	Building vertebrate facial shape diversity from a conserved embryonic framework	Marketa Kaucka
09:50 - 10:15 S08.3	The Pteraspid Paradox and the evolution of the vertebrate cranial dermal skeleton	Joseph Keating
10:15 - 10:40 S08.4	Fundamental Reorganization of the Amniote Facial Developmental Framework in Mammalian Evolution	Hiroki Higashiyama
10:40 - 11:05	Coffee Break, in Hunter Halls	
11:05 - 12:30 "Theory" in Humanities Lecture Theatre (255)		
C06- Evolution through simplification and loss		
11:05 - 11:19 C06.1	“Less, but more”: evolutionary innovation following massive gene loss in *Oikopleura dioica*	Cristian Cañestro
11:19 - 11:33 C06.2	How to lose your gut: the genomic mechanisms of parasitism loss in lampreys	Arne Jacobs
11:33 - 11:47 C06.3	Faster and smaller: heterochronic shift in gene expression leads to larval onset of spermatogenesis and miniaturized males	Alice Rouan
11:47 - 12:01 C06.4	Decoding the developmental genetic basis of song loss in rapidly evolving crickets	Jae Walker
12:01 - 12:15 C06.5	Morphological and molecular underpinnings of developmental truncation and loss of pectoral fins across life stages in the pipefish *Nerophis ophidion*	Ralf Schneider
12:15 - 12:29 C06.6	Developmental mechanisms underlying the evolution of visual system degeneration	Johanna Kowalko

WEDNESDAY 10TH JUNE 2026 – SECOND DAY		
11:05 - 12:30 “Molecular” in Bute Hall		
C02- From cell type- to tissue-evolution		
11:05 - 11:19 C02.1	Comparative transcriptomics reveals dynamic cell-type evolution in teleost gonads	Francisca Hervas-Sotomayor
11:19 - 11:33 C02.2	Hierarchical determination of organ identity - examples from arthropods	Ariel D. Chipman
11:33 - 11:47 C02.3	Endometrial cell innovations underlying the acquisition of menstruation in primates	Eulalie Liorzou
11:47 - 12:01 C02.4	The Role of Body Size in the Evolution of Tissues and Organs	Arsham Nejad Kourki
12:01 - 12:15 C02.5	How do complex organs arise? Exploration of the pancreatic-like cell composition of a sea urchin by differential single-cell transcriptomics	Maria Lorenza Rusciano
12:15 - 12:29 C02.6	The genetic basis of a cell type expansion in the mammalian placenta	Marcin Falis
11:05 - 12:30 “Physiology” in Kelvin Gallery		
C04- Mechanical Forces in EvoDevo		
11:05 - 11:19 C04.1	Using fly wings to study the evolution of morphogenesis	Jana Fuhrmann
11:19 - 11:33 C04.2	Tissue mechanics reveal evolutionary divergence of inner ear morphogenesis across vertebrates	Shuhei Horiguchi
11:33 - 11:47 C04.3	Morphogenesis establishes directional transport in the gastrovascular network of the jellyfish Aurelia aurita during development	Laureline Julien
11:47 - 12:01 C04.4	Rhythmic contractions of the yolk of goldfish, domesticated ornamental fish with diverse dorsoventral patterning traits	Paul Gerald Layague Sanchez
12:01 - 12:15 C04.5	Coordinating organismal and organ repair in a hydrozoan medusa	Chiara Sinigaglia
12:15 - 12:29 C04.6	Evolution and mechanics of retinal-pigmented epithelium morphogenesis during optic cup folding	François Agnès

WEDNESDAY 10TH JUNE 2026 – SECOND DAY		
11:05 - 12:30 “Specific Systems” in East Quad Lecture Theatre		
C08- Integrative Perspectives on Craniofacial Evo-Devo		
11:05 - 11:19 C08.1	Origin of the ground-borne sound perception in snakes	Taro Nojiri
11:19 - 11:33 C08.2	Prenatal cranial allometry reveals developmental adaptations to laryngeal echolocation in bats	Laura Wilson
11:33 - 11:47 C08.3	Sensory-skeletal integration and the origin of asymmetric facial bone fusions in blind cave-dwelling fish	Joshua Gross
11:47 - 12:01 C08.4	Genetic integration of craniofacial shape and eye size in Lake Malawi cichlids	Irene Soto Bajo
12:01 - 12:15 C08.5	Synchrotron microtomography reveals unusual vascular cartilage in an Ordovician vertebrate	Richard Dearden
12:15 - 12:29 C08.6	Elemental and Molecular Mapping Reveal Cartilage Cell Organization and Differentiation During Development	Tatjana Haitina
12:30 – 13:30	Lunch Break in Hunter Halls WO1 – Publishing workshops, in East Quad Lecture Theatre (226)	
13:30 - 15:10 "Theory" in Humanities Lecture Theatre (255)		
S13- Leveraging evo-devo for conservation biology and agroecology		
13:30 - 13:55 S13.1	Microbial symbionts and the flourishing of ecosystems	Scott Gilbert
13:55 - 14:20 S13.2	Evolutionary divergence of neural crest cells in Icelandic three-spined stickleback (*Gasterosteus aculeatus*) thermal ecotypes	Matthew Brachmann
14:20 - 14:45 S13.3	Developmental plasticity as a buffer against extinction: experimental insights from rotifers	Emily Harmon
14:45 – 15:10 S13.4	Early-life stress induces brain-region-specific epigenetic and micro-RNA dynamic changes across ontogeny in chickens	Carlos Guerrero-Bosagna
13:30 - 15:10 “Molecular” in Bute Hall		
S14- Role of Soma-to-Germline Inheritance in Evolutionary Change		
13:30 - 13:55 S14.1	Understanding the ‘sperm RNA code’ for epigenetic inheritance with emerging tools	Qi Chen
13:55 - 14:20 S14.2	Darwin’s Pangenesis in relation to evolution, development and soma-to-germline inheritance	Yongsheng Liu
14:20 - 14:45 S14.3	How Germ Cells Respond to Stress - and What Remains to Be Discovered	Vincent Fischer
14:45 – 15:10 S14.4	Somatically acquired sperm miRNAs initiate embryonic regulatory cascades underlying paternal epigenetic inheritance	Colin Conine

WEDNESDAY 10TH JUNE 2026 – SECOND DAY		
13:30 – 15:10 "Physiology" in Kelvin Gallery		
S22- Forty years of molecular paleontology and beyond		
13:30 - 13:55 S22.1	The diversification of land plant body plans through the lens of molecular palaeobotany	Alexander Hetherington
13:55 - 14:20 S22.2	Unraveling the Deep Evolutionary History of Microbial Life	Edmund Moody
14:20 - 14:45 S22.3	Molecular Palaeobiology of Echinozoan skeletons	Jeffrey Thompson
14:45 - 15:10 S22.4	Convergent genome evolution shaped the emergence of terrestrial animals	Jialin Wei
13:30 – 15:10 "Specific Systems" in East Quad Lecture Theatre		
S01- Evo-Devo Principles of Plant Specialized Metabolism		
13:30 - 13:55 S01.1	Development of a flower as a false female in the sexually deceptive orchid *Ophrys sphegodes*	Philipp M. Schlüter
13:55 - 14:20 S01.2	Complex Evolution in the Cellulose Synthase Protein Family: From Core Cell Wall Assembly to Enzymatic and Non-enzymatic Functions in Specialized Metabolism	Asaph Aharoni
14:20 - 14:45 S01.3	The evolution of plant specialized metabolism and specialized cell types	Maite Colinas
14:45 - 15:10 S01.4	Allopolyploidy-mediated innovation of gain-of-function defenses in a plant-insect arms race	Emmanuel Gaquerel
15:10 – 15:30	Coffee Break, in Hunter Halls	
15:30 – 16:40 "Theory" in Humanities Lecture Theatre (255)		
C20- Open Symposium (patterning)		
15:30 - 15:44 C20.1	Cnidarian Embryonic Aggregates Reveal an Alternative Developmental Morphospace of Initial Body Plan Formation	Kerim Anlas
15:44 - 15:58 C20.2	New meiofaunal genomes reveal the diversity of animal Hox clusters	Madeleine Aase-Remedios
15:58 - 16:12 C20.3	Hopeful Monsters in Action? Repeated Axial Evolution Across African Cichlid Fish Radiations	Callum Bucklow
16:12 - 16:26 C20.4	An Evolutionarily Conserved Genetic Framework for Pnant Root Cortex Patterning	Raffaele Dello Ioio
16:26 - 16:40 C20.5	Building branches: mechanisms of meristem duplication during dichotomous branching	Vicky Spencer
16:40 – 18:15	Poster Session: Odd numbers, in Hunter Halls // H01- Historical artifacts, in Concert Hall	
18:15 – 19:15	Keynote – Bute Hall – K2 Beverley Glover, *University of Cambridge* How to build a (fake) fly: the convergent evolution of sexually deceptive pollination	

WEDNESDAY 10TH JUNE 2026 – SECOND DAY		
15:30 – 16:40 "Molecular" in Bute Hall		
C14- Role of Soma-to-Germline Inheritance in Evolutionary Change		
15:30 - 15:44 C14.1	Intergenerational shifts in innate odour preferences upon odour feeding or odour injections in *Bicyclus anynana* butterfly larvae	Antonia Monteiro
15:44 - 15:58 C14.2	Germline consequences of a multigenerational metabolic disruption	Violeta de Anca Prado
15:58 - 16:12 C14.3	Epigenetic Inheritance of DNA methylation in an Early-Branching Animal	Lan Xu
16:12 - 16:26 C14.4	Developmental Dynamics of Annelid Methylation	Meghan Yap-Chiongco
16:26 - 16:40 C14.5	The genetic and epigenetic architecture of thermal plasticity in the threespine stickleback	Corin Stansfield
15:30 – 16:40 "Physiology" in Kelvin Gallery		
C22- Forty years of molecular paleontology and beyond		
15:30 - 15:44 C22.1	Towards a philosophy of reproduction for evo-devo: an organismal and relational approach	David Cortés-García
15:44 - 15:58 C22.2	Developmental morphological rates and the emergence of animal body plans: insights from early metazoan evolution	Zhiqing Guo
15:58 - 16:12 C22.3	Stepwise evolution of vertebrate median bony structures: from dorsal scutes to fin rays	Kazuhide Miyamoto
16:12 - 16:26 C22.4	Bayesian timetree inference using Hamiltonian dynamics	Laura Mulvey
16:26 - 16:40 C22.5	The first quantitative evidence of ontogenetic growth in the early Cambrian ecdysozoan worm *Cricocosmia jinningensis* reveals the earliest energy reallocation	Xiaomei Shi
15:30 – 16:40 "Specific Systems" in East Quad Lecture Theatre		
C20- Open Symposium (neuro)		
15:30 - 15:44 C20.1	Developmental evolution of brain monoaminergic systems: the outcomes of the mao mutation in blind Mexican cavefish	Alessandro Alunni
15:44 - 15:58 C20.2	Adaptive significance and evolutionary origin of the Turbanate Eye in Cloeon dipterum: a case of sensory innovation in mate recognition	Tòt Senar-Serra
15:58 - 16:12 C20.3	Astrocyte evolution in vertebrates: a tale of two fishes	Anna Box
16:12 - 16:26 C20.4	Tail muscle development of Oikopleura dioica: a neuromesodermal origin and the evolution of the appendicularian free-swimming lifestyle	Marc Fabregà-Torrus
16:26 - 16:40 C20.5	Sexual Dimorphism and Lifestyle Transition: from Sex Chromosome Turnover to Brain Remodeling in a Mayfly	Sophie Tandonnet

THURSDAY 11 JUNE 2026 – THIRD DAY		
08:00	Registration, The Concert Hall	
09:00 – 10:40 "Theory" in Humanities Lecture Theatre (255)		
S16- Modularity, robustness and evolvability		
09:00 - 09:25 S16.1	The process-specific GP maps and their interaction may explain micro-to-macro divide	Mihaela Pavlicev
09:25 - 09:50 S16.2	Modularization of the plant body plan and the rise of flowering plants	Rainer Melzer
09:50 - 10:15 S16.3	Modularity and morphological innovation : bridging master genes with developmental systems properties	Sophie Pantalacci
10:15 - 10:40 S16.4	Multi-scale devo-evo in a bite: nesting variation and evolvability of complex dentitions and beyond	Roland Zimm
09:00 – 10:40 "Molecular" in Bute Hall		
S19- Integrating Gene Regulatory Networks into Evo-Devo: Linking Molecular Mechanisms to Trait Evolution		
09:00 - 09:25 S19.1	A network-centric view on the evolution of developmental processes - Insights from eye size variation in *Drosophila*	Nico Posnien
09:25 - 09:50 S19.2	Gene regulatory networks driving neuronal diversification in development and in evolution	Nuria Flames
09:50 - 10:15 S19.3	Making diverse "breathing" pores - gene regulatory network evolution and the morphodiversity of plant stomata	Michael T. Raissig
10:15 - 10:40 S19.4	Effects of foreign transcription factors in vertebrate gene regulatory networks	Marta Portela
09:00 – 10:40 "Physiology" in Kelvin Gallery		
S05- The Impacts of Microbiota on Animal and Plant Development		
09:00 - 09:25 S05.1	Natural variation in *Arabidopsis thaliana* root responses to microbiota-derived *Pseudomonas*	Charles Copeland
09:25 - 09:50 S05.2	Decoding microbiota-driven plasticity in the vertebrate intestine	John Rawls
09:50 - 10:15 S05.3	A role of environmental microbes in triggering wound healing and regeneration	Azra Atabay
10:15 - 10:40 S05.4	Understanding how the microbiome shapes nervous system development	Helen Vuong

THURSDAY 11 JUNE 2026 – THIRD DAY		
09:00 – 10:40 “Specific Systems” in East Quad Lecture Theatre		
S11-Pixels, patterns, and perception: integrative eco–evo–devo perspectives on biological colour		
09:00 - 09:25 S11.1	How do girls make diamonds? The developmental basis of a female-limited color pattern polymorphism	Nathalie Feiner
09:25 - 09:50 S11.2	How the mouse got its stripes: formation and evolution of color patterns in rodents	Ricardo Mallarino
09:50 - 10:15 S11.3	Machine-learnt variation among butterfly photographs: new quantitative tools for evolution and development	Jennifer Hoyal Cuthill
10:15 - 10:40 S11.4	From shallow larvae to the abyss: extraordinary visual adaptation in deep-sea fishes	Zuzana Musilova
10:40 – 11:05	Coffee break	
11:05 - 12:30 “Theory” in Humanities Lecture Theatre (255)		
C16- Modularity, robustness and evolvability		
11:05 - 11:19 C16.1	Evolutionary tuning of Nodal mobility ensures robust embryonic patterning across temperatures	Daniel Čapek
11:19 - 11:33 C16.2	Developmental heterochrony underlies the evolution of extreme body axis segmentation in the Japanese eel	Ali Seleit
11:33 - 11:47 C16.3	Role of plasticity and developmental robustness in the evolutionary process and insights into genetic assimilation	Atsuko Sato
11:47 - 12:01 C16.4	Regulatory dominance promotes robustness and evolutionary innovation	Noa Ottilie Borst
12:01 - 12:15 C16.5	Environmental Noise and the Evolution of Evolvability	Masahito Tsuboi
12:15 - 12:29 C16.6	Developmental system drift as a consequence of the evolution of modularity	Áine McColgan
11:05 - 12:30 “Molecular” in Bute Hall		
C19- Integrating Gene Regulatory Networks into Evo-Devo: Linking Molecular Mechanisms to Trait Evolution		
11:05 - 11:19 C19.1	The roads to superorganisms: an evo-devo perspective from termites	Bitao Qiu
11:19 - 11:33 C19.2	The role of Fat-Hippo signaling in the origin and diversification of beetle horns	Yongsoo Choi
11:33 - 11:47 C19.3	Tracing the evolutionary origin of eyespot center cell types on butterfly wings with single-cell genomics	Suriya Narayanan Murugesan
11:47 - 12:01 C19.4	Redeployment of an ancestral gene regulatory network drives the origins of a novel cell type in Poeciliid fish	Sophie Kraunsoe
12:01 - 12:15 C19.5	Single-nucleus Profiling Highlights the All-Brain Echinoderm Nervous System and the Gene Regulatory Signature of Postmetamorphic Cell Types and Organs	Periklis Paganos
12:15 - 12:29 C19.6	Integrating Developmental Timing and Transcriptomics to Uncover Insect Brain Diversification	Christine Mau

THURSDAY 11TH JUNE – THIRD DAY		
11:05 - 12:30 "Physiology" in Kelvin Gallery		
C05- The Impacts of Microbiota on Animal and Plant Development		
11:05 - 11:19 C05.1	A Microbial Perspective on Animal Development and Behaviour	Thomas Bosch
11:19 - 11:33 C05.2	Microbes as manipulators of egg size and developmental evolution	Tyler Carrier
11:33 - 11:47 C05.3	Microbiome dynamics across the life cycle of *Platynereis dumerilii*	Giulia Ghisleni
11:47 - 12:01 C05.4	Synergistic holobiont plasticity in the sea urchin *Arbacia lixula*: Maternal effects and symbiotic microbiome under ocean acidification	Andres Rufino Navarro
12:01 – 12:29 C05.5	Discussion time	
11:05 - 12:30 "Specific Systems" in East Quad Lecture Theatre		
C11- Pixels, patterns, and perception: integrative eco–evo–devo perspectives on biological colour		
11:05 - 11:19 C11.1	How the lizard changed its spots: using computational models of development to predict evolutionary changes in colour patterns	Halley Cano-Fernández
11:19 - 11:33 C11.2	The evolution, genetics and development of butterfly structural colour	Nicola Nadeau
11:33 - 11:47 C11.3	The lncRNA–miRNA gene *ivory:mir-193* controls adaptive melanism in a polymorphic moth	Luca Livraghi
11:47 - 12:01 C11.4	Sketching Vertebrate Color Evolution through the Reconstruction of Reptile Chromatophore Development	Pierre-Yves Helleboid
12:01 - 12:15 C11.5	Non-iridescent structural color emergence and diversification controlled by keratin self-assembly during feather development	Rita Afonso
12:15 - 12:29 C11.6	How sexes diversify: Molecular drivers underlying sexual color dimorphism in cichlids	Muktai Kuwalekar
12:30 – 13:30	Lunch Break, in Hunter Halls RT01 – KLI Round Table, in East Quad Lecture Theatre (226) EED Council Meeting, in Humanities Lecture Theatre (255)	

THURSDAY 11 JUNE 2026 – THIRD DAY		
13:30 – 15:10 "Theory" in Humanities Lecture Theatre (255)		
S17- Micro-Evo-Devo: Developmental Origins of Within-Population Variation		
13:30 - 13:55 S17.1	Variation lost in translation between micro- and macroevolution	Jukka Jernvall
13:55 - 14:20 S17.2	Epigenetic changes alter developmental variance in isogenic mouse models	Rebecca Green
14:20 - 14:45 S17.3	Innovation and di versification of and through sexual dimorphism: insights from horned beetles	Erica Nadolski
14:45 - 15:10 S17.4	Changing developmental signal has an effect on developmental bias: Wnt mutation modifies phenotypic co-variation patterns for cell fates in the nematode vulva	Katie Pelletier
13:30 – 15:10 "Molecular" in Bute Hall		
S18- Evo-Devo of plant forms & patterns		
13:30 - 13:55 S18.1	Conserved modules, novel forms: Investigating the mechanisms underlying grass morphological diversity	Katie Jeal
13:55 - 14:20 S18.2	Stay hydrated – Deciphering the development and physiology of leaf succulence	Heike Lindner
14:20 - 14:45 S18.3	Unusual Morphogenesis in *Monophyllaea*: Indeterminate Leaf Meristems and the Absence of a Shoot Apical Meristem	Hirokazu Tsukaya
14:45 - 15:10 S18.4	From spots to stripes: interplay between a reaction-diffusion system and its prepatterns	Yaowu Yuan
13:30 – 15:10 "Physiology" in Kelvin Gallery		
S09- Nutritional regulation of developmental processes		
13:30 - 13:55 S09.1	Metabolic control of planarian regeneration	Cristina Gonzalez-Estevez
13:55 - 14:20 S09.2	A stem cell zoo uncovers intracellular scaling of developmental tempo across mammals	Jorge Lazaro Farre
14:20 - 14:45 S09.3	Maternal diet influences embryonic developmental trajectory and offspring phenotype via non-genetic factors in *Drosophila melanogaster*	Krittika Sudhakar
14:45 - 15:10 S09.4	Nutritional control of body plasticity across biological scales in a sea anemone	Patrick Steinmetz

THURSDAY 11TH JUNE 2026 – THIRD DAY		
13:30 - 15:10 "Specific Systems" in East Quad Lecture Theatre		
S15- Evolution and development of digestive systems: from specialised cell types to complex ecosystems		
13:30 - 13:55 S15.1	A blastoporal organizer in a ctenophore	Andreas Hejnol
13:55 - 14:20 S15.2	Comparative transcriptomics reveal the common anteroposterior molecular blueprint of adult bilaterian guts	Stefano Davide Vianello
14:20 – 14:45 S15.3	A new animal model for dissecting drug transporter functions during gut maturation and host-microbe interactions	Catherine Schrankel
14:45 - 15:10 S15.4	Gut evolution, from cell types to gene regulatory networks	Maria Ina Arnone
15:10 – 15:30	Coffee break , Hunter Halls	
15:30 – 16:40 "Theory" in Humanities Lecture Theatre (255)		
C17- Micro-Evo-Devo: Developmental Origins of Within-Population Variation		
15:30 - 15:44 C17.1	Organismal-level regulation of phenotypic variability in *Drosophila*	Luisa F. Pallares
15:44 - 15:58 C17.2	Determining hotspots of cellular adaptation in the polychaete *Platynereis dumerilii*	Tobias Gerber
15:58 - 16:12 C17.3	Morphometric quantification of temperature stress effects on embryonic development at single-cell resolution	Chiara Castelletti
16:12 - 16:26 C17.4	Investigating the genetic and developmental bases of compound eye size variation among populations, species and temperatures	Alistair P. McGregor
16:26 - 16:40 C17.5	Can annual fish become a model for micro-evolution and adaptation of development?	Tom Van Dooren
15:30 – 16:40 "Molecular" in Bute Hall		
C18- Evo-Devo of plant forms & patterns		
15:30 - 15:44 C18.1	Lignin patterning and evolutionary transitions in explosive fruit	Aurelia Emonet
15:44 - 15:58 C18.2	Modelling The Interplay Between Pre-Patterning and Morphogenesis in *Hibiscus trionum* Petals	Steven Oud
15:58 - 16:12 C18.3	Checkerboards and the chess flower: evolution of colour patterns in Fritillaria (Liliaceae)	Laetitia Carrive
16:12 - 16:26 C18.4	How plants grow cups: Investigating the Development and Plasticity of *Marchantia polymorpha* Gemma Cups	Leonie Kraska
16:26 - 16:40 C18.5	The evolutionary origins of apical growth in plants	Joseph Sardina

THURSDAY 11TH JUNE 2026 – THIRD DAY		
15:30 - 16:40 “Physiology” in Kelvin Gallery		
C19- Integrating Gene Regulatory Networks into Evo-Devo: Linking Molecular Mechanisms to Trait Evolution (continued)		
15:30 - 15:44 C19.1	Regulatory tinkering in the evolution of vertebrate olfaction	Demian Burguera
15:44 - 15:58 C19.2	Evolution of the photoreceptor developmental program in nocturnal geckos	Emily M. Dong
15:58 - 16:12 C19.3	Building a jellyfish muscle: Characterization of Myogenesis in *Pelagia noctiluca*	Clara Deleau
16:12 - 16:26 C19.4	Tracing the evolution of male pregnancy and brood pouch complexity in syngnathids through comparative transcriptomics	Tarek Gerhard
15:30 - 16:40 “Specific Systems” in East Quad Lecture Theatre		
C20- Open Symposium (cell-tissue)		
15:30 - 15:44 C20.1	Tracing the evolutionary origin and function of fluorescent pigmented cells in sea cucumbers	Rossella Annunziata
15:44 - 15:58 C20.2	EvoDevoBase: an open database for evolutionary developmental biology	Bruno Vellutini
15:58 - 16:12 C20.3	Late Vascular Development in Chondrichthyans Reveals a Conserved Programs of Venous Valve Formation	Virginia Panara
16:12 - 16:26 C20.4	Spatial transcriptomics reveals "temporal modularity" in gap and pair-rule gene patterning in the early Tribolium embryo	Ezzat El-Sherif
16:26 - 16:40 C20.5	Transient Receptor Potential (TRP) Channel diversity and localization in Choanoflagellates points to a role in cellular behavior	Jeffrey Colgren
16:40 – 18:15	Poster Session, even numbers Hunter Halls	
18:15 – 19:15	Keynote – Bute Hall – K3 Laura Nuno de la Rosa, Spanish National Research Council, Institute of Philosophy Reproduction as a Mediator of Development and Evolution	

FRIDAY 12TH JUNE 2026 – FOURTH DAY		
08:00	Registration, the Concert Hall	
09:00 - 10:40 "Theory" in Humanities Lecture Theatre (255)		
S21- Advances in theoretical evo-devo		
09:00 - 09:25 S21.1	The evolutionary implications of genotype-phenotype map structure – from molecular examples to general principles	Nora S. Martin
09:25 - 09:50 S21.2	The Extended Life Cycle	Nayely Velez-Cruz
09:50 - 10:15 S21.3	A theoretical perspective on evolvability	Thomas Hansen
10:15 - 10:40 S21.4	Constructive Internal Selection: a neglected factor in developmental evolution	Günter Wagner
09:00 - 10:40 "Molecular" in Bute Hall		
S12-Genomics in Evo-Devo - And what next?		
09:00 - 09:25 S12.1	Gene duplication and co-option originated a male specific visual system in mayflies	Isabel Almudi
09:25 - 09:50 S12.2	An integrative Eco-Evo-Devo approach to colour pattern variation in cichlid fishes	Emília Santos
09:50 - 10:15 S12.3	The evolution of new vertebrate cell types and organs	Margarida Cardoso-Moreira
10:15 - 10:40 S12.4	Simulating the evolution of development: current and future approaches	Renske Vroomans
09:00 - 10:40 "Physiology" in Kelvin Gallery		
S07- Evo-Devo of complex life histories: agametic development, regeneration and clonality		
09:00 - 09:25 S07.1	Probing clonal evolution in planarians	Jochen Rink
09:25 - 09:50 S07.2	Complex lifecycles and polyploidy in a cnidarian model species	Catriona Munro
09:50 - 10:15 S07.3	Using sponge whole-body regeneration to investigate early animal evolution	Roger Revilla-i-Domingo
10:15 - 10:40 S07.4	Conserved neurogenic functions of Notch pathway during regeneration and growth in the annelid *Platynereis*	Eve Gazave
09:00 - 10:40 "Specific Systems" in East Quad Lecture Theatre		
S10- Skin Deep: Common Threads in the Evolution of Bilaterian Ectodermal Appendages		
09:00 - 09:25 S10.1	Sensory origins of vertebrate teeth and the evolutionary links between odontodes and ectodermal sensory structures	Yara Haridy
09:25 - 09:50 S10.2	Armoured catfish evolved skin denticles that recapitulate teeth the structural, developmental, and genetic level	Carlos Rivera Rivera
09:50 - 10:15 S10.3	Ectodermally derived exoskeletons in molluscs: from signalling to seashells	Victoria Sleight
10:15 - 10:40 S10.4	Development and evolution of the whisker pattern	Denis Headon

FRIDAY 12TH JUNE 2026 – FOURTH DAY		
10:40 – 11:05	Coffee break	
11:05 - 12:30 “Theory” in Humanities Lecture Theatre (255)		
C21- Advances in theoretical evo-devo		
11:05 - 11:19 C21.1	Linking Developmental Dynamics and Statistical Models of Evolutionary Variation	Lisandro Milocco
11:19 - 11:33 C21.2	Why are multicellular differentiation programs directional?	Somya Mani
11:33 - 11:47 C21.3	A mathematical synthesis of genetics, development, and evolution	Mauricio González-Forero
11:47 - 12:01 C21.4	What happens to the questions and answers of evolutionary theory when we can model the genotype-phenotype map and its evolution	Isaac Salazar-Ciudad
12:01 - 12:15 C21.5	Loss, persistence and reversal of phenotypic traits	Giobbe Forni
12:15 - 12:29 C21.6	The evolution of tissue shape and differentiation in a computational model of plant morphogenesis	Pjotr L. van der Jagt
11:05 - 12:30 “Molecular” in Bute Hall		
C12- Genomics in Evo-Devo - And what next?		
11:05 - 11:19 C12.1	An ancient gene is a novel regulator of cell proliferation in the spider Parasteatoda tepidariorum	Zoe X. Schultz
11:19 - 11:33 C12.2	Uncovering the molecular basis of bat wing development using comparative genomic and single-cell approaches	Francisca Real
11:33 - 11:47 C12.3	From 52 to hundreds of species: integration of bulk and single-cell transcriptomics across animal diversity in Bgee	Marc Robinson-Rechavi
11:47 - 12:01 C12.4	The genomic and developmental basis of eyestalks in the stalk-eyed fly Teleopsis dalmanni	Victoria Lloyd
12:01 - 12:15 C12.5	Extensive transcriptomic turnover facilitates evolutionary novelty in brown algae	Jaruwatana Sodai Lotharukpong
12:15 - 12:29 C12.6	Functional genetics and genomics of egg development speed in the red flour beetle	Caspar Schmeits

FRIDAY 12TH JUNE 2026 – FOURTH DAY		
11:05 - 12:30 "Theory" in Humanities Lecture Theatre (255)		
C07- Evo-Devo of complex life histories: agametic development, regeneration and clonality		
11:05 - 11:19 C07.1	Large-scale reprograming of differentiated larval tissues establishes adult body plan during sea cucumber metamorphosis	Laurent Formery
11:19 - 11:33 C07.2	Multi-stage single-cell atlas of agametic reproduction in the colonial ascidian *Botryllus schlosseri* unveils transcriptomic reprogramming and possible dedifferentiation of bud founder cells	Marie Lebel
11:33 - 11:47 C07.3	Exploring zooid polymorphism in siphonophores (Cnidaria:Hydrozoa) at single cell resolution	Namrata Ahuja
11:47 - 12:01 C07.4	Food availability impacts developmental trajectory in the asexually reproducing flatworms	Ludwik Gąsiorowski
12:01 - 12:15 C07.5	Single-cell analysis of the hemichordate head blastema provides insights into the early deuterostome origins of regeneration	Ivan Candido-Ferreira
12:15 - 12:29 C07.6	The sea anemone *Nematostella vectensis* in laboratory vs. natural-like growth environments – molecular, cellular, developmental and life cycle effects	Uri Gat
11:05 - 12:30 "Specific Systems" in East Quad Lecture Theatre		
C10- Skin Deep: Common Threads in the Evolution of Bilaterian Ectodermal Appendages		
11:05 - 11:19 C10.1	Genetic erosion of the scale genetic network forced an evolutionary return to bony armor	Joost Woltering
11:19 - 11:33 C10.2	The unreasonable effectiveness of computational models in skin scale patterning and morphogenesis	Michel Milinkovitch
11:33 - 11:47 C10.3	Genomic and signaling network evolution underlying hedgehog spine formation	Anna Alessandra Monaco
11:47 - 12:01 C10.4	Arthropod's next top model: *Oxidus gracilis*	Alexandra Weber
12:01 - 12:15 C10.5	Scale cell type specification on butterfly wings	Joseph Hanly
12:15 - 12:29 C10.6	Developmental Mechanisms for Morphological Novelty in Early Osteichthyan Dentitions	Plamen Andreev
12:30 – 13:30	Lunch Break, Hunter Hall ERC workshop, in East Quad Lecture Theatre (226)	

FRIDAY 12TH JUNE 2026 – FOURTH DAY		
13:30 - 15:10 "Theory" in Humanities Lecture Theatre (255)		
C20-Open Symposium (theory)		
13:30 - 13:44 C20.1	Not your average archaeon: CCA diversity of Heimdallarchaeota as a window into early eukaryogenesis	Ella Cassidy
13:44 - 13:58 C20.2	Investigating the biological significance of evolutionary conflict using convergent traits in plants as case studies	Holly Robertson
13:58 - 14:12 C20.3	Learning developmental trajectories from time-lapse embryo imaging using unsupervised deep learning	Hernan Morales Navarrete
14:12 - 14:26 C20.4	Multimodal study of the convergent evolution of molar shape in rodent molars	Marie Sémon
14:26 - 14:40 C20.5	Modularity and Canalisation Shape Early Craniofacial Divergence in Arctic Charr	Kalina Kapralova
13:30 - 15:10 "Molecular" in Bute Hall		
C02- From cell type- to tissue-evolution (continued)		
13:30 - 13:44 C02.1	Transcription factor family expansions and the origins of novel cell types	Alison G. Cole
13:44 - 13:58 C02.2	How do novel cell types evolve? Co-option of fibroblast activation in the origin of uterine decidualization	Stefan Pavloski
13:58 - 14:12 C02.3	Developing AI models to reconstruct cell type evolution across animal phylogeny	Jacob Musser
14:12 - 14:26 C02.4	Using closely related planarian species and single-cell transcriptomics to study cell type evolution	Jordi Solana
14:26 - 14:40 C02.5	Complementary roles of alternative splicing and gene expression in bilaterian tissue evolution	Federica Mantica
13:30 - 15:10 "Physiology" in Kelvin Gallery		
S03- Bioelectric signals as a new frontier in EvoDevo		
13:30 - 13:55 S03.1	Living electronic interfaces to unravel Hydra bioelectricity	Claudia Tortiglione
13:55 - 14:20 S03.2	Integrating live voltage imaging and pharmacological manipulation to link bioelectric states to genetic control in ascidian morphogenesis	Josep Marti-Solans
14:20 - 14:45 S03.3	The Forces That Shape Us	Elias H Barriga
14:45 - 15:10 S03.4	Discussion time	

FRIDAY 12TH JUNE 2026 – FOURTH DAY		
13:30 - 15:10 “Specific Systems” in East Quad Lecture Theatre		
C20- Open Symposium (arthro)		
13:30 - 13:44 C20.1	A developmental mechanism producing dimorphism in ovary size between queen and worker ants	Claire Ramsay
13:44 - 13:58 C20.2	Evolutionary Flexibility of CpG Methylation and Histone Modifications in Beetles	Elisa Israel
14:12 - 14:26 C20.4	How to build a powerful water conserving system: the cryptonephridial complex of the beetle *Tribolium*	Robin Beaven
14:26 - 14:40 C20.5	Macroevolution of nutritional plasticity in exaggerated dung beetle forelegs and its underlying developmental mechanism	Ebony M Argaez
14:40 – 15:10	Discussion time	
15:10 – 15:30	Coffee break	
15:30 – 16:30	Poster Session, All numbers, in Hunter Halls	
16:30 – 17:25	EED Society Business Meeting – Poster Prizes – EED Society Medal – Bute Hall	
17:25 – 18:25	Keynote – Bute Hall Abderrahman Khila, CNRS Selection, plasticity and variation: any connection?	
18:25 – 18:30	Closing Remarks	
19:15 – 01:00	Conference Dinner Glasgow Science Centre	

POSTER PRESENTATIONS

Poster Number	Abstract Title	Presenter
P002	Braiding in the developmental hourglass model	Bassel Arnaout
P003	Unravelling ancient thyroid hormone signalling roles during larval and metamorphosis stages of the marine annelid Platynereis dumerilii	Samuel H. Tambunan
P004	Cryptic persistence of truncated abdominal legs in insects and the origin of novel structures	Heather Bruce
P005	Mechanical induction in metazoan development and evolution: from earliest multi-cellular gut-characterized organisms to modern animal embryos	Emmanuel Farge
P006	Defining multiciliated cell types in Platynereis larvae: Dispersed vs. Aligned ciliary arrays	Steffanie Mutiara
P007	Investigating germ cell source and sex differentiation in juvenile P. dumerilii	Julianna Escudero
P008	The origin and diversification of a cellular patterning mechanism: Lateral inhibition governs ancestral cellular specification in fossil and extant land plants	Josep Mercadal
P011	Using the sensory lateral line to explore enhancer evolutionary co-option between fish and mammals	Aurélie Hintermann
P012	Big Questions from a Small Nervous System. Dissecting Neuronal Cell Types in Sea Urchin Larvae	Olga Zueva
P013	Cellular origins of complex and variable traits: integrating genome-wide association studies and single-cell transcriptomics to investigate the cellular basis of craniofacial variation and congenital conditions across development.	Gianmarco Cavalli
P014	Ready, set, glia: Single-cell RNA sequencing reveals novel populations of glia cells in larvae of the annelid worm Owenia fusiformis	Lily Winkler
P015	Lunar rhythms impact on cell states in marine invertebrates	Maria Cocurullo
P016	Heterochrony diversifies accessibility to tissue volumetric growth across species during homologous organ morphogenesis	Shunya Kuroda
P017	Vitamin D elicits tissue-specific isoform expression in Atlantic salmon muscles	C. Darrin Hulsey
P018	Inter-species large synthetic replacements suggest transcriptional and developmental robustness at the Hoxd cluster	Silvia Aldrovandi
P019	Expansion of isoform diversity in key developmental transcription factors at the origin of vertebrates	Nuria P Torres-Aguila
P020	How to Build a Head: Developmental Divergence in Vertebrate Embryogenesis	Louk W.G. Seton
P021	Convergence of Emergent Mechanical Traits	Robert Bellow
P022	Molecular signature, heterogeneity, and evolutionary conservation of the vertebrate perichondrium	Linda Kappes
P023	Manipulation of a polyphenism-switch network reveals the molecular basis for canalization of alternative forms	Justin Braynt
P024	Single-cell sequencing in flies and beetles reveals regulators of neural stem cell specification as novel candidates for evolutionary modification of cellular brain composition	Gregor Bucher
P025	An ancient monoaminergic signaling system coordinates contractility in a nerveless sponge	Rong Xuan Zang

P026	From Mantle to Shell: Cellular Foundations of Structural Colour Patterning in the Blue-Rayed Limpet Patella pellucida	Nathaniel Crook
P027	Evolutionary drift in biomineralization processes questions the nature of ‘osteocytes’ and ‘chondrocytes’ in early vertebrates	Melanie Debiais-Thibaud
P028	Morphological innovation without gene co-option: the Drosophila sex comb evolved via changes in developmental tempo and energy metabolism	Ben Hopkins
P029	Heterochronic activation of TGF-β signaling drives the diversity of the avian sterna	Seung June Kwon
P030	Variability of asexual development trajectories in clonal zooids of the ascidian Botryllus schlosseri	Lucia Manni
P031	Evolution of Novel Contacts in Orthologous Regulatory Elements	Vasileios Papadogiannis
P032	Metabolic control of multicellular transition in a close unicellular relative of metazoa	Eudald Pascual-Carreras
P033	Evolutionary modulation of the peripheral nervous system gene regulatory network in the ascidian Molgula appendiculata	Cécile LEBLOND
P034	Post-embryonic development networks underlying trait evolution in teleost fishes	Agneesh Barua
P035	Novel RA-Wnt-Nr2f1 Conserved Regulatory Networks in vertebrate atrial development	Madisyn Hildenbrand
P036	Redrawing the Prosomeric Map: Hypoprethalamus as primordial ontogenetic territory in tetrapods.	Ruth Morona
P037	Co-option of conserved retinal cell types by a male-specific doublesex-related gene underlies the evolutionary origin of the mayfly Turbanate eye	Maria Rossello
P038	The Hes/her gene deadpan, a paralog of hairy, plays a partially redundant role in Drosophila segmentation.	Maciej Zurowski
P039	The structure of tooth evo-devo: Trp63 binding targets in the embryonic face	Julia Boughner
P040	Nuclear targeting enables highly efficient transgenesis in the milkweed bug Oncopeltus fasciatus	Yu Shirai
P042	Mechanisms specifying invariant ovariole and testiole number across insects	Daniella A. Haber
P044	Evolution of vertebrate facial morphogenesis	Tristan Wilhelm Jockheck
P045	Mayflies, an emerging model to investigate the origin of insect wings	Rafath Chowdhury
P046	A whole-organism single-cell perspective on larval haematopoiesis and the evolution of innate immunity in molluscs	Camilo Muñoz Schuler
P047	An Integrated Framework for Thermosensation in Platynereis dumerilii	David Hug
P048	Transcriptomic analysis of novel kidney-related genes in lesser spotted catshark (Scyliorhinus canicula) embryos	Nadeen Dai
P049	Developmental and evolutionary origins of the tetrapod neck	Heude Eglantine
P050	Development of transgenic reporters in Parhyale hawaiensis	Mowgli Dandamudi
P052	Begonia, a promising evo-devo model for studying the role of duplication in the diversification of the leaf developmental pathway	Lucy Turnbull
P053	Phylogenetic diversity and symbiotic associations of photosymbiotic acoels	Li-Jung Kao
P054	Genomic insights into latitudinal variation in reproductive diapause in Drosophila triauraria	Takako Fujichika
P055	Emergence and evolution of phototransduction: lessons from early animals	Filippo Nicolini
P056	Comparing early embryogenesis among teleost species from a mechanical perspective	Jun-Ru Lee
P057	Developmental functions of zebrafish isl2a and its enhancer in relation to the evolution of paired fins	Min-Chen (Janet) Wu
P059	GRN feedback dynamics in the Tribolium embryo across maternal–zygotic and extraembryonic–embryonic spatiotemporal contexts.	Kristen Panfilio

P060	From insertion to integration: the impact of regulatory landscapes in transposable element co-option	Manuel Fernández-Moreno
P061	Sauropsid embryonic brains lack the mammalian temporal transcriptional program	Sara Jimenez
P062	Early regulatory repatterning underlies lizard-snake craniofacial divergence	Caleb Northam
P063	How Development Shapes Mutational Rates and Directions: An Organismal View of Genotype Space Exploration	Paco Majic
P064	Early Tetrapod Pallial Neurogenesis Revealed in Xenopus laevis: Implications for the Evolutionary Origins of the Cerebral Cortex	Nerea Moreno
P065	The temporal skull region is shaped by the jaw musculature through development	Antonio Ballell
P066	Evolution of complete metamorphosis in insects	Yuichiro Suzuki
P067	Environmental perturbation increases and unmasks genetic regulation for gene expression variability	James Phipps-Tan
P068	Deciphering the ontogeny of cell types during embryonic development in the common octopus using trajectory analyses	Enora Geslain
P069	Did the homeotic function of AGAMOUS genes evolve from ancestral shoot regulation?	Andrew Plackett
P070	Distinct mechanisms decommission redundant enhancers to facilitate phenotypic evolution	Areej Said Ahmad
P072	Modularity, robustness and the evolvability of body plans	Frietson Galis
P073	Protocol variation and cross-species comparisons: A conceptual discussion	Yoshinari Yoshida
P074	Differential Evolution of Immunoglobulin Heavy Chain Variable Genes between Teleosts and Mammals	Shengfeng Huang
P075	Selective Hox Gene Loss and Axial Patterning in Early-Branching Flatworms	Denise Klinkenbuß
P076	The Evolutionary Origin of TGM1 Mediated Epidermal Cornification in Tetrapods is found in Non-Cornified Skin of Aquatic Ancestors	Attila Placido Sachslehner
P077	Investigating cell type-specific gene expression in Salpingoeca rosetta	Maria Eleonora Rossi
P078	Dissecting the role of TGFβ signaling in connective tissue precursors for skeletal muscle patterning	Ludivine Bertonnier-Brouty
P079	Phenotypic plasticity versus phenotypic determinism. Variations of colour in Ephemeroptera Cloeon dipterum.	María Morán-Saiz
P080	Deep conservation of cis-regulatory elements and chromatin organization in echinoderms uncover ancestral regulatory features of animal genomes	Nacho Maeso
P081	Towards a Developmental Atlas of Oncopeltus fasciatus: A Single-Cell RNA-seq Time-Course	Ariel Bar-Lev Viterbo
P082	Tooth-row patterning and heterodonty in lizards	Ida-Maria Aalto
P083	A Conserved BMP-Nfatc1-Wnt regulatory network controls the formation of valve interstitial cells in vertebrates	Sophia Prawiradilaga
P084	A single-cell approach to study axial patterning in Annelida	Allan Carrillo-Baltodano
P085	Linking Environmental and Mutational Robustness Across Mouse Phenotypes	Larisa Kaija
P086	Exploring the evolution of vertebrate telencephalon patterning: a comparative study across distantly related species	Dana Fakhreddine
P087	Divergence of neural patterning programs between larval and adult stages of indirect developing hemichordates	Brennan McDonald
P088	Does regeneration re-use embryonic gene regulatory networks?	Gillian Forbes
P089	Reconstructing The Evolution of Neuro-Sensory-Secretory Cell Types by Cross-Species Larval Single-Cell Transcriptomics	Imran Luqman

P090	Single-cell transcriptomics reveals early Wolbachia infection dynamics in Drosophila melanogaster	Jodie Jacobs
P091	Evolution of cis-regulatory landscapes in limb bone elongation: a comparative genomics approach focusing on bipedal rodents	Alexander Liu
P093	The Role of Divergent Disordered Domains in the Developmental Activity of Bicoid	Pinar Onal
P094	Genomic structural changes and the evolution of novelty in animals	Yi-Jyun Luo
P095	Large Scale Mouse Mutagenesis Reveals High modularity and plasticity in the mammalian sternum	Elizabeth Webb
P096	Studying the shark embryo at the single cell level to understand the cellular origin and evolution of the vertebrate telencephalon	Idoia Quintana-Urzainqui
P097	Photic ecology and the evolution of octopod rhodopsin	Giacinto De Vivo
P098	On the stability of dental complexity	Aida Kaffash-Hoshiar
P099	Nectar Spurs: on the development of an evolutionary key innovation in flowering plants.	Jean Watelet
P100	Identification of melanogenesis-disrupting variants in squamate reptiles reveals species-specific effects of conserved pigmentation genes	Pierre Beaudier
P101	Blurring the line: blade-sheath boundaries and the extended auricle1 mutant	Heather Jones
P102	Microbes Support Neural Circuit Resilience under Prolonged Starvation in Hydra	Ehsan Sakib
P103	Quantitative pleiotropy drove the convergent emergence of anterior fin spines	Simone Giulio Bordignon
P104	Establishing CRISPR/Cas9 mutagenesis in annelids with Vitellotag	Jingcheng Wei
P105	Characterising the cuticle striation patterns in Hibiscus trionum petals	Herrera-Ubaldo Humberto
P106	Heritable plasticity in Arabidopsis flowers promotes a pollination mode switch	Sofya Koblova
P107	The influence of developmental mechanisms on phenotypic variation and evolutionary outcomes	Christoph Netz
P110	And growth on form? How tissue expansion disrupts Turing-like patterns and enables eukaryotic species to be more and/or less visible by generating novel shapes and colours	Pierre Galipot
P111	Time-gated patterning in the Drosophila blastoderm establishes distinct expression boundaries downstream of moving stripes	Matthew French
P112	The molecular origins of phloem tissue in plants	David J. Hoey
P114	Single-cell evidence for neural crest hyper-function in the evolution of a phenotypic syndrome	Robin Pranter
P115	Mapping the evolution of phyllotaxy in lycophytes	Diego Sánchez-Ganfornina
P116	Gene expression variability, polymorphism, and evolvability in chordates	Samantha K. Smith
P117	Assessing the Identifiability of Complexity-Generating Regulatory Networks from Time-Series Expression Data	Szabolcs Zakany
P118	The evolution of the polar lobe in Spiralia	Jimena Montagne
P119	Interspecies Differences in Developmental Mechanisms Shaping the Mammalian Ovarian Reserve	Karolina Svobodova
P120	Smed-cbp-3 is essential for progenitor cells lineage progression and gene regulation in planarians	Anna Guixeras-Fontana
P121	A morphospace of fly ageing	Laura Campama Val
P122	“Heads or tails?”: The evolution of trunk growth heterochronies in animal larvae	Angelica Miglioli
P123	Splicing regulates gene dosage during limb development	Sean Driscoll

P124	The genetic basis of convergent placenta evolution	Diego Safian
P125	An intrinsically disordered glycoprotein and a glycan-binding protein enable strong underwater adhesion in Hydra vulgaris	Mario Kanetscheider
P126	Investigating the ancestral function of the known phloem-developmental regulator, BREVIS RADIX gene, in non-vascular plants.	Ciara Parratt
P127	Gene loss and the evolution of the open pangenome in the Mytilus species complex	Dona Kireta
P128	Characterisation of neural progenitors and their fate potency in the postnatal shark retina	Nicolás Vidal-Vázquez
P129	Uncoupling regeneration: Gut cell reprogramming via morphallactic tissue remodeling can progress even when epimorphic blastema-based regeneration fails in a freshwater annelid	Duygu Ozpolat
P130	Revealing the genetic innovations during the evolution of holometaboly	Lena Reim
P131	Inferring the ancestral wing developmental program across major insect lineages	Alba Almazán
P133	Genomic resources to unravel sex determination and regenerative plasticity in Syllis prolifera	Paula Moreno-Martín
P134	Do larval and juvenile skeletons share a common gene regulatory network in sea urchins?	Noé Wambreuse
P135	The contribution of transposable elements to mammalian organ development and evolution	Rocio Enriquez Gasca
P136	Birth and death of monoterpene synthases underlie chemodiversity evolution in Tanacetum vulgare (Asteraceae)	Marvin Hildebrandt
P137	The highly heterozygous European amphioxus (Branchiostoma lanceolatum) at the edge of panmixia	Marina Brasó-Vives
P138	Rab repertoire as a tool to understand critical evolutionary steps in intracellular trafficking	Ugo Coppola
P139	Early Developmental Scaling Drives Axial Diversification in Lake Malawi Cichlids	Alisha Dijkerman
P140	Evolution of neuronal diversity in the insect visual system	Elisavet Iliopoulou
P141	Regrowing the growth zone: metamorphosis kickstarts regeneration in the annelid, Capitella teleta	Alicia Boyd
P142	Mapping Divergence in a Sexually Selected Ornament in Lake Malawi Cichlids	Joel Elkin
P143	Evolution and development of grass organs	Annis Richardson
P144	Impact of Gene Regulatory Network Architecture on Developmental Robustness : Insights from Ascidian Gastrulation	Mona Mazouzi
P145	Homologies of gastrulation initiation across vertebrates	Sumit Garai
P146	Imaging neurogenesis during the pelagic-to-benthic transition in Platynereis dumerilii using a modular Flamingo light sheet microscope	Beke Lohmann
P147	Distinct cellular routes to budding in tunicates: epithelial reprogramming and lineage-restricted progenitors	Stefano Tiozzo
P148	Investigating postnatal oocyte formation in the naked mole-rat ovary	Ipathi Rao Urma
P149	Mechanisms of Cell Division Orientation in the brown alga Sphacelaria	Chloe Beziat
P150	Developmental insights into distal skeletal transformation during the fin-to-limb transition through Hoxa13 regulatory changes	Caroline Schwaner
P151	Keep an eye on the gland: size variation and comparative anatomy of the Harderian gland across squamates.	Killian Leblanc
P152	From local cell interactions to pigment pattern diversity: Insights from an agent-based model of egg-spot development in cichlids	James Malone
P153	Redefining the development of the freshwater sponge canal system using 4D optical coherence microscopy	Kathryn Hebert

P154	The Role of Thyroid Hormone Signalling in the Divergence of Pigmentation Patterns in East African Cichlid Fishes	Tereza Maxerová
P155	Cell type diversities underlying body plan evolution in sponges.	Dominica Cao
P156	Evolutionary and developmental origin of pancreatic endocrine cells through neural gene co-option	Liana Penteskoufi
P157	Mobile elements drive episodic genome expansion and functional novelty in obligate chemosynthetic symbionts of marine bivalves	Camryn Morey
P158	Does signaling network interconnectivity explain the phylotypic conservation?	Wenxin Zeng
P159	How acoels evolved to live with algae: a single-cell transcriptomic perspective	Tosuke Sakagami
P160	Homogeneous cell lines concentrate the signal of wMel infection, revealing evidence of nutritional complementation and thwarted reproductive manipulation	Shelbi Russell
P161	Distinct functions of splice variants of Ultrabithorax in Camponotus floridanus	Birgül Çolak-Al
P162	Neuronal Differentiation in Oncopeltus fasciatus: Insights from Late-Embryonic RNAi and Single-Cell Transcriptomics	Nitzan Alon
P163	Linking micro- and macroevolution through a developmental genotype-phenotype map	Pascal Hagolani
P164	How insects hacked their BMP toolkit to build a better wing	Anqi Huang
P165	Genomic and Transcriptomic Mechanisms Underlying the Evolution of Feeding Ecology in Phytophagous True Bugs	Nirakar Panda
P166	Single-cell RNA-seq analysis of ovarian reserve in mammalian species	Minh Triet Le
P167	Peptidergic integration of behavior in Placozoa	Jules Duruz
P169	The patterning mechanisms underlying shoot development in lycophytes	Róisín Fattorini
P170	A novel progenitor cell type in the embryonic mouse hippocampus suggests an ancient origin for cortical folding mechanisms	Tokiharu Takahashi
P171	The large ovarian reserve in the naked mole-rat – a unique adaptation or a general characteristic?	Lenka Gahurova
P172	Regulation of the delayed meristemoid-to-GMC transition and cell division patterns during stomatal development in succulents	Lidia Hoffmann
P173	Deciphering the emergence of a novel olfactory neuron population via gene module co-option in tetrapods	Ludovica Ciampi
P174	Not all noise is bad: Selection for high developmental noise depends on population size	Csenge Petak
P175	The Study of Phylogeny and Morphological Character Evolution in the Genus Zingiber	Peeraphat Thanasoot
P176	Quantitative characterization of heart development in Tribolium	Shannon Taylor
P177	Starvation induces an anaerobic metabolic program in the sea anemoneNematostella vectensis	Kathrin Garschall
P178	A continuum-mechanical framework for morphogenesis	Ebrahim Jahanbakhsh
P179	Syncytins: Shaping placentation? Identifying endogenous retroviral envelopes in 103 bat genomes	Amy O Brolchain
P180	Geographic variation in the microbiome of benthic phase Platynereis worms	Alvin Han
P181	Pre-bilaterian roots of the chordate body plan	Rohin Sengupta
P182	Developmental modularity facilitates the evolution of vertebral number over long, but not short, evolutionary timescales.	James Hammond
P183	Environmentally dependent developmental induction as a potential driver of heart evolution	Nina Kraus

P184	Hox gene evolution and patterning of the miniaturized annelid Ophryotrocha labronica	Eloïse Defourneaux
P185	Unveiling the genes underlying molecular patterns of development conservation across the tree of life	Stefan Manolache
P186	With a Hand in your heart. Functional analysis of Hand1/2 knockdown in the evolutionary deconstructed cardiopharyngeal gene regulatory network of Oikopleura dioica.	Constantino Andrés Molina
P187	Developmental shifts shape the evolution of color vision in phyllostomid bats	Taro Nojiri
P188	Looking for the origin of neurons by dissecting the peptidergic nervous system of ctenophores	Maria Sachkova
P189	10-plex imaging of segmentation gene expression in Drosophila and Tribolium embryos	Tom Pettini
P190	Flexibility of cell fates and functions across sex determination systems revealed by comparative single-cell analyses	Rafael D. Acemel
P191	Exploring the role of Mohawk homeobox A in the zebrafish musculoskeletal system	Anup Kumar Mani
P192	Dynamic roles of embryonic glial cells in octopus brain development	Mark Lassnig
P193	Evolution of FYVE_2 exocytic transport regulators at the transition to animal multicellularity	Antonia Heinrich
P194	Centromeres in the nematode Meloidogyne species share conserved epigenetic and genetic determinants	Damira Veseljak
P195	Centromere organization in Meloidogyne shaped by reproductive strategy and ploidy	Evelin Despot-Slade
P197	New genome assemblies from neglected phyla illuminate the evolution of Ecdysozoa	Alessandro Formaggioni
P198	Contrasting histories of gene and genome duplications shape biosynthetic and flower developmental pathways in Ranunculales	Le-Han Roessner
P199	Building a cell-type atlas of the larval apical sensory organ in the model sea anemone, Nematostella vectensis.	Sophie den Hartog
P200	Comparison of single nucleus transcriptomics between sea urchin juvenile and larval mesodermal corresponding cell types reveals transcriptional similarities but also novel cell clusters	Carmen Andrikou
P201	Dpp-dependent gene regulation in early embryogenesis of the carpenter ant Camponotus floridanus	Nihan Sultan Milat
P202	Sustained ectopic neurogenesis as a mechanism of evolutionary brain expansion in Octopus vulgaris	Maxence Lanoizelet
P203	Developmental evolution of feeding morphology in cave-dwelling Eurycea	Henry Camarillo
P204	Evolution of Coloniality and Invertebrate Allorecognition	Crystal Tang
P205	Haplotype-resolved genomes reveal pervasive structural polymorphism in the planktonic chordate Oikopleura dioica	Gaspar Sánchez-Serna
P206	A reaction-diffusion mechanism patterns the wing vein branching of Bicyclus anynana butterflies	Tirtha Banerjee
P207	Evolutionary conserved transcriptional regulators control monoaminergic neuron development	Roberto Feuda
P208	Brain regeneration in the marine chordate: insights from sulfation dynamics of dermatan and chondroitin sulfate	Taynan Motta Portal
P209	The Impact of Vertebrate Whole Genome Duplications on RNA Binding Protein Evolution	Robert Hussain
P210	Stepwise cellular and tissue-level remodeling underlies the evolution of viviparity in a reptile	Hongxin Xie
P212	Reconstructing the habit, development, and ecology of the 407-million-year-old species Rhynia gwynne-vaughanii	Sophie Burne

P213	Decoupling phenotypic from genetic pleiotropy during evolution on a complex genotype-phenotype-fitness map	Théotime Grohens
P214	Developmental variability in serial homologues: dissociability, developmental noise, and standing phenotypic variation	Daniela Rossoni
P215	Shedding Light on Beetle Circadian Rhythms	Alexandros Pantagias
P216	Ordered Chaos: Photoreceptor Mosaics in the Turquoise Killifish (Nothobranchius furzeri) Retina Across Life Stages	Nicole C. L. Noel
P217	Developmental origin of an evolutionary innovation in a water strider	Arnaud Badiane
P218	Developmental single-cell transcriptomics of the lamprey: insights into ancestral vertebrate cell types	Laura Hernandez-Hernandez
P219	Patterns of odour and colour biosynthesis in sexually deceptive Ophrys orchids	Aylin Janina Bersch
P220	Developmental basis of the diversification of an evolutionary innovation.	Carlyne Golding
P221	Testing for the origin of seed gene networks in the fern archegonium	Bethany J Pettifor
P224	Shared logic between learning and evolution: new approaches toward predictive evo-devo	Jason Cheok Kuan Leong
P225	A Mayfly Model for Studying the Origin of Insect Wings	Masaki Takenaka
P226	Beyond model species: a collection of living planarians for the comparative analysis of regeneration	Miquel Vila-Farré
P228	Decoding regulatory evolution across developmental lineages of skeletogenic cells	Mariam Museridze
P229	From Cells to Structure: Spatial gene expression during plug formation in medaka wild relatives	Alina Schüller
P230	Conserved roles of nitric oxide in regulating cnidarian larval behaviour and developmental progression.	Vengamanaidu Modepalli
P231	From head to tail: How an unusual annelid builds its trunk	Tom Frankish
P233	How did the mouse upper molar evolve a new shape?	Angélique Préaubert
P234	Deciphering unique bat wing development with comparative genomics and transcriptomics at the cell population level	Christian Feregrino
P235	Comparative single-cell transcriptomics across deeply divergent sponge species	Laia Montes-Espuña
P236	Deep Evolutionary Conservation of Hmx Transcription Factors and their Role in Vertebrate Sensory Ganglia	Alessandro Pennati
P237	Vertebrate-like Hox gene regulation in annelid worms	Billie E. Davies
P238	Integration and evolution of the cichlid pharyngeal skeleton: Tinkering with a palimpsest	Leticja Flamuraj
P239	Modulation of Hydra vulgaris microbiota through Targeted Phage-Based Photodynamic Therapy	Vittorio De Felice
P240	Reproduction emerges from ecological interactions at the onset of multicellularity	Alexandre Porcher Fernandes
P242	Non-linearities, cell signalling and the paradox between robustness and evolvability: A mechanism for safely exploring novel directions of shape change during development	Craig Jacobs
P243	Developing a Visually Detectable Biosensor in the Fern Pteris vittata to monitor soil arsenic contamination	Cristina Caissutti
P245	Boundary issues: investigating the role of LIGULELESS 2 in maize leaf development	Ed Bridge
P246	Dating the growth hormone gene duplication event in passerine birds	Rachel Warnock
P247	Molecular Evolution of Floral Diffraction Gratings in Hibiscus species	Yan Zhu
P249	Nuclear envelope breakdown in the ichthyosporean Chromosphaera perkinsii	Baukje Hoogenberg

P250	Evolution of developmental gene regulatory networks with an emphasis on flowering plant carpels	Theresa Sophie Uhlig
P251	Cell type atlas of the Mnemiopsis apical organ	Sanja Jasek
P252	Does the tail bud contribute to posterior gut formation in chordates? Evidence from amphioxus	Matteo Bozzo
P253	Tracing the roots of Neural Crest in Amphioxus	Pratyasha Biswas
P254	Cell autonomy, plasticity and patterning evolution: fish ionocytes as an experimental paradigm	Javier Vazquez Marin
P255	Cataloguing diversity in transcriptional processing mechanisms across diverse animal species and cell types	John Chamberlin
P256	Testing the link between DNA methylation and phenotypic variation in the water strider Microvelia longipes	Mirjam Urb
P257	Auxin, ARFs, and leaf architecture: The role of ZmARF28 in leaf initiation and development in maize	Bianca Ferreira
P258	Temporal modularity and evolvability of postembryonic development in Orthoptera	Jakke Neiro
P259	Sensory organ adaptation in polychaete larvae with vastly different developmental modes	Maria Belyaeva
P260	Identifying the mechanisms of phenotypic robustness using color pattern asymmetry in cichlid fish	Claudius Kratochwil
P261	Exploring chemodiversity in natural populations of Solanum dulcamara	Kruthika Sen Aragam
P262	Deciphering the role of cytoskeletal forces and mechanical buckling in developing butterfly scale architecture	Eleanor Gilbert
P263	Black flies as emerging Eco-Evo-Devo models: linking river ecology, morphological innovation, and genomics	Rodrigo Nunes-da-Fonseca
P264	An active role of yolk in Tribolium castaneum morphogenesis	Mariia Golden
P265	The role of evolvability and modularity in shaping sexual dimorphism in cranial morphology of simiiform monkeys (Primates, Haplorrhini)	Lucas Camacho
P266	Genetic underpinnings of the proximo-distal elaboration of pectoral appendages during the fin-to-limb transition	Sayoni Chatterjee
P267	Comparative Cell Type Atlases of Sea Anemones	Ava TRUE
P268	The hoja loca1 mutation disrupts mediolateral patterning in vegetative phytomer development	Robert Mason
P269	Towards understanding the mechanisms behind petal epidermal cell shape patterning and its diversity across angiosperm species	Yuxi Liang
P270	How well does phenotypic covariation predict evoltuionary potential? A mammal-wide comparison of G- and P-matrices.	Bárbara Costa
P271	Genomic adaptations of self-domestication across the populations of the yellow fever mosquito Aedes aegypti	Irma Lozada-Chavez
P272	Evolution of inhibitory interneurons contributes to vertebrate forebrain diversity	Giacomo Gattoni
P273	A Balanced Inversion Polymorphism Exhibits a Dominance Reversal at the Gene Expression Level that Depends on Developmental Context	Sebastian Kittelmann
P275	Comparative insect embryogenesis under changing climate	Martin Planke
P276	Incubation temperature alters stripe formation and head colouration in American alligator hatchlings and is unaffected by estradiol-induced sex reversal	John Mulley
P277	Exploring the Molecular Principles of Heterochrony between C.elegans and C. briggsae	Kwok Yin Man
P279	Developmental and genomic insights into umbel evolution in Apiaceae	Jakub Baczynski

P280	Understanding the Mechanism of Floral Trichome Patterning in Hibiscus trionum	Min Kim
P281	Comparative Analysis of the Genomic Signatures of Miniaturised Annelids	Pia Merete Eriksen
P283	"Molecular mechanisms and phenotypic consequences of paralog divergence in insects and chelicerates"	Dorothea Grillmayer
P284	Neuronal Dynamics in the Trochophore larva of Platynereis dumerilii	Benedikt Dürr
P285	Temporal Alignment in the Omics Age: Goal-Relative Comparability Across Developmental Time	Cassandra Zie Yang
P286	The role of developmental plasticity in the patterning and modularity of fins.	Thomas Stewart
P287	A conserved gene regulatory circuit underlies sensory neuron identity across chordates	Diego Dolgetta-Garcia
P288	Insights from Suncus murinus for mammalian tooth evolution	Wataru Morita
P290	From Polyp to Jellyfish. Transcriptomic profiling of the chemically induced strobilation in the jellyfish Aurelia sp.	Ernesto Maldonado
P291	Multiplexed Single-Cell Long-Read Transcriptomics Using SPLiT-seq and Oxford Nanopore Sequencing in to Study Planarian Regeneration	Olena-Maria Balta
P292	Investigating developmental genetics of panicle architecture and pulvinus-controlled branch angle in Eragrostis tef	Grace Chapman
P293	Translation control of developmental tempo: Evidence for a gastrulation-stage checkpoint in the chick embryo	Yuri Takahashi
P294	Evolution of the cell-surface protein Tartan reshapes genital morphogenesis and reproductive interactions during Drosophila speciation	Maria Daniela Santos Nunes
P295	Extreme intermittent fasting: cellular and microbial mechanisms of starvation tolerance in cavefish	Nicolas Rohner
P296	The lamprey habenula provides an extreme example for the temporal regulation of asymmetric development	Lucile Guichard
P297	The genomic mechanisms of melanism in a jawless vertebrate	Ziggy Macnaughton
P298	Cephalo-pelvic covariation and sexual dimorphism are disrupted in hybrid mice: implications for the human obstetrical dilemma	Eva Zaffarini
P299	Orientation of metachronal waves in prototroch multiciliated cells of Platynereis dumerilii	Marzia Matejcek
P300	Does regeneration re-use developmental gene regulatory networks?	Namrata Namrata
P301	A Comparative Study of Abdominal Development in Drosophila	Pedro Gaspar
P302	A reaction-diffusion process patterning the veins in a BMP-signalling competent section of early Bicylus anyana wings	Javen Yih Ruay Tan
P303	Shell-ular Biology: Single nuclei-resolved epithelial gene expression changes during moulting in Litopenaeus vannamei	Nicholas Wade
P304	Tracing the cellular basis of chordate evolution using single cell approaches	Alberto Pérez-Posada
P305	Thyroid hormone coordinates rotation with musculoskeletal development of the pectoral fins	Nicolas Cumplido
P306	Characterisation of a deeply conserved prohormone convertase identifies neuroendocrine cells regulating lipid metabolism in larval development	Vera Terblanche Hunnekuhl
P307	Interrogating intra-genomic coevolution to identify loci linked to phenotypic shifts	Carlos J Rivera Rivera
P308	Divergent visual-system adaptations in two sturgeon species revealed by single-nucleus analysis	Kristyna Eliasova
P309	Investigating the MADS-box gene CRM3 of the fern Ceratopteris richardii and its association to reproductive functions by overexpression and CRISPR-Cas9-mediated knockout	Robin Schulz
P310	SEM characterization of mechanoreceptors in the skin of adult Elasmobranchs	Maria Elena de Bellard

P311	An NKX2-5 homolog is required downstream of BMP signaling to pattern the sensory-adhesive organ of a tunicate larva	Christopher Johnson
P312	Lamprey Single-Cell Transcriptomics and Spinal Cord Evolution	Katia Jindrich
P313	Cucurbitaceae: A novel model system for pigment biosynthesis	Hannah Elisa Busch
P314	Cellular and molecular dynamics of anterior regeneration in Pristina leidyi	Irene del Olmo

ABSTRACTS (TALKS)

Keynote Lectures

K1- Keynote Lecture 1

Evolution Evolving: The Developmental Origins of Adaptation and Biodiversity

Kevin Lala, Biology, University of St Andrews, St Andrews, UK

Two major dynamical processes – evolution and development – underpin virtually all biology. That evolution shapes development is obvious and uncontentious, and this premise underpins the thriving field of evo-devo, yet the reciprocal claim, that development shapes adaptive evolution, remains steeped in controversy. In this talk, I draw on the latest findings in evolutionary genetics and evo-devo, as well as novel insights from studies of epigenetics, symbiosis, and inheritance, to examine the central role that developmental processes play in evolution. I will describe how evolutionary biology is currently coming to terms with a rush of data that challenge the orthodox narrative. New findings, particularly from evo-devo, suggest that natural selection is not something that just happens to organisms: the development of organisms, their activities and behaviours, contribute to whether and how it happens. The form that natural selection takes depends critically on the mechanistic details of how each organism develops. Types of inheritance, different capabilities to modify environments (a.k.a. 'niche construction') and to develop flexibly (a.k.a. 'phenotypic plasticity'), differ greatly across organisms, generating variation in evolvability. As organisms evolve and acquire new traits, the evolutionary process itself evolves. There is considerable, largely untapped, potential for the emergence of a vigorous field of 'devo-evo', as the intellectual offspring of 'evo-devo'.

K2- Keynote Lecture 2

How to build a (fake) fly: the convergent evolution of sexually deceptive pollination

Beverley Glover, Department of Plant Sciences, University of Cambridge, Cambridge, UK

Sexually deceptive pollination has been little studied from a developmental point of view because it is difficult to dissect in the non-model plant systems in which it is found and because most clades contain only all deceptive or all non-deceptive species, preventing comparative approaches. We have developed *Gorteria diffusa* Thunb., the only known sexually deceptive daisy, as a model system in which to address this developmental and evolutionary problem. Uniquely, the *Gorteria* species complex consists of multiple sexually deceptive, non-deceptive and intermediate floral forms, existing as geographically isolated populations. These "morphotypes" provide the material for comparative approaches, and are enabling the dissection of the development and genomic basis of this complex trait. However, they provide a second exciting opportunity – because we have established their phylogenetic relationships we can also map developmental transitions and their genetic regulation onto the evolutionary history of the species, to understand not only how sexual deception develops but how it evolved too. I will present our recent work on this fascinating pollination system.

K3- Keynote Lecture 3

Reproduction as a Mediator of Development and Evolution

Laura Nuño de la Rosa, Institute of Philosophy, Spanish National Research Council, Madrid, Spain

Although heredity has long been central to evolutionary theory, reproduction itself has often been reduced to genetic transmission and framed primarily as a strategy for maximizing fitness. This gene-centered, adaptationist perspective overlooks the complex relational and material dimensions of reproduction, a limitation shared by much of evo-devo, which has historically focused on internal embryological processes generating morphological characters. Recent work in evo-devo, however, enables the theorization of reproductive characters and reproductive modes in relational and developmental terms, capturing diversity across lineages. In this presentation, I examine how interorganismal reproductive relations (between parents and between parents and offspring) play a critical role in bridging development and evolution. Rather than treating reproduction merely as a vehicle for heredity or fitness optimization, I argue that it should be understood as the material link between generations, mediating plastic interactions with the environment and shaping the evolutionary potential of lineages through constraints and affordances embedded in reproductive modes.

K4- Keynote Lecture 4

Selection, plasticity and variation: any connection?

Abderrahman Khila, **Institut de Génomique Fonctionnelle de Lyon, CNRS UMR5242, Ecole Normale Supérieure de Lyon, Université Lyon1, Lyon, France**

Secondary sexual traits are often exaggerated and highly variable. Classical models of sexual selection explain the exaggeration but provide limited explanation for the persistence of the extreme variation often observed in these traits. In this talk, I will present data showing how the interaction between sexual conflict, phenotypic plasticity and directional sexual selection can generate and maintain extreme phenotypic variation in an exaggerated sexually selected trait. In some water striders, males wield extravagant weapons, used as weapons in male–male competition, and exhibit striking variation in these weapons. Reaction norm experiments reveal that nutrition contributes to this variation; however, phenotypic plasticity alone cannot account for the full extent of variation observed in natural populations. I will present molecular and functional evidence demonstrating that nutritional effects on weapon size are mediated through changes in gene expression. Unexpectedly, experimental evolution under directional sexual selection further amplified phenotypic variation beyond the initial distribution. At the same time, selection for male weapon size imposed substantial fecundity costs on females, highlighting the role of sexually antagonistic selection in preventing fixation of the exaggerated phenotype. I will conclude by proposing a model in which the combined effects of condition dependence, directional sexual selection, and sexual antagonis inflate phenotypic variation beyond expectations based on genetic variance alone.

Symposia and Contributed Sessions

1 | Evo-Devo Principles of Plant Specialized Metabolism

Symposium S01: Wednesday 10 June, 13:30 – 15:10

Organisers: Shuqing Xu (University of Mainz, Germany, Emmanuel Gaquerel (University of Strasbourg, France)

Plants synthesize an immense diversity of specialized metabolites, yet the principles that determine where, when, and how this chemical diversity arises remain fragmented across ecology, development, and evolution. This symposium proposes an evo-devo framework that links evolutionary mechanisms (such as gene duplication and neo/subfunctionalization, pathway assembly, and metabolic gene clustering) with developmental patterning across cell-type, tissue, and life stages to explain the origin and diversification of plant chemical novelty. We will highlight how specialized metabolites function not only as ecological mediators but also as developmental signals that influence cell fate and organ identity, and how shifts in regulatory networks can rewire flux through emerging pathways. We welcome contributions that integrate single-cell multi-omics, metabolomics, comparative and population genomics, and functional genetics to connect genomic innovations to cell-resolved expression, biochemical activity, and ecological performance. By comparing convergent innovations across lineages (e.g., trichomes, roots, flowers), we aim to distill general design principles of metabolic innovation and identify points for prediction and responsible engineering of new chemistries. We anticipate that this symposium will create a shared research agenda that unites chemical ecology and evo-devo to map selection pressures onto developmental programs, advancing our understanding of how plant specialized metabolism evolves.

S01.1 | Evo-Devo Principles of Plant Specialized Metabolism

Development of a flower as a false female in the sexually deceptive orchid *Ophrys sphegodes*

Mattia Alessandrini[1], Noa L. A. Schwabe-Model[1], Chengwei Wang[1], Aylin Bersch[1], Shuqing Xu[2], **Philipp M. Schlüter[1]**

[1]Dept. of Plant Evolutionary Biology, University of Hohenheim, Stuttgart, Germany, [2]Institute of Organismic and Molecular Evolution, Johannes Gutenberg University of Mainz, Mainz, Germany

Sexually deceptive orchids use specialised floral metabolites along other floral traits to attract pollinators with high specificity. Beside flower shape and trichomes on the adaxial labellum epidermis, biosynthesis of anthocyanins and alkene hydrocarbons play important roles in pollinator interaction. These pollination-relevant features are produced in different regions of the flower's labellum and at different times throughout its development. We present data on the adaptive role of several of these flower features, show spatial patterns of different epidermal cell types and present reference stages of labellum development in *Ophrys sphegodes*. Finally, we characterise the transcriptomic changes during labellum development, and also with a view to specific cell types, so as to understand how metabolic and developmental traits are integrated at the molecular level.

S01.2 | Evo-Devo Principles of Plant Specialized Metabolism

Complex Evolution in the Cellulose Synthase Protein Family: From Core Cell Wall Assembly to Enzymatic and Non-enzymatic Functions in Specialized Metabolism

Asaph Aharoni

Plant and Environmental Sciences, Weizmann Institute of Science, Rehovot, Israel

Specialized metabolites represent a large portion of the plant metabolic repertoire counting thousands in an individual plant. Generating such chemical complexity requires continues evolution of genes encoding proteins producing novel metabolites with selective advantage in a particular environmental niche. Genes with new function in specialized metabolism often arise following duplication of genes involved in primary/core metabolites formed across all species. In the presentation, I will portray several different molecular mechanisms wherein genes of core, primary metabolic pathways were 'hijacked', providing a template for the evolution of new enzymatic functions. I will focus on our findings that protein of the cellulose synthase family (i.e. Cellulose Synthase-Like; CSLs) evolved novel activities in different plant families. While we initially identified CSLs associated with triterpenoid saponin biosynthesis, recent results showed that in some plant families such proteins lack enzyme activity and serve as structural elements critical for other pathway proteins function. This example of CSLs and proteins of other classes reported recently, highlight the prevalence and importance of non-catalytic proteins to proper functioning of pathways generating specialized metabolites.

S01.3 | Evo-Devo Principles of Plant Specialized Metabolism

The evolution of plant specialized metabolism and specialized cell types

Maite Colinas[1], Joshua C. Wood[2], Ketil Krabbe[1], Chloée Tymen[1], Clara Morweiser[1], Lorenzo Caputi[1], C. Robin Buell[2], Sarah E. O'Connor[1]

[1]Department of Natural Product Biosynthesis, Max Planck Institute for Chemical Ecology, Jena, Germany, [2]Center for Applied Genetic Technologies, University of Georgia, Athens, USA

The continuous coevolution of plants with their environment has led to an enormous range of chemically diverse specialized metabolites. They are widely known to mediate beneficial and adverse biotic interactions. Moreover, many bioactive specialized metabolites are pharmaceutically relevant and continue to play an important role in the field of modern drug discovery. While some specialized metabolites are widespread among many species, others are highly species-specific. Interestingly, pathways for even chemically complex specialized metabolite scaffolds have evolved independently multiple times. Noteworthy, the biosynthesis of specialized metabolites is commonly restricted to specific cell types. In many cases these are specialized cell types that are dedicated to the production of specialized metabolites and have likely coevolved with the pathways, for example glandular trichomes or idioblasts. In this presentation I will exemplify these aspects by discussing our research on the evolution of iridoid (cyclised monoterpenoids) and iridoid-derived ipecac alkaloid biosynthesis. While iridoids are ancestral to the asterid clade of flowering plants and continue to be widespread, their conversion into ipecac alkaloids occurs only in three genera of two distinct asterid orders. The discovery of ipecac alkaloid biosynthetic enzymes showcases how nature can capitalize on highly reactive starting substrates and the manner in which multistep pathways can arise independently. Recently, we have been increasingly including single-cell multi-omics techniques in our research, which has accelerated enzyme discovery, and moreover enables us to start studying the evolution and development of specialized cell types involved in iridoid and iridoid-derived alkaloid biosynthesis.

S01.4 | Evo-Devo Principles of Plant Specialized Metabolism

Allopolyploidy-mediated innovation of gain-of-function defenses in a plant-insect arms race

Emmanuel Gaquerel[1,2], David Elser[1], Ludivine Malherbe[1], Aura Navarro Quezada[2]

[1]Institut de Biologie Moléculaire des Plantes, CNRS / University of Strasbourg, Strasbourg, France, [2]Centre for Organismal Studies, University of Heidelberg, Heidelberg, Germany

The evolution of defensive innovations is central to plants' adaptation to diverse ecological niches. Specialized herbivorous insects actively respond to these innovations by evolving biochemical and behavioral counter-adaptations, thereby imposing additional selection pressure on their host plants. The genus *Nicotiana*, in which half of the species are allopolyploids, provides a compelling framework for exploring the role of allopolyploidization as an evolutionary driver of specialized metabolite diversification for defense. A recent evolutionary metabolomics analysis from our group investigated chemodiversity innovations linked to allopolyploidization in *Nicotiana*, highlighting *N*-acyl-nornicotines (NANNs) as defenses strongly modulated by allopolyploidy events (Elser et al. Science Advances, 2023: 10.1126/sciadv.ade8984). NANNs are derived from the ancestral nicotine state of the genus. Nicotine is known to be a potent defense, although certain herbivores, such as the tobacco hornworm, have developed tolerance, likely through efficient nicotine excretion. Using the evolutionary trajectory of NANNs as a case study, my talk will illustrate how allopolyploidization in *Nicotiana* section *Repandae* has, through Evo-Devo shifts, transformed trichome-based NANN production into a gain-of-function defense against specialized insects. Specifically, I will present findings on the discovery of NANN biosynthetic genes, their evolutionary origin in light of allopolyploidy events, and their novel mode of action in circumventing nicotine tolerance.

2 | From cell type- to tissue-evolution

Symposium S02: Wednesday 10 June, 09:00 – 10:40
Contributed Session C02: Wednesday 10 June, 11:05 – 12:30
Contributed Session C02 Continued: Friday 12 June, 13:30 – 15:10

Organisers: Mihaela Pavlicev (University of Vienna, Austria), Günter P. Wagner (Yale University & University of Vienna, US/Austria)

Organismal complexity evolves through the origin of novel cell types, the organization of cell types into supracellular units, such as tissues, and tissues into organs, the structure which ultimately represent clade-specific body plans. While this overall scheme is broadly accepted, the mechanisms underlying each of these steps are largely unknown. What is known is that they involve evolution of cooperation between related and unrelated cell types. Recent progress in single cell technology and metabolomics has enabled a deeper look into the molecular mechanisms behind cell type identity, the causes of tissue organization, and organ development. Insights into the evolution of genomic rearrangements, cellular communication and mutual metabolic complementation open new avenues for a refined understanding of the evolutionary origin of higher-order levels of organisation. This symposium aims at a review of current progress, and identification of open questions.

S02.1 | From cell type- to tissue-evolution

Principles of cell type and tissue evolution: Origin and centralisation of the nervous system

Detlev Arendt

Developmental Biology, EMBL, Heidelberg, Germany

We study the evolution of animal body plans and nervous systems from a cell type perspective, with particular emphasis on nervous system origins and centralization. For this, we have generated whole-body single cell atlases for a broad range of slow-evolving invertebrate and vertebrate species, sharing different important ancestors. Exemplifying this approach, we have chosen the marine annelid *Platynereis dumerilii* as a powerful model for comparative studies across and beyond bilaterians and recently built the *PlatyBrowser,* which establishes a link between cellular transcriptomes and subcellular morphologies for all cell types of the entire body for the six-days-old young worm. Leveraging these resources, we are tracing cell types and tissues through animal evolution and thus reconstruct the cellular and tissue composition of different ancestors, with special emphasis on the urbilaterian. Comparing cell types between closely related species and populations, we also attempt to unravel cellular adaptations governing evolutionary change.

Building on this, I will outline basic principles of cell type and tissue evolution, taking the nervous system as a case in point. I will explain how the step-wise emergence of coregulons, sets of coregulated genes, has driven the hierarchical diversification and individuation of cell types and tissues. I will present the concept of deneuralisation, which posits that non-neural tissues emerged from neural tissues (and not vice versa). Finally, I will attempt to explain the emergence of organs from the local interaction and coordination of diverse cell types and tissues, as manifest in the nervous system.

S02.2 | From cell type- to tissue-evolution

Evolutionary assembly of vertebrate digestive organs

Arun Chavan[1], Parastou Yaghoubi[1], Naomi Philip[1], Kejue Jia[2], Kun-Lung Li[3], Jr-Kai Yu[3], Jacob Musser[2], Ruslan Medzhitov[1]

[1]Department of Immunobiology, Yale University, New Haven, CT, USA, [2]Department of Molecular, Cellular, and Developmental Biology, Yale University, New Haven, CT, USA, [3]Institute of Cellular and Organismal Biology, Academia Sinica,Taiwan

The vertebrate digestive system comprises morphologically and functionally distinct organs—liver, pancreas, intestine—derived from an ancestral multifunctional gut tube. Here we investigate how these organs arose through comparative analysis of chordate digestive systems. First, we show that vertebrate organs are composed of cell types from distinct cell type families. These include a parenchymal cell type and accessory cell types—stromal cells, endothelial cells, and tissue-resident macrophages. Then, using single-cell transcriptomics and spatial gene expression analysis in a cephalochordate (amphioxus, *Branchiostoma floridae*) and a tunicate (*Ciona robusta*), we identify cell types homologous to vertebrate parenchymal cells, namely, hepatocytes, enterocytes, pancreatic exocrine cells, and enteroendocrine cells. Crucially, these cell types form spatially coherent tissue-like clusters, suggesting pre-vertebrate origin not only of these parenchymal cell types but also of the tissue units they compose. In amphioxus, we also identify cells associated with hepatocytes in the midgut diverticulum that are functionally similar, but not homologous, to vertebrate Kupffer cells. While their parenchymal cell types are homologous across chordates, ancestral gene expression programs are partitioned differently—between sister parenchymal cell types in amphioxus versus between parenchymal and accessory cell types in vertebrates—suggesting that hepatic and pancreatic tissues were organized through distinct evolutionary routes in different lineages. We discuss implications of these findings for the homology and evolutionary origin of animal tissues.

S02.3 | From cell type- to tissue-evolution

Metabolic complementation between cells drives the evolution of tissues and organs

Mihaela Pavlicev[1,2,3], **James DiFrisco[4]**, Alan Love[5], Gunter Wagner[1,3,6]

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Although evolutionary transitions in individuality have been the subject of much discussion, little attention has been paid to the origin of levels of organization within organisms. How and why do specialized cells become organized into specialized tissues or organs? I present a hypothesis for this kind of evolutionary transition based on two features of cellular metabolism: metabolic constraints on functional performance, and the capacity for metabolic complementation between parenchymal and supporting cells. These features suggest a scenario whereby pre-existing specialized cell types are integrated into tissues when changes to the internal or external environment favour offloading metabolic burdens from a primary specialized cell type onto supporting cells. This process of 'supra-functionalization' is illustrated using the nervous system and pancreas.

S02.4 | From cell type- to tissue-evolution

Whole genome duplication drove cell-type evolution in the vertebrate brain

Yuanzhen Zhu[1], Shuai Zhang[2], Jiankai Wei[1], Diego Dolgetta-Garcia[1], Katia Jindrich[1], Chenggang Shi Shi[2], Gunter Wagner[3,4], Peter Holland[1], Guang Li[2], Sebastian Shimeld[1], Huimin Liu[2], Rongrong Pan[2], Yuwei Chen Chen[2], Yan Xu[2], Qiye Li

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Vertebrate brains contain far more cell types than those of their closest invertebrate relatives, yet the evolutionary mechanisms driving this expansion remain poorly understood. Early vertebrate evolution was marked by whole-genome duplications (WGDs), which generated duplicated genes (ohnologues), but their role in cell-type innovation has been unclear. Using single-cell transcriptomic data from the brains of five chordates—human, mouse, lizard, lamprey, and amphioxus—we identify numerous vertebrate-specific cell-type families defined by conserved core transcription factors which do not show 1-to-1 homology with amphioxus. We show that ohnologues, particularly those originating from the first WGD, contributed more strongly to vertebrate cell-type innovation than small-scale duplication paralogues. By reconstructing ancestral cell-type states, comparing them with amphioxus, and experimentally analysing macroglia development, we provide mechanistic evidence that ohnologues played a key role in early vertebrate cell-type diversification. Our results prompted us to propose a formal evolutionary framework for cell-type classification in which names explicitly encode the phylogenetic position where they originated. A Linnaean-style binomial nomenclature provides a simple and coherent system that captures homology, innovation, and levels of comparison, offering an extendable language for future comparative analyses and evolutionary developmental biology.

C02.1 | From cell type- to tissue-evolution

Comparative transcriptomics reveals dynamic cell-type evolution in teleost gonads

Francisca Hervas-Sotomayor[1], Jérôme Montfort[1], Aurélie Le Cam[1], Alexandra Depincé[1], Amaury Herpin[1], Florent Murat[1]

Fish Physiology and Genomics Institute, National Research Institute for Agriculture, Food and the Environment, Rennes, France

We aim to understand how gonadal cell-type diversity evolved in teleost fishes, a group characterized by a remarkable reproductive diversity and a dynamic history of genome duplication. By leveraging these features, we investigate the interplay between cell-type diversity, genomic history, and evolutionary forces. We are generating quantitative and spatially resolved single-cell transcriptomic atlases and integrating them with anatomical data at cellular and tissue levels to link molecular profiles with phenotypes. We have characterized cell diversity in species with varying levels of sperm competition (zebrafish, medaka, and pipefish, with ongoing work in goldfish and stickleback), identifying diverse germline and somatic cell types in both gonads. Cross-species comparisons of the testis reveal heterogeneous evolutionary rates among germline and somatic tissues. Germ cells retain a conserved overall molecular core, particularly at early stages of spermatogenesis, yet we don't find clear one-to-one cell-type correspondences across species, suggesting rapid cell-type evolution and regulatory rewiring. Somatic tissues appear more dynamic. While some cell types, such as peritubular cells, are highly conserved, Sertoli cells are among the most divergent. Consistently, we identify multiple Sertoli types per species, a contrasting cell diversity with other vertebrates. Spatial transcriptomics shows that these cell types occupy distinct anatomical zones within the testis, suggesting functional specialization. Current analyses of the ovaries indicate a consistent diversification for granulosa cells, the supporting cells of the ovary. Ongoing work explores whether this rapid cell-type evolution is associated with teleost genome duplication events, and whether species-specific cell diversity correlate with testicular structure and sperm competition.

C02.2 | From cell type- to tissue-evolution

Hierarchical determination of organ identity - examples from arthropods

Ariel D. Chipman

Ecology, Evolution & Behavior, The Hebrew University of Jerusalem, Jerusalem, Israel

Organs are hierarchically organized structures, composed of constituent parts, usually tissues, which in turn are composed of cells and extracellular matrix. Intuitively, it should follow that organ identity is determined by the identity of its constituents. However, different organs can be composed of similar constituents, differing in the spatial arrangement of these constituents. Organ identity is thus determined by the developmental signals that organize its structure. These developmental signals are themselves hierarchical, acting in a sequence of gradual fate determination that actually runs in opposite sequence to that of the hierarchical components. General characteristics of the organ are determined first, followed by its more specific identity, with the differentiation into different cell types occurring at the final stage. As an example of this process, I will discuss the determination of segment identity in arthropods. A given segment, e.g. the third thoracic segment, has an identity as a segment, an identity as a thoracic segment and an identity as the third thoracic segment. Embryonic fields determining tagma identity are the earliest identity signals, followed by the segmentation cascade and axial identity determination. The many cell types comprising the segment are determined gradually, and only after it has adopted its segmental identity.

C02.3 | From cell type- to tissue-evolution

Endometrial cell innovations underlying the acquisition of menstruation in primates

Eulalie Liorzou[1], Axelle Brulport[1], Anthony Lepelletier[1], Maëlle Daunesse[1], Claire Lavergne[1], Camille Berthelot[1]

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Women's uterine physiology is rhythmed by periodic shedding of the superficial uterine lining together with blood, which defines menstruation. Menstruation remains an evolutionary enigma because it is observed in only a few mammals, notably within the primate lineage. Current literature has proposed a unified biological basis for menstruation, suggesting that it is causally related to the acquisition of spontaneous decidualisation—the differentiation of endometrial stromal cells into endocrine cells that contribute to the maternal side of the placenta. However, evidence for spontaneous decidualisation remains poorly supported across menstruating primates, and the contribution of other endometrial cell types to the acquisition of menstruation is unknown.

In this study, we leveraged single-cell transcriptomics to characterize endometrial cell populations present at the end of the endometrial cycle in three menstruating primates (human, macaque, and baboon) and three non-menstruating primates, including two New World species (marmoset, squirrel monkey) and a lemur (mouse lemur). Cellular and molecular evidence confirms that humans, macaques, and baboons undergo spontaneous decidualisation. Surprisingly, our results highlight that molecular markers of spontaneous decidualisation are not restricted to menstruating primates, suggesting a gradual acquisition of this trait during primate evolution. The identification of a vascular cell population specific to menstruating primates further suggests that the emergence of menstruation involved vascular innovation within the primate endometrium. Together, our results depict the evolutionary history of endometrial cell types across primates and refine cellular hypotheses for the acquisition of menstruation.

C02.4 | From cell type- to tissue-evolution

The Role of Body Size in the Evolution of Tissues and Organs

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The evolution of tissues and organs is often discussed in terms of division of labour or transitions in individuality, yet why core biological functions come to be performed at higher organisational levels remains poorly understood. We test the hypothesis that increases in body size impose physical constraints that render ancestral cellular mechanisms for function performance ineffective, favouring the reorganisation of functions at tissue- or organ-level scales. Using a large comparative dataset spanning unicellular organisms to large metazoans, we analyse body-size distributions associated with different organisational modes of locomotion, circulation, and digestion. Across all three domains, higher-level implementations—such as muscular propulsion, closed and centralised circulatory systems, and extracellular digestion with specialised glands—are associated with significantly larger characteristic body sizes. These results support a view of organisational evolution as the preservation of biological functions across changing size regimes, linking biophysics, comparative biology, and the origin of complex anatomical organisation.

C02.5 | From cell type- to tissue-evolution

How do complex organs arise? Exploration of the pancreatic-like cell composition of a sea urchin by differential single-cell transcriptomics

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The digestion of food sources depends on the coordinated activity of digestive enzymes and hormones involved in maintaining metabolic homeostasis. In mammals, these processes are largely controlled by a specialized complex organ: the pancreas. While the formation of a specific pancreatic organ is a mammalian innovation, cells that show a highly conserved expression of canonical pancreatic genes are widely distributed in the digestive tract of many invertebrates. However, how such an organ arose during evolution remains unresolved. Despite the evident conserved expression of pancreatic genes in the digestive tract cells of most deuterostomes, most studies have focused primarily on the exocrine and digestive cells, leaving the broader endocrine pancreatic “landscape” unknown and its evolutionary origins unexplored. Here, using single-cell transcriptomics, we investigate for the first time the pancreatic-like cell type composition of the Mediterranean sea urchin Paracentrotus lividus larva reared under different feeding regimes. We focus on feeding-dependent changes in gene expression, we identify and characterize its exocrine and endocrine cell populations and place these findings in an evolutionary context by comparing such cells to other bilaterian species.

C02.6 | From cell type- to tissue-evolution

The genetic basis of a cell type expansion in the mammalian placenta

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The emergence of novel cell types is an important mechanism underlying the evolution of organismal complexity. Yet, we lack empirical evidence of how new cell types arise and diversify across species. The mammalian placenta – a recently evolved, morphologically diverse organ – offers a unique opportunity to investigate the evolution of novelty. Although the placenta has been long considered a reservoir of cell type innovation, the evolutionary relationships amongst its cell types remain unexplored.

Here, we reconstructed the evolutionary history of placental cell types using single-cell transcriptome data. By profiling and comparing ~380,000 cells from six mammals (mouse, rat, human, marmoset, guinea pig, rabbit), we discovered a lineage-specific cell-type expansion of trophoblasts. We identified three novel cell types that evolved in the common ancestor of murid rodents, effectively doubling the number of trophoblast cell types in this lineage. We found that the emergence of the novel trophoblasts was accompanied by a functional division of labour, whereby the novel cell types took up functions performed ancestrally by a single multifunctional cell type. By coupling gene expression with chromatin accessibility, we reconstructed the gene regulatory networks underlying murid-specific cell types and found that their multinucleated syncytiotrophoblasts evolved as sister cell types. Lastly, we found that the syncytiotrophoblasts of non-murid mammals did not evolve convergently, but constitute a rapidly evolving, homologous cell type. Our results provide rare empirical examples of cell type innovations in mammals and shed light into the molecular mechanisms underlying the origins of novel cell types.

Transcription factor family expansions and the origins of novel cell types

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After the emergence of multicellularity, cell types diversified, acquiring distinct physiological traits. The mechanism(s) driving this diversification over evolutionary time remain unclear. One hypothesis is that the appearance of new cell types correlates with duplication of regulatory genes and subsequent co-option of pre-existing gene modules. We test this idea by integrating chromosome-level genome assemblies and gene trees with single-cell multiomic data. As the sister group to Bilateria, cnidarians offer critical insights into metazoan cell type evolution: they are composed of canonical animal cell types (e.g., muscles and neurons) as well as clade-specific types (cnidocytes). We constructed life cycle- and tissue- resolved cell type atlases for two cnidarian species that diverged over 500 million years ago. These data reveal expansions within the bHLH family of transcription factors that correlate with the emergence of putatively novel cell types. Conserved expression profiles across cnidarians suggest that the origin of cnidocytes, a synapomorphy of the clade, coincided with expansion of the Myc family. We also experimentally demonstrate that a member of an anthozoan expansion of a gene clade related to the Paraxis/Twist-family (cPaTH) contributes to a unique cell type integral to tentacle anatomy and function in the sea anemone. Another notable case is a medusa-specific, acetylcholine-producing neuron that coincides with an expansion of the Atoh8 family. We discuss these examples in the broader context of cell type evolution.

How do novel cell types evolve? Co-option of fibroblast activation in the origin of uterine decidualization

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A key innovation that enabled eutherian pregnancy is the origin of a novel cell type - the decidual stromal cell. These cells differentiate from endometrial stromal fibroblasts under the influence of progesterone and prostaglandin E2 during pregnancy, in a process called decidualization. The resulting tissue is necessary for successful implantation and pregnancy. Decidualization shares similarities with the process of fibroblast activation- a response of fibroblasts to injury. Fibroblast activation entails the transformation of fibroblasts to myofibroblasts, which contributes to wound healing, and when chronically activated, to scarring. While decidualization initially follows fibroblast activation, it neither results in wound healing nor in scarring, but in differentiation to a decidual stromal cell. To explore whether decidualization may have arisen from co-opting and modifying fibroblast activation, we are comparing the two processes using published single-cell transcriptomes of human decidualization and wound healing. As expected, the comparison of gene expression trajectories between the two processes shows clear parallels in some of the known central factors. The two trajectories, however, differ strikingly with respect to the involvement of inflammatory genes- they are a key part of fibroblast activation in wound healing, but less so in decidualization. Downregulation of immune genes may be the modification of the process of fibroblast activation during its co-option in the evolution of decidualization, enabling a novel stable tissue that is able to support implantation.

Developing AI models to reconstruct cell type evolution across animal phylogeny

Jacob Musser, Kejue Jia

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Cell types diversified extensively during animal evolution, but comparing them across large evolutionary distances remains challenging because cell type-specific expression programs and protein sequences are often highly divergent. We are developing new AI models that integrate protein language models with single-cell transcriptomic data to identify remote gene homologs, align cell types across species, and reconstruct the evolution of cell type-specific molecular machinery. Using protein language models, we annotate previously unannotated cell type-specific genes in non-model organisms and recover homologous relationships not detected by conventional pipelines. These improved gene homology mappings enable more robust cross-species comparisons of single-cell atlases. We then extend this by predicting protein-protein interactions and their binding interfaces using a novel protein language model trained via a directed masking scheme, and use these predictions to generate hypotheses about the evolutionary assembly of cell type-specific machinery. As a case study, we apply this approach to the evolution of the neuronal presynapse, finding preliminary evidence suggesting that it evolved through the origin of new scaffolding interactions linking vesicle release machinery to calcium channels and sensors at cell junctions. Our results show how AI models provide a powerful new platform for investigating the origin and diversification of animal cell types.

Using closely related planarian species and single-cell transcriptomics to study cell type evolution

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Understanding how cell types evolve is a central question in evolutionary biology. While many cell types, such as muscle and neurons, are deeply conserved across animals, others appear to be lineage-specific, raising fundamental questions about how novel cell identities emerge. Proposed models include duplication and sub-specialisation of ancestral cell types, or the co-option of new gene expression programmes, yet the underlying mechanisms remain poorly understood. A major obstacle has been the difficulty of comparing cell types across species. The advent of single-cell transcriptomics (scRNA-seq) now enables unbiased classification of cell types based on whole-transcriptome profiles, offering unprecedented opportunities to study their evolution.

Here, we generate single-cell atlases of five planarian species and compare them in a multi-species framework. Focusing on closely related species allows us to refine computational approaches for cross-species cell type comparison under the expectation of substantial shared cell identities. We characterise conserved cell types across all species, identify lineage-specific cell types, and provide a detailed analysis of sexual cell types. Using established and novel graph-based analyses, we compare cell types and gene expression modules across species.

Strikingly, we identify a cell type present in one clade whose characteristic gene expression modules are distributed across distinct multiple cell types in another clade. This finding suggests that novel cell types may arise as hybrid entities assembled from pre-existing gene expression programmes. Together, our results provide a fine-grained view of cell type diversification among closely related species and introduce new conceptual and computational frameworks for studying cell type evolution.

Complementary roles of alternative splicing and gene expression in bilaterian tissue evolution

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Repurposing of ancestral genes for specialized biological roles is a pervasive process in animal evolution. Tissue-specific alternative splicing (TS-AS) and tissue-specific gene expression (TS-GE) represent two regulatory mechanisms shaping transcriptomes through conceptually analogous modifications of conserved genes. However, their relative contributions have never been systematically compared across multiple tissues and deep evolutionary distances.

Here we characterize TS-AS and TS-GE landscapes across ~7,000 ancestral gene families, using bulk RNA-seq data from eight homologous tissue types in twenty bilaterian animals. Almost 75% of these conserved gene families exhibit tissue-specific regulation, yet TS-AS and TS-GE act on largely non-overlapping gene sets with distinct molecular and functional properties that differentially shape tissues, species, and evolutionary lineages.

Specifically, TS-AS genes are universally longer, less duplicated, encode proteins with higher intrinsic disorder, and preferentially target the same intracellular machinery across tissues, whereas TS-GE mainly drives tissue-defining functions in nuclear or extracellular compartments. Across the phylogeny, TS-AS predominates in neural and muscle while TS-GE is overall more prominent in other tissues, with chordates showing the strongest impact of both regulatory modes. Importantly, although TS-GE regulation is notoriously more conserved than TS-AS across animals, we suggest that TS-AS nonetheless played a comparatively bigger role in shaping tissue-specific transcriptomes during key evolutionary events, such as the emergence of the vertebrate and insect brains. Altogether, our work uncovers complementary yet distinct evolutionary roles for TS-AS and TS-GE in shaping tissue-specific transcriptomes throughout bilaterian evolution.

3 | Bioelectric signals as a new frontier in EvoDevo

Symposium S03: Friday 12 June, 13:30 – 15:10

Organisers: Josep Martí-Solans (CNRS, France), Elias Barriga (Dresden University of Technology, Germany)

Bioelectricity is a pivotal, yet underappreciated, layer of control in morphogenesis, growth, and differentiation. This fundamental phenomenon is driven by the movement of charged ions across the cell membranes of all cells, not just excitable ones, creating subtle voltage differences essential for large-scale tissue patterning. Recent years have seen a surge of interest in how these bioelectrical cues coordinate complex developmental events and guide the cell movements required for tissue remodeling and repair. We are now at a critical point. Advances in bioelectronics, genetic editing, and high-resolution live imaging allow us to rigorously investigate how these endogenous bioelectric signals regulate core developmental processes like cell fate specification and morphogenesis. Evidence confirms bioelectricity's role as an evolutionarily conserved signal at both tissue and organ levels, found across vast phylogenetic distances, including plants, cnidarians, and vertebrates. This confluence of technological maturity and deep evolutionary significance establishes bioelectricity not merely as a biological curiosity, but as a tractable, high-impact frontier for understanding the construction of biological form. The aim of this session is to create a platform for discussing how bioelectric signals function as an instructive cue that shapes an organism's development. By convening researchers working on different model organisms, we will highlight both the conserved and divergent roles of bioelectric signals in regulating processes such as cell migration, tissue morphogenesis, and organ regeneration.

S03.1 | Bioelectric signals as a new frontier in EvoDevo

Living electronic interfaces to unravel Hydra bioelectricity

Claudia Tortiglione

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Bioelectrical signaling, in combination with biochemical and genetic regulatory networks, is increasingly recognized as a fundamental and indispensable source of information that coordinate and guides not only neuronal functions but also crucial biological processes such as tissue development, regeneration, and cellular differentiation. Conventional methods to record electrical activity in living organisms are limited by technical difficulties in precise, stable and non-invasive measurement, constraining our understanding of vital processes such as how bioelectrical networks encode information for tissue organization, or neuronal functions. Here we show the possibility to record and stimulate Hydra electrical activity by developing a custom-fabricated chip (Hydra on Chip, HoC) supporting the animal's morphology and spontaneous behaviour. This configuration enables reliable positioning and stabilization of the polyp on the electrode surface, allowing reproducible and non-invasive recordings of bioelectrical activity along the body column [1]. Using this approach, we observed distinct electrophysiological patterns evoked in *Hydra* in the presence of diverse stimulations, and studied the impact of conductive interfaces spontaneously produced in Hydra [2, 3]. These structures, acting as living electrodes, may advance fundamental understanding of biological processes governed by bioelectrical signalling, from neural signalling to regeneration, both in simple organisms and applied research in bioelectronics.

[1] Blasio M, *et al*. Unraveling Hydra bioelectrical activity on multielectrode array. *Front Bioeng Biotechnol*. 2026; 13:1736024.

[2] G. Tommasini, *et al*. New Perspectives on Semiconducting Conjugated Oligomers for Neuromodulation in *Hydra vulgaris*." *Adv. Mater. Interfaces* 2025, 12, 20: 2400962.

[3] G.Tommasini *et al.* Seamless integration of bioelectronic interface in an animal model via in vivo polymerization of conjugated oligomers, *Bioactive Materials*, 2022, 10: 107-116.

S03.3 | Bioelectric signals as a new frontier in EvoDevo

Integrating live voltage imaging and pharmacological manipulation to link bioelectric states to genetic control in ascidian morphogenesis

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Bioelectricity serves as a fundamental regulatory layer in morphogenesis, yet the functional dialogue between membrane potential and gene regulatory networks remains largely unexplored. Beyond excitable tissues, bioelectric signaling stems from ion flux across all cellular membranes, creating voltage gradients that orchestrate large-scale tissue patterning and guide cellular movements during embryonic remodeling. Despite its importance, the interface between electrophysiology and genomic control is poorly understood due to the traditional separation of these disciplines. The ascidian *Phallusia mammillata*, a basal chordate with a transparent embryo and stereotyped development, provides an ideal model to bridge this gap. Utilizing synthetic voltage-sensitive dyes, we performed high-resolution live imaging of voltage dynamics throughout ascidian development. Our mapping revealed dynamic bioelectric signatures characterizing specific embryonic domains, where distinct voltage gradients precede major morphological shifts. This suggests that electrical states provide a vital spatial scaffold for downstream developmental events. To test functional necessity, we employed pharmacological perturbations targeting specific ion channels. Initial data indicate that disrupting endogenous bioelectric states leads to significant morphological deviations and altered expression of key developmental genes. By integrating these high-resolution voltage maps with 3D spatial gene expression analysis, we provide a comprehensive view of the relationship between ion channel activity and gene regulatory states. This research establishes a clearer mechanistic link between bioelectric signaling and the genetic blueprints governing chordate development.

S03.4 | Bioelectric signals as a new frontier in EvoDevo

The Forces That Shape Us

Elias H Barriga

Cluster of Excellence Physics of Life, TU Dresden, Dresden, Germany

Robust morphogenesis is fundamental to organismal development, tissue regeneration, and disease progression. In this talk, I will share our recent advances in understanding the mechanisms that synchronize morphogenetic transitions during both development and regeneration. Drawing on examples from our biomechanical, bioelectrical, and molecular frameworks, I will discuss how the integration of these processes is essential for achieving robust tissue morphogenesis.

4 | Mechanical Forces in EvoDevo

Symposium S04: Wednesday 10 June, 09:00 – 10:40
Contributed Session C04: Wednesday 10 June, 11:05 – 12:30

Organisers: Anaïs Bailles (Max Planck Institute of Molecular Cell Biology and Genetics, Germany), Bruno Vellutini (Federal University of Minas Gerais, Brazil), Charlotte Manser (Department of Mathematics, King's College London, UK), Chiara Sinigaglia (Observatoire Océanologique de Banyuls-sur-Mer, CNRS, France), James Hammond (Department of Genetics, University of Cambridge, UK).

Morphogenesis is a biophysical process that gives rise to tissues and organs by integrating genetic and environmental cues during embryonic development. While the role of genes has been thoroughly studied over the years, it was only in the last decade that the molecular and cellular mechanisms underlying mechanical forces during development began to be uncovered. The field of Mechanobiology revealed that mechanical forces are not only crucial for driving tissue movement but also an essential source of information for embryonic cells during development. Cells can sense physical stimuli during development and respond with changes in behavior and gene regulation, a feedback loop that ultimately determines the tissue patterning and shape. Physical processes are therefore crucial for driving and shaping embryonic development. However, the role mechanical forces play in the evolution of development remains unclear. Do the mechanical properties of tissues constrain or enable evolutionary innovation? How does mechanical information fit into our current genetic-based view of developmental processes? Answering these questions is fundamental to understanding the interplay between development and evolution. Our symposium aims at bringing together pioneering researchers working at the interface of evo-devo and the biophysics-oriented framework of Mechanobiology. We will feature talks from a diversity of approaches and model organisms to provide an interdisciplinary overview of the role of cellular and tissue mechanics in evo-devo. We aim to raise awareness of the emerging field of MechanoEvoDevo and to provide a platform for fostering new ideas and collaborations within the evo-devo community.

S04.1 | Mechanical Forces in EvoDevo

Mechanics of the cleavage pattern of the brown alga *Fucus* embryo

Bénédicte Charrier[1], Marie Zilliox[1], Tanguy Dufourt[1], Bernard Billoud[1], Mayeul Collot[2], Charlotte Rivière[3]

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Fucus is a brown alga belonging to an evolutionary lineage that is distant from animals, fungi and other plants (including red and green algae). Despite foundational observations by W. Nienburg in 1931, the cleavage patterns and regulatory mechanisms of Fucus early embryogenesis have remained largely unexplored.
This study investigates the mechanisms that govern the organisation of the first cell divisions in Fucus embryos. We have developed novel fluorescent vital probes that enable the real-time, 3D visualisation of cell division up to the 20-cell stage. We observed that all cells divide perpendicular to the previous cell division plane, either equally or unequally depending on their orientation relative to the embryo's main axis. To identify the cues that control these cell division orientations, we performed two experimental manipulations. First, we subjected the embryos to external mechanical constraints using a PDMS-based squeezing device. Second, we treated the embryos with Ciliobrevin, an inhibitor of the molecular motor dynein. In animals, dynein mediates the cortical and cytoplasmic pulling of astral microtubules, displacing the centrosomes and ultimately the cell division plane. Both perturbations altered the cleavage pattern, indicating that Fucus embryonic division is regulated by a combination of external mechanical forces and intracellular, dynein-dependent processes.
This talk will present a more detailed study of how these factors impact the positioning of each cell division. In particular, the role of cell shape will be tested using the Cytosim software that simulates centrosome displacement based on pushing or pulling mechanisms.

S04.2 | Mechanical Forces in EvoDevo

The mechanics of crocodile and tortoise head scale patterning

Rory Cooper[1,2], Gabriel N. Santos-Durán[1], Ebrahim Jahanbakhsh[1], Grigorii Timin[1], Michel Milinkovitch[1]

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Amniote skin appendages are diverse micro-organs, including feathers, hair, and scales, that typically develop as genetically controlled units via a self-organized chemical Turing process. The mechanical patterning of crocodile head scales is an exception to this paradigm. However, the nature of the mechanical stress field driving their patterning remains unclear. Here, using injections of epidermal growth factor protein, we generate Nile crocodile embryos with convoluted head skin, and hatchlings with smaller polygonal head scales resembling those of caimans. We use light-sheet microscopy to quantify tissue-layer geometry, collagen architecture and proliferating cells, and build a mechanical growth model that recapitulates normal and experimentally modified patterning. Additionally, we show that tortoise head scales arise from sequential chemical and mechanical processes. Overall, crocodile and tortoise head scales self-organize through compressive folding arising from differential skin growth and material properties. Variations in these parameters provide an evolutionary mechanism to produce diverse reptilian head-scale patterns. Therefore, in addition to chemical Turing type processes, mechanical developmental mechanisms also contribute to the diversity of amniote skin appendage patterning.

S04.3 | Mechanical Forces in EvoDevo

The origin and evolution of animal morphogenesis: insights from choanoflagellates

Thibaut Brunet, Diede De Haan, Maite Freire-Delgado, Uzuki Horo, Mathilde Dura

Evolutionary Cell Biology and Evolution of Morphogenesis Unit, Institut Pasteur, Paris, France

All animals are characterized by a recognizable, species-specific adult shape, which results from a controlled process of morphogenesis during embryonic development. Morphogenesis relies on a small core set of cellular mechanisms, including cell adhesion, migration, division, deformation, and apoptosis. These mechanisms did not always exist but gradually evolved as single-celled ancestors successively acquired multicellularity, development, and cell differentiation more than 600 million years ago. While the origin of animals has left few fossil traces, some key steps can be reconstituted through the comparative study of modern groups. In recent years, important insights have emerged from the study of choanoflagellates, the closest living relatives of animals. Choanoflagellates are aquatic micro-eukaryotes that display multiple features informative for understanding the origin of animal development. Their polarized cell architecture is reminiscent of epithelial cells, their genomes encode a partial animal-like developmental toolkit (including cadherins, integrins, collagens, and actomyosin), and they can facultatively form multicellular colonies resembling early animal embryos. Our work combines functional genetics, cell biology, and fieldwork to investigate the emergence of cellular and multicellular shapes across several choanoflagellate species, and to place their life history in its natural ecological context. I will discuss our latest findings and their implications for early animal evolution.

S04.4 | Mechanical Forces in EvoDevo

Evolution of gastrulation as a mechanically constrained process

Steffen Lemke

Zoology, University of Hohenheim, Stuttgart, Germany

Living systems must reliably transform genetic information into physical form. In multicellular organisms, this transformation depends on the coordination of molecular, cellular, and mechanical processes under constraints imposed by size, geometry, and material properties. How such coupled systems evolve to generate diverse morphogenetic outcomes remains poorly understood. To address this question, we take a comparative approach and study gastrulation across a phylogenetically broad panel of fly species, combining quantitative in toto live imaging with targeted perturbations. This allows us to link changes in gene regulatory architecture to differences in tissue dynamics and mechanical behavior. Across systems, we find that evolutionary modifications of gene expression are associated with distinct modes of morphogenesis, while lineage-specific genes alter cytoskeletal organization and tissue material properties, expanding the range of physically accessible developmental trajectories. Collectively, our observations suggest that gastrulation can be understood as a continuous negotiation across scales and physical limits, in which genetically encoded programs give rise to cells with specific mechanical properties. In this context, mechanical constraints may not only respond to genetic change but also bias the evolution of morphogenesis.

C04.1 | Mechanical Forces in EvoDevo

Using fly wings to study the evolution of morphogenesis

Jana Fuhrmann, Benjamin Prud'homme

IBDM, Aix Marseille Univ, CNRS, Marseille, France

In flying insects, the wings typically retain the same basic aerodynamic, flat, double-layered shape despite differences in appearance and color. In contrast, the morphogenesis that leads to this bilayer geometry can be very different.

For example, the wings from Lepidoptera grow and morph in plane of the bilayer, whereas in Diptera, the wing imaginal discs can differ dramatically from the adult wing shape, necessitating a complex 3D shape change at pupariation. Among Diptera, wing development is best characterized in Drosophila melanogaster, where the wing disc grows into a stereotypically folded monolayer before undergoing unfolding and bilayer formation at pupariation. Yet this folded wing disc geometry is not universal.

We find that the wing discs of the scuttle fly Megaselia abdita have no folds, but undergo a similar monolayer to bilayer transition at pupariation. Utilizing this naturally occurring geometric divergence, we address the constraints on cell fate specification, growth, and tissue mechanics that result in the evolution of different wing disc morphologies between Drosophila and Megaselia. We then ask how subsequent morphogenesis and tissue mechanics are adapted to wing disc shape, to converge towards a similar final wing geometry.

This comparative approach between the wing discs of Drosophila and Megaselia provides a powerful tool to elucidate the general question of how developmental constraints during evolution manifest in different tissue shapes and how morphogenesis adapts to geometry.

C04.2 | Mechanical Forces in EvoDevo

Tissue mechanics reveal evolutionary divergence of inner ear morphogenesis across vertebrates

Shuhei Horiguchi, Shunya Kuroda, Satoru Okuda

Nano Life Science Institute, Kanazawa University, Kanazawa, Japan

The vertebrate inner ear is a conserved sensory organ for balance. Its morphogenesis follows a two-phase program: (i) expansion of the otic vesicle—a closed epithelial monolayer enclosing a fluid-filled lumen—and (ii) topological transition from a closed vesicle to a toroidal structure of the semicircular canals. Although this two-phase process is broadly conserved, previous studies have reported divergence in molecular mechanisms and tissue mechanics between zebrafish and mouse during phase (ii). This raises two questions: how do distinct mechanisms yield a common morphology, and how did such divergence evolve?
To address these questions, we focus on phase (i)—otic vesicle expansion—in zebrafish and mouse, combining pharmacological perturbations with mathematical modeling. We find that zebrafish vesicles expand primarily through fluid influx into the lumen, whereas mouse vesicles expand through coupled fluid influx and mechanosensitive tissue growth. In zebrafish, limited tissue growth reflects restricted cell growth rather than reduced proliferation.
Extending our analysis to 12 vertebrate species sampled broadly across the phylogeny, we identify two distinct vesicle-expansion modes and corresponding cell-growth programs, exemplified by zebrafish and mouse. We propose that divergence in these morphogenetic processes reflects the adaptive evolution of developmental duration, accompanied by shifts in cell-growth programs.

C04.3 | Mechanical Forces in EvoDevo

Morphogenesis establishes directional transport in the gastrovascular network of the jellyfish Aurelia aurita during development

Laureline Julien[1], Xinbo Li[2], Aaron Winn[2], Daisuke Noto[2], Thibault Chasserau[2], Léna Zig[1], Alain Ponton[1], Bérengère Abou[1], Eleni Katifori[2], Annemiek JM Cornelissen[1]

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Jellyfish Aurelia aurita possesses a gastrovascular system composed of stomachs connected to a canal network that evolves during development. At the juvenile stage, this network consists of separate canals in which fluid motion shows back-and-forth flow. During development, canals connect, and directional circulation emerges. We investigate the development of transport mechanisms that give rise to flow directionality in this system.

We identify two candidate drivers: 1) peristaltic pumping generated by muscle contractions used for swimming, and 2) beating cilia lining the canal walls. To separate the contributions of cilia and peristalsis, we track food particles in canals. Under anesthesia, contractions cease, and flow is driven solely by cilia. Cilia beat in coordinated waves and are oriented in the direction of the adult flow in all canals, from the juvenile stage.

Comparing velocities in anesthetized animals shows that cilia maintain a baseline flow, while muscular contractions accelerate transport at all phases of the swimming cycle. Using a model that maps body deformation to fluid propulsion, we show that swimming kinematics can control flow magnitude and direction. In addition, canal fluid contains mucus with a shear-thinning behavior facilitating transport by lowering resistance under pumping.

In growing canals, we observe planar cell polarization aligned in the direction of the growth. Our results suggest that cilia do not require net flow to get orientation, and that directionality of flow is set by tissue growth.

This study links growth of a network with mechanics of transport that induces circulation and distribution inside the gastrovascular system.

C04.4 | Mechanical Forces in EvoDevo

Rhythmic contractions of the yolk of goldfish, domesticated ornamental fish with diverse dorsoventral patterning traits

Paul Gerald Layague Sanchez[1,2,3], Chen-Yi Wang[1], Ing-Jia Li[1], Kinya G. Ota[1]

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While the yolk is commonly considered as a passive nutritional source for fish post-hatching, evidence show that the yolk in fact plays an active role in early fish embryo development and patterning. Here, we report peculiar movements of the yolk in eggs and embryos of goldfish Carassius auratus, a domesticated ornamental fish with diverse traits. We quantify these contractions and record robust rhythmic yolk movements that are not seen in closely-related Cyprinus carpio and Danio rerio. These contractions do not require fertilization nor cell division, and they notably emerge at a precise time — suggesting a capacity to measure elapsed time from what could be egg activation. We further show that these contractions persist even in yolk in isolation. As the yolk itself is known to confer critical cues for dorsoventral (DV) patterning of teleost embryos, we hypothesize that its contractions in goldfish may influence the patterning of this species. Indeed, we find that embryos of the naturally more ventralized twin-tail goldfish strain Oranda display altered yolk contraction dynamics. We also present that the period of contractions is independent of chds, a key gene involved in DV patterning and linked to the twin-tail phenotype, but is instead a trait that is maternal in origin. We aim to uncover whether the yolk contractions happening during early development of domesticated goldfish are the licensing process which permit the emergence of novel patterning phenotypes naturally-observed in this species and which instead have not been found among closely-related species whose yolks do not contract.

C04.5 | Mechanical Forces in EvoDevo

Coordinating organismal and organ repair in a hydrozoan medusa

Chiara Sinigaglia

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Regenerative capacities vary widely across metazoans, raising fundamental questions about their evolution. Although several conserved mechanisms have been identified across diverse species, we still lack a clear framework for defining relevant evolutionary characters. This is a direct consequence of the type of questions asked, as data refer to very different scales and body parts, and of the fact that several mechanisms are involved in multiple processes, notably during embryogenesis or asexual reproduction.

The medusa stage of the hydrozoan cnidarian *Clytia hemisphaerica*, with its simple anatomy and robust regenerative capacity, provides a valuable model for investigating whole-body re-patterning and the reorganization of progenitor cells into new structures. Our previous work showed that *Clytia* can restore form and function through widely conserved mechanisms, further suggesting that smooth epitheliomuscular cells could have an organizing role.

We are undertaking an integrative approach, focusing in parallel on processes occurring at the organismal scale, at the tissue and organ level, and at the level of progenitor cell dynamics. Our research program combines omics, imaging, functional perturbations, and biophysical modeling, and explores how different components of the medusa body—the stem cell system (i-cells), the smooth muscle-epithelial cells, and the extracellular matrix (mesoglea)—contribute to shape re-emergence, and how these features are coordinated at the systemic level.

This work provides insight into the multiscale coordination of cellular behaviors, tissue mechanics, and organismal architecture underlying shape restoration. It offers a framework for integrating cellular, mechanical, and organismal perspectives in the comparative study of regeneration across metazoans.

C04.6 | Mechanical Forces in EvoDevo

Evolution and mechanics of retinal-pigmented epithelium morphogenesis during optic cup folding

François Agnès, Mathilde Pain, Dimitri Vérité, Parisa Zia, Estelle Giry, Sylvie Rétaux

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The morphogenesis of the optic cup provides a robust system for studying how two opposing epithelial monolayers with distinct properties fold and stretch in a coordinated manner to form the primordial eye. While retinal neuroepithelium invagination has been extensively studied, the spatial organization and expansion of the retinal pigment epithelium (RPE) remain less understood. The teleost fish *Astyanax mexicanus*, which exists as two ecomorphotypes with natural variation in eye development, offers a unique comparative model to address this question. Using quantitative 3D imaging of optic cups at various stages from both morphs, we show that RPE morphogenesis is characterized by transient, graded, and anisotropic cell stretching that patterns the epithelium during optic cup shaping. Analyses of RPE nuclear spacing and cell morphology predict that stretching progresses along the proximo-distal axis, with maximal tension in distal RPE cells that are elongated and aligned along optic cup meridians. Nuclear volumes and apical surface areas scale along the same axis independently of endoreplication, suggesting that transient cell size increase may reflect increased metabolic activity that supports region-specific deformation. In cavefish, RPE expansion is delayed by over six hours, and proximal stretching exhibits altered isotropy, indicating disrupted temporal coordination and modified mechanical constraints. Together, these findings establish RPE morphogenesis as a spatiotemporally heterogeneous process offering new insights into the study of the biomechanical principles of eye development in vertebrates.

5 | The Impacts of Microbiota on Animal and Plant Development

Symposium S05: Thursday 11 June, 09:00 – 10:40
Contributed Session C05: Thursday 11 June, 11:05 – 12:30

Organisers: Thomas C.G. Bosch (University of Kiel, Germany), Tyler Carrier (University of North Carolina at Charlotte, USA)

Understanding how animals reproduce and develop has remained a central tenet of biology for nearly two centuries. Early studies focused on how eggs divide, cells differentiate, and tissues form three-dimensional structures. These morphological investigations shifted a half-century ago to determine the genomic basis of embryonic and adult body plans, how regulatory networks change over evolutionary time, and how the environment shapes the phenotype. Conceptually integrating embryology, evolution, and ecology has allowed development to be studied in a more natural setting. The last three decades has featured the rise of another discipline within the life sciences: host-microbe symbiosis. The principles of host-microbe symbiosis, however, have been slow to be integrated into the framework of embryology, despite that it has become increasingly difficult to differentiate between the developmental processes that are exclusively performed by the host and those that are performed in concert with a specific microbial symbiont or diverse microbiome. This symposium will feature the diverse ways that microbes are integrated into the reproductive and developmental programs of plants and animals. It will also feature model and non-model systems from across the tree of-life that are derived from both the developmental and symbiosis worlds.

Natural variation in *Arabidopsis thaliana* root responses to microbiota-derived *Pseudomonas*

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Plant root system architecture (RSA) shows extreme developmental plasticity based on the integration of multifactorial cues from the complex soil environment. The microbial community associated with roots (the microbiota) influences RSA; however, the molecular mechanisms underlying plant interactions with the microbiota and how these interactions affect root developmental pathways remain unclear. To identify genetic determinants in plant-microbiota interactions, we screened a panel of natural *Arabidopsis thaliana* accessions grown in mono-association with microbiota-derived *Pseudomonas* bacteria, and observed variation in RSA responses among the accessions. While many accessions including Col-0 showed only subtle responses to *Pseudomonas* isolate R569, some accessions, such as Van-0, responded with reduced primary root growth and increased lateral root emergence. In addition to the RSA changes, Van-0 roots show transcriptional downregulation of genes with cell wall-related functions. Using a population of recombinant inbred lines, we identified a quantitative trait locus (QTL) associated with the RSA changes. Through crispr-Cas9 gene editing, we identified multiple genes within the QTL that contribute to the phenotypic variation. In Van-0, the exocyst subunit *EXO70E2* is required for strong root responses. In contrast, the root responses are suppressed by the Col-0 haplotype at the *OAKL* cluster, which encodes malectin-like and leucine-rich-repeat containing receptor-like kinases. The *OAKL* cluster has greatly diversified in *A. thaliana*, showing at least 3 major haplotypes with overlapping geographic distribution. Our results hint at a signaling pathway through which plants adapt their root growth according to the local rhizosphere microbial environment.

Decoding microbiota-driven plasticity in the vertebrate intestine

Courtney Rivet-Noor, Jennifer Heppert, Matthew Tillman, James Davison, Colin Lickwar, **John Rawls**

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Animal genomes encode developmental and physiological programs with remarkable capacity to sense and adapt to environmental stimuli, including microorganisms. Nowhere is this plasticity more evident than in the intestine, which harbors complex microbial communities that shape epithelial development, metabolism, and immunity. Yet the evolutionary origins of these host–microbiota relationships and the genomic mechanisms that enable such plasticity remain incompletely understood. Comparative genomics hold the potential to uncover the history of intestinal symbiosis by identifying host genomic features shaped by microbial interactions over deep evolutionary time. Focusing on vertebrate lineages that diverged ~420 million years ago, I will show how coding sequences, transcriptional programs, and cis-regulatory DNA regions encode signatures of conserved host–microbe dialogue. First, comparative analysis of protein-coding gene repertoires reveals the evolution of molecular tools for sensing and managing microbes, including conserved and lineage-specific components of innate immunity and epithelial defense. Second, cross-species comparisons of microbiota-dependent transcriptional responses identify conserved symbiotic programs governing metabolism, epithelial renewal, and immune function. Third, mapping microbiota-responsive genes to their cis-regulatory elements can identify the broader transcriptional networks that translate microbial cues into coordinated host responses. I will describe a case study demonstrating how integrating comparative expression profiling, enhancer logic, and transcription factor biology uncovered a conserved role for the nuclear receptor HNF4A in mediating intestinal epithelial responses to the microbiota. Together, these approaches provide a framework for reconstructing ancestral host–microbe interactions and defining regulatory networks that shape intestinal plasticity and disease susceptibility.

A role of environmental microbes in triggering wound healing and regeneration

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ENS de Lyon, Biological Sciences, The Institute of Functional Genomics of Lyon (IGFL), CNRS and ENS de Lyon, Lyon, France

For wound healing and organ regeneration to occur, an injury must be sensed by the tissues surrounding the site of injury. However, the mechanisms by which animals sense injuries and mobilise cells to regenerate missing structures are poorly understood. Our goal is to unravel the mechanisms behind these early events, following an injury, using the crustacean *Parhyale hawaiensis* as an experimental model. *Parhyale* are genetically tractable and can regenerate their legs after amputation. Upon injury, the breached cuticle allows environmental microbes to come in contact with blood cells and tissues. Blood cells make a clot, and that is followed by the formation of a melanised scab. Using antibiotics, we have demonstrated that the presence of environmental bacteria is required for wound melanisation and for triggering regeneration. Our results suggest that melanin formation generates reactive oxygen species (ROS) at the site of the wound, which we hypothesise serves to signal wounding and trigger regeneration.

S05.4 | The Impacts of Microbiota on Animal and Plant Development

Understanding how the microbiome shapes nervous system development

Helen Vuong

Pediatrics, University of Minnesota, Minneapolis, USA

Neurodevelopment is shaped by genetic and environmental inputs. Recent evidence in animal studies reveal the gut microbiota as an important environmental regulator of brain development including blood brain barrier formation, microglia structure and function, neurotransmitter and receptor expression, axonogenesis and consequently social, cognitive, and sensory behaviors in adult animals. However, how the microbiota shapes other aspects of brain development such as neocortical development, and what are the cellular and molecular mechanisms mediating this interaction remains to be understood. In this project we describe how the maternal gut microbiota composition during gestation influences neocortical lamination and cell cycle dynamics. We discover depleting the maternal gut microbiota significantly alters neoorticogenesis, suggesting maternal microbes and metabolites contribute to development of the neocortex. Conventionalizing the maternal gut microbiota during early- to mid-gestation can prevent abnormal neocortical deelopment. Additionally, maternal microbiota interactions with maternal nutritional deficiency contributes to neocortical cell type development. Together, this data shows the microbiome during gestation provides environmental cues that inform cell organization within the neocortex.

C05.1 | The Impacts of Microbiota on Animal and Plant Development

A Microbial Perspective on Animal Development and Behaviour

Thomas Bosch

Zoological Institute, Kiel University, Kiel, Germany

Animals have evolved within the framework of the microbes and are constantly exposed to diverse microbiota. This dominance of the microbial world is forcing all fields of biology to question some of their most basic premises, with developmental biology being no exception. While animals under laboratory conditions can develop and live without microbes, they are far from normal, and would not survive under natural conditions, where their fitness would be strongly compromised. Since much of the undescribed biodiversity on Earth is microbial, any consideration of animal development in the absence of the recognition of microbes will be incomplete. Here, using the ancestral metazoan Hydra as model, I will show that animal development and behaviour may never have been autonomous, rather it requires transient or persistent interactions with the microbial world. I propose that to formulate a comprehensive understanding of embryogenesis and post-embryonic development, we must recognize that symbiotic microbes provide important developmental signals and contribute in significant ways to phenotype production. By integrating the microbiome perspective into our understanding of evolution and function, we gain richer insights into the dynamic interplay that drives health, adaptation, and the very fabric of life itself.

Keywords: Animal-microbe, Evolution, Hydra, Microbiome, Symbiosis

Microbes as manipulators of egg size and developmental evolution

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Marine invertebrates mainly reproduce by energy-poor eggs that develop into feeding larvae or energy-rich eggs that develop into non-feeding larvae. The factor(s) responsible for driving transitions in reproductive strategy and developmental mode remain elusive despite having been studied in detail. Microbes may be one such factor, as they are functionally beneficial to animal reproduction and have evolved mechanisms to override components of this host program. This talk identifies the first suspected reproductive manipulator in the ocean and then presents theoretical models that support the premise that this type of microbe could serve as the selective agent of these developmental transitions. Specifically, these models outline the predicted circumstances by which these microbes may spread within populations. They then predict that microbial manipulators would create a sperm-limited environment that selects for larger eggs by shifting the host's sex ratio towards female dominance and, as a result, serve as an evolutionary driver of transitions in the developmental mode for marine invertebrates. Lastly, this talk will suggest candidate marine invertebrate genera from throughout the world's oceans that fit the framework of a microbe-induced switch between these predominate reproductive strategies.

Microbiome dynamics across the life cycle of *Platynereis dumerilii*

Giulia Ghisleni[1, 2], Alvin Han[3], Tatiana Gorojankina[2], Ivan Zubcic[3], Maurizio Casiraghi[1], Detlev Arendt[3], Guillaume Balavoine[2], Antonia Bruno[1]

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Microbial symbioses are increasingly recognized as key contributors to animal development and evolution. The marine annelid *Platynereis dumerilii* is a widely used model in evolutionary developmental biology, yet its associated microbiome has remained unexplored. Here, we present the first longitudinal characterization of the microbiome of laboratory-cultured *P. dumerilii* across its entire life cycle, including major culture subenvironments. Using 16S rRNA gene sequencing, we describe the composition, diversity, and temporal dynamics of host-associated and environmental microbial communities. We identify a persistent host-associated core microbiome supported by temporal continuity of specific taxa, alongside strong sample type-specific microbial signatures. Shifts in culture conditions triggered a pronounced microbiome restructuring event, marked by high taxonomic turnover and the collapse and reassembly of microbial interaction networks. Source-tracking and diversity analyses indicate that the worm microbiome is primarily maintained through temporal persistence, with transient environmental contributions from the microbial mat during feeding stages. Network analyses further reveal structurally central taxa, such as *Vibrio*, *Fluviicola*, *CL500-3* (family *Phycisphaeraceae*), *Algimonas*, and members of the family *Flavobacteriaceae*, suggesting functional organization despite compositional variability. Comparative analyses with the sibling species *Platynereis massiliensis* and other *P. dumerilii* cultures highlight substantial microbiome divergence, reflecting both species-specific traits and environmental context. Overall, our results establish *P. dumerilii* as a tractable holobiont model and provide a foundation for integrating host-microbe interactions into evo-devo research and experimental reproducibility frameworks.

Synergistic holobiont plasticity in the sea urchin *Arbacia lixula*: Maternal effects and symbiotic microbiome under ocean acidification

Andres Rufino Navarro[1], Sara González Delgado[1,2,3], Vanessa Arranz[2,3], Tyler Carrier[4,5], Rocío Pérez Portela[2,3], José Carlos Hernández[1]

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The resilience of the sea urchin *Arbacia lixula* to naturally acidified ecosystems offers a model for understanding adaptation to ocean acidification (OA) among marine calcifiers. By utilizing a volcanic CO2 vent in La Palma (Spain), this research evaluates the interplay between maternal investment, microbiome plasticity, and host-symbiont functionality. Adult females from vent habitats demonstrated a shift in reproductive strategy, yielding fewer eggs characterized by increased jelly coat thickness and a lipid profile high in saturated fatty acids. This provisioning mechanism is hypothesized to reinforce membrane stability, thereby improving offspring viability in low-pH environments. Additionally, the composition of the larval microbiota displayed significant environmental plasticity. Functional assays involving the pre-exposure of embryos to distinct microbial pools prior to OA stress confirmed the protective role of these symbionts. Larvae colonized by vent-associated bacteria exhibited higher developmental success and survival rates under acidification than those exposed to ambient microbes or filtered seawater. Ultimately, the capacity of *A. lixula* to endure future OA scenarios depends on the synergistic integration of bioenergetic maternal traits and beneficial microbial partnerships.

6 | Evolution through simplification and loss

Symposium S06: Wednesday 10 June, 09:00 – 10:40
Contributed Session C06: Wednesday 10 June, 11:05 – 12:30

Organisers: Madeleine E. Aase-Remedios (Copenhagen University, Denmark), Meghan Yap-Chiongco (Copenhagen University, Denmark), Ludwik Gąsiorowski (University of Warsaw, Poland), Nikolaos Papadopoulos (University of Vienna, Austria)

The decrease in organismal complexity over evolutionary time (i.e., simplification) through morphological reduction or loss, is an important driver of diversification in eukaryotes. Simplification can occur via several mechanisms (e.g., genome reduction/compaction, gene gain/loss, and changes to gene regulation) and is often associated with major evolutionary transitions. The proposed symposium will cover varied examples of simplification in life history, ecology, habitat, and environment. Our invited speakers have diverse backgrounds, with both evo-devo and comparative genomics perspectives representing both animals and plants. They will discuss the signatures of miniaturization and their phenotypic and developmental consequences for adaptation to life in the marine meiofauna; the comparative genomics of flatworms, particularly in the context of the evolution of sexual traits; the successive losses of nuclear genes and plastids characterising the adaptation to parasitism in plants; or the genomic signatures of miniaturization in vertebrate evolution.

S06.1 | Evolution through simplification and loss

Stepwise loss of recombination preceeded the origin of asexuality in *Schmidtea mediterranea*

Jeremias N. Brand[1,4], Ajinkya Bharatraj Patil[1], Luca Pandolfini[2], Kira Zadesenets[3], Nikolay Rubtsov[3], Laura Robledillo[5], Meng Zhang[5], André Marques[5], Jochen C. Rink[1,6]

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The emergence of sex chromosomes is traditionally hypothesized to occur when a sex-determining locus acquires recombination suppression that subsequently expands. An alternative model suggests that recombination suppression occurs first, with the sex-determining locus emerging secondarily. Chromosome 1 of the hermaphroditic planarian *Schmidtea mediterranea* has been proposed as such a "sex-primed" autosome, characterized by a >300 Mb inversion with suppressed recombination. While *S. mediterranea* typically reproduces sexually, asexual strains also exist. This shift in sexual system is linked to a conspicuous heteromorphic chromosomal translocation also involving the same chromosome, but due to the lack of genomic resources the underlying genomic architecture could not be elucidated. Here we use a highly contiguous, haplotype-phased genome assembly to reconstruct the genomic rearrangements preceding the origin of asexuality. We identify the genomic breakpoint of the translocation to a large tandem-repeat gene desert, suggesting no direct gene loss. But beyond this visible translocation, we uncover pervasive chromosomal restructuring across the entire genome. Genomic analyses of wild populations and laboratory strains reveal that these rearrangements are linked to elevated heterozygosity, confirming they acted as barriers to recombination. Our results suggest that successive rearrangements in a sexual ancestor, potentially driven by inbreeding pressure and local adaptation, rendered large portions of the genome functionally asexual prior to the transition to obligate asexuality. These findings support a model where the gradual loss of recombination facilitates shifts in reproductive systems and provides a framework for understanding the diverse sexual systems observed in flatworms.

Evolution through simplification and loss – A panarthropod perspective

Ralf Janssen

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Intuitively, it is understandable why the gain of morphological and/or genetic novelties is a strong driver of diversification and thus evolution. Less obvious, however, is the opposite.

In the context of simplification and loss, changes in the environment and behaviour of an organism are the main drivers of diversification, along with which morphological and genetic changes go hand in hand.

Arthropods represent a group of invertebrate animals that have inhabited almost all ecological niches on our planet, including some very specific and peculiar places; some examples will be presented. Often these examples refer to cases of parasitism and/or miniaturization, and such extreme specialization is often accompanied with the loss of certain morphological and genetic features that are no longer needed.

The closest relatives of arthropods are tardigrades and onychophorans, both of which show substantially less morphological variability than the arthropods. Tardigrades especially are famous for their extreme life style, including cryptobiosis. Beyond that, tardigrades appear to have lost a large part of their body, and genetic and fossil evidence suggests that they evolved from much larger Cambrian ancestors through miniaturization and indeed the loss of the posterior trunk. Possibly, these drastic changes of their body architecture were prerequisites for the later evolution of novel, tardigrade specific traits, along with the maintenance of ancestral panarthropod traits.

In my talk, I will present some key examples of arthropod miniaturization and accompanied genetic and morphological losses, and will discuss specifically the case of Tardigrada.

S06.3 | Evolution through simplification and loss

Small but mighty: How miniaturization shapes metazoan evolution

Katrine Worsaae, Alessandro Formaggio, Madeleine Aase-Remedios, Meghan Yap-Chiongco, Alice Rouan, Shoyo Sato

Department of Biology, University of Copenhagen, Copenhagen, Denmark

Microscopic body size has evolved repeatedly across the Animal Tree of Life with one-third of the ~32 animal phyla being primarily meiofaunal (<1mm long) and with multiple examples of miniaturization occurring within macrofaunal lineages. These genomically unexplored, microscopic lineages offer a unique window into how reduction in scale may act as a generative force in shaping animal diversity and evolutionary innovations.

With new protocols optimized for ultra-low input DNA/RNA we can generate high-quality genomes from single meiofaunal organisms. Our phylogenomic analyses suggest a microscopic ancestor of major clades both within Ecdysozoa and Spiralia (e.g., Scalidophora and Gnathifera), and potentially at the base of these entire groups, which encompass >90% of all animal species. However, our comparisons of architecture, gene losses/gains, and Hox cluster compositions show severe losses/contractions even at deeper nodes, e.g. of Gnathifera. These genomic signatures of miniaturization indicate that Gnathifera, and potentially most meiofaunal phyla, have undergone miniaturization secondarily, and that this evolutionary process is a major driver of metazoan evolution. Further, our comparative transcriptomic studies reveal that miniaturization can be initiated by heterochronic shifts in gene expression during development, causing early sexual maturation and arrest of somatic growth, resulting in microscopic adults.

Our advancements in meiofauna genomics provide new insights into the interplay between size, development, and genomic properties in metazoan evolution. We herewith address the fundamental biological questions: Is there a microscopic ancestry of major animal groups? How have meiofauna evolved across the animal kingdom? What are the genomic mechanisms and outcomes of miniaturization?

S06.4 | Evolution through simplification and loss

Loss-driven genomic remodeling enables a freshwater–marine transition in a teleost

Salman Kolbadinezhad[1], André Machado[1], André Gomes-dos-Santos[1,2], João Moutinho[1,2], Elsa Froufe[1], Alex Quijada-Rodriguez[3], Miguel Cordeiro[1], Raquel Ruivo[1,2], Jonathan Wilson[3], **Filipe Castro[1,2]**

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Major ecological transitions test the evolutionary limits of physiological systems. Among teleost fishes, transitions from freshwater to marine habitats are rare, reflecting both the severity of the osmotic challenge imposed by seawater and the constrained capacity of most lineages to expand salinity tolerance. Lineages that nevertheless cross this boundary offer powerful models for understanding how complex physiological systems are reconfigured during major ecological change. The striped eel catfish, *Plotosus lineatus*, represents one such transition. Although descended from freshwater ancestors, it is fully marine and exhibits an unusual osmoregulatory phenotype, including hyperosmotic urine production and a salt-secreting anatomical innovation, the dendritic organ. To investigate the genomic basis of this transition, we combined whole-genome sequencing, comparative analyses across catfishes, and organ-level transcriptomics. Rather than simple modification of ancestral freshwater machinery, we find evidence for extensive genomic simplification affecting core components of renal ion reabsorption and diluting pathways. These losses coincide with structural and functional remodelling of the kidney and are paralleled by the emergence of a distinct molecular program supporting salt secretion in the dendritic organ. Together, our results suggest that marine colonization in this lineage did not arise through incremental tuning of freshwater physiology, but through targeted loss and systems-level rewiring. This case illustrates how simplification—via gene loss and architectural reduction—can facilitate major ecological transitions, expanding our understanding of the evolutionary routes available to complex physiological systems.

C06.1 | Evolution through simplification and loss

“Less, but more”: evolutionary innovation following massive gene loss in *Oikopleura dioica*

Gaspar Sánchez-Serna[1,2,3], Alfonso Ferrández-Roldán[1,2], Marc Fabregà-Torrus[1,2], Nuria Torres-Águila[1,2], Biel Cassà-Garcia[1,2], Esteve Escolà-Calero[1,2], Josep Martí-Solans[1,2,4], Ricard Albalat[1,2], Johannes N. Wibisana[3], **Cristian Cañestro[1,2]**, Michael J. Mansfield[3], Charles Plessy[3], Nicholas M. Luscombe[3], Cristian Cañestro[1,2]

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Gene losses are pervasive across the tree of life and often occur more frequently than gene gains. Rather than representing merely neutral or regressive processes, gene loss can also promote adaptive evolution, a concept known as the “less is more” hypothesis. Work from our laboratory shows that the appendicularian tunicate *Oikopleura dioica* provides an attractive Evo-Devo model for investigating how gene loss reshapes gene regulatory networks (GRNs). Our analyses reveal that appendicularians have undergone extensive dismantling of several key developmental signalling pathways, including retinoic acid (RA), FGF, and Wnt pathways, as well as the partial “deconstruction” of the cardiopharyngeal GRN, following massive ancestral gene losses. Strikingly, these losses are frequently accompanied by bursts of gene duplication, revealing an evolutionary scenario that we describe as “less, but more”. In this context, gene loss is not associated with phenotypic simplification but rather with the emergence of evolutionary innovations. Our findings suggest, for example, that extensive gene loss may have facilitated the transition from an ancestral tunicate with an ascidian-like sessile lifestyle to the evolution of a complex free-swimming lifestyle in appendicularians.

C06.2 | Evolution through simplification and loss

How to lose your gut: the genomic mechanisms of parasitism loss in lampreys

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Lampreys are ancestral parasitic vertebrates, but parasitism has been repeatedly lost across lineages, accompanied by changes in life-history and development. Compared to parasitic species, non-parasitic lampreys skip the juvenile feeding phase entirely, which is associated with intestinal degradation and metabolic rewiring during metamorphosis - making them the only vertebrates that never feed as adults. Yet, the genomic mechanisms of this fascinating evolutionary change in life-history and development remain unknown. We integrate comparative genomics, population genomics, and developmental transcriptomics of natural populations to identify the genomic basis of recent parasitism loss in European lampreys (*Lampetra* spp.).

We found that parasitism loss mapped to a small number of major-effect genomic regions, some co-localising with intra- and interspecific chromosomal rearrangements. Strikingly, these regions differed across more divergent lamprey lineages despite convergent developmental outcomes, suggesting that the loss of parasitism can be achieved through distinct genomic routes. The implicated regions encompassed hundreds of genes involved in core developmental pathways, neural development, and metabolism. Transcriptomic analysis across multiple tissues during metamorphosis in parasitic and non-parasitic ecotypes, and their natural hybrids, revealed that divergent genes show strong, tissue-specific differences in expression trajectories between ecotypes, which correlated with divergence in developmental patterns.

Together, our results show that complex developmental changes associated with the recent loss of parasitism are encoded by relatively few genomic regions driving tissue-specific regulatory divergence. They further highlight the power of integrating population genomics and developmental transcriptomics in natural populations for uncovering the genomic mechanisms of recent trait evolution or loss.

C06.3 | Evolution through simplification and loss

Faster and smaller: heterochronic shift in gene expression leads to larval onset of spermatogenesis and miniaturized males

Alice Rouan[1], Madeleine E. Aase-Remedios[1], Flora Rasmussen[1], Norio Miyamoto[2], Katrine Worsaae[1]

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Many microscopic animal lineages (meiofauna) are hypothesized to descend from macroscopic ancestors, but direct evidence of miniaturization mechanisms is scarce. Heterochrony, a change in the timing of developmental events, is suggested as the prevailing evolutionary route to an underdeveloped or paedomorphic body plan. Here, we investigate the molecular developmental basis of the microscopic, paedomorphic males of the annelid *Osedax japonicus* known to havebone-eating macroscopic females. We find that males start their sexual maturation extremely early with testes already developing in the larval stage and cease their somatic growth soon after metamorphosis. Heterochronic shifts in gene expression explain this male paedomorphosis through early onset of germline development, sexual determination, and maturation in concert with early offset of somatic growth. Our findings provide direct developmental evidence for how heterochrony can drive extreme miniaturization, shedding new light on the evolutionary origins of microscopic animal lineages.

C06.4 | Evolution through simplification and loss

Decoding the developmental genetic basis of song loss in rapidly evolving crickets

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Courtship song is crucial for mate attraction in many insect species – so how could this be lost? In the Hawaiian field cricket (*Teleogryllus oceanicus*) a silent, X-linked male morph, flatwing, has arisen under pressure from the acoustically-orientated parasitoid fly *Ormia ochracea*. Silent males have lost the wing structures that allow for acoustic signal production. In addition, multiple other sound-reducing wing morphs have recently evolved – suggesting rapidly evolving underlying gene regulatory networks. Transcriptional profiling indicates the sex-differentiation pathway gene *doublesex* is involved.

Here, we explored the hypothesis that core developmental genes like *doublesex* interact with a broader network of venation specification genes as *T. oceanicus'* wings develop, such that numerous slight adjustments to this network result in convergent song-loss venation patterning.

First, we established and compared timing schemas of embryonic and nymphal development for wild-type (normal-wing) and flatwing genotypes of both sexes. Both sexes have ten nymphal instars and visible, sexually divergent morphological trajectories are initiated at instar four. However, normal-wing males take significantly longer than flatwing males and females of either morph to reach adulthood.

We discuss the consequences of protogyny in this species, and the potential for morph-specific differences in generation time that we discovered, focusing on advantages this may confer to flatwing males who reach maturity at the same time as females.

Intraspecific differences in development time among segregating genotypes may have significant consequences on the rate of mutation accumulation and evolutionary responses to select, with developmental heterogeneity potentially driving unexpected impacts on adaptive evolution and diversification.

C06.5 | Evolution through simplification and loss

Morphological and molecular underpinnings of developmental truncation and loss of pectoral fins across life stages in the pipefish *Nerophis ophidion*

Ralf Schneider, Tarek Gerhard, Olivia Roth

Biology, Kiel University, Kiel, Germany

Throughout tetrapod lineages, limb-loss contributed to highly derived body plans, including that of the limb-less snake, or the whale and manatee - both only retaining their forelimbs. In actinopterygian fish, the shape and number of body appendages vary considerably, and fin-loss occurred in many lineages. Among paired fins, pelvic fins have been lost much more frequently than pectoral fins, likely as pectoral fins take a more active part in locomotion of most species. Syngnathids (pipefish and seahorses) all lack pelvic fins, but several of their lineages have lost additional fins. Here, we study the pipefish *Nerophis ophidion* whose planktonic larvae uses pectoral fins and a caudal fin fold for locomotion, while its adult stage features neither morphology and lives a demersal life. We show on a skeletal level how a heterochronic shift leads to developmentally truncated - but functional - pectoral fins in larvae that are lost later during metamorphosis. We explore underlying gene expression patterns using comparative transcriptomics and in situ hybridization, identifying pronounced changes in shh-signalling in the neotenic developing pectoral fin compared to normally developing fins. Given shh-signalling's central involvement in early differentiation in pectoral fins, we explored gene sequence divergence of genes involved in shh-signalling, but conclude that changed shh-signalling patterns are likely the result of regulatory evolution. Pectoral limb-loss in *Nerophis ophidion* may thus have evolved as a parallelism to fore-limb-loss in snakes, illustrating how also highly conserved and pleiotropic gene regulatory pathways can be tinkered with by evolution.

C06.6 | Evolution through simplification and loss

Developmental mechanisms underlying the evolution of visual system degeneration

Brooke Coonrod[1], Itzel Sifuentes-Romero[2], Robert Kozol[2], Stefan Choy[1], Erik Duboue[2], **Johanna Kowalko[1]**

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Brain development is a complex process shaped by genetics, sensory experience, and activity-dependent refinement of neural circuits. Given this complexity, how is development altered when the brain evolves in response to different environments? We investigate this question in the small freshwater fish, *Astyanax mexicanus*. *A. mexicanus* exists in two extant forms, a sighted surface form and a blind form that has adapted to life in caves. While the mechanisms underlying eye degeneration in cavefish of this species have been studied extensively, much less is known about the visual processing centers of the brain. We have investigated the developmental and genetic mechanisms that underlie changes in the development of the optic tectum, the primary visual processing center of the fish brain, in cavefish. Our work suggests that eye degeneration directly impacts the development of optic tectum. Moreover, we have investigated the developmental mechanisms by which the eye influences the development of the optic tectum. Together, this work provides insight into how loss of a sensory system influences the development of the brain.

7 | Evo-Devo of Complex Life Histories: Agametic Development, Regeneration and Clonality

Symposium S07: Friday 12 June, 09:00 – 10:40
Contributed Session C07: Friday 12 June, 11:05 – 12:30

Organisers: Lucas Leclère (Banyuls-sur-Mer, CNRS/Sorbonne Université, France), Stefano Tiozzo (Villefranche-sur-Mer, CNRS/Sorbonne Université, France, Chiara Sinigaglia (Banyuls-sur-Mer, CNRS/Sorbonne Université, France)

The majority of the studies in evolution, ecology, and development focus on unitary organisms that undergo sexual reproduction and embryonic development. However, organisms with complex life cycles, including asexually reproducing phases and potentiality for extensive repair, are fundamental components of global biodiversity, occurring in plants, fungi, and metazoans. Nearly all existing phyla contain multicellular species that can reproduce clonally by agametic propagation and thus can develop a fully functional body without forming a zygote. Some of these species produce populations of separate clonal "individuals", while many others form colonies of interconnected individuals or develop clusters of genetically identical modules, with either similar or phenotypically distinct body types. Often, this plasticity is accompanied by extensive repair capacities - with some organisms capable of regenerating their entire body in response to injury - and/or the potential for indefinite growth. The links between development, agametic reproduction, repair and growth remain unclear, but represents an active area of multidisciplinary research. The life histories of organisms have direct consequences on their distribution and potential for evolution. This symposium seeks to bring together scholars studying different model species, investigating a range of experimentally or theoretically distinct aspects of agametic development, whole-body regeneration, clonality, coloniality and their evolution.

S07.1 | Evo-Devo of Complex Life Histories: Agametic Development, Regeneration and Clonality

Probing clonal evolution in planarians

Jochen Rink

Tissue Dynamics and Regeneration, Max Planck Institute of Multidisciplinary Sciences, Goettingen, Germany

Planarian flatworms are renowned for their remarkable whole-body regeneration, which enables complete individuals to regenerate from tiny tissue fragments. This capacity also allows asexual reproduction by fission followed by regeneration as an alternative to sexual reproduction. Trade-offs between these reproductive modes have been proposed as important drivers shaping the evolutionary dynamics of regeneration in planarians. At the cellular level, planarian reproduction is closely linked to abundant pluripotent—and likely totipotent—adult stem cells known as neoblasts. Neoblasts are the only division-competent somatic cells and continuously renew all cell types and tissues. They not only rebuild missing structures during regeneration but also maintain and regenerate the germ line in sexually reproducing strains and species. Because individual neoblasts can contribute directly to the next generation—either by residing within a regenerating fission fragment or by differentiating into gametes during sexual reproduction—the boundary between soma and germ line is unusually blurred. The continuity of neoblast lineages across individuals and populations, together with the absence of a single-cell bottleneck enforcing somatic clonality during asexual reproduction, raises intriguing evolutionary questions. Do neoblast lineages compete for representation in the next generation? How genetically mosaic are asexually reproducing planarians? And how are multi-level selection processes—acting on cells, individuals, and populations—governed in such a dynamic system? In this talk, I will present our recent efforts to address these questions in the planarian model species and related taxa, exploring how cellular lineage dynamics shape intra- and inter-organismal population processes in organisms capable of perpetual regeneration.

S07.2 | Evo-Devo of Complex Life Histories: Agametic Development, Regeneration and Clonality

Complex lifecycles and polyploidy in a cnidarian model species

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Polyploidy, which can lead to stably-inherited whole genome duplications, is found across the tree of life and is thought to be a common mode of evolution and speciation in plants, but is much rarer in animals. Thus the pathways to whole genome duplication are much better understood in plants than animals. While polyploidy is common, ancient whole genome duplication events in plants and animals are relatively rare, suggesting that most polyploidy events are 'evolutionary dead ends'. In certain environmental conditions, polyploidy can be advantageous for the individual, but sexual reproduction, particularly reductional meiotic divisions, present a significant bottleneck for transmission of polyploidy to the next generation due to a greater risk of cytological defects. Here, we present data from the cnidarian model species *Clytia hemisphaerica*, an animal with a complex lifecycle (asexual colonial polyp stage, sexual jellyfish stage, and larval stage). We obtained neotriploid colonies from diploid parents as a result of knocking out key meiosis gene Spo11 (Munro et al. Science Advances. 2023). Spo11 KO resulted in loss of double stranded breaks, and thus loss of crossovers between homologous chromosomes, leading to non-reduced diploid eggs after a failed first meiotic division. Unexpectedly, these triploids are fertile, despite the challenges posed by meiosis. In *Clytia*, thanks to the complex lifecycle, we can analyse the cellular consequences of polyploidy through their lifecycle and through successive generations. We explore the hypothesis that a triploid polyp colony may provide a triploid bridge to tetraploidy, a pathway enabled by their multi-stage lifecycle.

S07.3 | Evo-Devo of Complex Life Histories: Agametic Development, Regeneration and Clonality

Using sponge whole-body regeneration to investigate early animal evolution

Roger Revilla-i-Domingo

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The evolution of animal multicellularity has been proposed to involve the repurposing of temporal cell-state transitions in unicellular ancestors into stable differentiation programs in stem-like cells of early animals. To test this and alternative hypotheses for the evolution of multicellularity, our group investigates the gene regulatory networks controlling stem cell differentiation in a basal metazoan, the sponge *Suberites domuncula*.

This research leverages the experimental accessibility of a whole-body regeneration system, which provides a powerful framework for dissecting the cellular and molecular mechanisms underlying stem cell differentiation. Using single-cell transcriptomics, chromatin accessibility and transgenic reporter assays, we infer and validate candidate regulatory interactions. In addition, collaboration with the Simakov laboratory has enabled the application of chromosome conformation capture approaches (Micro-C) to characterize the three-dimensional organization of the sponge genome.

Contrary to earlier reports, our findings provide no evidence for distal enhancer-driven gene regulation in the sponge. On the other hand, our work reveals deeply conserved regulatory functions of the transcription factor Myc in animal stem cells.

To assess whether these conserved regulatory interactions could have contributed to the transition from unicellular to multicellular life, we are reconstructing gene regulatory networks underlying temporal cell-state transitions in unicellular relatives of Metazoa. This effort has benefitted from a collaboration with the lab of Iñaki Ruiz-Trillo and Elena Casacuberta, which has provided us with access to key unicellular model organisms.

We propose that some conserved functions of Myc may represent animal-specific innovations that contributed to the emergence of stem cell systems during early animal evolution.

S07.4 | Evo-Devo of Complex Life Histories: Agametic Development, Regeneration and Clonality

Conserved neurogenic functions of Notch pathway during regeneration and growth in the annelid *Platynereis*

Loïc Bideau, Pierre Kerner, **Eve Gazave**

CNRS, Universite Paris Cite, Institut Jacques Monod, Paris, France

Notch signaling is an evolutionarily conserved pathway known to orchestrate neurogenesis by regulating the transition from progenitors to neurons and glia, and by directing neurite outgrowth and axon guidance in many species. Although extensively studied in vertebrates and ecdysozoans, the role of Notch in spiralians remains unexplored, limiting our understanding of its conserved functions across bilaterians. In a recent study, our group focused on the segmented annelid *Platynereis dumerilii*, a model organism in regeneration and neurobiology, to investigate Notch signaling functions during post-embryonic developmental processes. We showed that Notch pathway components are expressed in neurogenic territories during both posterior elongation and regeneration, two post-embryonic processes requiring sustained neurogenesis. Through chemical inhibitions of the pathway and RNA-seq profiling, we find that Notch signaling regulates neural progenitor specification, differentiation, and overall neurogenic balance in the regenerating and elongating posterior part. Disruption of Notch signaling activity leads to severe defects in pygidial and central nervous system organization, including abnormal axon guidance and impaired neurite outgrowth. Altogether, our results support the hypothesis that Notch has multifaceted conserved functions in neurogenesis across bilaterians, shedding light on the ancestral functions of this critical pathway during post-embryonic processes.

C07.1 | Evo-Devo of Complex Life Histories: Agametic Development, Regeneration and Clonality

Large-scale reprograming of differentiated larval tissues establishes adult body plan during sea cucumber metamorphosis

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Most of our understanding of animal body plan formation come from the study of a handful of selected model organisms. These model species are predominantly direct developers, in which the adult body plan arises directly from embryogenesis. However, this developmental strategy is not broadly representative of animal diversity. In many taxa – and particularly in marine invertebrates – the formation of the adult body plan is postponed by one or several larval stages, a strategy known as indirect development. How an adult body plan arises through the transformation of a pre-existing larva, rather than as a direct outcome of embryogenesis, remains poorly understood, biasing our view of animal body plan evolution. To address this gap, we have started investigating the molecular and cellular mechanisms underlying adult body plan formation in the indirect-developing sea cucumber *Apostichopus parvimensis*. Larval and juvenile stages in this species are completely transparent, making it amenable for live imaging through metamorphosis. Using cell tracking, fluorescent in situ hybridization and single-cell RNA sequencing, we show that metamorphosis in *A. parvimensis* involves extensive recycling of larval tissues into adult structures. In particular, portions of the larval ciliary band give rise to the adult central nervous system. These findings challenge the existing dogma of metamorphosis, in which most larval tissues are presumably simply discarded. Instead, it suggests that cellular reprogramming might be an important – but underappreciated mechanism when building an adult body plan from a larval stage. Our approach also demonstrates how harnessing animal biodiversity with new modern molecular tools and outside of traditional model species can reveal new features of animal development and evolution.

C07.2 | Evo-Devo of Complex Life Histories: Agametic Development, Regeneration and Clonality

Multi-stage single-cell atlas of agametic reproduction in the colonial ascidian *Botryllus schlosseri* unveils transcriptomic reprogramming and possible dedifferentiation of bud founder cells

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Whole body regeneration and agametic reproduction rely on somatic cells to produce new individuals. In many metazoans species, the cell sources are still being debated, and while some have been shown to rely strictly on adult pluripotent stem cells, others appear to use lineage-restricted stem cells, dedifferentiation and/or transdifferentiation. In the colonial ascidian *Botryllus schlosseri,* new individuals (zooids) are constitutively being formed through agametic reproduction, or "budding". The bud emerges as a fold of the peribranchial epithelium and overlaying epidermis, but the nature of the cellular and molecular mechanisms underpinning bud initiation remain elusive. Two hypotheses have been proposed for primary cell sources: circulating stem cells giving rise to all tissues, or commited cells from the peribranchial epithelium, implying local reprogrammation.

To address this question, we generated a multi-stage single-cell transcriptomic atlas of budding, capturing initiation and development of new zooids.This atlas contains all known major cell types and a branching topology pointing to their developmental trajectories. We identified the bud founders as a subpopulation of the peribranchial epithelium cells, which appear to gain developmental potential through possible dedifferentiation, while no early contribution of mesenchymal cells to the early bud could be inferred. The bud founders specifically express a *Sumo2* post-translational modifier, a *Stip1-like* chaperone co-factor as well as a *Mbnl1-like* splicing regulator, all known to be involved in the regulation of stemness. This atlas provides a foundational resource to understand the cellular origin and mechanisms underlying agametic development in a chordate, and opens the door for comparison with other species.

C07.3 | Evo-Devo of Complex Life Histories: Agametic Development, Regeneration and Clonality

Exploring zooid polymorphism in siphonophores (Cnidaria:Hydrozoa) at single cell resolution

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Siphonophores (Cnidaria:Hydrozoa) are pelagic, gelatinous animals characterized by their coloniality and functionally specialized bodies (zooids). They have the largest diversity of polymorphic zooids, including those for feeding, swimming, defense, and reproduction. There are 190 described siphonophore species, with multiple species displaying unique colonial arrangements, and some with novel zooids. To understand how zooids arise and become differentiated for tasks, we sequenced single-cell RNA from multiple siphonophore species, including *Nanomia septata, Physalia physalis, Agalma elegans* and *Agalma okenii*. We identified multiple cell types such as cnidocytes, neurons, muscles and gland cells that form distinct clusters. To validate and explore marker genes belonging to these cell clusters, we used hybridization chain reactions (HCR) to look at their spatial expression in *Nanomia septata*. We also explored the expression of zooid gene markers that were found to be differentially expressed in specific zooid types over others. We found that these gene markers are scattered across multiple cell types, with no clear zooid-specific cluster for each zooid type. In contrast, we found a clear cluster marking the pneumatophore, a novel structure made up of chitin and multiple cell types, such as epithelial and gland cells. These results show that zooids arise through a reorganization and combination of multiple cell types to form distinct bodies.

C07.4 | Evo-Devo of Complex Life Histories: Agametic Development, Regeneration and Clonality

Food availability impacts developmental trajectory in the asexually reproducing flatworms

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Microscopic catenulid flatworms reproduce primarily asexually through paratomy. In this process, new head structures are inserted in the trunk of the fully developed worm, followed by the transverse fission that separates the offspring. In some species, however, the regular paratomy might be altered – the new individuals (zooids) are produced, but do not separate. This leads to the development of so-called chains – linear conglomerates of multiple zooids that remain connected tail-to-head. While this phenomenon has been known for over a century, the cellular and molecular underpinnings of alternation between asexual reproduction and chain formation remain unknown. Here, we tested whether chain formation can be triggered by different feeding regimes in the common catenulid genus *Stenostomum*. By manipulating the concentration of the prey ciliate *Paramecium bursaria*, we were able to stimulate chain formation in four species of Stenostomum. Using in vivo measurements, staining of mitotically active cells, and transcriptomic analyses, we show that increased food availability accelerates cell division and longitudinal growth, but it does not affect head morphogenesis. Effectively, in the over-fed animals, the longitudinal growth rate exceeds the head morphogenesis rate, enabling the initiation of new head formation before the first round of paratomy concludes. Altogether, our results suggest that asexual reproduction and chain formation do not represent two independent developmental modalities, but rather two solutions of the same developmental program that depends on the environmental input.

C07.5 | Evo-Devo of Complex Life Histories: Agametic Development, Regeneration and Clonality

Single-cell analysis of the hemichordate head blastema provides insights into the early deuterostome origins of regeneration

Ivan Candido-Ferreira[1,2], Eric Ross[2], Albert Martí i Sabarí[1], Paul Bump[1], Blair Benham-Pyle[2], Chris Lowe[1], Alejandro Alejandro Sánchez Alvarado[2]

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The hemichordate *Ptychodera flava* is the closest known relative of vertebrates capable of fully regenerating their heads. These animals are at a key phylogenetic position for understanding the origins and evolution of the vertebrate body plan. In addition to displaying high conservation of ectodermal patterning along the anteroposterior axis, hemichordates also possess three nervous system signaling centers homologous to the vertebrate anterior neural ridge, zona limitans intrathalamica, and isthmic organizer. Such exquisite conservation of brain patterning programs deep in the evolution of the deuterostome lineage illustrate the importance of hemichordates in testing hypotheses of vertebrate evolution. Here, we have conducted an unbiased profiling of the transcriptional and cellular regulators of head regeneration in *P. flava* at single-cell resolution. Pseudotime trajectory and RNA velocity analyses suggest an heterogenous population of highly proliferative Myc-expressing cells as the blastema cell source. Remarkably, these cells display autonomous regulation thyroid hormone (TH) metabolism in response to injury, suggesting a central role for TH to the formation of the head blastema. This systems biology approach will provide a genome-wide characterization of adult nervous system regeneration across multiple scales, from tissues to molecules, and will reveal how multiple regulatory layers interact to shape regenerative outcomes.

C07.6 | Evo-Devo of Complex Life Histories: Agametic Development, Regeneration and Clonality

The sea anemone *Nematostella vectensis* in laboratory vs. natural-like growth environments – molecular, cellular, developmental and life cycle effects

Uri Gat[1], Itamar Kozlovski[2], Adva Mizrahi[1], Yehu Moran[2]

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The sea anemone *Nematostella vectensis* (*Nve*) is a basal cnidarian model organism for developmental and evolutionary studies, a close sister clade to bilaterian animals. In laboratory settings, *Nve* is cultured in glass or plastic containers, in contrast with its natural habitat, where adult polyps are embedded in sediment. Animals in their natural state are significantly smaller and reproduce asexually at a much faster rate. This raises questions about the molecular and genetic basis of these differences and the traits that distinguish animals between the two environments.

To address these questions, we established long-term *Nve* cultures on various types of sediment, including several types of sand and mud sourced from the native *Nve* habitat. These natural-like conditions allow the animals to display native like phenotypes, enabling us to investigate the influence of the matrix on phenotypic traits and monitor changes during transitions between the two states.

To gain an unbiased and comprehensive understanding of the differences between these cultures, we conducted an RNA-Seq experiment, comparing *Nve* cultured on glass in the regular laboratory settings and several types of sediments. Over-representation analysis of significantly differentially expressed gene categories revealed interesting major differences between artificial condition and all sediment types.

Here, I will shortly portray the findings regarding the main differences between the culture conditions and their possible relevance to the phenotypes observed. Our studies revealed changes across all biological levels from the general animal morphology to cellular and developmental biology pathways, leading to insights into the basic nature of *Nve's* structure, function, behavior and life cycle.

8 | Integrative Perspectives on Craniofacial Evo-Devo

Symposium S08: Wednesday 10 June, 09:00 – 10:40
Contributed Session C08: Wednesday 10 June, 11:05 – 12:30

Organisers: Hiroki Higashiyama (The Graduate University for Advanced Studies SOKENDAI, Japan, Robert Cerny (Charles University in Prague, Czech Republic)

How did complex forms such as our own bodies come to be? This is one of the most fundamental questions in evolutionary developmental biology. Yet, the long-standing challenge of understanding how diverse cell populations coordinate to construct complex anatomical structures—and how these developmental processes themselves have evolved—still stands before us as a major frontier. In this symposium, we focus on one of the most complex regions among vertebrates—the rostral end of the head, the craniofacial region—and present the latest advances in its morphological evolution from various perspectives, ranging from palaeontology to developmental genetics. The program includes topics such as the evolutionary transformation of facial morphogenetic patterns that gave rise to mammals, the unexpected contribution of the endoderm to the craniofacial region that overturns traditional views, new palaeontological insights into the skulls of early vertebrates, and the genomic/transcriptomic/cellular mechanisms driving macroevolutionary transitions in the brain and skull. We hope this symposium will foster new synergy in understanding complex morphology through the integrated perspectives of evolution and development.

S08.1 | Integrative Perspectives on Craniofacial Evo-Devo

Evolutionary transition of the vertebrate pituitary from a pharyngeal gland revealed in embryos of basal fishes

Agata Horckova[1], Zuzana Klacikova[1], Jesus Emmanuel Jimenez-Jimenez[1], Ann Huysseune[1,2], Peter Fabian[3], Martin Minarik[1], Lenin Arias-Rodriguez[4], Robert Cerny[1]

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The pituitary is a master endocrine gland in the vertebrate head with long-standing controversies on its embryonic origin and evolution. The front lobe of the pituitary develops from the rostral placode as the vertebrate specific acquisition of our New Head. However, classic theories proposed the evolution of pituitary from an ancient pre-oral pharyngeal exocrine gland, as known from amphioxus. This radical developmental transformation from endodermal to ectodermal, and from exocrine to endocrine gland still presents a major challenge for our understanding of the vertebrate head evolution. Here, we show that in embryos of basal fishes (bichirs, sturgeons, and gars) early pituitary formation is intimately linked with pre-oral pharyngeal glands. In these embryos, pituitary migrates on the surface of the pre-oral gut endoderm and temporarily merges with it forming one ecto-endodermal domain. In all three fishes the endodermal component of this domain gives rise to ephemeral exocrine glands (cement or hatching gland). Moreover, pituitary in bichir embryos forms a temporal palatal exocrine gland, and in gar embryos we identified a new exocrine gland of mixed pituitary-pharyngeal origin. Finally, the shared gene expression between pituitary and ephemeral glands of basal fishes supports the evolutionary scenario where both of them evolved from a single gland of pre-vertebrates. Basal fish embryos thus reveal the pharyngeal counterpart of the pituitary that have presumably disappeared in the majority of modern vertebrates.

S08.2 | Integrative Perspectives on Craniofacial Evo-Devo

Building vertebrate facial shape diversity from a conserved embryonic framework

Marketa Kaucka, Louk Seton, Amor Damatac II, Andrea P Murillo-Rincon, Stella Kyomen, Elio Escamilla Vega

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Vertebrates display extraordinary morphological diversity, much of which is concentrated in the face, a structure tightly linked to ecological adaptation. Despite its evolutionary significance, uncovering the mechanisms that generate facial diversity has remained challenging. Craniofacial development is a multi-stage process marked by increasing cellular and structural complexity, making it difficult to pinpoint when, where, and how divergence emerges. At the same time, craniofacial development utilizes conserved cellular sources and developmental signaling, raising the question of how these systems are calibrated to generate variability. A key framework for understanding developmental evolution is the hourglass model of embryogenesis. During mid-development, known as the phylotypic period, vertebrate embryos transiently converge on conserved gene expression programs. The "waist" of the hourglass represents the most constrained phase of development and a window most resistant to evolutionary change. Therefore, much of the phenotypic diversity observed across vertebrates arises after this phase. We will revisit this pattern and show that it emerges as a sum of properties of individual cellular lineages. Further, we set out to explore the developmental origins of craniofacial divergence by dissecting the genomic, transcriptomic, and cellular mechanisms that operate during and after the phylotypic period. By integrating comparative omics with cellular-resolution analyses across species, we aim to determine when developmental divergence first becomes detectable, what the cellular sources are, and how conserved gene expression programs are subsequently altered to generate species-specific facial morphologies. These findings provide insight into the fundamental principles governing morphological evolution and reveal the origins of vertebrate diversity.

S08.3 | Integrative Perspectives on Craniofacial Evo-Devo

The Pteraspid Paradox and the evolution of the vertebrate cranial dermal skeleton

Joseph Keating, Philip Donoghue

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The evolution of a mineralised cranial dermal skeleton transformed the development of the vertebrate head, imposing fundamental constraints on morphogenesis. In living bony vertebrates (Osteichthyes), these constraints are overcome by dynamic skeletal remodelling, in which bone is resorbed and redeposited to permit continuous, allometric growth. However, the origin of this capacity, and how early skeletonising vertebrates grew in its absence, remains unclear. We investigate skeletal growth in pteraspid heterostracans, Silurian–Devonian jawless stem-gnathostomes that represent some of the earliest vertebrates with an extensively mineralised dermal skeleton. Their cranial dermal plates form size series consistent with ontogeny and display concentric surface invaginations interpreted as arrested growth lines. Yet their internal histology lacks evidence of incremental mineralisation, creating a long-standing paradox. Comparative histology of specimens spanning multiple growth stages from a single population supports a new model of dermal skeletogenesis. Mineralisation begins with a superficial sheet of dentine tubercles, followed by apposition of underlying trabeculae. Lateral expansion proceeds by marginal accretion of the superficial layer, with delayed mineralisation of the middle layer. The basal layer mineralises last, allowing discrete plates to fuse during ontogeny. Plate growth thus occurred through a coordinated appositional process rather than remodelling. Ancestral state estimation indicates that dynamic remodelling evolved stepwise along the gnathostome-stem: extensive resorption predates the origin of jaws, whereas secondary osteons are restricted to jawed vertebrates. These results identify appositional growth as the primitive mode of craniofacial skeletogenesis and suggest that the diverse dermal plate organisations of fossil jawless vertebrates represent alternative developmental solutions to growth in the absence of remodelling.

S08.4 | Integrative Perspectives on Craniofacial Evo-Devo

Fundamental Reorganization of the Amniote Facial Developmental Framework in Mammalian Evolution

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How evolutionary novelties arise is a central question in evolutionary developmental biology. The mammalian face, characterised by an independent nose and thick, muscular lips bearing vibrissae, represents one of the most striking examples of novelty within the complex craniofacial region, yet the developmental basis for its emergence remains uncertain. To address this problem, we compared the three-dimensional morphology of tetrapod embryos, including mouse (*Mus musculus*), echidna (*Tachyglossus aculeatus*), gecko (*Paroedura picta*), chicken (*Gallus gallus*), and frog (*Rana japonica*), using histological reconstructions and synchrotron radiation X-ray micro-computed tomography. Our results demonstrate a profound reorganisation of embryonic facial prominences in mammals. In sauropsids and amphibians, the medial nasal prominence forms the teeth-bearing rostral tip of the upper jaw. Sauropsids with apparently centralised but not morpho-functionally independent noses, such as the alligator (*Alligator mississippiensis*) and soft-shelled turtle (*Pelodiscus sinensis*), also follow the typical sauropsid developmental pattern. In contrast, in mammals the medial nasal prominence is redeployed to form the nose, while the maxillary prominence expands to constitute the entire upper jaw. These findings indicate that mammalian facial evolution does not represent a mere modification within the ancestral amniote developmental framework; rather, the topographical relationships among conserved facial prominences were fundamentally altered in the synapsid lineage. Our analyses also point to a mammalian-specific developmental trajectory of the facial prominences during the late pharyngula stage, indicating that mammalian evolutionary novelty should have originated earlier in development than the morphogenetic processes that generate facial diversity in other amniotes.

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Origin of the ground-borne sound perception in snakes

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Vertebrates have colonized diverse ecological niches through multiple-time gain-and-loss of traits. In snakes, this was achieved by the iconic modification of existing sensory and locomotory organs, through complete loss of the limbs and tympanic membrane. The loss of the tympanic membrane and outer ear canal have led to the subsequent change of the sound signal from air to ground-borne sound and of the sound receiver from ear to mandible. In snakes, ground-borne sound signals are propagated into the central nervous system via the articulation between the mandible and quadrate, supratemporal, and prootic bones. Due to the paucity of fossil records that show intermediate state between tympanate and non-tympanate snakes and relevant previous studies, origin of the transition into the ground-borne sound perception has been little resolved. Here we integrate comparative embryology, geometric morphometrics, and finite element analysis for the simulation of sound propagation with broadly collected 70 extinct and extant reptilian species. By examining the ossification pattern and phylomorphospace of the jaw-ear articulation, we revealed that the distinct configuration of the jaw-ear articulation in snakes is shaped by an enlarged, rostrally repositioned prootic bone, acquired through an accelerated (heterochronic) and rostrally shifted (heterotopic) ossification center relative to other reptiles. We suggest that the ground-borne sound perception of snakes has been driven by the spatio-temporal change of the inner ear development and its origin can be traced back to end-Cretaceous stem-snakes. These findings show how heterochrony and heterotopy can result in the functional innovation through targeted remodeling of a single cranial element.

Prenatal cranial allometry reveals developmental adaptations to laryngeal echolocation in bats

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Bats (Order Chiroptera) represent approximately a fifth of modern mammalian species diversity. The ability of laryngeal echolocation, which characterises these flying mammals, has impacted cranial diversity and evolution in the group. Growth of the cranium in bats is poorly understood, especially in the prenatal period when most bony elements differentiate, shaping adult morphological diversity. In this study, ontogenetic trajectories of cranial elements from foetal to adult stages of the three major bat lineages (i.e., pteropodids, rhinolophoids, and yangochiropterans) were analysed and compared to those from other boreoeutherian mammals to observe whether allometric changes in prenatal growth occurred across bat phylogeny. We present novel data on an unparalleled sample of 350 crania of foetuses and neonates from 17 bat and 11 non-bat boreoeutherian mammal species. These wet specimens were 3D scanned, and all cranial elements were individually segmented. Volumetric data were extracted to quantify cranial growth rate. Principal component analysis and ancestral state of cranial bone growth were used to reconstruct developmental patterns and evolution of bone growth within bat phylogeny. Our results show yangochiropterans experience slower bone growth compared to pteropodids and rhinolophoids. Different developmental patterns to shape the skull are observed in yangochiropterans compared to other bats, resulting in alternative approaches to emit echolocation calls and providing additional evidence of the convergent evolution of laryngeal echolocation between yangochiropterans and rhinolophoids.

Sensory-skeletal integration and the origin of asymmetric facial bone fusions in blind cave-dwelling fish

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During development, diverse tissues must be properly integrated to generate a functional organ system. The mechanosensory lateral line system necessitates the integration of sense organs (neuromasts) with bone and neural tissues. The mechanisms that govern this integration remain largely unknown. Evidence of this integration, however, has been noted for over a century. Classical researchers suspected the positions of certain neuromasts co-localize to primary ossification centers. These centers are associated with deeply conserved facial bones across vertebrates, including the lacrimal, jugal and sphenoid bones. In this study, we examined the timing and signaling mechanisms that underlie this colocalization. Our prior work revealed that facial bones commonly fuse in cave morphs of the Mexican tetra, but never fuse in surface-dwelling morphs. We tracked the position of neuromasts until the first appearance of bone and discovered that fusion events are predicted by the positioning of canal neuromasts (and associated ossification centers) in cave morphs. Longitudinal analyses revealed that bone morphogenetic protein is expressed at the precise positions of neuromasts just prior to appearance of bone. Although chemical and mechanical ablation of neuromasts had little impact, lesioning the afferent nerve fibers that innervate neuromasts led to impaired bone formation. This work provides novel insight to the nature of sensory-skeletal integration, which is governed in part by nerve fibers. Comparing conspecific cave- and surface-dwelling morphs provides insight to the evolutionary lability of this sensory-skeletal integration, and the longstanding mystery of cranial asymmetry in cave-dwelling fish. US National Science Foundation Award IOS-2205928.

Genetic integration of craniofacial shape and eye size in Lake Malawi cichlids

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Studying the molecular basis of morphological diversification is essential to improve our understanding of evolution. To explore this, we focused on the Great African Lakes cichlid radiation, which produced thousands of species of diverse forms and ecologies - including a remarkable range of craniofacial morphologies. We examined the basis of craniofacial variation in two divergent Malawi cichlids, a generalist omnivore (*Astatotilapia calliptera)* and a benthic carnivore (*Aulonocara stuartgranti*), plus a hybrid cross of the two species.

We identified quantitative differences in morphology between the species via geometric morphometrics, including larger eye diameter and a convex cranial profile in *A. stuartgranti*. F2 hybrids displayed intermediate phenotypes, with principal components of variation mirroring interspecific differences. Interestingly, eye diameter and profile shape were significantly correlated, displaying morphological integration.

Similarly, embryos presented significant quantitative differences in craniofacial structure between species since early cartilage deposition, which reflected adult variation. Developmental trajectories suggest heterochrony may contribute to these differences.

QTL mapping in hybrids produced multiple significant associations between craniofacial features and genomic regions. Remarkably, eye size and profile shape mapped to overlapping regions, revealing a genetic basis for their morphological correlation. Such integration between craniofacial features may have facilitated the rapid adaptation of several cichlids to benthic environments. Additionally, we are examining a single-cell RNA-seq dataset spanning three developmental stages in both species. We aim to explore expression of candidate loci in cell populations involved in cranial ontogeny, like the neural crest.

Overall, our results suggest polygenic effects, heterochrony and genetic integration between traits underlie craniofacial divergence in closely related cichlids.

Synchrotron microtomography reveals unusual vascular cartilage in an Ordovician vertebrate

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The origins of vertebrate mineralised tissues lie in the early Palaeozoic, with the first skeletonising fishes being found in the Ordovician. The anatomy of these early armoured vertebrates remains obscure beyond their outer skeleton of dermal scales and plates, leaving the structure and tissue-makeup of their endoskeletons largely unknown. The sole known exception is a specimen of *Eriptychius americanus*, an armoured vertebrate from the Sandbian (458.4–453.0 Ma) Harding Sandstone of Colorado, USA, which preserves an articulated head incorporating dermal and endoskeletal structures. The tissue forming the endoskeletal skull in *Eriptychius* is poorly understood but is assumed to be cartilage, despite the fact that it is pervaded by a dense network of branching tubules. We used synchrotron microtomography to image the cranial endoskeleton of Eriptychius at high resolution. These new data reveal the tissue comprising the endoskeletal mineralisations of Eriptychius to be a form of globular calcified cartilage, comprising globules surrounding chondrocyte spaces. Structurally, however, it is unlike other globular calcified cartilages, with the tissue becoming more densely mineralised in proximity to the edges of the cartilage elements and where the cartilage tissue is pervaded by the extensive tubular network. In this sense this early cartilage contrasts with cartilage in living vertebrates which is typically avascular, although bears comparison with pervasive vascularisation of cartilage during long bone development. This suggests that early in their evolution vertebrates explored a greater diversity of tissue structures than has survived to the present day.

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Elemental and Molecular Mapping Reveal Cartilage Cell Organization and Differentiation During Development

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The emergence of cellular cartilage embedded within a dense extracellular matrix was a key evolutionary event that provided structural rigidity and enabled extensive variation in the shape and geometry of the vertebrate head.

Cartilage is a skeletal tissue exhibiting a complex set of differentiation, maturation, and cellular arrangement processes and often serves as a transitional tissue that precedes bone formation. Cartilages pattern and support the pharyngeal arches of the vertebrate head, where chondrocytes display a zonal arrangement that depends on the size of the skeletal elements and proximity to the articulating surfaces of the skeletal joints. While chondrocytes differentiate from mesenchyme and progress through proliferative, prehypertrophic, and hypertrophic cell states, articular chondrocytes derived from the joint interzone do not undergo hypertrophy and, early during embryonic development, suppress the chondrogenic program.

We aimed to characterize the co-localization of molecular markers and conduct elemental mapping of skeletal cells in the craniofacial region of zebrafish larvae. We performed cell marker co-localization analysis by flow cytometry, single-cell RNA sequencing, and chemical element analysis by synchrotron X-ray fluorescence microscopy in wild-type zebrafish larvae and gene mutants generated with CRISPR/Cas9 genome editing. Our results demonstrate different marker localization and chemical element distribution in the cartilage and joint interzone cells at various differentiation stages. These findings provide new insight into how cartilage and joint interzone cell states can be distinguished during development and offer a framework for comparing skeletal tissues during the evolution of vertebrates.

Authors thank Beata Filipek-Górniok at DanioReadout facility for generating knock-in and knock-out lines.

9 | Nutritional regulation of developmental processes

Symposium S09: Thursday 11 June, 13:30 – 15:10

Organisers: Eudald Pascual-Carreras (Institute of Evolutionary Biology (CISC - Universitat Pompeu Fabra), Barcelona, Spain / EMBL Barcelona, Barcelona, Spain), Kathrin Garschall (University of Bergen, Michael Sars Centre, Bergen, Norway)

Metabolism provides energy and biomaterials for growth, repair, and shapes how organisms interact with their environment. Increasingly, metabolic signals, that reflect the energy and nutritional state of cells and organisms, are recognized as key regulators of cell cycle progression, quiescence and differentiation. Despite the far-reaching implications for developmental processes, most of our current understanding comes from cell culture systems, which fail to reflect the metabolic complexity of whole organisms and the evolutionary context. This symposium brings together researchers who study how metabolism shapes aspects of organismal development across diverse branches of the tree of life, to explore metabolic diversity and its evolution. Topics include the role of metabolism during planarian regeneration and organismal growth in the cnidarian Nematostella, transgenerational metabolic reprogramming in Drosophila, and a mammalian “stem cell zoo” perspective on the metabolic regulation of developmental rates. Together, these talks offer a comparative and integrative perspective on metabolism as a driver of developmental evolution. We aim to foster dialogue across disciplines and inspire new approaches in evo-devo research.

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Metabolic control of planarian regeneration

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Planarians, particularly *Schmidtea mediterranea*, are powerful models for studying regeneration due to their remarkable ability to rebuild complete body structures, including the brain, from small tissue fragments. This regenerative capacity depends on neoblasts, a population of adult stem cells capable of giving rise to all differentiated cell types. Importantly, planarians retain strong regenerative potential even under prolonged starvation, highlighting a close relationship between metabolic state and stem cell function.

We are exploring the contribution of major metabolic pathways, including glycolysis, oxidative phosphorylation (OXPHOS), and lipid metabolism, to the regulation of regeneration. While carbohydrate-based metabolism has been extensively studied in stem cell systems, the role of lipids in regenerative contexts remains less understood. Preliminary findings suggest that lipid metabolic changes may occur early after injury and could contribute to the regulation of regenerative processes. These observations point toward a potential involvement of lipid metabolism in supporting neoblast activity and successful tissue remodeling.

Overall, this work seeks to define how shifts between glycolytic, oxidative, and lipid-dependent metabolism influence neoblast behavior and whole-body regeneration. By dissecting the metabolic framework that underpins tissue renewal, we aim to provide new insights into how energy utilization and metabolic state shape stem cell-driven regeneration.

A stem cell zoo uncovers intracellular scaling of developmental tempo across mammals

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Differential speeds in biochemical reactions have been proposed to be responsible for the differences in developmental tempo between mice and humans. However, the underlying mechanism controlling the species-specific kinetics remains to be determined. Using in vitro differentiation of pluripotent stem cells, we recapitulated the segmentation clocks of diverse mammalian species varying in body weight and taxa: marmoset, rabbit, cattle, and rhinoceros. Together with mouse and human, the segmentation clock periods of the six species did not scale with the animal body weight, but with the embryogenesis length. The biochemical kinetics of the core clock gene HES7 displayed clear scaling with the species-specific segmentation clock period. However, global cellular metabolic rates did not show an evident correlation. Instead, individual metabolic activities showed a selective effect on segmentation clock processes. This indicates that the scaled kinetics of the segmentation clock across species may result from combined selective metabolic modulators shaped by evolutionary constraints. Altogether, our stem cell zoo uncovered general scaling laws governing species-specific developmental tempo.

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Maternal diet influences embryonic developmental trajectory and offspring phenotype via non-genetic factors in *Drosophila melanogaster*

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Parental nutrition influences offspring development, consistent with the Developmental Origins of Health and Disease (DOHaD) hypothesis, which proposes that maternal conditions shape long-term health outcomes. In *Drosophila melanogaster*, where embryogenesis proceeds independently after egg laying, such effects suggest that environmental information is pre-encoded within the oocyte.

To investigate this, we exposed female flies to low-, medium-, or high-sugar diets for 10 days before mating with males maintained on a control diet. We measured body weight, lifespan, fecundity, and triglyceride (TAG) levels in mothers and their F1 offspring. A low-sugar diet reduced maternal lifespan, while a high-sugar diet increased maternal TAG levels. Notably, female F1 offspring from both low- and high-sugar mothers exhibited increased starvation resistance compared to medium-sugar controls.

To uncover underlying mechanisms, we performed single-embryo RNA sequencing and metabolomics using approaches developed in our laboratory. The kynurenine (Kyn) pathway emerged as a recurrent candidate. We are testing its functional role through dietary supplementation and pharmacological inhibition. Our results reveal diet-dependent differences in embryonic metabolite deposition, accompanied by altered stress resistance in adult offspring. Ongoing single-cell transcriptomic analyses aim to further dissect the molecular basis of these effects.

Together, these findings demonstrate that maternal nutrition induces lasting metabolic and phenotypic changes in offspring, supporting DOHaD principles even in oviparous species such as *Drosophila*.

Nutritional control of body plasticity across biological scales in a sea anemone

Patrick Steinmetz

Michael Sars Centre, University of Bergen, Bergen, Norway

Many animals adjust growth and body size in response to fluctuating nutrient availability. Despite its ecological importance, the cellular basis and evolutionary origins of environmentally controlled body plasticity remain poorly understood. The sea anemone Nematostella vectensis, which inhabits coastal environments with extreme fluctuations, provides a powerful system to study how nutritional inputs are translated into coordinated responses across biological scales.

At the organismal level, feeding promotes growth through increased cell proliferation, whereas starvation induces rapid body shrinkage accompanied by extensive cell loss. At the cellular level, I will show how nutrient availability modulates cell cycle dynamics and TOR signalling, with a particular focus on a population of Vasa-/Piwi-expressing stem-like cells. Prolonged starvation drives these cells into a deep quiescent state, resulting in delayed cell cycle re-entry upon refeeding. At the tissue level, I will show that starvation-induced cell loss involves epithelial cell extrusion in the epidermis, a process that shares striking morphological and molecular similarities with bilaterians. Using quantitative image analysis and perturbation experiments, we find that apoptosis is necessary but not sufficient for extrusion, and that ERK1/2 signalling constrains extrusion density. Extrusion rates increase during starvation and parallel overall cell loss. The presence of cell debris and activated TOR signalling in the vicinity of extruded cells suggests a potential contribution to nutrient recycling.

Together, these findings establish a mechanistic link between environmental inputs, stem-like cell dynamics, and epithelial remodelling, and suggest that key features of these responses are evolutionarily ancient.

10 | Skin Deep: Common Threads in the Evolution of Bilaterian Ectodermal Appendages

Symposium S10: Friday 12 June, 09:00 – 10:40
Contributed Session C10: Friday 12 June, 11:05 – 12:30

Organisers: Dr Carlos Rivera Rivera (University of Manchester, UK), Dr Joseph Keating (University of Bristol, UK)

The ectoderm is a dynamic germ layer that uniquely interfaces with the external environment. In vertebrates, it gives rise to a remarkable diversity of evolutionarily significant appendages, including teeth, denticles, feathers, and hair. Across invertebrate phyla, ectodermal sensory structures such as cuticular papillae and setae further illustrate ectodermal innovation. Cutting-edge advances in developmental genetics, computed tomography, and comparative phylogenetics are transforming our understanding of how these structures originate and diversify. This symposium brings together researchers harnessing these methods from across disciplines to foster integrative discussions on ectodermal appendage evolution. Speakers will include developmental biologists, palaeontologists, and comparative morphologists working across vertebrate and invertebrate systems. By synthesizing these perspectives, we will explore how developmental innovations have fueled the evolution of ectodermal appendages across animals and what this reveals about the interplay between development, form, and function.

Sensory origins of vertebrate teeth and the evolutionary links between odontodes and ectodermal sensory structures

Yara Haridy

Organismal Biology and Anatomy, University of Chicago, Chicago, USA

The origin of vertebrate teeth and their tissues is tied to the much earlier emergence of odontodes. These dentine-based mineralized ectodermal appendages first appeared in the exoskeleton of early vertebrates. However, the timing, function, and evolutionary drivers of the earliest odontodes remain unresolved. To assess the first instance of dentine in the fossil record, we combine high-resolution synchrotron tomography with comparative histology across fossil and extant taxa. Cambrian fossils previously interpreted as the first odontodes (Anatolepis) are revealed to possess microstructures diagnostic of arthropod sensory sensilla rather than vertebrate dentine. This reinterpretation removes the only Cambrian occurrence of vertebrate odontodes and shifts the origin of dentine-bearing tissues to the Middle Ordovician. Analysis of early vertebrate dermal odontodes reveals anatomical features consistent with sensory function, including large dentine tubules and open pulp cavities. To test whether sensory integration persists in living systems, we examined external odontodes in extant fishes using tissue clearing and immunofluorescence. These analyses demonstrate extensive innervation associated with dermal denticles, indicating that sensory integration is a conserved property of odontode tissues. Together, these results support a model in which dentine evolved as part of a sensory exoskeleton rather than as a purely protective or feeding adaptation. In this framework, vertebrate teeth represent a later redeployment of an ancestral sensory ectodermal appendage system, linking odontodes, dermal denticles, and teeth within a shared evolutionary and developmental context.

Armoured catfish evolved skin denticles that recapitulate teeth the structural, developmental, and genetic level

Carlos Rivera Rivera[1,2,3], Nadezhda Guevara Delgadillo[1], Ilham Bahechar[1], Claire Shea[1], Juan Montoya-Burgos[1,2,4]

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The first vertebrate mineralised skeleton was an external bony armour coated with dental structures. The subsequent emergence of a mineralised endoskeleton and of teeth are considered key innovations in the diversification of vertebrates. Although time clouds our understanding of the initial evolution of these mineralised elements, recent re-emergences of similar structures may help clarify the evolutionary and developmental processes underpinning the emergence of the vertebrate mineralised skeleton. Loricarioid catfishes comprise several lineages that, much like those ancestral vertebrates, bear a denticle-clad bony armour from head to tail. Loricarioid denticles (LDs) and oral teeth are very similar in superstructure. Here we show through in situ hybridisation techniques that this similarity extends into their developmental and genetic machineries. This machinery in itself has been previously described as a highly conserved battery of co-expressed genes which are the base of the development of teeth in a large diversity of vertebrate lineages (the odontogenic gene regulatory network - oGRN). Keeping in mind that LDs are an independent emergence of extra-oral dental structures, we also present here an in-depth developmental-phylogenetic literature review to compare the developmental cues of LDs to those of 16 other vertebrate dental and non-dental ectomesenchymal appendages and find that the developmental signature of LDs is identical to that of teeth in other teleosts and in mouse, but unlike placoid scales in sharks, pointing at an independent re-activation of the (oral) dental program, as opposed to a retention or atavistic re-emergence of the ancestral denticulate armour.

Ectodermally derived exoskeletons in molluscs: from signalling to seashells

Molly L. Rivers[1], Maryna P. Lesoway[2], Susan C. Fitzer[3], **Victoria A. Sleight[1]**

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Molluscs are the second most speciose animal phylum and owe part of their evolutionary success to their iconic exoskeletons – shells. Mollusc shells have strikingly diverse morphologies that are exquisitely preserved in the fossil record, detailing evolutionary change since the early Cambrian. Shells are produced by the mantle tissue, an ectodermally derived specialized epithelium that covers the dorsal body wall. While the mantle and shell are deeply homologous features of the molluscan body plan, the extracellular matrix proteins that make-up the shell are rapidly evolving and lineage specific, highlighting differing degrees of homology across levels of biological organisation. To understand the evolutionary mechanisms determining vast shell diversity across the phylum, developmental processes must be understood, yet to date, mollusc shell evolution studies have focussed primarily on adult stages. Here, we took an evo-devo approach to shell morphological evolution by examining shell development in four closely related species of calyptraeid gastropods (genus: *Crepidula*). We combined electron microscopy, histology, bulk and single-cell transcriptomics, in situ hybridisation and confocal microscopy to describe and compare shell development between species. By integrating phenotypic descriptions with transcriptomic comparisons through developmental time, we uncovered gene expression signatures for specific signalling pathways that correlate to between-species morphological differences in the mantle and shell. Together, this works highlights the role of developmental processes in shaping evolutionary diversification of mollusc shells and presents a powerful system (*Crepidula*) to decipher ectodermal appendage evolution in a remarkably diverse but understudied phyla.

S10.4 | Skin Deep: Common Threads in the Evolution of Bilaterian Ectodermal Appendages

Development and evolution of the whisker pattern

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Whiskers form a stereotypical sensory array on the face of many mammals. Small mammals usually have many well-organised whiskers while larger terrestrial mammals have fewer whiskers, with less regular arrangements. We explored the developmental basis of whisker pattern formation, identifying the operation of a Turing-type patterning system influenced by anatomical cues on different parts of the developing face. These inputs tune the output of the patterning system to vary whisker size and spacing across the snout. The operation of this system upon a growing domain, together with the reading of anatomical landmarks, yields the highly accurate and reproducible pattern of whiskers on the mouse. This patterning mode appears to be ancestral to therians but lends itself to biased patterns of whisker loss in large mammals.

C10.1 | Skin Deep: Common Threads in the Evolution of Bilaterian Ectodermal Appendages

Genetic erosion of the scale genetic network forced an evolutionary return to bony armor

David Walter, Simone Giulio Bordignon, Ming Li, Axel Meyer, **Joost Woltering**

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An evolutionary arms race drove modern fishes to replace the heavy armor that encased ancestral vertebrates with lightweight scales that optimize maneuverability. Armored catfishes (e.g. *Corydoras*) represent a paradox as they re-evolved heavy bony plates from naked skin - millions of years after their modern squamation was secondarilly lost. In a scaled teleost fish we show that breaking the repressive BMP loop of the placode forming Turing network elicits catfish-like scutes by eliminating dorsoventral inter-placodal spacing, while anteroposterior spacing follows a myotomal pre-pattern. Developing *Corydoras* skin exhibits attenuated BMP signaling and convergent genomic degeneration of *bmp* genes occurred across catfishes. We predict that genetic erosion during an evolutionary window of scalesness compromised the ability to re-evolve discrete scales. As a result of this evolutionary "one-way street" catfishes resorted to a simpler pre-patterning mechanism to re-mineralize their skin, embarking on a trajectory towards archaic armor. These findings demonstrate the heuristic value of Dollo's law that complex traits cannot re-evolve unchanged, and highlight the necessity of interpreting evolutionary trajectories through the dual lens of ecological adaptation and developmental constraint.

The unreasonable effectiveness of computational models in skin scale patterning and morphogenesis

Michel Milinkovitch, Ebrahim Jahanbakhsh

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We have shown that reaction-diffusion-taxis models are particularly effective for understanding the patterning of hair, feather, and scale primordia (placodes) —as well as skin colour patterning— without the need to parametrise the profusion of variables at the microscopic scales. We have also shown that three-dimensional mechanical models, integrating growth and material properties of embryonic skin layers, capture most of the patterning and morphogenetic dynamics of head scales in crocodiles and tortoises. Here, I demonstrate how the spectacular morphogenesis of strongly overlapping snake scales can be recapitulated by a mechanical model integrating tissue plasticity and active material properties. Together, these results suggest that even complex morphogenetic processes can be understood within a unified quantitative framework. More broadly, they indicate that biology, despite its 'messy' nature —with its unmanageable profusion of cellular and molecular variables— can be efficiently and quantitatively investigated using mathematical and computational approaches, including simple phenomenological models.

Genomic and signaling network evolution underlying hedgehog spine formation

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Hedgehog spines are an iconic example of diversification in mammalian skin appendages. Like hair, spine development begins with placode formation, but the two primordia diverge in size and internal structure. While these remarkable adaptations are encoded in the genome, the genetic mechanisms that drive spine rather than hair formation remain elusive.
Here, we investigate how the hedgehog *Atelerix albiventris* evolved spines from hair by combining evolutionary, computational, and molecular approaches. Genomic sequence comparisons between the *Erinaceidae* lineage and other Insectivora identify hedgehog-specific gene loss and changes in non-coding regulatory landscapes. In parallel, we used epigenetic and single-cell transcriptomic profiling of ventral and dorsal skin at stages of early placode development to identify hedgehog- and mouse-specific changes in gene expression and regulation. These data provide the basis for the functional dissection of placode formation in spine *versus* hair.

This integrative approach revealed species-specific alterations of key components of Turing networks that control placode patterning. We observe increased activity of classic activator pathways – such as Shh and Wnt – at the expense of inhibitor pathways like BMP in spine-generating skin. Additionally, stronger activation of dorso-ventral patterning genes in hedgehog suggests the establishment of stricter body plan boundaries, leading to the division in spine- and hair-producing skin.
Uncovering the genetic origin of hedgehog spines will advance our understanding of the evolutionary mechanisms underlying morphological repurposing and, more broadly, of developmental patterning control.

Arthropod's next top model: *Oxidus gracilis*

Alexandra Weber, Heather Bruce

Zoology, University of British Columbia, Vancouver, Canada

There are four major extant groups within Arthropoda: Chelicerata, Myriapoda, Crustacea and Insecta. Of these groups, Myriapoda (millipedes and centipedes) is the only not to have an established lab tractable model, despite scientific interest. Much of this interest is due to their phylogenetic position between Chelicerates and Pancrustacea (Crustaceans and Insects), and unique traits like cyanide-based defensive glands, distinctive neural patterning, tracheal systems, and extreme take on Arthropoda's characteristic segmented body plan. We are developing the garden millipede, *Oxidus gracilis,* as the first lab tractable myriapod model with a complete lab life cycle, sequenced and annotated transcriptome, and CRISPR Cas-9 gene editing. In contrast to other myriapod species examined to date, *Oxidus* is an ideal model system: it thrives in lab environments; breeds year-round; produces clutches of >200 eggs; has a short generation time of ~4 months; and is readily available worldwide. Pest control is the biggest issue, with nematodes, mold and phoretic mites all posing threats to the health of the animals. We will employ *Oxidus* to investigate the origins and relationships of seemingly "novel" ectodermal structures across Arthropoda, such as the tracheal system of myriapods. The first step of this investigation is to develop an embryonic staging manual for *Oxidus*. A complete developmental series of embryos from 8 hours post laying to hatching has been collected, stained and imaged. We found that, like insect trachea, millipede trachea also forms on the embryonic leg, suggesting that there may be more to the story than just convergence of unrelated structures.

Scale cell type specification on butterfly wings

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Butterfly wings are covered in scales, which act like pixels on a screen to make patterns. Each scale is a specialised, non-innervated Sense Organ, and is one of multiple specific types which combine pigmentary and ultrastructural features to create different functional outputs. To investigate the mechanisms by which these scale cell types become specified, we generated a multi-species single nucleus RNAseq time series, allowing us to study the transcriptomic trajectory of scale types as they are specified, and the cellular mechanisms by which they generate colour and structure.

Developmental Mechanisms for Morphological Novelty in Early Osteichthyan Dentitions

Plamen Andreev[1], Qiang Li[1], Wenjin Zhao[2], Lijian Peng[1], Federica Marone[3], Martin Rücklin[4], Min Zhu[2]

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Isolated teeth and jaw fragments present some of the oldest unequivocal evidence for jawed vertebrates and are indicative of unexpected disparity among early osteichthyans. These fossil remains preserve critical information about the tooth replacement patterns and phenotypic diversity of osteichthyan dentitions, which have largely been informed by living species. We analysed with synchrotron X-ray tomography the dental elements of the oldest known osteichthyans from the lower Silurian (c. 440 million years ago) of China, represented by serially repeated medial bones (coronoids and tooth 'cushions') of the lower jaw. These belong to two species with distinct mandibular anatomy influenced by the medial jawbones. Tooth 'cushions' are not in contact with other dermal bones and are supported by bulbous processes of the meckelian endoskeleton. Our data demonstrate that during ontogeny tooth 'cushions' fuse into compound elements and bring about the formation of labio-lingual rows of meckelian processes. The coronoid bones display typical contact surfaces with the dentary, however random tooth addition within these elements does not produce the expected vertical stacking of successive tooth generations previously reported in stem and crown osteichthyans. This manifests as an absence of fixed tooth positions and an uncharacteristically toothed labial surface of the coronoid. The results reveal previously unsuspected developmental discrepancy in the dentitions of early osteichthyans and single out medial jawbones as the site of rapid morphological change during the initial evolutionary radiation of these lineages.

11 | Pixels, Patterns, and Perception: Integrative Eco–Evo–Devo Perspectives on Biological Colour

Symposium S11: Thursday 11 June, 09:00 – 10:40
Contributed Session C11: Thursday 11 June, 11:05 – 12:30

Organisers: Emília Santos (University of Cambridge, UK, Hannah Weller (University of Helsinki, Finland)

Colour and pattern are among the most visible outcomes of evolution, enabling researchers to link molecular mechanisms to ecological and behavioural function. Colour pattern evolution is an exceptionally well-poised system for connecting the genetic origins of variation to their evolutionary consequences. In addition to more classical genetic and behavioral experiments, the field has approaches for modeling both pattern development and receiver psychology. These modeling tools enable researchers to extend experimental insights from a few species to test hypotheses over wider taxonomic and evolutionary scales than would be feasible experimentally. This symposium will explore the evolution and development of biological colour through an eco–evo–devo lens – integrating genetic, developmental, ecological, and perceptual processes across biological scales, from genes to fitness. We will highlight studies combining genomics, developmental biology, quantitative modelling, and visual ecology to understand how colour patterns originate, evolve, and are perceived. By bridging molecular mechanisms and ecological consequences, this symposium aims to connect disciplines that traditionally work in isolation.

S11.1 | Pixels, Patterns, and Perception: Integrative Eco–Evo–Devo Perspectives on Biological Colour

How do girls make diamonds? The developmental basis of a female-limited color pattern polymorphism

Tyra Westelius[1], Robin Pranter[1,2], Corin Stansfield[1,3], Natalia Zajac[2], **Nathalie Feiner[1,2]**

[1]Department of Biology, Lund University, Lund, Sweden, [2]Max Planck Institute for Evolutionary Biology, Ploen, Germany

The presence of multiple discrete colour patterns within a single species has captivated evolutionary biologists for more than a century, especially when such polymorphism is confined to one sex. In the brown anole lizard *Anolis sagrei* a dorsal color pattern polymorphism is controlled by allelic variation at the autosomal *CCDC170* locus, yet it manifests only in females. How is this sex-specific expression achieved? We propose a threshold model in which *CCDC170* acts only in females because it is co-expressed with the neighboring gene *Estrogen Receptor 1*, which is highly transcribed in female but not male embryos. Experimentally manipulating estradiol levels revealed that genetic males can be induced to display the female-specific patterns, whereas estradiol depletion in genetic females (via fadrozole) produces the monomorphic male pattern regardless of the *CCDC170* genotype. RT-qPCR confirmed that estradiol and fadrozole directly modulate *CCDC170* and *ESR1* expression, supporting our threshold model. By linking a morph-determining locus to the sex-differentiation pathway, our work illustrates how hormonal context can change the phenotypic consequences of genetic variation. Our results suggest that the *CCDC170-ESR1*-locus is part of a mechanistic link between the morph-determining and the sex-differentiation system and provide a causal explanation for the developmental basis of a sex-limited color polymorphism.

S11.2 | Pixels, Patterns, and Perception: Integrative Eco–Evo–Devo Perspectives on Biological Colour

How the mouse got its stripes: formation and evolution of color patterns in rodents

Ricardo Mallarino

Molecular Biology, Princeton University, Princeton, USA

Due to their visual prominence, extensive diversity, and prevalence across species, animal color patterns provide a powerful framework for studying the developmental mechanisms that govern biological organization. In mammals, periodic color patterns arise through two distinct processes. The first, pattern establishment**,** occurs during embryonic development and specifies the positional boundaries of the eventual pattern. The second, pattern implementation, generates and maintains color differences during hair growth and cycling. My laboratory has developed a wide range of genomic resources and molecular tools in the African striped mouse (Rhabdomys pumilio), a rodent whose coat consists of alternating dark and light stripes, to investigate the mechanistic basis of color pattern formation at these two levels. In this talk, I will discuss how we use an integrative approach—combining experimental embryology, mathematical modeling, multi-omic analyses, and functional experiments—to uncover the mechanisms underlying the formation and evolution of rodent color patterns. Taken together, our work provides insights into how biological organization is established and into the mechanisms by which phenotypic novelty arises.

S11.3 | Pixels, Patterns, and Perception: Integrative Eco–Evo–Devo Perspectives on Biological Colour

Machine-learnt variation among butterfly photographs: new quantitative tools for evolution and development

Jennifer Hoyal Cuthill[1], Nicholas Guttenberg[2], Blanca Huertas[3]

[1]School of Life Science, University of Essex, Colchester, UK, [2]Cross Labs, Cross Compass, Tokyo, Japan, [3]Department of Science, Natural History Museum, London, UK

Machine learning methods to learn “embedding” distances between images have great potential for evolutionary and developmental biology as they can quantify biological variation at new scales of diversity and complexity. In previous work, we have shown that machine learnt image distances can be strongly correlated with independent genetic signals of phylogenetic relatedness on divergent axes of variation, and able to capture conflicting signals of convergence (Hoyal Cuthill et al., Science advances 5.8 (2019): eaaw4967). Recently, we have demonstrated in the sexually dimorphic birdwing butterflies that machine learning can also detect spectra of variation in males or females, from striking phenotypic divergence in males to cryptic diversity among females (Hoyal Cuthill et al., Communications Biology 7.1 (2024): 774). This talk will give an overview of these emerging tools for biological image analysis, including case studies detecting contrasting patterns of within-individual, between-sex and inter-species variation across butterfly clades.

S11.4 | Pixels, Patterns, and Perception: Integrative Eco–Evo–Devo Perspectives on Biological Colour

From shallow larvae to the abyss: extraordinary visual adaptation in deep-sea fishes

Zuzana Musilova[1], Fabio Cortesi[2], Zuzana Konvičková[1], Camilla Kruse Kidmose[1]

[1]Department of Zoology, Charles University, Prague, Czech Republic, [2]School of the Environment and Queensland Brain Institute, University of Queensland, Brisbane, Australia

Deep-sea fishes experience dramatic shifts in their visual environment during development. While adults inhabit the dim and spectrally restricted deep ocean, their larvae live in shallow, well-lit waters before undergoing metamorphosis and descending to depth. This ontogenetic transition is accompanied by profound changes in the visual system. Using genomic, transcriptomic, and single-cell RNA sequencing data, we investigate molecular adaptations in genes underlying vision, with a particular focus on opsins. Across many deep-sea fish families, larvae predominantly express cone opsins of the RH2 family for vision in brighter environments, whereas adult retinas are dominated by the rod photopigment rhodopsin (RH1) better suited to extremely low light levels. Although most fishes possess a single rhodopsin gene, several deep-sea lineages show extensive gene duplications. Spinyfins (Diretmidae) are the most extreme cases with 38 rod opsins tuned to blue-green wavelengths, and our results suggest that these rhodopsins play different roles across life stages. In larvae, rhodopsin complement the dominant cone opsin, indicating that multiple rhodopsins in the retina are beneficial to both larval and adult stages. Pearleye fishes (Scopelarchidae) display another unusual configuration by having photoreceptors with intermediate characteristics that blur the classical distinction between rods and cones. Using single-cell RNA sequencing, we investigate the molecular identity of these cells focusing on the rod and cone opsins, as well as the typical cell markers and phototransduction cascade genes. Together, these systems provide an exceptional opportunity to explore how developmental transitions and gene duplication shape the evolution of vertebrate visual systems under extreme conditions.

C11.1 | Pixels, Patterns, and Perception: Integrative Eco–Evo–Devo Perspectives on Biological Colour

How the lizard changed its spots: using computational models of development to predict evolutionary changes in colour patterns

Halley Cano-Fernández[1], Guannan Wen[1], Tobias Uller[2], Nathalie Feiner[1]

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Development, natural selection and genetic drift jointly determine the direction of phenotypic change. An open question is whether "developmentally biased" directions actually happen more frequently during evolution. In other words, does evolution go where development leads? The particularly high variation in dorsal colour patterns of the Mediterranean wall lizards (genus *Podarcis*) provides an excellent opportunity for answering this question. In this study we combined an empirical and a computational approach. In our empirical approach, we took standardized photographs of 4093 individuals from 134 populations and 6 wall lizard species that live in archipelagos. Dorsal colour patterns were extracted from the images and quantified using a custom pipeline. We observed that some patterns were very frequent, particularly on large islands, while other patterns were rare and almost only appeared on very small islands. In our computational approach, we developed a mathematical model of colour pattern development that included a reaction-diffusion system, scale formation, tissue growth and developmental noise. This model was able to quantitatively recapitulate the phenotypic variation observed in wall lizards. Using this model, we simulated hundreds of random walks in the parameter space. We showed that some patterns were produced by a higher number of parameter combinations than others, and that some transitions between patterns were more likely to happen than others, again matching the empirical observations. Finally, our two approaches combined suggest that development can explain why some colour patterns appear more frequently than others during evolution.

C11.2 | Pixels, Patterns, and Perception: Integrative Eco–Evo–Devo Perspectives on Biological Colour

The evolution, genetics and development of butterfly structural colour

Victoria Lloyd[1], Anupama Prakash[1, 2], Melanie Brien[1, 3], Juan Enciso Romero[1, 4], Patricio Salazar Carrion[1, 5], **Nicola Nadeau[1]**

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Some of the brightest and most striking colours found in nature are produced not by pigments but through coherent scattering of light by nanostructures. Despite the importance of these colours for animal and plant signalling and communication, very little is known about their genetic control. Within the *Heliconius* butterflies, structural colour has evolved several times and there is within-species variation, making this an excellent study system to understand the evolution and genetic basis of this trait. We have been using genomic approaches combined with transcriptomics and proteomics to understand how structural colour has evolved repeatedly in the *Heliconius* butterflies. We have combined this with super-resolution imaging to understand how specific genes may control nanostructure formation. We have found that different genomic regions control structural colour in different species. The genes involved appear to control the actin cytoskeleton, cuticle composition and membrane curvature. Our results provide first steps in understanding the genetic and developmental control of structural colour and how this fascinating trait evolves.

C11.3 | Pixels, Patterns, and Perception: Integrative Eco–Evo–Devo Perspectives on Biological Colour

The lncRNA–miRNA gene *ivory:mir-193* controls adaptive melanism in a polymorphic moth

Luca Livraghi[1], Jordan L. Cassily[1], Joseph J. Hanly[1], Mallory Brayer[1], Alexander T. Carter[1], Jasmine D. Alqassar[1], Sheina B. Sim[2], Scott M. Geib[2], Arnaud Martin[1]

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Melanic pigmentation underlies camouflage in most moths, and genetically controlled colour polymorphisms provide powerful systems for linking molecular mechanism to ecological function and perception. Here we leverage natural variation in the velvetbean caterpillar moth, *Anticarsia gemmatalis*, to identify the genetic basis and developmental mechanism of adaptive melanism used for camouflage. Genome-wide association mapping implicates the *ivory:mir-193* non-coding RNA locus as a major determinant of colour, with deeply divergent haplotypes differentiating Light and Dark morphs. Using long-read sequencing and de novo assembly of multiple haplotypes, we uncover extensive sequence divergence across the *ivory:mir-193* region between morph-associated haplotypes, consistent with a large, structurally complex regulatory region. Expression analyses show that *ivory:mir-193* expression in developing wings predicts adult melanic scales, supporting cis-regulatory differences that drive morph-specific deployment. To test causality, we used CRISPR to independently disrupt the *ivory* promoter and the *mir-193* hairpin. Strikingly, each perturbation abolishes melanic scale pigmentation, demonstrating that this lncRNA/pri-miRNA module is required for dark-scale differentiation and the production of camouflaging melanism. Together, these results establish *ivory:mir-193* as a major-effect developmental switch underlying camouflage variation and highlight non-coding regulatory architecture as a recurrent substrate for colour-pattern evolution in Lepidoptera.

C11.4 | Pixels, Patterns, and Perception: Integrative Eco–Evo–Devo Perspectives on Biological Colour

Sketching Vertebrate Color Evolution through the Reconstruction of Reptile Chromatophore Development

Pierre-Yves Helleboid, Athanasia Tzika

Laboratory of Artificial & Natural Evolution (LANE), Department of Genetics, University of Geneva, Geneva,Switzerland

Pigmented cells have been extensively studied in teleost fishes, particularly zebrafish, where interactions among brown melanophores, yellow-to-red xanthophores, and reflective iridophores generate color patterns. These chromatophore types are also conserved in squamates, which diverged from teleosts approximately 400 million years ago. Squamates display a wide diversity of hues and patterns; however, unlike teleosts, they do not undergo developmental metamorphosis. Thus, they provide a valuable model to explore the evolution and development of vertebrate pigment cell diversity.

We used single-cell transcriptomics to reconstruct the development of chromatophore lineages in two reptilian model species, the corn snake and the bearded dragon. In the corn snake, the differentiation of chromatophores resembles that of zebrafish chromatophores. In the bearded dragon, we uncovered two previously undescribed chromatophore populations related to melanophores and xanthophores that share a common transcriptional program and contribute to regional patterning. Furthermore, ASIP, a gene implicated in countershading in mice and zebrafish, is likely to play a similar role in the bearded dragon. In addition, we investigated the role of major chromatophore transcription factors in the differentiation process and could highlight their central and evolving role in tetrapod color pattern evolution. We then integrated our corn snake dataset with published zebrafish and amphibian single-cell datasets, revealing that while differentiated pigment cell lineages rely on conserved molecular actors, progenitor populations display greater transcriptional diversity.

C11.5 | Pixels, Patterns, and Perception: Integrative Eco–Evo–Devo Perspectives on Biological Colour

Non-iridescent structural color emergence and diversification controlled by keratin self-assembly during feather development

Rita Afonso

CIBIO, Research Centre in Biodiversity and Genetic Resources, University of Porto, Porto, Portugal

Animals produce colors through light-absorbing pigments or light-scattering structural mechanisms. While the major animal pigmentation systems are generally well characterized, our understanding of the genetic and developmental basis of structural colors is lagging behind. In birds, opaque blues and violets emerge from the physical self-assembly of keratin nanoscale architectures during feather morphogenesis. To date, the molecular mechanisms driving the formation and diversification of these non-iridescent optical structures remain largely unexplored. Thanks to the availability of many artificially-selected structural color mutations with simple inheritance patterns and large phenotypic effects, captive bird species represent powerful resources to tackle these questions. Here, we integrate genetic mapping, single-cell transcriptomics, gene expression and functional analyses, high-resolution microscopy, and photonics to characterize the molecular mechanisms involved in structural blue color emergence and variation in lovebirds. We identified a single gene encoding a keratin cross-linking transglutaminase as a key initiator of photonic structure self-assembly during feather development. Moreover, we show that modulating transglutaminase expression alters the kinetics of keratin polymerization, thus changing photonic matrix conformation and fine-tuning structural color hues. The generalization of our findings to non-parrot species suggests that the function of this gene may be broadly conserved across birds, potentially contributing to a vast proportion of natural avian color diversity. Our work reports a novel molecular mechanism of vertebrate structural coloration, contributing to our understanding of animal color evolution beyond classical pigmentary pathways.

C11.6 | Pixels, Patterns, and Perception: Integrative Eco–Evo–Devo Perspectives on Biological Colour

How sexes diversify: Molecular drivers underlying sexual color dimorphism in cichlids

Muktai Kuwalekar[1,2], Nidal Karagic[1,2], Hannah Weller[1,2], Dimitri Meistermann[1,2], Claudius Kratochwil[1,2]

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Sexual dimorphism is a widespread phenomenon where conspecific sexes exhibit distinct traits such as coloration. In some animals, juveniles display monomorphic coloration, but dimorphic coloration emerges only at the onset of sexual maturity. For instance, *Chindongo saulosi,* an African cichlid fish where both sexes are yellow as juveniles and only the males undergo yellow to blue color change during sexual maturity. To investigate the genetic and mechanistic basis of this sexual dimorphism, we hybridized *C. saulosi* and its closely related, but monomorphic blue species *C. demasoni*, and performed QTL mapping. We identified a sex-linked locus associated with color dimorphism and reveal a tandem duplication of *gsdf* (*gonadal soma-derived factor*) gene acting as a Y-allele in a XY sex-determination system. Additionally, we identified loci involved in juvenile yellow coloration that plays a role in sex-specific color change. Electron and light microscopy reveal that the yellow-to-blue transition is driven by the emergence of one type of pigment cells: the blue, iridescent iridophores. Both sexes of the blue monomorphic species show epidermally localized iridophores early in development. These iridophores are absent in yellow females and juvenile males of dimorphic species, but appear in color changing males. Using single cell RNA-sequencing, we show that the dimorphic species has a higher abundance of pigment cell progenitors that likely drives the epidermal iridophore differentiation. Taken together, we propose that a heterochronic shift in iridophore differentiation that is influenced by a combination of a sex-linked and autosomal loci underlies this conspicuous sexual dimorphism.

12 | Genomics in Evo-Devo - And what next?

Symposium S12: Friday 12 June, 09:00 – 10:40
Contributed Session C12: Friday 12 June, 11:05 – 12:30

Organisers: Nico Posnien (Developmental Biology, University Goettingen, Germany), Natascha Turetzek (Evolutionary Ecology, LMU Munich, Germany)

Evo-Devo grew from the synthesis of comparative anatomy and embryology and gained increasing attention with the advent of molecular genetics, providing tools to mechanistically link genotype to phenotype across evolutionary timescales. The omics revolution driven by technological advances in short- and long-read sequencing applicable for many species has the potential to lift Evo-Devo research on yet another level of mechanistic understanding. Comparative genomics and transcriptomics are currently extensively employed to identify candidate genes, networks and processes underlying the evolution of developmental programs. We argue, however, that omics approaches must be placed in a robust phylogenetic context to deduce meaningful patterns of genome evolution, such as conservation, divergence, duplication, and rearrangement of genes and regulatory regions. Moreover, we advocate for a thorough integration of comparative omics data with underlying differences in cellular and developmental processes. To this end, we must accelerate the development and adoption of novel functional tools for gain- and loss-of-gene-function and imaging approaches. Eventually, the use of omics data to reconstruct gene regulatory networks and to train predictive mathematical models hold the potential of propelling Evo-Devo research beyond the state-of-the-art. With our symposium, we want to highlight the power of omics data analysis for Evo-Devo research, and we want to outline its potential and challenges for a meaningful integration with multi-modal data on organismic development and evolution.

Gene duplication and co-option originated a male specific visual system in mayflies

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Mayflies are a key order to understanding the evolution of insects due to their phylogenetic position. Within this lineage, the family of Baetidae has evolved a striking sexual dimorphism: in addition to the lateral compound eyes, males develop an extra pair of extremely large dorsal, turban-shaped eyes. Thus, by comparing males versus females, these insects are a privileged system to understand the origin and integration of new visual structures. For this, we have successfully established the mayfly *Cloeon dipterum* as a model species with a continuous culture in the lab and a high-quality reference genome that allows as describe the development of the eye and its integration with the optic lobes of male and female *Cloeon* nymphs using confocal and electronic microscopy. Furthermore, we compare sex-specific gene expression in nymphal heads at single cell resolution, to show a set of candidate genes specifically expressed in male cell populations. Together with ATAC-seq and functional assays we uncover the GRN responsible for the origin of this new visual system, which constitutes a great advantage for males to detect other objects and individuals in the swarms that adults form to mate.

S12.2 | Genomics in Evo-Devo - And what next?

An integrative Eco-Evo-Devo approach to colour pattern variation in cichlid fishes

M. Emília Santos

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How morphological variation originates and is maintained in natural populations is a fundamental question in evolutionary biology. We address this using cichlid fishes as a model system, focusing on egg-spots — brightly pigmented markings on male anal fins that vary remarkably within and across ~1,500 species and are subject to sexual selection through female choice and male-male competition. We use intra- and inter-specific comparative genomics, combined with phenotypic plasticity studies and trait development characterisation, to identify factors shaping egg-spot variation and evolution. Candidate loci play a role in pigment cell development, and we present progress on the functional dissection of their role in pattern formation. Strikingly, loci underlying intraspecific variation do not overlap with those driving interspecific divergence, suggesting a highly polygenic trait where standing variation within populations does not repeatedly fuel divergence between species. To move from description toward prediction, we use visual modelling to contextualise colour variation in an ecological and sensory framework, and mathematical modelling to reconstruct developmental dynamics underlying egg-spot patterning. Whilst these frameworks are still under development, we discuss the potential of integrating comparative omics with developmental data — and brainstorm what it would take to move Evo-Devo from identifying patterns of variation to predicting them.

S12.3 | Genomics in Evo-Devo - And what next?

The evolution of new vertebrate cell types and organs

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How do new cells, new tissues, and entire new organs arise during evolution? Our lab investigates these questions using an organ that has evolved independently many times and is remarkably diverse: the placenta. The placenta forms through the fusion of embryonic and maternal tissues and enables the transfer of nutrients, waste, and more during gestation. Placentas have evolved more than 100 times independently among vertebrates, including once in mammals. Within a family of small live-bearing fishes known as Poeciliidae, at least nine independent origins of a placenta have occurred. This makes them excellent models for studying how new organs emerge during evolution. We combined whole-genome sequencing, single-cell expression and chromatin accessibility, and imaging to uncover the molecular, cellular, and developmental basis of five independent origins of a placenta in Poeciliids. We found that the evolution of a novel cell type unique to Poeciliids allowed this family to transition from egg-laying to live-bearing. This cell type was subsequently and independently co-opted five times to form the placenta in the five species. Across these independently evolved placentas, we observe strong convergence at the molecular level, with specific genes and pathways repeatedly recruited during placental evolution. Together, our findings provide empirical support for a model in which cell-type innovation drives the emergence of new organs and demonstrate that major life history transitions can follow predictable developmental and molecular trajectories.

S12.4 | Genomics in Evo-Devo - And what next?

Simulating the evolution of development: current and future approaches

Pjotr van der Jagt[1,2], Steven Oud[1,2], Alexandre Porcher Fernandes[1,2], Enrico Sandro Colizzi[1,3], **Renske Vroomans**[1,2]

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Both animals and plants have evolved their developmental program over 500 million years, resulting in a great diversity of sizes, shapes and colours.

In my team, we develop computational models in which the genetic information that governs gene expression and cell differentation can evolve, to study changes in developmental processes at different time scales.

With a model of petal pattern evolution, we predict the emergence of a cryptic cell type as a side-effect of selection for a distinct bullseye pattern and show how gene expression noise drives the functionalisation of this cell type.

In a model of plant stem cell niche evolution, investigate the neutral change of developmental gene regulatory networks over longer time scales. Here, we identify a core network of gene interactions that are deeply conserved and turn over much more slowly than other parts of the developmental mechanism.

By coupling cellular gene expression to cell growth and division, we can now simulate the evolution of organ morphogenesis together with differentiation, to understand the role of feedbacks between these two processes in the emergence of robust development.

Finally, I will discuss the direction in which we would like to take these developmental models, moving away from fixed selection pressures towards “constructive” emergence of selection through environmental interactions.
I will illustrate this with a model of the evolution of multicellularity -- the origin of development itself.

An ancient gene is a novel regulator of cell proliferation in the spider Parasteatoda tepidariorum

Zoe X. Schultz[1], Grace Blakeley[2], Madeleine E. Aase-Remedios[1], Tom Frankish[3], Gemma Sutton[4], Daniel J. Leite[1], Chema Martin-Duran[3], Matthias Pechmann[5], Ralf Janssen[6], Alistair P. McGregor[1]

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Precise control of cell proliferation is fundamental to ensure the correct development of animal embryos. Comparative studies and screening in model organisms have revealed a 'tool-kit' of genes that regulate development is conserved across metazoans and has shed light on overarching mechanisms controlling cell proliferation during embryogenesis and organogenesis. Expanding to a wider range of animals can reveal key components of the developmental toolkit potentially missed by interrogating only a handful of model organisms, and in turn can provide a more robust understanding of animal developmental regulation and evolution. This is now readily achievable by leveraging unbiased genomic approaches. Using single-cell sequencing, we discovered an ancient regulator of progenitor cell division during embryogenesis in the spider *Parasteatoda tepidariorum*, which we have named *beer-belly* (*be-be*). In *P. tepidariorum* embryos, *be-be* is expressed in ectodermal cells in the developing brain, leg-bearing segments, and the segment addition zone where it overlaps with *Wnt8* expression. Knockdown of *be-be* results in an excess of ventral cells during segment addition, leading to disorganisation and enlargement of the opisthosoma. Curiously, although *be-be* has been lost in mammals and holometabolous insects, it is found in most other major animal lineages. We have found that in other arthropods *beer-belly* orthologues are expressed in similar patterns to *P. tepidariorum* and this gene is also expressed during embryogenesis in annelids and zebrafish. We hypothesise that *beer-belly* is an ancient and conserved modulator of Wnt signalling.

Uncovering the molecular basis of bat wing development using comparative genomic and single-cell approaches

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Bats are the only mammals capable of self-powered flight, an evolutionary innovation based on the transformation of forelimbs into wings. The bat wing is characterized by an extreme elongation of the second to fifth digits and a wing membrane called chiropatagium connecting them. This adaptation enabled bats to conquer the aerial environment, contributing to the massive diversification of this taxonomic group. Yet, the molecular underpinnings of this evolutionary innovation remain obscure. In this study, we have explored the molecular basis of wing formation through a suite of genomic tools and single-cell analyses in bats and mice. We found that despite the substantial morphological differences between the species, there is an overall conservation of cell populations and gene expression patterns, including interdigital apoptosis. To trace the cellular origin of the chiropatagium, we performed single-cell and label transfer analyses on manually micro-dissected cells and identified a distinct fibroblast population, independent of the apoptosis-associated interdigital cells, as the source of this tissue. These distal cells express a conserved gene program including the transcription factors *MEIS2* and *TBX3*, which are commonly known to specify and pattern the early proximal limb. Transgenic ectopic expression of *MEIS2* and *TBX3* in mouse distal limbs resulted in the activation of genes expressed during wing development and phenotypic changes related to wing morphology, such as the retention of the interdigital tissue. Our results elucidate fundamental molecular mechanisms of bat wing development and illustrate how drastic morphological changes can be achieved through repurposing of existing developmental programs during evolution.

From 52 to hundreds of species: integration of bulk and single-cell transcriptomics across animal diversity in Bgee

Frederic Bastian[1, 2], Julien Wollbrett[1, 2], Alessandro Brandulas Cammarata[1, 2], Sébastien Moretti[1, 2], Anne Niknejad[1, 2], Sara Carsanaro[1, 2], Sagane Dind[1, 2], Marion Nyamari[1, 2], **Marc Robinson-Rechavi**[1, 2]

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Single-cell transcriptomics has become a widely used technology to study heterogeneous and dynamic biological systems. The complexity of these datasets makes them particularly challenging to find, access, and reuse; and to be integrated and compared over multiple experiments. In primary repositories such as the Sequence Read Archive (SRA), cell type information is mostly unstructured, and critical information for allowing reanalyzes is often missing. The Bgee database (https://www.bgee.org/) aims at standardizing scRNA-seq dataset annotations across experiments and species for improving data reproducibility and integration. It also aims at making the resulting expression data comparable between experiments and species. Moreover, Bgee is one the groups leading the scFAIR consortium (https://www.sc-fair.org/), to coordinate curation work and agree on best practices among major single-cell international resources. As a result, Bgee is recognized as a Global Core Biodata Resource and as an ELIXIR Core Data Resource. Finally, Bgee integrates single-cell with bulk RNA-seq to provide a unified answer to where and when a gene is expressed. Bgee is now extending its model to integrate all animal species with expression data across organs, developmental stages, or cell types. We are also developing new visual interfaces to compare the expression of homologous genes between homologous anatomical structures (curated from the literature), and dynamic use of single-cell data from diverse species on the web and in R. We will present how manual curation and standardized integration allow to easily answer evolutionary questions over thousands of transcriptome experiments, fulfilling the promise of genomics for Evo-Devo.

The genomic and developmental basis of eyestalks in the stalk-eyed fly Teleopsis dalmanni

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Exaggerated traits deviate from typical allometric scaling relationships resulting in disproportionately large and morphologically striking features. Despite the prevalence of exaggerated traits in nature the genes and regulatory pathways underlying the origin and elaboration of such traits remain understudied. Stalk-eyed flies have evolved highly exaggerated eyestalks through the lateral expansion of the head capsule. The developmental mechanisms and regulatory architecture underlying their evolution from the conserved Dipteran headplan remain largely unknown. Here, we use single-cell RNA sequencing coupled with comparative and functional genomics to investigate the developmental and genomic basis of eyestalk formation in *Teleopsis dalmanni*. We generated a single-cell RNAseq atlas for the developing Stalk-eyed fly larval eye-antennal disc. Together with developmental imaging, we find that the major cell types and gene expression programs are largely conserved with *Drosophila*. However, several core regulators of head development show expanded expression domains associated with eyestalk formation. Functional analyses confirm that these genes contribute to eyestalk development, revealing how changes to conserved developmental networks drive trait exaggeration and evolutionary innovation.

Extensive transcriptomic turnover facilitates evolutionary novelty in brown algae

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Over the course of eukaryotic evolution, complex multicellularity emerged independently in only animals, plants, fungi, red algae and brown algae. Despite their relatively recent origin, brown algae have diversified into over 2,000 species with extraordinary variation in morphology, sexual systems, and life cycles. Yet, the molecular blueprint underlying their developmental complexity and trait divergence remain largely unknown. Here, we present a comprehensive, stage- and tissue-resolved atlas of gene expression spanning the life cycle across 15 species representing the complete brown algal radiation. The atlas captures developmental trajectories from unicellular stages to complex multicellular forms, resulting in a unified comparative framework for brown algal development. We uncover rapid divergence in gene expression and co-expression patterns across both shallow and deep evolutionary scales. Evolutionarily young genes, including those of giant viral origin, are recurrently and preferentially deployed in a highly tissue- and stage-specific manner, particularly within motile unicellular cell types. These brown algal specific changes may be driven by shifts in genomic context through the acquisition of novel regulatory motifs. This rapid evolution of gene expression patterns contrasts with the conservation of linear genomic synteny across brown algae, providing a molecular basis for sexual and morphological diversification. Together, our results suggest that the interplay of conserved and novel genes and regulatory programs play a role in shaping the emergence and evolution of complex multicellularity in an independent eukaryotic radiation.

Functional genetics and genomics of egg development speed in the red flour beetle

Caspar Schmeits[1], Sharon Singh[1], Olivia Schalekamp[1], Daphne Aerts[1], Maurijn van der Zee[1]

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Climate change likely affects insect species that overwinter as eggs by putting selection pressure on the speed of embryonic development. To investigate the developmental pathways that are involved in egg development speed, we are investigating universal targets of selection using the red flour beetle (*Tribolium castaneum*) as a model. By performing a genome-wide association study (GWAS) on pooled whole-genome sequencing data from beetles that were artificially selected for slow embryonic development, we identified a narrow region of SNPs associated with slow development upstream of the Sik3 gene, which encodes a kinase involved in metabolism and insulin signaling. ATAC- and FAIRE-seq data indicate that the region upstream of Sik3 is accessible for transcription factors, suggesting a role for *cis*-regulatory mechanisms. Using quantitative PCR, we found that Sik3 expression is significantly reduced in the slowly developing lines compared to wild-type around 76 hours into development, while nearby genes are not affected. Additionally, RNAi against Sik3 significantly increases the time until hatching. We are currently investigating which alleles exist at this region in our populations, and once identified, will utilize CRISPR-Cas9 to artificially alter the alleles and measure the effects on the expression of Sik3 and on the speed of egg development. In the near future, an analysis of chromatin conformation (Hi-C) may provide us with a wider scope of the functional changes that occurred during selection.

13 | Leveraging evo-devo for conservation biology and agroecology

Symposium S13: Wednesday 10 June, 13:30 – 15:10

Organisers: Kevin Parsons (University of Glasgow, UK)

Evo-devo has contributed fundamental insights into the evolutionary process. However, given the maturity of the field it is curious that it has not been widely applied to tackle the problems of a changing world. For example, evolutionary and population genetics is regularly applied to conservation or agricultural issues as a way to manage biological diversity on the basis of allelic diversity, and allele frequency change. While commonplace, this approach does not recognize that changing environments also change development and in turn the genetic (and non-genetic) mechanisms that underlie phenotypic variation. This provides an opportunity for evo-devo to provide a vital role in understanding the evolutionary potential of populations, and how biodiversity and agrobiodiversity can persist in the future. In short, if we can understand how variation arises, a central focus of evo-devo research, we can provide improved approaches to agroecological and conservation strategies in different environmental and sociocultural contexts. This symposium will bring together researchers who are focused on gaining an understanding of how developmental processes are impacted and impact by biodiversity and agrobiodiversity conservation, in relation to ongoing and future challenges

S13.1 | Leveraging evo-devo for conservation biology and agroecology

Microbial symbionts and the flourishing of ecosystems

Scott Gilbert[1]

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The ecological developmental biology of symbiotic relationships is critical for understanding ecosystem dynamics. First, microbial symbionts are biological species that need to be conserved. The holobiont nature of animals and plants demands that there be symbionts to provide specific functions to the developing and adult organisms. In many instances, a single bacterial species is known to provide a unique service that allows an organism to develop in its particular environment. The rhizosphere surrounding plant roots may be a particularly important place where this occurs. Second, the collaboration between symbionts and humans may be crucial for the remediation and flourishing of the biosphere. For instance, the larvae of the waxworm moth contains microbes that can digest plastics, and certain bacteria produce antibiotics that can counter the fungal strains that are decimating amphibian populations across the globe. Third, these symbionts must become objects of knowledge and funding if we are to understand the processes that allow the initiation and mainenence of obligate and facultative symbioses. Of particular importance are the algal symbionts of coral that are released from their host cells at high temperatures.

Evolutionary divergence of neural crest cells in Icelandic three-spined stickleback (*Gasterosteus aculeatus*) thermal ecotypes

Matthew Brachmann[1], Kevin Parsons[1]

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Climate change is rapidly causing temperature increases in freshwater habitats and an organisms ability to adapt to changing climatic conditions is dependent on the amount of phenotypic variation arising through development. Neural crest cells promote variation in craniofacial traits throughout development and can be phenotypically plastic in response to novel environmental cues. Icelandic threespine stickleback have adapted to both geothermally warmed and cold water environments, showing heritable phenotypic divergence in craniofacial traits and phenotypic plasticity. To understand the role of neural crest cells in facilitating divergence and plasticity in craniofacial development, we used single cell RNA-seq on geothermal and ambient ecotypes, and their reciprocal hybrids, raised under 12 and 18°C. We targeted developmental stage 17 in the developmental series as there is high neural crest cell activity during the development of somites and formation of mesodermal tissue in the head. In total, we sequenced 64654 cells comprised of 28430 genes that made up 29 distinct clusters of cell populations. Neural crest cells appear to plastically differentiate geothermal and ambient ecotypes in response to temperature and hybridization dramatically affects cell-cell communication between neural crest cells and other cell populations. Neural crest cells appear to be a developmentally dynamic population of cells that facilitate phenotypic divergence originating through development.

Developmental plasticity as a buffer against extinction: experimental insights from rotifers

Emily Harmon[1,2], David Pfennig[1]

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Phenotypic plasticity – the ability of organisms to modify traits in response to environmental conditions – is a fundamental developmental mechanism that allows populations to cope with environmental variation. Understanding this capacity is critical in conservation biology, as populations capable of such plasticity may be less prone to extinction and better able to adapt to novel stressors. We used experimental evolution in the rotifer *Asplanchna brightwellii* to test these ideas, exposing 33 clonal lines with varying plasticity to over 20 novel environments for up to 40 overlapping generations. Lines expressing greater plasticity in morphology and life-history traits persisted longer and were less likely to go extinct. This plasticity-mediated persistence also bought time for evolution: more plastic lines not only survived but exhibited stronger evolutionary responses. These results demonstrate that plasticity can enhance population resilience and facilitate adaptation in changing environments. They also highlight the value of integrating evo-devo perspectives into conservation biology, linking developmental mechanisms directly to population survival under environmental stress.

Early-life stress induces brain-region-specific epigenetic and micro-RNA dynamic changes across ontogeny in chickens

Carlos Guerrero-Bosagna[1], Farnaz Sourani[1], Michela di Criscio[1], Fábio Pertille[1], Camille Montalcini[2], Matthew Petelle[2], Michael J. Toscano[2]

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Early-life-stress (ELS) is increasingly recognized as a major factor shaping long-term physiological and behavioural phenotypes. However, the temporal and regional dynamics through which ELS remodels the vertebrate brain remain poorly understood. Here, we investigated the long-term molecular consequences of ELS in laying hens using a cross-sectional multi-omics analysis integrating DNA methylation and micro-RNAs across development.

One-day old chickens were exposed to transportation stress (TPS) and compared with on-farm-hatched controls (OFH) across multiple life stages spanning embryogenesis, juvenile development, sexual maturation, and peak and decay of reproduction. DNA methylation was profiled in five brain regions using GBS-MeDIP sequencing, while micro-RNA expression was analysed in two limbic brain regions and red blood cells with RNA-seq.

Unsupervised analysis revealed that methylomic variation was strongly brain-region-specific, showing their independent epigenetic trajectories across ontogeny. Supervised analyses revealed ontogenic methylation responses in the hippocampus suggestive of epigenetic resilience, characterized by early hypermethylation followed by compensatory changes later in life. In contrast, the nidopallium and hypothalamus showed methylomic effects during juvenile development, whereas the amygdala displayed comparatively limited methylation effects.

Micro-RNA responses were also highly stage- and tissue-specific, with the strongest alterations occurring in red blood cells early after stress exposure, and later-life micro-RNA changes emerging predominantly in hippocampal regions. Although no common micro-RNA was identified between blood and brain tissues, common targets were observed between tissues and timepoints, suggesting that circulating RNAs act as mediators between ELS and later-life consequences.

The results highlight the importance of developmental timing and neural compartmentalization for stress response in vertebrates.

14 | Role of Soma-to-Germline Inheritance in Evolutionary Change

Symposium S14: Wednesday 10 June, 13:30 – 15:10
Contributed Session C14: Wednesday 10 June, 15:30 – 16:40

Organisers: Daniel Phillips (University of Oxford, UK), Atsuko Sato (Ochanomizu University, Japan)

Evolution relies on phenotypic variability, which is at least partially heritable. The stability of DNA combined with its capacity to accumulate spontaneous mutations that modulate phenotypes satisfied these requirements and endowed DNA as the material basis of heredity and proper locus of evolutionary change. However, in the last decade it has been shown that other mechanisms of inheritance exist, including microbiome transfer as well as somatic RNAs that can directly transmit environmentally-induced adaptations across generations, but if and how such soma-germline communication might influence evolution has not yet been addressed. To address this gap, our speakers will present how somatic RNA modify epigenetic information in sperm; introduce the concept of a sperm "RNA code" and emerging tools used to understand how it mediates epigenetic inheritance; describe the role of environmental stress-induced phenotypes and how they are communicated to the gametes and offspring via somatic RNA; and argue why these ideas revive Darwin's theory of "Pangenesis" and how they reveal new directions for evolutionary biology.

S14.1 | Role of Soma-to-Germline Inheritance in Evolutionary Change

Understanding the ‘sperm RNA code’ for epigenetic inheritance with emerging tools

Qi Chen[1]

[1]Department of Human Genetics, and Division of Urology Department of Surgery, University of Utah School of Medicine, Salt Lake City, USA

Recent discoveries have revealed a novel layer of inheritance, showing that mammalian sperm transmit not only DNA but also diverse RNA molecules that mediate the intergenerational effects of paternal condition (e.g., diet, stress, aging, and exercise). Expanding knowledge of sperm RNAs—including miRNAs, tsRNAs, rsRNAs, and lncRNAs—together with their modifications and spatial compartmentalization, supports the emerging concept of a ‘sperm RNA code’ that programs offspring phenotypes during development. I will discuss the challenges and opportunities in establishing this field, including identifying the key sperm RNAs responsible for paternal phenotype transmission, uncovering their native expression and modification profiles with advanced sequencing tools, and understanding the molecular events they trigger in embryos. Complete deciphering of the sperm RNA code under different environmental conditions could transform our understanding of epigenetic inheritance and open paths toward translational applications and precision medicine.

Darwin’s Pangenesis in relation to evolution, development and soma-to-germline inheritance

Yongsheng Liu

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Evolution is a process of inheritance and variation, in which development is involved. Charles Darwin held that inheritance includes both the transmission and the development of characters, and variation is a change in the developmental process. He devoted his later years to seeking a unified theory that would strengthen natural selection and explain diverse phenomena. He extended the cell theory and advanced Pangenesis, a developmental theory of inheritance and variation. He assumed that cells of an organism could “throw off” numerous granules or molecules called gemmules during each stage of development. These gemmules are capable of self-multiplication, diffusion among cells or circulation throughout the system, being modified by changed conditions of life as well as the use or disuse of organs, union with both somatic and germ cells, and transmission from parent to offspring. When first published in 1868, however, it was severely criticized by his contemporaries. Today there has been increasing evidence for the inheritance of acquired characters, graft hybridization and other previously doubtful phenomena which Darwin’s Pangenesis was formed to explain. The chemical existence of Darwin’s hypothetical gemmules has also been vindicated by the discovery of extracellular vesicles as well as circulating DNA and RNAs, and his Pangenesis seems to be surprisingly modern in many respects. This short talk provides unique insights into Darwin’s Pangenesis in relation to soma-to-germline inheritance, graft-induced heritable changes and horizontal gene transfer, regenerative medicine, and other subjects pertaining to genetics, evolutionary and developmental biology, and the history of biological science.

S14.3 | Role of Soma-to-Germline Inheritance in Evolutionary Change

How Germ Cells Respond to Stress - and What Remains to Be Discovered

Vincent Fischer[1,2], Katharina Gapp[1,2]

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In this talk, I will summarize what is currently known about how stress can change molecular features in the germline, when these changes appear, and how they have been reported to affect offspring in mice. I will compare these results with findings from other environmental exposures and from different species to show where the evidence overlaps and where it differs. I will also point out patterns that may apply universal, discuss the main limitations of current experimental approaches, and outline what future studies should address.

Somatically acquired sperm miRNAs initiate embryonic regulatory cascades underlying paternal epigenetic inheritance

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Paternal environmental conditions prior to conception can influence offspring development, implicating epigenetic information in sperm as a conduit for transmitting non-genetically inherited phenotypes at fertilization. In mammals, sperm acquire small RNAs somatically during post-testicular maturation in the epididymis through extracellular vesicle-mediated RNA transfer. Among these somatically acquired RNAs, sperm microRNAs (miRNAs) are particularly notable for their reproducible sensitivity to environmental exposures and their ability to transmit inherited phenotypes to offspring. However, how sperm miRNAs function after fertilization to influence embryonic development and offspring phenotype has remained unresolved. Using dose-controlled zygotic microinjection of individual sperm miRNAs across physiologically relevant ranges, combined with stage-resolved single-embryo RNA-seq, we find that modest variation in sperm miRNA abundance is sufficient to elicit reproducible and sequence-specific gene expression responses that quantitatively scale with miRNA dose and propagate across preimplantation development. To directly identify miRNA targets in early embryos, we developed AGO2-REMORA, an Argonaute-coupled RNA editing strategy compatible with single embryos, providing direct molecular evidence that sperm miRNAs repress target transcripts immediately after fertilization. Together, these findings support a model in which somatically acquired sperm miRNAs initiate embryonic regulatory cascades that mediate paternal epigenetic inheritance.

Intergenerational shifts in innate odour preferences upon odour feeding or odour injections in *Bicyclus anynana* butterfly larvae

Antonia Monteiro[1], Yan Ling Chua[1], Gowri V[1], Ian Chan[1], Suriya Murugesan[1], Preethi Prakash[1]

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How lepidopterans learn to prefer novel plant odours and transmit odour preferences acquired during the larval stage to their offspring is unknown. *Bicyclus anynana* butterfly larvae can learn to prefer a banana-smelling odour, isoamyl acetate (IAA), via feeding on coated leaves, or simply via haemolymph transfusions from an IAA-fed animal, and transmit this preference to their naive offspring. Different concentrations of IAA injected directly into the haemolymph impact odour learning and transmission of learned preferences or learned avoidances. Injections of IAA at low concentrations led to an increase in preference for IAA, whereas higher concentrations led to an increase in avoidance. Naive offspring inherited the odour preferences/avoidances of their parents. Feeding on IAA-coated leaves led to hundreds of differentially spliced genes in the larval brain but few differentially expressed genes. We establish that the same molecule (IAA) can illicit both a preference as well as an aversive reaction when eaten or directly injected into the haemolymph, and eating IAA triggers a complex splicing cascade in the brain, but IAA does not seem to be directly implicated in the intergenerational inheritance.

Germline consequences of a multigenerational metabolic disruption

Violeta de Anca Prado[1], Josep C. Jiménez-Chillarón[2,3], Carlos Guerrero-Bosagna[1]

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In recent years, transgenerational phenomena have been described in a variety of experimental models following exposure to certain environmental insults. However, not much is known regarding the dynamics followed by the genome and the germ line epigenome, in the generations when such exposure takes place. This is relevant for understanding the inheritance across generations. In this study was used a murine model of early life metabolic disruption by litter size reduction following the paternal germ line. Control dams nursed 8 pups, whereas overnutrition dams nursed 4 pups throughout lactation. This exposure was maintained for three generations, and sperm from adult males was collected after sexual maturation in each generation. We found no evidence of founder effect in the study, supporting the robustness of the model. Genomic analyses showed genetic similarity of two unrelated families from the exposed group, that the exposure explained part of the genetic variance between exposed and control animals, and the exposure was associated with the emergence and expansion of CNV events in the exposed group. These CNV were enriched in LINEs and LTRs, suggesting the involvement of transposable elements in their emergence. Although global methylome analyses did not show a strong treatment impact, regional differential analyses revealed altered sperm DNA methylation patterns in the exposed lineage, including in the same classes of TE enriched within CNV regions. Together, these findings suggest that metabolic challenge can alter both genome and methylome paternal germ line, with transposable elements as a candidate link between the two.

Epigenetic Inheritance of DNA methylation in an Early-Branching Animal

Lan Xu[1], Alex de Mendoza[1]

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DNA sequence mutations are the classical substrate of heritable variation, yet epigenetic states can also be transmitted across generations. DNA methylation, as an epigenetic mark, is frequently proposed as a potential mediator of phenotypic plasticity and transgenerational inheritance, but its role remain largely unclear in animals. Here we use the cnidarian *Nematostella vectensis* to study the function and inheritance of DNA methylation. We experimentally disrupted DNA methylation and tracked its consequences across somatic development, germline formation and transmission to the next generation. Loss of methylation produced viable embryos with limited transcriptional disruption but triggered widespread chromatin opening and spurious transcription, particularly from intragenic transposable elements. Methylation recovery was strongly lineage-dependent: somatic tissues showed limited restoration, whereas partial re-establishment occurred in the germline, revealing a fundamental asymmetry between soma and germline in the maintenance and restoration of epigenetic information. Because early embryos lack extensive epigenetic reprogramming, the aberrant methylation states can be inherited across generation. Remarkably, a proportion of demethylated individuals developed as hermaphrodites, producing both eggs and sperm. Hermaphroditism has never been reported in this species as they normally produce distinct males and females. Even more strikingly, some offspring produced through self-fertilisation also developed as hermaphrodites, indicating the intergenerational inheritance of this reproductive phenotype. Together, our findings demonstrate how incomplete epigenetic resetting in germline can allow epimutations to pass through generations, generating heritable regulatory and phenotypic variation with potential evolutionary consequences.

Developmental Dynamics of Annelid Methylation

Meghan Yap-Chiongco[1], Éloïse Defourneaux[1], Dorthea Brix Bovbjerg[1], Marie Høybye[1], Flora Lind-Rasmussen[1], Alessandro Formaggioni[1], Katrine Worsaae[1]

[1]Department of Biology, Marine Biology Section, University of Copenhagen, Copenhagen, Denmark

Methylation of cytosines in DNA (5mC methylation) is a highly conserved, epigenetic modification that has been shown to be a modulator of diverse biological processes. While studies have been largely restricted to vertebrate and invertebrate models, recent investigations focusing on segmented worms (Annelida) show diverse methylation landscapes from hypermethylation to complete loss in the miniaturized *Dimorphilus gyrociliatus*. 5mC is likely an important factor in early development as we see erosion of DNA methylation across ontogeny and functional studies disrupting 5mC in early development results in altered phenotype and/or death. To further understand the role of methylation in annelid development, we have sequenced time-course transcriptomes across the life history of the miniaturized, direct-developing annelid *Ophryotrocha labronica*. We then functionally tested the role of 5mC through treatment of embryos with cytidine analogs (zebularine and 5-azacytidine) to chemically impair DNA methylation. We find the core methyltransferase genes highly expressed in early development with a dip in the transitionary period between trochophore-like and juvenile stages. When treated with demethylating agents, we also see a dose-dependent effect in larvae resulting in embryo death and morphological defect. Our data supports past studies and highlights the necessity of 5mC during annelid development. We will further explore methylation patterning in the miniaturized *O. labronica* with base-resolution methylation sequencing and future functional studies to investigate the ability of 5mC to modulate developmental timing and pathways.

The genetic and epigenetic architecture of thermal plasticity in the threespine stickleback

Corin Stansfield[1], Kevin Parsons[1], Ana Costa[1,2], Gabriel Lawson-Duck[1], Bjarni Kristjansson[1,3], Skuli Skulason[1,3]

[1]School of Biodiversity, One Health and Veterinary Medicine, University of Glasgow, Glasgow, UK, [2]Rosenstiel School of Marine, Atmospheric, and Earth Science, University of Miami, Miami, USA, [3]Department of Aquaculture and Fish Biology, Holar University, Holar, Iceland

Climate change imposes extreme selection pressures on freshwater fishes, forcing many populations to adapt or face extirpation. How populations will adapt could involve plasticity, genetic, and epigenetic change, requiring a range of underlying mechanisms for phenotypic variation to change. The precise changes involved, and the relative contribution of these mechanisms is currently unknown. Here, we utilise Icelandic populations of the threespine stickleback displaying parallel patterns of rapid adaptive evolution in response to geothermal warming. We use a multi-generational hybrid cross to investigate the genetic and epigenetic architecture of thermally responsive traits and thermal plasticity. QTL analysis elucidated the genetic architecture of thermally responsive traits, and a novel statistical approach allowed the genetic architecture of within-generational plasticity (WGP) and transgenerational plasticity (TGP) for each trait to be determined. Our results provide candidate genes potentially underlying both forms of plasticity and show that TGP and WGP have distinct genetic architectures and involve different biological functions. Incorporating methylation data further allowed the genetic architecture of temperature-induced epigenetic variation to be elucidated, with candidate genes identified. We also show that the genetic architecture of traits is strongly influenced by both the parental and offspring thermal environments, suggesting a role for cryptic genetic variation underlying thermal plasticity. Our approach leverages the multiple routes that development can generate variation to gain insight into mechanisms underlying thermal plasticity and responses to a warming world.

15 | Evolution and development of digestive systems: from specialised cell types to complex ecosystems

Symposium S15: Thursday 11 June, 13:30 – 15:10

Organisers: Carmen Andrikou (Department of Biology, University of Patras, Greece, Rossella Annunziata (Stazione Zoologica Anton Dohrn Napoli, Italy, Maria Lorenza Rusciano (Stazione Zoologica Anton Dohrn Napoli, Italy)

The emergence of extracellular digestion and organised digestive systems is a fundamental innovation in animal evolution. Whether the ancestral digestive tract consisted of one end (blind gut) or two ends (through gut) is still debated. A through gut, possessing both a foregut and a hindgut, is only found in members of bilaterians, among which, great variations are witnessed. Despite morphological diversity, however, gut development is governed by conserved gene regulatory and morphogenetic programs, as confirmed by single-cell omics, which point to a common origin of gut cell types. Recent findings also highlight the gut as a central communication hub, linked to the brain and hosting complex microbiomes that influence host physiology and dietary adaptation. This symposium explores the origin and evolution of gut development and function across the animal tree, from non-bilaterians to chordates

S15.1 | Evolution and development of digestive systems: from specialised cell types to complex ecosystems

A blastoporal organizer in a ctenophore

Stanislav Kremnyov[1], Tatiana Lebedeva[1], Grigory Genikhovich[2], **Andreas Hejnol**[1]

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The embryonic organizer plays a fundamental role in early animal development by coordinating body axis formation and guiding tissue differentiation during embryogenesis. In a landmark experiment in 1924, Hilde Mangold und Hans Spemann demonstrated that the dorsal blastopore lip of amphibian embryos functions as an organizer capable of inducing a secondary body axis. This discovery established that specific embryonic regions can regulate large-scale patterning of the embryo. Later studies revealed that cnidarians also possess a blastoporal organizer, suggesting that organizer-based axial patterning predates the origin of Bilateria. However, the evolutionary origin of the organizer itself remains unresolved. Here we show that the blastopore lip of the ctenophore *Mnemiopsis leidyi* possesses organizing activity. Transplantation of gastrula-stage blastoporal lip tissue efficiently induces ectopic pharynx and mouth formation in host embryos, directly linking organizer activity to the establishment of the embryonic digestive opening. Furthermore, in a trans-phyletic transplantation experiment, the blastopore lip of *Mnemiopsis* is able to induce a secondary body axis in embryos of the cnidarian *Nematostella vectensis*. Pharmacological and molecular analyses indicate that TGFβ ligands acting through TGFβ type I receptors likely mediate the inductive capacity of the ctenophore blastoporal organizer tissue. These findings support deep homology of blastoporal organizers across ctenophores, cnidarians, and vertebrates, suggesting that the blastopore lip functioned as an organizing center in the last common ancestor of Metazoa. Our results therefore link the evolutionary origin of the embryonic organizer to the emergence of the blastopore and the establishment of the first digestive opening in animal embryos, highlighting organizer-based axial patterning as a key innovation underlying the evolution of animal body plans.

S15.2 | Evolution and development of digestive systems: from specialised cell types to complex ecosystems

Comparative transcriptomics reveal the common anteroposterior molecular blueprint of adult bilaterian guts

Stefano Davide Vianello[1,2,3], Ching-Yi Lin[4], Wahyu Cristine Pinem[5,4], Han-Ru Li[4], Kun-Lung Li[4], Grace Sonia[4], Shu-Hua Lee[1], Szu-Kai Wu[1], Vincent Laudet[1,6,7], Yi-Hsien Su[4], Jr-Kai Yu[1, 4], Stephan Q Schneider[4, 5]

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(EARLY), Okinawa Institute of Science and Technology Graduate University, Onna son, Japan
A through-gut is one of the major features of bilaterians. Comparative work among bilaterians has identified common molecular mechanisms during early gut patterning, but the primordial gut later often undergoes different degrees of reorganization in each lineage to form a fully differentiated adult gut with specializations along its anteroposterior (AP) axis. Yet, how the conserved embryonic gut AP pattern relates to the adult guts in diverse bilaterians after metamorphosis is still poorly understood. To unravel the molecular subdivisions of adult guts, we investigated the gut through transcriptomic analyses of five phylogenetically informative species: an annelid, a sea urchin, a hemichordate, a cephalochordate, and a vertebrate. We identified bipartite transcriptional programs defining the AP functional subdivisions. Patterning systems composed of Hox, ParaHox, and, surprisingly, other transcription factors (TFs) known to be involved in gut formation in sea urchin larvae are maintained in these adult tissues. Using unbiased analyses, we identified five conserved TF modules corresponding to the AP compartments of the guts that are elaborated or shifted in different species. Our study inferred conserved and modified adult AP patterning modules along bilaterian guts enabling the reconstruction of ancestral bilaterian features with profound implications for the evolution of the bilaterian body plan.

A new animal model for dissecting drug transporter functions during gut maturation and host-microbe interactions

Catherine Schrankel

Department of Biology, San Diego State University, San Diego, CA USA

The transition from endoderm progenitors to a functionally differentiated intestinal barrier requires the precise timing of molecular mechanisms that will allow the host to distinguish between symbiotic or pathogenic "non-self." To investigate how the building gut establishes this equilibrium, we utilized a transgenic sea urchin model, *Lytechinus pictus*. This transparent organism allows for high-resolution imaging of the gut as it undergoes primary specification to terminal differentiation within 48 hours.

We show that a novel, yet critical component of the endoderm toolkit is the ATP-binding cassette (ABC) transporter, ABCB1/P-glycoprotein. While it is known that loss of ABCB1 activity drives dysbiosis and inflammatory bowel disease (IBD) across vertebrate gut epithelia, the developmental point at which gut cells require ABCB1 function to prevent microbe-mediated inflammation is unknown. In the developing *L. pictus* embryo, ABCB1 is enriched to the apical membrane as the endoderm matures into a simple tube. Using homozygous knock-out lines, we identified that in the absence of ABCB1, developing gastrulae exhibit premature epithelial inflammation and a failure to organize a competent gut barrier. Paired 16S rRNA sequencing reveals that without the functional transporter, feeding larvae also fail to recruit a healthy microbial assemblage. The commensal community of ABCB1-KO larvae remains reduced in richness and diversity during further larval development up to metamorphosis.

We propose that the ABCB1-mediated export of metabolites, lipid intermediates, and inflammatory molecules is already active while maturing endoderm cells transition from a naive state to terminal gut cell types. New tools in our *L. pictus* model now enable us to directly test the genetic and cellular mechanics of when and how differentiating epithelia use xenobiotic transporters to permit healthy colonization while still resisting potential "foes".

S15.4 | Evolution and development of digestive systems: from specialised cell types to complex ecosystems

Gut evolution, from cell types to gene regulatory networks

Maria Ina Arnone[1], Giovanna Benvenuto[1], Periklis Paganos[1], Lorenza Risciano[1]

[1]Biology and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrm, Napoli, Italy

All living organisms depend on their cells' ability to take in and process molecules in order to obtain energy. As multicellularity emerged during evolution, specific cell types and organs took on the role of catching food particles, decomposing them, and absorbing nutrients. The development of the animals' gut is controlled by an intricate network of gene regulatory systems and morphogenetic processes that direct the differentiation of each compartment. Recent single cell omics techniques have nicely verified that these mechanisms exhibit a considerable degree of conservation among animals, suggesting a common origin of the various gut cell types. Sea urchins, non-vertebrate deuterostome animals displaying a variety of body plans and lifestyles—both planktonic and benthic—that are able to feed and develop while interacting with their environment, are key to study gut evolution. Specifically, we use single-nucleus transcriptomics to examine the extent to which the sea urchin pluteus larva and the juvenile that emerges from it during metamorphosis share developmental gene regulatory networks and cell types. Focusing on the digestive system, and integrating single cell transcriptomics, spatial gene expression analysis, and high-resolution electron imaging, we identified a wide variety of gut cell types, including pancreatic-like ones that predate the emergence of the pancreas as a distinct organ.

16 | Modularity, robustness and evolvability

Symposium S16: Thursday 11 June, 09:00 – 10:40
Contributed Session C16: Thursday 11 June, 11:05 – 12:30

Organisers: Roland Zimm (Dublin City University, Ireland, Frietson Galis (Naturalis Biodiversity Center, the Netherlands)

The purpose of this symposium is to promote dialogue among researchers on the three core concepts of evo-devo: modularity, robustness and evolvability. Our aim is to explore various approaches to clarify the relationships between these concepts. Understanding these relationships should connect Evo with Devo. To this end, we have selected speakers who can shed light on various aspects of these relationships, in the hope that this will lead to a more comprehensive synthesis.

For example, they will discuss the relationship between sex determination, robustness and evolvability, and providing a perspective on robusness research in plants more generally; they will present their research on tooth modularity and evolvability; they will discuss the integration of Evo-Devo and population genetics, because modularity and robustness (canalization) were the first organismal Evo-Devo concepts that were studied using using population genetical approach; finally they will provide a morphometric and computational evo-devo perspective.

S16.1 | Modularity, robustness and evolvability

The process-specific GP maps and their interaction may explain micro-to-macro divide

Mihaela Pavlicev

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The potential for evolutionary change is deeply anchored in the amount and pattern of heritable phenotypic variation that organisms can produce, and thus in the way that genetic predispositions translate into the phenotype, the GP map. Thinking about the structure of the GP map and its consequences is not new. Especially in the context of evolvability, robustness and modularity immediately come to mind. In my talk, I will suggest that these principles, while key, are not sufficient to explain patterns of evolution. I will propose that the GP map is not unitary, but instead a mosaic of processes with different GP patterns, and process-specific evolvability, including both the genetically varying and also the conserved traits. The latter often create the boundary conditions for variation in the former. I will illustrate this on vertebrates, where metamorphosis, growth, and maintenance can be seen as such different sub-GP maps. It is when we think of these together, and in particular how they mutually influence each other, that we may be able to understand the micro- and macroevolutionary patterns.

Modularization of the plant body plan and the rise of flowering plants

Rainer Melzer

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Plants and animals differ fundamentally in their development, with plants possessing higher degrees of plasticity and a lower level of robustness than most animals. There is however one structure in plants that is an exception from this general observation: the flower. Variations in the floral bauplan are often diagnostic for larger taxonomic groups and smaller alterations in the structure of the flower are used to delineate species boundaries. This shows that the flower possesses a high degree of evolutionary and developmental robustness. At the same time flowering plants are also by far the most species-rich and morphologically diverse group of land plants. Non-flowering plants possess reproductive structures that also fulfil vegetative functions like photosynthesis and thus require plastic responses to environmental variation. This is different for flowering plants: the flower as an evolutionary novelty has primarily a reproductive function and is developmentally clearly separated from vegetative parts of the body plan. With the separation of reproductive and vegetative functions plants evolved the capacity to respond with plasticity to changing environments and simultaneously pursue robust reproductive strategies. Here, I argue that this increased modularization and specialization of the plant body plan was a major factor in the success of flowering plants.

Modularity and morphological innovation : bridging master genes with developmental systems properties

Océane Chanel[1], Angélique Préaubert[1], Claudine Corneloup[1], Theotime Grohens[1], Pascal Hagolani[1], Marie Sémon*[1], **Sophie Pantalacci**[1]

[1]LBMC, ENS Lyon, Lyon, France

Mammals exhibit a wide diversity of molar shapes, with varying degree of similarity between lower and upper molar. Paleontological data show how this degree of similarity fluctuates along given mammalian lineages. While the ancestral state in mammals is to have very different lower and upper molar shapes, the common ancestor of mice and hamster displayed much more similar shapes, with two different outcomes in the mouse and hamster lineage: golden hamster kept similar molars, while the mouse upper molar evolved a radically new morphology with two additional cusps. By combining timeseries data in embryos, transcriptomic data (bulk and single-cell) and *in silico* evolutionary simulations, we ask how modularity evolves in this developmental system, coping with pleiotropy and still enabling morphological innovations. We characterized an ancestral modularity in molar development of mouse and hamster, likely dating back to early mammals. This modularity involves a handful of conserved master genes with strict expression specificity and conserved dynamics of molar development along the bucco-lingual and antero-posterior axes. This results in a transitory developmental stage, during which upper and lower molars can clearly be identified. This stems from earlier differences in relative abundance of the molar germ cell types with limited differences in cell type identity. We further show how the new mouse morphology evolved in line with this ancestral modularity, but also involved pleiotropic developmental changes. Our work characterizes modularity as a developmental system property, in which the conservation and evolution of organ-specific master genes is just the tip of the iceberg.

Multi-scale devo-evo in a bite: nesting variation and evolvability of complex dentitions and beyond

Roland Zimm[1,2]

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Functional complex traits often depend on the fine-tuned coordination of developmental mechanisms that are hierarchically nested in time and space. A notorious example are vertebrate dentitions that generate hyper-diverse and complex phenotypes with reiterative features - from minute cusplets and serrations within individual teeth to jaw-level differences (dignathic heterodonty) - by sequential activation of partially coopted gene regulatory networks (GRNs). Such a hierarchically nested developmental architecture suggests that potentially adaptive morphological variation can originate at different ontogenetic levels. Combining novel morphometric tools and genomic information, I leverage comprehensive phenomic data from shark dentitions across all extant clades to show that intermediate morphological scales give rise to more ecologically relevant variation than large or small scales that emerge, respectively, early and late in development. While a functional explanation cannot be ruled out for periodic ectodermal organs, general *in silico* modular genotype-phenotype maps under open-ended artificial evolution suggest a systems-independent pattern linking intermediate developmental stages to increased evolvability. Interestingly, this finding appears to emerge from long-term selection of development itself and showcases the reciprocal relationship between developmental structure and its macro-evolution.

Evolutionary tuning of Nodal mobility ensures robust embryonic patterning across temperatures

Daniel Čapek[1-4], Hernán Morales-Navarrete[5], Thomas Thumberger[6], Johanna Bihler[1], Oleksandr Riabtsev[1], Theresia Eich[1], Baubak Bajoghli[7], Jochen Wittbrodt[6], Patrick Müller[1,2,8]

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Poikilothermic embryos must maintain stable body plan formation despite large environmental temperature fluctuations. The Japanese ricefish medaka (*Oryzias latipes*) develops across a thermal range of nearly 20 °C, accelerating in warm and slowing in cold conditions. To preserve correct germ layer patterning, Nodal-dependent morphogen gradients must therefore rescale with developmental rate. Classical biophysical principles predict that biochemical reactions scale exponentially with temperature, whereas diffusion changes only marginally. How morphogen-based patterning remains robust under these asymmetric constraints remains unclear. Using fluorescence decay after photoconversion (FDAP), fluorescence correlation spectroscopy (FCS), and fluorescence recovery after photobleaching (FRAP), we quantified temperature-dependent changes in morphogen degradation and mobility in medaka embryos. While developmental speed, morphogen production, and degradation scaled in an Arrhenius-like manner, effective Nodal mobility remained remarkably constant across temperatures. Despite proportional rescaling of gradient dimensions, diffusion did not measurably increase. These findings indicate that medaka embryos operate in a reaction-dominated regime in which strong ligand–receptor interactions constrain Nodal mobility, minimizing the contribution of diffusive transport to gradient scaling across temperature ranges. We propose that evolutionary tuning of Nodal mobility represents a quantitative adjustment of biophysical parameters that enhances developmental robustness in thermally variable environments. Such tuning may arise through changes in ligand–receptor interactions, receptor abundance, or tissue-level physical constraints on diffusion, illustrating how robustness can evolve through coordinated parameter shifts across molecular and tissue scales.

Developmental heterochrony underlies the evolution of extreme body axis segmentation in the Japanese eel

Ali Seleit[1,2], Kazuharu Nomura[3], Leon Hilgers[4,5], Michael Hiller[4,5], Kiyoshi Naruse[6], Yukinori Kazeto[7], Alexander Aulehla[1]

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Vertebrate body plan evolution is driven by heterochrony, yet the underlying molecular and cellular mechanisms remain poorly characterised. Here, we show that extreme axis segmentation in *Anguilla japonica* is driven by an extension of somitogenesis. This process relies on a temporally expanded stemness program coupled to a tightly controlled tissue scaling regime that operates under the constraint of minimal axis growth. We show that this mode of segmenting does not depend on changes in the segmentation clock tempo or topology; instead, delayed *Hox13* activation sustains *Oct4* expression, extending stemness in time. And distinct pools of neuromesodermal progenitors in the tail—a cellular innovation—extend stemness in space. These findings reveal how the spatiotemporal modulation of stemness drives extreme vertebrate morphological evolution. Taken together, our results highlight how the modularity of developmental processes drives evolutionary innovation, allowing heterochronic shifts to uncouple somitogenesis from overall developmental progression. We propose that the dissociability of developmental modules represents a fundamental strategy for morphological evolution in vertebrates.

Role of plasticity and developmental robustness in the evolutionary process and insights into genetic assimilation

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The role of developmental plasticity and/or genetic assimilation in the evolutionary process has been a central interest for evolutionary biologists for decades. However, since the transgenerational culture of animals for many generations is not feasible apart from a few exceptions, testing related hypotheses is no easy task. To address this, we compared two sister species of tunicates, Ciona robusta (type A) and Ciona intestinalis (type B), which shows different levels of robustness to thermal stress. By comparing the gene network of the two sibling species and compare plasticity to thermal stress in both species during embryogenesis, we investigated role of plasticity in the evolutionary process. The distinction of high robustness and low robustness (<80% normal development after thermal stress at neurula stage) was clearly recognized by Random Forests, a Machine Learning technique. Previous study showed that robustness is maternally inherited; however, the classification was done more precisely by neural stage transcriptome data, rather than oocyte transcriptome data, suggesting that maternally controlled epigenetic regulation is involved in determining robustness. Using Differential Gene Co-Expression analysis, we found that robust network contains more network modules. The gene network of higher robustness contains larger variation in gene expression. Furthermore, we found changes of gene network in common with changes between the gene network of sibling species. We suggest that robust network has more plasticity that can be assimilated to the evolutionary change.

Regulatory dominance promotes robustness and evolutionary innovation

Noa Ottilie Borst[1], Timothy Fuqua[1,2], Fabian Ruperti[1,3], Justin Crocker[1]

[1]Developmental Biology, European Molecular Biology Laboratory (EMBL), Heidelberg, Germany, [2]Institute of Ecology and Evolution, University of Bern, Bern, Switzerland, [3]School of the Environment, University of Queensland, Brisbane, Australia

Developmental enhancers drive precise gene expression patterns, yet mutations often substantially alter regulatory output, raising a paradox: how can such fragile regulatory elements evolve while maintaining function? Here, we dissect the evolutionary trajectories of a pleiotropic developmental enhancer in *Drosophila*. A genotype–phenotype map between *D. melanogaster* and *D. simulans* enhancer sequences reveals extensive epistasis, and many homozygous evolutionary paths reduce transcriptional output. In heterozygous genotypes, however, regulatory dominance masks variants that reduce gene expression, potentially relaxing constraints on evolutionary trajectories. Using allele-specific reporters and imaging, we show that this dominance arises from interallelic interactions (also known as transvection) reinforced by transcriptional hubs. Importantly, we find that this enhancer dominance is cell-type specific, raising the possibility of it concealing deleterious effects in essential tissues while revealing novel, ectopic activity in others. Thus, enhancer dominance in diploid regulatory architectures may reconcile robustness with evolvability.

Environmental Noise and the Evolution of Evolvability

Masahito Tsuboi[1], Lisandro Milocco[2], Masato Yamamichi[3], Nobuto Takeuchi[4], Saburo Tsuru[5]

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Modularity and robustness are central to evo-devo, yet their evolutionary origins remain unresolved. A classic observation is that developmental architecture — which generates modularity, robustness, and evolvability — often remains stable over long evolutionary timescales. Why does phenotypic variation consistently occupy a restricted, low-dimensional space, and how does such robustness persist despite drift and changing external environments? I present empirical evidence that axes of developmental bias within populations are systematically aligned with evolutionary divergence across timescales. In insect wings, phenotypic variance arising from local environmental perturbations (fluctuating asymmetry) predicts both short-term evolutionary responses and macroevolutionary diversification. In sperm morphology, within-ejaculate variability mirrors patterns of among-male and among-species differentiation. Parallel results from gene expression studies suggest that this congruence between micro- and macroevolutionary variation may be widespread. Revisiting classical theories of canalization, I argue that these observations can be explained if environmental noise — subtle but persistent variation in external conditions — is a key driver of variational structure. Rather than merely perturbing development, environmental fluctuations may favor genetic canalization in dimensions repeatedly exposed to stochastic variation, thereby stabilizing developmental architecture. This process generates robustness along some axes while leaving others comparatively labile. By embedding canalization theory within a quantitative genetic framework, this perspective connects robustness, modularity, and evolvability as emergent properties of population-level dynamics under environmental noise. If so, stochastic environmental variability is not a statistical nuisance to be removed from evolutionary explanations, but a fundamental driver of the evolvable structure of development.

C16.6 | Modularity, robustness and evolvability

Developmental system drift as a consequence of the evolution of modularity

Áine McColgan[1], James DiFrisco[1]

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Developmental system drift (DSD) occurs when the genetic basis for homologous traits diverges over time despite conservation of the phenotype. Although DSD is known to be driven by neutral molecular change and by phenotypic robustness, it can also be a natural consequence of the evolution of modularity.

Modularity can be understood as *limited pleiotropy* across functionally distinct traits. When there are conflicting selection pressures on pleiotropically linked traits, genetic systems can be expected to respond by reducing pleiotropy or compensating for deleterious pleiotropic effects. This response to selection re-shapes developmental modules to align with selection gradients, but a by-product of this process is re-wiring of regulatory connections and compensatory changes that may not visible at the level of the phenotype. Traits that have remained stable through this process stay conserved while their genetic underpinnings have changed.

This presentation will provide a conceptual model of how DSD arises as a consequence of the evolutionary re-partitioning of developmental modules. It also explores how DSD may influence evolvability. Phenotypic robustness allows populations to explore genotype space without deleterious fitness effects, thereby accessing genotypes that, when combined with environmental changes or further genetic changes, can give rise to potentially adaptive phenotypes. I present the theory behind these processes and outline the systems biology methodologies that can be implemented to study them.

17 | Micro-Evo-Devo: Developmental Origins of Within-Population Variation

Symposium S17: Thursday 11 June, 13:30 – 15:10
Contributed Session C17: Thursday 11 June, 15:30 - 16:40

Organisers: Robin Pranter, (Max Planck Institute for Evolutionary Biology, Germany), Lisandro Milocco (Department of Zoology and SciLifeLab, Stockholm University, Sweden)

Understanding evolution requires understanding the variation on which selection acts. Classical evo-devo has shown how developmental rules shape differences between species but has rarely addressed how developmental processes generate subtle phenotypic variation within populations. This emerging "micro-evo-devo" perspective is key to integrating developmental biology with population-level evolution and to advancing evo-devo toward a predictive science relevant to rapid environmental change.

Recent advances in high-resolution imaging, single-cell and spatial transcriptomics, computational modeling and theory now make it possible to connect developmental mechanisms directly to population-level variation. Together, the talks in this symposium will outline an interdisciplinary roadmap for connecting developmental processes to evolutionary change within populations.

Variation lost in translation between micro- and macroevolution

Jukka Jernvall[1, 2], Aida Kaffash Hoshiar[1], Otto Stenberg[1], Outi Hallikas[1]

[1]Institute of Biotechnology, University of Helsinki, Helsinki, Finland, [2]Department of Geosciences and Geography, University of Helsinki, Helsinki, Finland

The fossil record mammalian molar teeth provide many examples of large changes in morphology. Especially lineages specializing to eat fibrous vegetation have evolved molars with novel features. How new morphologies, ultimately linking micro- to macroevolutionary patterns, emerge within species remain difficult to study. One challenge is that novel morphological features distinguishing higher taxa may lack transitional forms due to development producing discontinuous variation. Here we will show how seemingly discontinuous variation in dental morphology can be linked to dental variation within populations. To do this, we use experimental mouse models whose expression levels of patterning genes are finely modified. Analytically, data mining and machine learning approaches are then used to link discontinuous macroevolutionary changes to population level microevolutionary variation. These results suggest that without developmental biology insights, phenotypic analyses tailored for microevolutionary studies may not be directly translatable to macroevolutionary patterns.

Epigenetic changes alter developmental variance in isogenic mouse models

Rebecca Green[1], Justin Lopes[1], Christian Waronsky[1], Matthew Jones[1], Michelle Leong[2], Lucas LoVercio[2], Benedikt Hallgrimsson[2]

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Gene by environment and other epigenetic events can wreak havoc in some mouse models, leading to phenotypic variation and other variably penetrant birth defects. The A/J and A/WySn iso-genic mouse lines have altered methylation at the Wnt9b-IAP retrotransposon in subsets of embryos. These embryos display altered gene expression, increased phenotypic variance and develop cleft lip and palate. Mice with null mutations in B9d1, a transition zone gene in primary cilia, display a range of phenotypes, including variable midfacial narrowing. As cilia defects often lead to neural tube defects, and folic acid is a known modifier of neural tube closure, we were interested in testing the hypothesis that dietary folate would affect phenotypic variance in B9D1 null animals by altering DNA methylation and gene expression variance. This talk will highlight recent work on these two project, with emphasis on how 3D morphological quantification can help answer these micro-evo questions.

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

Erica Nadolski, Armin Moczek

[1]Biology, Indiana University, Bloomington, USA

Sexual dimorphism represents one of the most significant dimensions of intraspecific variation, with the potential to constrain or facilitate the diversification of novel traits and functions on both micro- and macro-evolutionary timescales. Yet, rather than exhbiting equal sexual dimorphism across all traits, organisms are better conceptualized as mosaics of tissues that vary in the degree to which they exhibit dimorphism. While the ecological and fitness relevance of sex-biased development is often well-characterized, the genetic and developmental mechanisms underlying sexual dimorphism and its evolution are not. Using a trio of closely-related horned beetle species, my work assesses the regulatory mechanisms that instruct sex-biased development along two critical axes of diversification: divergence among body regions and evolutionary diversification across species, with the ultimate goal of identifying genomic mechanisms by which sexual dimorphisms originate and diversify. Among others, I test the following hypotheses: that (i) evolutionary elaboration of sexual dimorphism is made possible through both the rapid acquisition of novel cis-regulatory elements (CREs) and the elaboration of sex- and trait-specific transcriptomes, and that (ii) the evolution of novel secondary sexual traits is facilitated by the evolution of novel regulatory interactions, rather than the re-use of pre-existing regulatory machinery. I discuss my results in light of fundamental questions regarding gene regulatory network evolution: are the networks that instruct the formation of a trait the same as those that facilitate its diversification, or is context-responsiveness decoupled from trait formation in development and evolution?

Changing developmental signal has an effect on developmental bias: Wnt mutation modifies phenotypic co-variation patterns for cell fates in the nematode vulva

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While mutation is mostly random at the DNA level, realized co-variation between traits following random perturbation can be strongly biased along some directions of phenotypic space. Development is often invoked as an explanation as to why some combinations of trait values are more probable to co-occur than others. However, studies that modify the accessible phenotypic space though a manipulation of development are lacking. We use variation in cell fate of nematode vulva precursor cells to ask if reduction of morphogen signal is sufficient to change the co-variation pattern of these cells. Previous work in the lab has demonstrated that in *Caenorhabditis*, the most posterior cell, P3.p, is sensitive to random genetic perturbations while the fate of the next most anterior cell, P4.p, is not (Picao-Osorio *et al* 2025). Posteriorly expressed Wnt signal, encoded by two genes, *egl-20* and *cwn-1*, is important for promoting the divided cell fate in Pn.p cells in *C. elegans*. We reduced the Wnt signal dose using a *cwn-1* deletionand assayed the accessible phenotypic space of this mutant. The large-effect *cwn-1* mutation results in a shift in the phenotypically accessible space, as P4.p becomes sensitive to random mutation, in contrast to P3.p being sensitive in the wild-type line. This is a direct example of how changes to a developmental signal can modify the realized co-variation of traits following perturbations.

C17.1 Micro-Evo-Devo: Developmental Origins of Within-Population Variation

Organismal-level regulation of phenotypic variability in *Drosophila*

Saudat Alishayeva, **Luisa F. Pallares**

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Within-population phenotypic variation is the raw material for evolution, yet its developmental origins remain poorly understood. In particular, whether developmental noise results in higher phenotypic variability through the genotype-phenotype map remains underexplored. To address this question, we used an outbred population of Drosophila melanogaster and collected genetic and phenotypic variation for ~1000 individual flies. The phenotypes spanned a range of ecologically and functionally relevant traits—including flight performance, wing shape and size, and fluctuating asymmetry as a proxy for developmental robustness.

We found that phenotypic variability is not a trait-specific property but an organismal property – that is, the degree of developmental robustness seems to propagate across morphological and functional traits within an individual. Using ~400,000 genome-wide SNPs, we mapped the genetic basis of trait mean and trait variability and found that, in contrast with trait means which tend to have an additive genetic architecture, trait robustness has instead a strong epistatic nature. The genomic loci associated with organismal-level robustness are enriched in developmental and morphogenetic processes, suggesting that the regulation of tissue differentiation and organ development may underlie the global control of phenotypic variability in Drosophila.

Using a micro-evo-devo approach, we show that phenotypic variability is an individual-level property with clear genetic basis distinct from the mean. This suggests that, at the within-population level, variability, as any other trait, could be subject to selection.

C17.2 Micro-Evo-Devo: Developmental Origins of Within-Population Variation

Determining hotspots of cellular adaptation in the polychaete *Platynereis dumerilii*

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Cosmopolitan organisms possess the ability to easily adapt to changing environments. Although organismal adaption occurs at the level of cell types and tissues the molecular mechanism and the microevolutionary processes involved in response to changing conditions such as anthropic pressure, remain poorly understood. We focus on the marine polychaete *Platynereis dumerilii* which inhabits a wide range of ecosystems and for which a comprehensive morphological and transcriptomic celltype atlas is available. By comparing at whole body scale transcriptomic differences across cell types at single-cell resolution, we seek to identify cell types that are prone to environment-driven change. In the framework of the TREC expedition we have sampled multiple *P. dumerilii* populations across European coastal sites, including the Atlantic, Med Sea and North Sea. Strikingly, worms from Sweden show a clear shift of cell type abundances when compared to other populations independent of the developmental timing. Utilizing transcriptomic data allowed the identification of many population-specific SNPs that can be assigned to cell types and used to determine which cell types are more or less affected by SNPs. Taken together, our data suggests that cell type specific adaptations have occurred on the genomic, transcriptomic and tissue level which will help to predict the effects of changing environments on cellular machineries and gene expression networks. While traditional population genetics rely exclusively on DNA variants to study diversity, structure, and selection, we aim to integrate scRNA-seq, allowing to identify the genetic composition of cells and at the same time understand their functional states.

C17.3 Micro-Evo-Devo: Developmental Origins of Within-Population Variation

Morphometric quantification of temperature stress effects on embryonic development at single-cell resolution

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[1]Michael Sars Centre, University of Bergen, Bergen, Norway, [2]Department of Biology, Center for Developmental Genetics, New York University, New York, USA

Embryonic development is programmed to produce a narrow range of phenotypes, yet increased environmental pressure can lead to deviations from canonical developmental outcomes. Temperature is a key environmental variable that strongly influences cell physiology and developmental dynamics. As ocean temperatures continue to rise, it is increasingly important to understand how thermal pressure affects development and whether it can give rise to novel phenotypes. Due to its highly stereotyped development and small cells number, the tunicate *Ciona intestinalis* represents an excellent model for detecting deviations from canonical development at single-cell resolution. Here, we profiled early embryonic development in *Ciona* at different temperatures to identify the limits at which development deviates from the canonical pattern, as well as the temperature-dependent scaling of developmental tempo. We acquired confocal 3D image stacks of embryos spanning from gastrula to tailbud stage at a reference permissive temperature, along with matched developmental time points at lower and higher temperatures. 3D reconstructions were generated through membrane and nuclei segmentation, and quantitative morphological parameters were extracted at multiple scales: single cell, tissue, and whole-organism levels. We find that although overall morphology becomes increasingly perturbed at high temperatures across scales, tissue identities are largely maintained. Sensitivity to thermal stress varies across tissue types, with the notochord and neural tube exhibiting the strongest defects. These findings suggest that thermal stress affects not only morphology but also tissue organization. By integrating this morphological dataset with transcriptomic data, we aim to identify molecular candidates explaining differential sensitivity to thermal stress during embryogenesis.

C17.4 Micro-Evo-Devo: Developmental Origins of Within-Population Variation

Investigating the genetic and developmental bases of compound eye size variation among populations, species and temperatures

Beñat Yañez Iturbe-Ormaeche[1], Heshika Hematilake[2], Javier Figueras Jimenez[1], Amber M. Ridgway[2], Madeleine E. Aase-Remedios[1], Maike Kittelmann[2], **Alistair P. McGregor**[1]

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Insect compound eye size shows striking variation between sexes, populations and species caused by differences in ommatidia number and size, which directly impact vision. For example, *Drosophila mauritiana* generally has larger eyes composed of wider ommatidia and consequently greater contrast sensitivity than *D. simulans* and *D. melanogaster*, but each of these species exhibit extensive intra-specific variation and plasticity in eye size. However, the genetic bases of inter- and intra-specific eye size variation and how developmental temperature impacts eye size regulation mechanisms are poorly understood. To identify shared and private pathways and genes that underly differences in ommatidium size and number within and among species, we compared RNA-sequencing data from eye imaginal discs of different populations of *Drosophila melanogaster*, *D. mauritiana* and *D. simulans*. We found that most intra-specific variation in gene expression is not shared between species, suggesting population-level differences in eye development regulation are species-specific. Consistent with this, more distantly related species showed more differentially expressed genes. These analyses also allowed us to identify and test differentially expressed candidate genes located within previously mapped QTLs for eye size variation. We also explored how developmental temperature affects eye size and gene expression during eye development, and found substantial variation across species. Interestingly, candidate genes for eye size thermal plasticity showed little overlap with candidates for population and species differences. In summary, although eye size is thought to be tightly controlled during development, the GRN harbours extensive variation underlying eye size differences within and between species, and environmental conditions.

C17.5 Micro-Evo-Devo: Developmental Origins of Within-Population Variation

Can annual fish become a model for micro-evolution and adaptation of development?

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We aim to understand how development generates variation, and how selection acts on it in changing environments. In order to have responses to selection across generations, some variation needs to be heritable.

Since 2000, different killifish species have become models for stress responses, ageing, hermaphroditism or diapause. We review the current state of knowledge on sources of developmental variation in annual killifish. Several studies have explained developmental variation as bet-hedging, maternal effects or phenotypic plasticity, but the existence of heritabilities due to genetic variation has not often been considered.
Variance components demonstrated in several studies could be attributed at least in part to quantitative genetic variation. We complement this with results of three studies explicitly designed to demonstrate additive quantitative genetic developmental variation.
The results: it exists and coexists with other sources of variation. Genetic variation for maternal effects, plasticity and environmental sensitivity remain to be investigated. However, with one exception, these demonstrations occurred in lab environments. Since about 1945, the study of natural selection in the wild has advanced our knowledge of short-term evolution in post-embryonic stages a lot. For eco-evo-devo to catch up, we will need to study developmental quantitative genetic variation in the field.

18 | Evo-Devo of plant forms & patterns

Symposium S18: Thursday 11 June, 13:30 – 15:10
Contributed Session C18: Thursday 11 June, 15:30 - 16:40

Organisers: Pierre Galipot (MNHN, France), Edwige Moyroud (Sainsbury laboratory, University of Cambridge, UK)

Objects of interest to humans due to their visual immediacy, plant forms and patterns participate in numerous biological functions at all scales. Despite the many constraints shaping their potential morphogenetic landscape, an immense diversity of shapes is displayed by plants, particularly on leaves and flowers, but also in terms of spatial arrangement of organs. The study of their formation -morphogenesis- and its evolution are historical disciplines, but the highly integrative nature of its objects means that many gray and unexplored areas remain. Thus, paradigms regularly need to be overcome, and new ways of approaching them seem necessary. To this end, this session will explore the diversity of current research in evo-devo of plant forms and patterns through a variety of biological models, approaches and scales.

S18.1| Evo-Devo of plant forms & patterns

Conserved modules, novel forms: Investigating the mechanisms underlying grass morphological diversity

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Evolution repeatedly generates novel forms by reusing and modifying conserved developmental programs, yet the specific mechanisms driving such innovation remain unclear. In the grass family (Poaceae), the lemma, comprising a lemma body and an awn, is homologous to the leaf sheath and leaf blade, respectively. Unlike the morphologically uniform leaves, lemma and awn diversity is vast, ranging from straight photosynthetic awns in barley to bent, hygroscopic awns in oats. This research tests whether the differential activation and positioning of conserved leaf developmental modules drives this morphological innovation. To identify the genetic and developmental mechanisms driving morphological diversity of lemmas we use barley (*Hordeum vulgare*) awn mutants. These mutants exhibit contrasting leaf and lemma phenotypes, demonstrating that conserved programs can be "decoupled" and independently regulated. By analysing developmental series of wild-type and mutant meristems, we pinpointed critical stages where developmental trajectories diverge. To determine if this mechanism governs family-wide diversity, we use the ligule doman at the blade-sheath boundary as a model domain. We contrast two species with divergent lemma morphologies: blackgrass Alopecurus myosuroides, which features proximal awn initiation and a predicted expanded ligule-like domain, and Brachypodium distachyon, where awns initiate at the distal tip. By manipulating genetic regulators of ligule development in these morphologically distinct species, we test whether suppressing conserved domains is sufficient to generate novel forms. This work investigates whether evolutionary innovation arises through modular reuse, providing insights into how the reuse of conserved developmental programs drives morphological diversity.

S18.2 | Evo-Devo of plant forms & patterns

Stay hydrated – Deciphering the development and physiology of leaf succulence

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Succulent species have evolved anatomical and physiological features to thrive in dry growth environments. Their succulent organs, like leaves, stems, or roots, can store- and remobilize water during water-scarce growth periods. In addition, most succulent plants use Crassulacean Acid Metabolism (CAM), the most water-use efficient mode of photosynthesis, where atmospheric carbon uptake is temporally decoupled from fixation into carbohydrates. Evolutionary and comparative studies have identified correlations between succulent anatomy and CAM establishment; however, a functional link remains elusive. Here, we established the constitutive CAM leaf succulent *Kalanchoë laxiflora* as an experimental model system. Strikingly, *K. laxiflora* shows a developmental transition from C3 photosynthesis in young leaves to CAM photosynthesis in mature leaves. This transition enabled the detailed description of anatomical, physiological, and metabolic differences between C3, transitioning, and CAM leaves within a single plant individual. We observed increasing succulent anatomy accompanied by a gradual transition from C3 to CAM photosynthesis, with succulent anatomy development preceding CAM physiology. This indicates that succulent anatomy not only enables water-storing capacity but might also be required for efficient CAM photosynthesis. We currently perform single-nucleus transcriptomics, gene editing, and forward genetics to identify developmental modules required for leaf succulence establishment. Through the manipulation of distinct anatomical leaf traits, we will be able to experimentally link anatomical aspects of leaf succulence to CAM physiology and, with that, understand strategies of succulents to survive prolonged drought periods.

S18.3 | Evo-Devo of plant forms & patterns

Unusual Morphogenesis in *Monophyllaea*: Indeterminate Leaf Meristems and the Absence of a Shoot Apical Meristem

Hirokazu Tsukaya[1]

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Angiosperm leaves exhibit an extraordinary diversity of morphologies. Among these, we are particularly interested in leaves with indeterminate growth. In most plant species, leaves have a determinate fate, with leaf meristem activity lasting only a few days. However, a limited number of species possess indeterminate leaves. Representative examples include the genus *Monophyllaea*, which has simple indeterminate leaves, and the genus *Guarea*, which develops compound indeterminate leaves. In this presentation, we focus on *Monophyllaea*. Members of the genus *Monophyllaea* (Gesneriaceae) exhibit multiple deviations from the canonical developmental system of the shoot. During the vegetative phase, these plants do not form a shoot apical meristem. Consequently, after cotyledon expansion, no additional leaves are produced until the transition to flowering. Moreover, the two cotyledons compete with each other, and only the dominant one undergoes indeterminate growth. As a result, depending on the species, a cotyledon that initially measures only a few millimeters can develop into a leaf several tens of centimeters in length. These unusual developmental processes give rise to the highly distinctive growth form characteristic of *Monophyllaea*. In this presentation, we discuss how modifications in developmental regulatory systems have led to the emergence of such unconventional morphological systems, based on insights obtained from genome and transcriptome analyses, as well as single-nucleus RNA sequencing (snRNA-seq).

S18.4 | Evo-Devo of plant forms & patterns

From spots to stripes: interplay between a reaction-diffusion system and its prepatterns

Mei Liang[1,2], Lee Ringham[3], Changning Ye[2], Xu Yan[2], Nathan Schaumburger[1], Mikolaj Cieslak[3], Michael Blinov[4], Przemyslaw Prusinkiewicz[3], **Yaowu Yuan**[1]

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Reaction-diffusion (RD) systems are widely assumed to account for many complex, self-organized pigmentation patterns in natural organisms. However, the specific configurations of these systems and how they interact with potential prepatterns to generate pattern diversity remain largely unknown. Here, we characterize an RD system underlying the formation of repetitive pigment spots and stripes in *Mimulus* flowers, and identify genetic changes contributing to the evolutionary transition from sparsely dispersed spots to longitudinal stripes through modulation of the RD kinetics and its prepatterns. Computer simulations with a newly developed mathematical model recapitulate the various pigmentation patterns resulting from experimental perturbations. Our findings highlight the importance of prepatterns for a realistic description of RD systems in nature and the value of natural variation in understanding pattern formation mechanisms.

C18.1 | Evo-Devo of plant forms & patterns

Lignin patterning and evolutionary transitions in explosive fruit

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Adaptations for seed dispersal can be found all around us – from the fleshy fruit you eat, to the dandelions you blow on. Cardamine species spread their seeds by explosive dispersal, a trait innovation that distinguishes this genus from the rest of the Brassicaceae family. Explosive dispersal relies on a unique pattern of lignin that is deposited asymmetrically in a single cell layer found on the adaxial side of the fruit valve called the endocarp *b*. Genetic analysis in *Cardamine hirsuta*, together with mathematical modelling and phylogenetic comparisons, have shown that this specific lignin pattern is necessary for seed pods to explode and is strictly associated with the evolution of this trait. To investigate the genetic basis of polar lignin patterning and whether such genes play a role in evolutionary transitions between non-explosive and explosive fruit, we developed *Cardamine chenopodiifolia* as a novel experimental system. *C. chenopodiifolia* is amphicarpic: above ground it bears explosive fruit with polar lignin deposition, while underground it develops non-explosive fruit with uniform lignification. We use this comparative system to identify differentially expressed transcripts between explosive and non-explosive fruit and potential gene functions in the evolution and development of explosive fruit.

Modelling The Interplay Between Pre-Patterning and Morphogenesis in *Hibiscus trionum* Petals

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Flowering plants display diverse petal pigmentation patterns that arise through coordinated spatial regulation of gene expression and tissue growth. In *Hibiscus trionum*, for example, a characteristic bullseye pattern is pre-patterned and maintained at a constant proportion during early petal development despite region-specific differences in growth dynamics. How gene regulatory networks (GRNs) governing tissue growth and patterning are coordinated to preserve bullseye proportions, and how evolutionary changes in these networks generate diversity in pattern size, remains poorly understood. To address this, we constructed a two-dimensional mechanical-molecular model of petal development that captures the interplay of molecular patterning and tissue morphogenesis. We use evolutionary simulations to evolve GRNs capable of transforming an early primordium with about 400 cells into a tissue with the geometry of a young petal (~12,000 cells) while also establishing a proportionally stable bullseye pattern throughout. Once GRNs capable of producing such petals have evolved, we can simulate genetic mutants and perform targeted ablation experiments to generate quantitative, experimentally testable predictions for how growth and patterning respond to developmental perturbations. More broadly, this model provides a computational platform for dissecting the mechanisms underlying robust pre-pattern formation in growing tissues, and a basis for understanding how evolutionary changes in GRNs shape floral pattern and morphological diversity.

C18.3 | Evo-Devo of plant forms & patterns

Checkerboards and the chess flower: evolution of colour patterns in Fritillaria (Liliaceae)

Laetitia Carrive[1], Pierre Galipot[2], Romain Garrouste[1], Agnès Schermann-Legionnet[2], Julie Zalko[3]

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The variety of colours and patterns displayed by flowers is one of the most visible expressions of their diversity. These traits, which serve as visual signals for pollinators, play a fundamental role in the reproduction—and thus the evolution—of flowering plants. One genus of Liliaceae in particular, *Fritillaria*, exhibits spectacular colours and contrasts, sometimes combined with a particularly striking and rare pattern: a checkerboard. When present, this pattern can be located on various parts of the perianth and can be made with various colours. These visual characters ancestral states were reconstructed with a phylogenetic framework based on published sequences, and putative correlations between the checkerboard pattern, colours and colour contrast were tested with standard PCM. The development of this pattern is a mystery since no morphogenetic process is known to generate checkerboards. One species (among ~170), *Fritillaria meleagris*, was chosen as a model to study the formation of the "chess squares". Anthetic flowers were collected in Brittany, dissected and observed. The pattern appears linked to tepal venation and to a putative Turing-like pattern, which serve as physical constraints and strong morphogenetic hypotheses are enabled by this study. Together, this shows the extent of the processes selected during evolution to generate complex forms and colors, and confirms the importance of studying morphogenesis with in-depth pattern analyses and through species diversity. Finally, by enabling strong hypotheses to be made about the morphogenesis of these patterns, it paves the way for the molecular identification of the morphogenetic processes at work.

How plants grow cups: Investigating the Development and Plasticity of *Marchantia polymorpha* Gemma Cups

Leonie Kraska[1], Ana Maria Bratu[1], Valentin Laplaud[1], Bapiste Castel[2], Stéphanie Drevensek[1], Pierre Marc Delaux[2], Arezki Boudaoud[1]

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The dispersal of clonal progeny constitutes a crucial challenge for the reproduction of sessile organisms. To overcome this, diverse lineages—including plants, fungi, and lichens—have evolved specialized structures known as splash cups, organs with a distinct concave geometry that harnesses the energy of raindrops to spread clonal propagules. However, how these organs acquire their particular shape remains poorly understood. Here, we integrate morphological analyses of overall cup shape with cellular-level tissue characterization to investigate the development and plasticity of cups in *Marchantia polymorpha*. Cup development requires differential circumferential growth, with higher growth rates toward the rim. The relative magnitude of this growth gradient determines the cup angle. We determine the spatiotemporal dynamics of cell division and elongation and identified distinct zones of cell orientation and cell division. We further investigate phenotypic plasticity by comparing *M. polymorpha* accessions and growth conditions, finding that cup angle differs between accessions, while growth temperature specifically affects cup wall length. Finally, fluid mechanical analyses using 3D-printed cup replicas revealed that drop size, cup angle, and impact position jointly determine the angle and velocity of propagule dispersal. These results suggest that cup shape contributes to fitness in a manner dependent on local rainfall conditions. Our work provides a mechanistic framework for understanding the tissue organization of cup-shaped plant organs and reveals how growth regulation underlies phenotypic plasticity, supporting both within-lifetime adaptive responses and evolutionary diversification.

C18.5 | Evo-Devo of plant forms & patterns

The evolutionary origins of apical growth in plants

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Approximately half a billion years ago, the transition of charophytic green algae to land marked a pivotal event in Earth's history, giving rise to land plants (embryophytes). This event coincided with the evolution of apical meristematic cells with altered division properties, likely driving the subsequent morphological radiation of embryophytes. Notably, two charophyte algal lineages (Charophyceae and Coleochaetophyceae) also grow primarily through the division of apical cells. However, these cells possess only one or two cutting faces, constituting 1- or 2-dimensional apical growth, respectively, and producing branching or disc-shaped body plans. Conversely, apical cells of embryophytes can be cleaved from three or more faces (3-dimensional growth), a key developmental feature underpinning the complex morphology of this group. Recently, the genetic mechanisms regulating 3-dimensional growth have begun to be uncovered. However, despite the key phylogenetic positions held by the charophyte algae, molecular genetic investigations in these lineages are lacking. Here, we investigate the genetic toolkit governing the apical meristematic cells of two representative charophyte algal taxa (*Chara braunii* and *Coleochaete scutata*) and a land plant (the moss *Physcomitrium patens*), shedding light on the origins of multidimensional growth and body plan complexity in plants.

19 | Integrating Gene Regulatory Networks into Evo-Devo: Linking Molecular Mechanisms to Trait Evolution

Symposium S19: Thursday 11 June, 09:00 – 10:40
Contributed Session C19: Thursday 11 June, 11:05 – 12:30
Contributed Session C19 (Continued): Thursday 11 June, 15:30 - 16:40

Organisers: Maria Rosselló (Universitat de Barcelona, Spain), Alba Almazán (Universitat de Barcelona, Spain), David A. Salamanca-Díaz (University of Exeter, UK)

Gene regulatory networks (GRNs) arise from interactions and regulatory relationships among gene products. These networks coordinate biological processes across all levels of organization. In developmental evolutionary biology, GRNs are central to understanding how cells, tissues, and organs acquire their identity, shape, size, and function. Traditionally, the causal effects governing the global behavior of these networks were attributed to a few key gene products. However, recent advances in next-generation sequencing, and comparative evolutionary genomics have revealed that phenotypic traits often emerge from the collective dynamics of many interacting factors, rather than from the control of one or few factors. Deciphering and integrating these complex regulation interactions is essential to understand how evolution shapes development and how ecological contexts influence gene network architecture. By uncovering how network structure, modularity, and connectivity evolve, we can begin to explain the origins of developmental robustness, phenotypic plasticity, and biological diversity. Ultimately, GRN research provides a crucial framework to bridge molecular mechanisms, evolutionary processes, and ecological outcomes, offering new insights into how biological complexity is wired through evolutionary time.

S19.1 | Integrating Gene Regulatory Networks into Evo-Devo: Linking Molecular Mechanisms to Trait Evolution

A network-centric view on the evolution of developmental processes - Insights from eye size variation in *Drosophila*

Nico Posnien

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Understanding the evolutionary forces that shape morphological diversity present in nature is one prerequisite to describe and maintain this biodiversity. Linking genetic variation to phenotypic differences is a crucial step towards this goal because information about the appearance of an organism is stored in its genome. New sequencing technologies fueled exciting advances in the identification of the genetic architecture of many organismal traits. However, a mechanistic understanding of the biological processes affected by these genetic variants remains largely elusive. Closing this gap is challenged by the fact that many organismal traits vary continuously in a population, and they are often affected by hundreds or even thousands of mutations spread throughout the genome with each of them having only a minor impact on the phenotype. It is therefore of general interest to improve our understanding of genotype-phenotype associations. As adult morphology is a result of embryonic and post-embryonic development, genetic variants associated with differences in adult organ size and shape must affect developmental and cellular processes as intermediate organismal phenotypes. Such developmental processes are coordinated by the action of gene products, which are often part of complex gene regulatory networks. Therefore, the integration of multi-omics data quantifying the regulation and expression of developmental genes represents a powerful intermediate molecular phenotype. I will present recent data on the genetic and developmental basis of eye size variation in *Drososphila* to propose an integrative framework to study the genetic basis of quantitative trait variation.

S19.2 | Integrating Gene Regulatory Networks into Evo-Devo: Linking Molecular Mechanisms to Trait Evolution

Gene regulatory networks driving neuronal diversification in development and in evolution

Andrea Millán-Trejo[1,2], Carlos Mora-Martínez[1,2], Adrián Tarazona-Sánchez[1,2], Carla Lloret-Fernández[1,2], Rafael Alis[1,2], Antonio Jordán-Pla[1,2], Arantza Barrios[3], **Nuria Flames**[1,2]

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How nervous systems balance the generation of robust neuron types with gene expression plasticity mechanisms and how these processes impact cell-type evolution is unknown. Here we use *Caenorhabditis* species to study neuron-type robustness, plasticity and evolution, using VC4 and VC5 cholinergic motoneuron types as models. In *C. elegans,* we found that epigenetic silencing through histone 3 lysine 9 methylation (H3K9me) is necessary to suppress the expression of the serotonin reuptake gene *mod-5/Sert* and a serotonergic phenotype in these cells. In contrast, we observed that VC4 and VC5 neurons in the Angaria group of *Caenorhabditis* species have evolved an intense serotonergic staining. This phenotype is caused by the emergence of a new enhancer in the *mod-5/Sert* locus, which has been recruited to the ancestral neuron-type gene regulatory network. Enhancer transfer from *C. angaria* is sufficient to impose a constitutive serotonergic fate in *C. elegans*. Remarkably, acquiring this new trait modulates egg-laying responses to high levels of exogenous serotonin, which could be found in specific nematode environments. Finally, we discovered that the repression of the serotonergic fate in *C. elegans* VC4 and VC5 neurons is indeed a plastic trait that can be adjusted in specific environmental growth conditions to elicit egg-laying behaviours similar to those observed in Angaria species. Our work uncovers a dual regulatory paradigm: epigenetic constraints harmonize neuron-type robustness and plasticity, while enhancer co-option enables evolutionary innovation. This mechanistic plasticity directly links regulatory adaptation to behavioral diversification.

S19.3 | Integrating Gene Regulatory Networks into Evo-Devo: Linking Molecular Mechanisms to Trait Evolution

Making diverse “breathing” pores - gene regulatory network evolution and the morphodiversity of plant stomata

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Stomatal complexes are breathing pores on plant leaves that enable plant-atmosphere gas exchange and are thus essential regulators of photosynthesis and water-stress resilience. Diverse stomatal morphologies have evolved in the plant kingdom, where the two central guard cells can be surrounded by subsidiary cells, like in Crassulaceae leaf succulents or grasses. Stomatal morphology is likely adaptive; the graminoid morphology of grasses, for example, is linked to faster stomatal movements–an advantageous trait reducing excessive water loss.

Both in grasses and leaf succulents, the stomatal transcription factor *MUTE* facilitates asymmetric, subsidiary-cell-forming divisions. Indeed, overexpressing KlaxMUTE in the leaf succulent *Kalanchoë laxiflora* induces ectopic asymmetric divisions. This is different from its role in the eudicot model *Arabidopsis thaliana*, where *AtMUTE* terminates asymmetric division potential. Accordingly, overexpression of *AtMUTE* in *A. thaliana* induces symmetric rather than asymmetric divisions. Strikingly, cross-species *MUTE* overexpression phenocopies the role of the endogenous MUTE, suggesting that the cis-regulated targetome has changed rather than protein function itself. In the more distantly related grasses, however, the protein seems to have neofunctionalized and cannot induce asymmetric divisions in *A. thaliana*.

To start decoding the role of MUTE and its induced targetome in the model grass *Brachypodium distachyon*, we generated a single-cell transcriptomics atlas of the developing grass leaf. Subsetting the stomatal lineages, pseudo-temporal trajectory and gene regulatory network analyses began to uncover the MUTE-specific targetome across different developmental stages. This atlas will lay the foundation for comparative studies to decipher how developmental modules were rewired to generate the morphodiversity of plant stomata.

S19.4 | Integrating Gene Regulatory Networks into Evo-Devo: Linking Molecular Mechanisms to Trait Evolution

Effects of foreign transcription factors in vertebrate gene regulatory networks

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The first appearance of new genes and proteins often implies an unexpected encounter between these new molecular entities and the cellular environment of the organism in which they originated. However, since only successful encounters are evolutionarily retained, general principles governing the earliest stages of molecular innovation remain difficult to infer. To assess how first-time interactions take place, we artificially generated unexpected encounters by putting in contact molecular structures that had never interacted before. In particular, we expressed mammal ARGFX and LEUTX and plant-specific YAB1 and WRKY40 transcription factors (TFs) in the completely foreign chromatin environment of zebrafish embryos, a novel heterologous context.

Through a multidimensional genomic approach, involving gene expression, chromatin accessibility and protein-chromatin binding, we show that these TFs possess an intrinsic capacity to engage with and interpret a foreign chromatin landscape. Across the diverse TFs evaluated, we identified intrinsic functional properties, including sequence-specific binding common to all factors, pioneering activity in the case of LEUTX, and gene repression, which was particularly pronounced among plant-derived TFs. We also reconstructed both the pre-existing zebrafish gene regulatory networks (GRNs) and the novel GRNs emerging from the ectopic TF activity. By defining transcription factor–context–specific interactions and systematically comparing network topology across conditions, we quantified the extent and direction of GRN rewiring. This allowed us to determine how newly formed regulatory connections relate to ancestral host networks and to the TFs' original regulatory logic, and ultimately how such rewiring could reshape cellular identity and development.

C19.1 | Integrating Gene Regulatory Networks into Evo-Devo: Linking Molecular Mechanisms to Trait Evolution

The roads to superorganisms: an evo-devo perspective from termites

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Termites are among the oldest social insects and exhibit diverse social systems in which worker developmental and reproductive potential declines with increasing colony-level (super-)organismality. However, the evolutionary history of termite social systems and the underlying developmental and genetic mechanisms remain poorly understood. Here, we employed comparative developmental transcriptomics across seven termite species that differ in worker reproductive potential. We reconstructed the evolutionary history of termite social systems and show that the totipotent false worker, which develops linearly toward winged reproductives (nymphal line), represents the ancestral state. Pluripotent true workers, which bifurcate early in development from the nymphal line, evolved independently at least three times, two of which subsequently gave rise to superorganisms with unipotent, sterile workers. Ancestral gene regulatory network (GRN) reconstruction reveals that totipotent workers resemble early developmental stages of hemimetabolous insects, whereas their larvae exhibit gene expression profiles characteristic of late embryogenesis. GRN comparisons show that the convergent evolution of pluripotent workers involved distinct genetic mechanisms but a conserved core developmental toolkit, including juvenile hormone (JH), ecdysone, and activin signaling pathways, whose regulatory roles differ from those in social Hymenoptera. Caste differentiation between nymphs and workers is further associated with distinct metabolic strategies that exhibit lineage specificity. Finally, the convergent evolution of superorganisms involved somatization of worker castes, recruitment of lineage-specific genes, and lineage-specific gains of JH response elements. Together, these findings reveal repeated, mechanistically diverse routes to reduced worker potency and highlight parallels between superorganism evolution in social insects and organismal evolution in metazoans.

C19.2 | Integrating Gene Regulatory Networks into Evo-Devo: Linking Molecular Mechanisms to Trait Evolution

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

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Understanding the origin of novel traits is a foundational challenge in evo-devo. A common mechanism enabling the evolution of novelty is the co-option of ancestral genetic modules into new developmental contexts. Yet the mechanisms enabling, biasing, or constraining co-option remain poorly understood. We investigated the function of Fat-Hippo signaling in the development of horns in *Onthophagus* beetles, a highly diversified, developmentally plastic, and evolutionarily novel trait. We focused on this pathway because (i) it is an evolutionarily conserved regulator of organ size; (ii) earlier work on other taxa suggested that this pathway may have been co-opted repeatedly into weapon formation; and (iii) our previous analyses of chromatin dynamics indicated enrichment of the binding motif of a key pathway member - Scalloped (Sd) - in open chromatin regions differentially accessible in male horn primordia. Using RNA interference, we have begun to systematically and comparatively assess gene function of key pathway members across multiple *Onthophagus* species. We find that Fat-Hippo signaling regulates the relative size, shape and orientation of head horns during the prepupal growth phase, and that these effects are conserved across onthophagine beetles. At the same time, we identified that this pathway also regulates the pupal remodeling phase of horn formation via modulating programmed cell death, resulting in sexually dimorphic horn resorption. We use our findings to explore how deeply conserved signaling pathways are able to fuel the origin and diversification of novel traits and contribute to the regulation of sexual dimorphism.

C19.3 | Integrating Gene Regulatory Networks into Evo-Devo: Linking Molecular Mechanisms to Trait Evolution

Tracing the evolutionary origin of eyespot center cell types on butterfly wings with single-cell genomics

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Novel complex traits might originate through co-option and redeployment of pre-existing gene-regulatory networks (GRNs) in new developmental contexts. In this scenario, two or more traitswill rely on shared genes and shared regulatory relationships during their development. Empirical evidence that non-homologous traits share such regulatory features, however, remains limited. Here, we use single-cell sequencing to identify butterfly embryonic cells whose gene expression profiles mostly resemble eyespot central cells in larval wings, and localize those embryonic cells to the base of a developing appendage.This work clarifies the origin of eyespots in nymphalid butterflies by showing that eyespot central cells are merely re-activating the GRN present in cells at the proximal end of a developing appendage, which is presumably unable to produce an outgrowth.More broadly, this study provides a bottom-up approach, grounded in cellular identity, for addressing how novel complex traits can arise through the reuse and modification of pre-existing regulatory programs.

C19.4 | Integrating Gene Regulatory Networks into Evo-Devo: Linking Molecular Mechanisms to Trait Evolution

Redeployment of an ancestral gene regulatory network drives the origins of a novel cell type in Poeciliid fish

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The evolution of complex biological structures involves the stepwise modification of simpler forms. However, empirically studying the emergence of evolutionary novelties is often challenging due to the ancient origins of most organs. An exception is the recent, independent evolution of placentas in the fish family Poeciliidae. In contrast to egg-laying teleosts, Poeciliids have evolved internal fertilisation and live-bearing. Fertilisation of the egg occurs without ovulation, with the ovarian follicle continuing to surround the embryo until birth. Remarkably, in some species, this novel interaction between the ovarian follicle and the embryonic yolk-sac has evolved into a placenta, with the ovarian follicle transitioning from an ancestral function of oocyte provisioning to transferring nutrition directly to the embryo. Combining single-cell RNA and ATAC sequencing with imaging, we characterised ovarian cell diversity across nine Poeciliids, covering five independent placental origins. We identified that the Poeciliid ovarian follicle is initially formed by granulosa and theca cells, as in other vertebrates. However, uniquely in Poeciliids, these granulosa cells subsequently differentiate into a novel Poeciliid-specific cell type that forms the maternal interface with the embryo. Therefore, this novel cell type appears to have evolved via 'terminal addition' onto the granulosa lineage and is specified by a gene regulatory network co-opted from an earlier progenitor cell type. Our findings suggest that the redeployment of an ancestral gene regulatory network in a novel cellular context enabled the transition from egg-laying to live-bearing and provided a novel cellular substrate subsequently co-opted multiple times to drive placental evolution.

C19.5 | Integrating Gene Regulatory Networks into Evo-Devo: Linking Molecular Mechanisms to Trait Evolution

Single-nucleus Profiling Highlights the All-Brain Echinoderm Nervous System and the Gene Regulatory Signature of Postmetamorphic Cell Types and Organs

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Metazoans comprise diverse tissues and cell types, each essential for the organismal survival. Most of these types are established early in embryogenesis and persist into adulthood. In indirectly developing sea urchins, however, the continuity between embryonic and adult stages is interrupted by a planktonic bilaterally symmetric larval stage that undergoes complete metamorphosis giving rise to an individual with pentradial symmetry. The identity of these cell types are dectated by distinct Gene Regulatory Networks (GRNs). While GRNs controlling sea urchin embryonic and larval lineages are well studied, the molecular and morphological identities of postmetamorphic cell types remain poorly understood. How different are the gene regulatory signatures of the pre- and post-metamorphic sea urchin cell types? How did specialized organs emerge during evolution? To address these questions we reconstructed the cell atlas of postmetamorphic *Paracentrotus lividus* juveniles and the adult tube feet organs using single-nucleus transcriptomics. We identified signatures of eight distinct cell type groups and analyzed 29 neuronal families, including 15 unique photoreceptor types in the sea urchin juvenile. By combining transcriptomics, spatial analysis, and ultrastructure, we show that adult organogenesis is both reusing larval gene regulatory singatures as well as adult unique ones. Most importantly we found that the echinoderm body plan is predominantly head-like, exhibits an "all-brain" organization, and possess highly sophisticated multifunctional photosensory centers/organs.

C19.6 | Integrating Gene Regulatory Networks into Evo-Devo: Linking Molecular Mechanisms to Trait Evolution

Integrating Developmental Timing and Transcriptomics to Uncover Insect Brain Diversification

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Evolutionary adaptations to diverse life strategies have shaped the brain's structure and function across species, yet the cellular and genetic mechanisms underlying these changes remain poorly understood. Here, we investigate how developmental timing and gene regulatory programs drive neural diversification in the vinegar fly *Drosophila melanogaster* and the red flour beetle *Tribolium castaneum*. Focusing on the *retinal homeobox* (*rx*) genetic lineage and higher-order brain centers such as the central complex (CX), we explore how heterochronic shifts contribute to the evolution of insect brain architecture. Comparative analyses reveal that *Drosophila* constructs most CX neuropils during late larval and metamorphic stages, while *Tribolium* initiates CX formation embryonically and extends it into larval development. Consequently, *Tribolium* exhibits a partially functional CX already in the early larval stage, whereas this is not the case in *Drosophila*. To resolve the molecular basis of these differences, we established a single-nuclei RNA sequencing (snRNA-seq) pipeline targeting *rx*-positive neurons from fixed, immunostained larval brains of both insect species derived from a protocol for whole embryos (Veloso, 2022, doi: 10.18452/24358). Nuclei sorted by FACS enable gene regulatory network reconstruction at single-cell resolution without requiring transgenic markers. By integrating transcriptomic data with morphological information from imaging lines and developmental staging, this approach links specific transcriptional programs to altered neurogenetic timing. Together, these methods provide an integrative framework for understanding how evolutionary modifications of developmental schedules and *rx*-mediated gene networks shape brain diversity across holometabolous insects.

Regulatory tinkering in the evolution of vertebrate olfaction

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Most vertebrates possess dozens to hundreds of chemosensory genes involved in odor detection. These genes are expressed in sensory neurons of the olfactory epithelium, which project their axons into the brain. Each neuron expresses only one receptor gene from the vast genomic repertoire, through multiple regulatory mechanisms acting at distinct molecular levels. In mammals, powerful enhancers (known as “Greek islands”) have been described that can overcome the strongly repressive chromatin environment of olfactory gene clusters. However, most odorant receptor genes in tetrapods belong to a particular subtype that is largely absent in aquatic vertebrates. Moreover, functional experiments in mice involving transcriptional regulators associated with Greek islands have reported stronger regulatory effects on this tetrapod-expanded subtype. Based on these observations, it has been proposed that the olfactory transcriptional mechanisms observed in mammals might have evolved after the water-to-land transition. Using a combination of transgenesis, single-cell sequencing, and CRISPR-guided mutagenesis, we identify and characterize non-coding elements involved in the transcriptional regulation of olfactory genes in zebrafish, as well as associated transcription factors. Finally, we reconstruct some of the evolutionary steps that led to the emergence of olfaction in vertebrates.

Evolution of the photoreceptor developmental program in nocturnal geckos

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Squamates are unusual in having repeatedly evolved rod or cone only retinas by transmutation, or the evolutionary transition whereby one photoreceptor class adapts to evolve features of the other. Transmutation was identified first by retinal anatomist Gordon Walls in the 1940's by morphology, and more recently by our group using molecular markers. Though it remains unresolved how these unique simplex retinas arise we do know that photoreceptor identity is tightly controlled by a network of transcription factors (including CRX, NRL, and NR2E3) during development. We hypothesized that constraint in these genes is the key driving force underlying photoreceptor transmutation in squamates. To address this, we have combined computational and experimental methods using an emerging developmental model, the mourning gecko. Despite being nocturnal, this gecko has a morphologically all-cone retina which lacks the rod-specific opsin *RH1*. Despite having a cone-only retina, rod-specific factors *NRL* and *NR2E3* are expressed in the eye and are a hot spot for non-synonymous substitutions. When tested in vitro, these substitutions identified by our computational analyses significantly reduce the induction of rod photoreceptor gene expression. Together, we infer that functional differences we observe in rod development driving transcription factors like NRL have led to transmuted photoreceptors in the squamate lineage. Our study is the first to apply a developmental perspective to photoreceptor transmutation and adds to a growing body of work aimed at understanding photoreceptor identity from fish to mammals.

C19.3 | Integrating Gene Regulatory Networks into Evo-Devo: Linking Molecular Mechanisms to Trait Evolution (Continued)

Building a jellyfish muscle: Characterization of Myogenesis in *Pelagia noctiluca*

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Muscle cell contraction is powered by interactions between actin and myosin filaments. Striated muscle is characterized by a highly ordered and repetitive arrangement of actin and myosin filaments forming contractile units called sarcomeres. While smooth muscle is present in both cnidarian and bilaterian lineages, striated muscle is observed only in medusozoans and bilaterians. Comparative analyses of genes expressed in striated muscles of medusozoans and bilaterians have led to the widely accepted hypothesis that striated muscle evolved independently in these two groups. However, recent data reveal striking similarities in sarcomere molecular composition in bilaterians and medusozoans. The transcription factors responsible for muscle cell development in medusozoans therefore remain unknown, and their identification would provide key insights into the development and evolution of medusozoan muscle. Unlike most jellyfish, *P. noctiluca* has lost the benthic life stage and develops functional striated muscles within only five days. This atypical life cycle, combined with tissue transparency and daily spawning availability, makes *P. noctiluca* a powerful model for studying myogenesis in medusozoans. A chromosome-scale genome assembly and a single-cell RNA-seq atlas were used to screen for transcription factors expressed in muscle precursor cells. Their expression patterns were validated by in situ hybridization to identify candidate myogenic genes in medusozoans. We also characterized the developmental trajectory of muscle cells from precursor states to fully differentiated contractile cells. This work provides the first developmental and molecular framework for muscle formation in a scyphozoan and supports the theory of independent evolution of striated muscles in medusozoans and bilaterians.

Tracing the evolution of male pregnancy and brood pouch complexity in syngnathids through comparative transcriptomics

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Syngnathidae (pipefish and seahorses) are a morphologically diverse clade of teleost fishes renowned for their diversity in body shapes and appendages and uniquely characterized by male paternal care. During syngnathid evolution, elaborate brooding structures surrounding developing eggs evolved, culminating in fully enclosed brooding pouches that support internal embryonic development in several lineages. A major evolutionary shift distinguishes the syngnathids two main clades: brood structures are positioned either ventrally at the trunk (Nerophinae) or tail (Syngnathinae), and both clades produced lineages with either simple basal brooding structures or derived and complex brood pouches . While significant insights into the morphology and brooding strategies of syngnathids have been made, the molecular underpinnings of brood pouch diversification and convergence remain virtually unexplored. To address this, we sampled syngnathid species representing both simple and complex brooding structures across both clades (trunk and tail brooders). For each species, we investigated transcriptome profiles across sexes, developmental stages, and body positions (trunk/tail) of brooding structures and corresponding non-pouched skin tissues. Using comparative transcriptomics, we analyzed gene expression profiles across these lineages and identified genes contributing to convergently evolved brood pouch phenotypes, as well as lineage-specific genes. Furthermore, we describe gene expression dynamics throughout several key stages of brood pouch development in these species and link them to morphological features using histological sections of sampled brooding structures. Finally, we explored the transcriptional underpinnings of brooding structure positioning (trunk/tail). Our work paves the way to understand the evolutionary transitions in the unique brooding organ facilitating male pregnancy in syngnathids.

20 | Open Symposium

Contributed Session C20 (Patterning): Wednesday, 10 June, 15:30 - 16:40
Contributed Session C20 (Neuro): Wednesday, 10 June, 15:30 - 16:40
Contributed Session C20 (Cell-Tissue): Thursday 11 June, 15:30 - 16:40
Contributed Session C20 (Theory): Friday 12 June. 13:30 – 15:10
Contributed Session C20 (Artho): Friday 12 June, 13:30 – 15:10

The Open Symposium will be organized by the Scientific Committee and will consist of contributed talks with a diversity of subject areas. If your abstract does not seem to fit within any of the other Symposia, please do not hesitate to submit to the Open Symposium.

C20.1 | Open Symposium (Patterning)

Cnidarian Embryonic Aggregates Reveal an Alternative Developmental Morphospace of Initial Body Plan Formation

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The basic animal body plan is established during gastrulation which entails the transformation of a simple collective of embryonic cells (ECs) into an axially patterned, multilayered structure. Beyond conserved gene networks, species-specific maternal pre-patterning and extra-embryonic environments play crucial roles in orchestrating associated events. Strikingly, in the cnidarian *Nematostella vectensis*, dissociation and re-aggregation of the gastrula in random spatial order still results in the formation of a functional animal, through alternative but viable developmental trajectories, distinct to the natural embryo. The study of this enigmatic, self-organized developmental morphospace can uniquely reveal how animal form may emerge and diversify as an interplay between inherent EC developmental flexibility and external embryonic context. However, taxon coverage of cnidarian EC aggregates and detailed molecular analyses are lacking. To tackle this, we have established blastula-stage aggregates in the anthozoan *Nematostella* and the distantly related hydrozoan *Clytia hemisphaerica* which exhibit different modes of gastrulation *in vivo*. Comparative imaging, single cell transcriptomics and targeted perturbations will reveal the degree of similarity in principles of self-organization between EC aggregates from these two species. Furthermore, we leverage the *Nematostella* system to investigate the role of cell contractility, adhesion and epithelial polarity in facilitating body plan formation via alternative developmental trajectories. We find that initial *Nematostella* EC population heterogeneity influences initial oral-aboral axis formation dynamics, since blastula-stage aggregates do not exhibit the associated collective migration events previously identified in gastrula-stage aggregates. Altogether, through these research lines, we aim to uncover universal principles governing the generation of basic embryonic body plans.

C20.2 | Open Symposium (Patterning)

New meiofaunal genomes reveal the diversity of animal Hox clusters

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The Hox cluster is a highly conserved group of developmental transcription factors involved in patterning along the primary body axis of most animals. Bilaterians share seven homologous Hox gene families, several of which underwent subsequent duplications following the splits between deuterostomes, spiralians, and ecdysozoans, such that some Hox genes constitute synapomorphies of these clades. This understanding was until recently based on surveys narrowly biased towards large, tractable model organisms. New genomes from early-branching, microscopic, and/or lesser studied spiralians and ecdysozoans now reveal instances of divergent Hox gene losses, duplications, and rearrangements, unveiling the complex evolutionary trajectory of bilaterian Hox clusters. We surveyed not only the Hox gene complement, but also the organization of Hox genes and conserved micro-RNAs known to regulate Hox gene expression in newly sequenced genomes from meiofaunal animals, to understand how cluster integrity and gene complement may reflect larger-scale evolutionary trajectories of animal body plan diversity and genomic organization. Several meiofaunal lineages represent instances of secondary miniaturization, some recent and others more ancient. Across independent events, species with more derived body plans tend to have undergone more changes to their Hox gene clusters, though this varies with each instance. Furthermore, some species that share ancient body plan modifications, i.e., miniaturization, now show interesting differences both in body size and complexity and Hox gene complement. Our survey of Hox clusters provides several interesting new avenues to explore the relationship between the genetics underlying developmental patterning and body plan evolution in the future.

C20.3 | Open Symposium (Patterning)

Hopeful Monsters in Action? Repeated Axial Evolution Across African Cichlid Fish Radiations

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The independent cichlid radiations of Lakes Tanganyika, Malawi, and Victoria, together with basal riverine lineages, provide a powerful framework for examining how developmental systems shape morphological diversification across evolutionary timescales. Here we focus on axial skeleton evolution, including total vertebral count, determined during somitogenesis, to investigate the developmental basis of body elongation. Riverine and lake cichlids occupy distinct regions of axial morphospace, with riverine taxa tending toward fewer vertebrae and deeper bodies and lake taxa toward increased vertebral counts and elongation. Across all three lake radiations evolution repeatedly explores the same axes of axial variation, with each radiation independently converging on similar elongate body forms. Rates of vertebral count evolution are accelerating and substantially higher in Lakes Victoria (x100) and Malawi (x10) than in the older riverine and Lake Tanganyika lineages, consistent with rapid evolutionary change in early adaptive radiations. Vertebral count intraspecific variation, a readout of somitic fidelity, is low and does not predictably scale with body elongation within species, indicating strong developmental canalisation. Vertebral count increases, therefore, are unlikely to arise through gradual selection on elongate individuals; rather, rare but developmentally viable shifts in segmentation may generate novel axial forms, exemplifying Alberch's reinterpretation of "*hopeful monsters*". Together, these findings suggest that axial diversification in cichlids is extremely rapid yet occurs despite segmentation being strongly canalised, highlighting how developmental constraints and opportunities interact to shape macroevolutionary outcomes.

C20.4 | Open Symposium (Patterning)

An Evolutionarily Conserved Genetic Framework for Plant Root Cortex Patterning

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Understanding how evolution generates diverse anatomical patterns is a central question in biology. Root cortical tissue provides an excellent model to address this problem, as the number of cortical layers varies widely among plant species, enabling adaptation to different ecological niches. However, the mechanisms underlying the formation of multiple cortical layers remain poorly understood. Here, we show that in species with a multilayered cortex—including *Cardamine hirsuta*, *Nicotiana benthamiana*, barley, and rice—the plant hormone cytokinin acts as a key and previously overlooked driver of cortical multilayer formation. In these species, cytokinin acts in coordination with the transcription factor SHORTROOT (SHR) to promote the expression of the cell-cycle regulator CYCD6;1, reinforcing cortical cell divisions. This regulatory module is absent in *Arabidopsis thaliana*, which develops only a single cortical layer. Remarkably, reintroducing this module is sufficient to restore the ancestral multilayered cortical pattern. Together, our results identify this cytokinin–SHR–CYCD6;1 module as a core developmental circuit underlying the evolutionary diversification of root cortical patterning.

C20.5 | Open Symposium (Patterning)

Building branches: mechanisms of meristem duplication during dichotomous branching

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By varying the activity and formation of new meristems, plants create elaborate and complex three-dimensional branching architectures that are specialised for light capture and reproduction. Many lineages produce new meristems by dichotomous branching, during which the meristem expands and separates to produce two daughter meristems. Despite the importance of dichotomy for understanding meristem function and evolution, little is known about how dichotomy is regulated. To uncover the molecular mechanisms controlling dichotomous branching, I am using the genetically tractable liverwort species, *Marchantia polymorpha*. I characterised the anatomical and gene expression changes that occur during dichotomy to create a quantitative framework for meristem duplication. Using an unbiased RNA-Seq approach, I identified the cytochrome P450 MpCYP78E1 as a novel regulator of dichotomy and will describe how MpCYP78E1 controls meristem processes to modulate the overall branching architecture of the plant.

C20.1 | Open Symposium (Neuro)

Developmental evolution of brain monoaminergic systems: the outcomes of the *mao* mutation in blind Mexican cavefish

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The vertebrate brain exhibits remarkable anatomical and functional diversity. While its overall organization is conserved, fine differences arise from distinct developmental processes and are often linked to species-specific behaviors and adaptations shaped by evolution. Monoamines, particularly serotonin, play a central role in the formation and function of brain circuits. They regulate mood, stress responses, motivation and social behavior. Importantly, monoamines also influence brain development by modulating neuronal proliferation, migration, and differentiation. The fish *Astyanax mexicanus* provides a powerful model to investigate these mechanisms. This species includes river fish and dark-adapted cavefish morphotypes. Cavefish carry a point mutation in the Monoamine Oxidase (*mao*) gene, reducing enzymatic activity and leading to elevated serotonin levels. This natural mutation offers a microevolutionary framework to explore how altered neuromodulation contributes to neuroanatomical and behavioral divergence. We hypothesized that *mao* genotype influences brain development. *Astyanax* with the *mao* mutation show increased sensitivity to stress, shown by behavioral changes and elevated cortisol levels. Since neurogenesis is linked to stress, we investigated interactions between *mao*, stress, and brain proliferation. We induced acute (single 10-minutes exposure) and chronic stress (5 minutes twice daily for five days) and measured cortisol levels in fry. The *mao* genotype influenced acute stress responses at larval stages, with effects differing from adults, but did not affect chronic stress responses. Chronic stress reduced cell proliferation in the hypothalamus but not in the telencephalon. Overall, *mao* modulates stress responses depending on stress type and developmental stage, and stress impacts brain proliferation in a region-specific manner.

C20.2 | Open Symposium (Neuro)

Adaptive significance and evolutionary origin of the Turbanate Eye in Cloeon dipterum: a case of sensory innovation in mate recognition

Tòt Senar-Serra[1,2], Maria Rossello[1,2], Núria Bonada[2,3], Lauren Sumner-Rooney[4], Pedro de la Villa[5,6], Ana Méndez[7], Isabel Almudi[1,2]

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Cloeon dipterum possess one of the most distinct visual system among the Arthropoda phylum. In addition to the pair of compound eyes and three ocelli common in both sexes, the males exhibit a remarkable sexually dimorphic morphological novelty: the turbanate eyes. Those eyes are located in the dorsal part of the head, and their development is belated compared with that of the other compound eye set. Only in their adult form, when the individuals emerge from the water, those eyes are fully functional. Anatomical data obtained by transmission electronic microscopy in adults suggest that in the turbanate eyes there is a switch from apposition optics, the most common among insects, to superposition, providing to this eye a more suitable ability to see in low-light conditions. Mayflies mate during dawn, where an increased light sensitivity will improve their ability to see in scarce light conditions. Moreover, we investigate the genes from the phototransduction cascade in Ephemeroptera. Interestingly, in the Baetidae family, where *C. dipterum* belongs, both ends of the cascade (opsins and transient-receptor potential or *trp*) have experienced a significant expansion. In the turbanate eye of *C. dipterum*, a *trp*, a UV and a blue-sensitive opsin are expressed specifically there and, by performing electrophysiological essays, we showed a specificity for the turbanate eye towards those wavelengths. Furthermore, we observed greater reflectance of those wavelength in the female body. These various results suggest that the appearance of the turbanate eye played a major role in the diversification of the Baetidae family.

C20.3 | Open Symposium (Neuro)

Astrocyte evolution in vertebrates: a tale of two fishes

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Astrocytes comprise among the most important and heterogenous cell types of the vertebrate central nervous system and are thought to have contributed to the high degree of specialisation seen in the amniote brain. However, the exact evolutionary origins of astrocytes in vertebrates are unclear, owing largely to limited information on the functional roles of astrocytes from anamniote and in particular basal gnathostome species. Here we present data from two sister chondrichthyan species – the elasmobranch epaulette shark (*Hemiscyllium ocellatum*) and the holocephalan elephant shark (*Callorhinchus milii*)– to comprehensively characterise the heterogeneity and role of astrocytes in the basal anamniote in both structurally conserved and divergent regions of the central nervous system. We use data from both adult and embryonic individuals of both species, in combination with histological and transcriptomic analysis at the single-cell level, to compare evolutionary differences of chondrichthyan astrocytes in the telencephalic injury response as well as in the establishment and maintenance of the blood brain barrier. Our work presents a comprehensive picture of astrocytes at the earliest stage of gnathostome lineage and provides evidence for the mechanisms by which astrocytes first arose in the vertebrate subphylum.

C20.4 | Open Symposium (Neuro)

Tail muscle development of *Oikopleura dioica*: a neuromesodermal origin and the evolution of the appendicularian free-swimming lifestyle

Marc Fabregà-Torrus[1,2], Alfonso Ferrández-Roldán[1,2], Gaspar Sánchez-Serna[1,2,3], Marcos Plana-Carmona[1,2], Beatriz Iralde Cárdenas[1,2], Cristian Cañestro[1,2]

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In tunicates, the sister group of vertebrates, the tail is typically present only during the larval stage and is reabsorbed during metamorphosis, giving rise to a sessile adult form. However, this is not the case in appendicularian tunicates, where the lifelong maintenance of a chordate-like tail, together with the evolution of complex tail behaviours, has accompanied the transition to a fully free-swimming lifestyle. Our current research on tail muscle development in the appendicularian *Oikopleura dioica* investigates the developmental changes underlying this evolutionary transition. We identified a muscle organization that differs strikingly from that of other tunicates, suggesting extensive remodelling of tail muscle identities. Within the muscle lineage of *O. dioica*, we uncovered a previously undescribed progenitor population located at the distal tip of the tail. This lineage differs in both origin and molecular profile from other muscle precursors and displays an unexpected combination of mesodermal and neural regulatory signatures, reminiscent of vertebrate neuromesodermal progenitors. In addition, analysis of the myofibrillar repertoire revealed a lineage-specific and massive expansion in appendicularians. Expression analysis of multiple paralogs of myofibrillar myosins and troponins uncovered a complex pattern of distinct muscle identities along the tail. Together, these findings provide new insight into how the evolution of axial musculature may have facilitated the transition to a fully free-swimming lifestyle in appendicularians.

C20.5 1| Open Symposium (Neuro)

Sexual Dimorphism and Lifestyle Transition: from Sex Chromosome Turnover to Brain Remodeling in a Mayfly

Sophie Tandonnet[1,2], Rafath Chowdhury[1,2], Maria Moran Saiz[1,2], Jessica Gomez Garrido[3,4], Tyler Alioto[3,4], Marta Gut[3,4], Maria Rossello[1,2], Aurora Ruiz Herrera[5,6], Ignacio Maeso[1,2], Isabel Almudi[1,2]

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Mayflies (Ephemeroptera) belong to an ancient lineage of winged insects and are characterized by a hemimetabolous development, in which metamorphosis occurs gradually. Within this order, Baetids display extreme sexual dimorphism, especially in the visual system of adults: males possess an extra-pair of dorsal “turbanate” eyes and, correspondently, have duplicated optic lobes (brain regions responsible for integrating visual inputs). Their life cycle is also marked by an ecological transition from aquatic nymphs to short-lived flying adults. All these characteristics provide a natural framework to study how sex-specific traits emerge during development and how lifestyle transition leads to neural remodeling.

We combine genomics with single-cell transcriptomics to dissect the genetic basis of sexual dimorphism, especially sex-specific brain remodeling across the ecological transition in the mayfly *Cloeon dipterum* (Baetidae).

We generated a chromosome-level male genome assembly using long-read and Hi-C sequencing, which revealed the presence of neo-sex chromosomes. The neo-Y chromosome carries a ~500kb inversion and a paralog of the key sex-determination gene *doublesex* within a Y-specific region absent in females. This previously undescribed configuration suggests recent sex chromosome emergence in a lineage with pronounced male-specific morphology.

To investigate how sexual dimorphism manifests in the brain (an organ displaying huge structural changes), we conducted brain-wide single-cell RNA sequencing of both sexes across four stages spanning the aquatic-to-aerial transition. Our preliminary results show that male and female brain transcriptomes progressively diverge during development. Moreover,

males exhibit stronger transcriptional remodeling during the transition to the aerial stage, coinciding with the maturation of male-specific visual structures.

Tracing the evolutionary origin and function of fluorescent pigmented cells in sea cucumbers

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Holothuroids represent a diverse and ecologically important group of echinoderms, yet the molecular and cellular mechanisms underlying their embryonic and larval development remain poorly explored. We established the Mediterranean species *Holothuria tubulosa* as an experimental model to investigate sea cucumber development from oocyte maturation to the juvenile stage. Using high-resolution microscopy and live imaging, we identified a previously undescribed fluorescent pigmented cell type localized along the larval ciliary band and tracked its emergence from the onset of pigment production. The pigment displays exceptional resistance to photo-bleaching enabling long-term imaging, and we are currently defining its chemical identity. These fluorescent pigmented cells share some features with echinoid pigment cells with known immune functions, including expression of mesodermal markers, and derive from the same embryonic lineage. However, transcriptomic profiling of FACS sorted fluorescent cells reveal a distinct molecular identity and, functional assays show that unlike in sea urchins, these cells do not migrate in response to bacterial exposure, suggesting a divergent function in holothuroids. Together, our findings illustrate how homologous cell types can be evolutionarily recruited for different functions and highlight the value of expanding the range of experimental systems to advance our understanding of animal biology and cell type evolution.

C20.2 | Open Symposium (Cell-Tissue)

EvoDevoBase: an open database for evolutionary developmental biology

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Evo-Devo has developed into a mature, interdisciplinary field that continues to integrate concepts and tools from other disciplines (e.g., metabolism and mechanics), extend the range of organisms under investigation, and expand its research community. This growth, however, makes it increasingly difficult to keep track of the trends across the field. EvoDevoBase is an open, curated database that aims to provide a panoramic overview of Evo-Devo research and its scientific community. The database aggregates data from papers, people, institutions, journals, and organisms from various online, publicly available sources. It enables users to follow the latest articles, discover overlooked works, explore studies by species or subfield, and identify specialists around the globe. It also lets users visualize trending organisms or topics, offering insight into the history of the field and how it continues to evolve. The database is designed to facilitate collaboration, surface underrepresented labs and organisms, and serve as both an entry point for researchers new to the field and a living platform built with and for the broader Evo-Devo community. Presented here as a prototype, EvoDevoBase is actively seeking input from evo-devo researchers and societies to steer its development.

C20.3 | Open Symposium (Cell-Tissue)

Late Vascular Development in Chondrichthyans Reveals a Conserved Programs of Venous Valve Formation

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Vertebrates are characterized by a closed vascular system that supports essential physiological functions, including respiration, nutrient transport, and waste removal. While vascular development has been extensively studied in traditional model organisms such as mice and zebrafish, comparatively little is known about the development of the vascular system outside of bony fish vertebrates, and particularly about the vascular remodeling and reorganisation following the formation of the main vessels.

Here, we investigate late-stage vascular development in the embryos of the small-spotted catshark *Scyliorhinus canicula* using an integrative approach combining morphological, histological, and molecular analyses.

X-ray microtomography revealed extensive remodeling of the vascular network during late development, including the formation of the renal portal system. High-resolution synchrotron microtomography enabled us to identify previously undescribed venous valves in the cranial vasculature, including parietal valves located within a single vessel lumen, a configuration previously thought to be absent in fishes. Finally, the analysis of venous valve development across late developmental stages uncovered a conserved molecular program of valvulogenesis in jawed vertebrates, involving key transcription factors such as *Prox1* and *Itga9*.

Together, these findings provide new insights into vascular remodeling and maturation in chondrichthyans and offer new perspectives on the origin of the venous valve system in the gnathostome ancestor over 450 mya.

C20.4 | Open Symposium (Cell-Tissue)

Spatial transcriptomics reveals "temporal modularity" in gap and pair-rule gene patterning in the early Tribolium embryo

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The omics revolution has transformed our understanding of gene regulatory networks (GRNs) underlying developmental and evolutionary processes. Yet conventional sequencing-based approaches require cell dissociation, sacrificing the spatial context essential for mechanistic insight into embryonic patterning. Imaging-based spatial transcriptomics overcomes this limitation by assaying gene expression directly in intact tissue sections or whole embryos at high spatial resolution, opening new frontiers for Evo-Devo research.
We apply sequential FISH (seqFISH) to simultaneously assay up to ten gap and pair-rule genes in the same early Tribolium castaneum embryo, with the approach readily expandable to the full repertoire of a GRN. By preserving native spatial architecture, seqFISH enables co-visualization of multiple genes across developmental time, a feat unachievable by conventional methods.
Recent work proposed that embryonic patterning is fundamentally a temporal process translated into space, exemplified by gap and pair-rule dynamics in short-germ insects. Our seqFISH data reveal a striking refinement: the temporal sequence of gene activation initiates in a defined order in the posterior, but is progressively and systematically altered toward the anterior. We term this "temporal modularity": the temporal logic of a GRN is incrementally reconfigured as it is translated into spatial gene expression. This observation, only possible through simultaneous multi-gene readout in individual embryos, is supported by computational modeling, and reshapes our understanding of the evolution of embryonic patterning in light of the contrasting spatial logic of gap and pair-rule regulation in Drosophila.

C20.5 | Open Symposium (Cell-Tissue)

Transient Receptor Potential (TRP) Channel diversity and localization in Choanoflagellates points to a role in cellular behavior

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Animals represent one of the few instances of complex multicellularity on Earth, displaying extraordinary diversity in morphology, tissues, and cell types. Yet each generation begins as a single cell that must rebuild this complexity. While genomic information provides the constraints for development, individual cells must detect and respond to nuanced environmental and intercellular cues, often mediated through bioelectric signaling, to coordinate morphogenesis. How such signaling systems emerged during the transition to animal multicellularity remains unresolved. Choanoflagellates, the closest living relatives of animals, provide a powerful system for investigating signaling mechanisms that preceded animal multicellularity. Previously, we identified complex electrical signaling in both unicellular and multicellular stages of the choanoflagellate *Salpingoeca rosetta*. These events correlate with distinct cellular behaviors, including ciliary arrest and rapid apical-basal contraction, which were coordinated across multicellular colonies. In order to better understand these signaling events, we invested various ion channels in choanoflagellates, focusing on the transient receptor potential (TRP) channel superfamily. Comparative analyses reveal a diverse TRP complement, including lineage-specific expansions and a previously undescribed family. In *S. rosetta*, TRPA, TRPC, and TRPV channels localize to distinct subdomains of the flagellar–collar apparatus, a multifunctional structure involved in feeding and environmental sensing. Together, these findings suggest that diversification, spatial organization, and combinatorial assembly of ion channels generate nuanced bioelectric signaling capable of coordinating cellular behavior in a close relative of animals. Such mechanisms may represent ancestral building blocks for the bioelectric control systems that later shaped early animal development and morphogenesis.

C20.1 | Open Symposium (Theory)

Not your average archaeon: CCA diversity of Heimdallarchaeota as a window into early eukaryogenesis

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CCA-adding enzymes are essential for tRNA maturation and, in consequence, translation. A long-standing dogma holds that archaea encode exclusively Class I CCA-adding enzymes, whereas bacteria and eukaryotes encode Class II enzymes, creating a perceived evolutionary divide between archaeal ancestors and eukaryotic descendants. Here, we show that this dichotomy is incomplete. We identify Class II CCA-adding enzymes that are widespread across multiple archaeal superphyla, including Asgard archaea. Functional assays demonstrate that these archaeal Class II enzymes can replace their Class I counterparts. Within Heimdallarchaeota, widely regarded as the closest known archaeal relatives and putative ancestors of eukaryotes, sequence analyses reveal distinct clusters with affinities to different bacterial lineages, consistent with multiple ancient acquisition events. Notably, one Heimdallarchaeota CCA-adding enzyme lineage shows strong similarity to Bacteroidetes/Chlorobi, an ancient bacterial superphylum previously implicated in scattered gene contributions to early eukaryotes. Early-branching eukaryotic CCA-adding enzymes share this deep bacterial signature, supporting a model in which these enzymes were inherited via Asgard archaea. Together, these findings challenge a central paradigm of CCA-adding enzyme evolution and provide molecular-level support for archaeal mediation of bacterial gene transmission during eukaryogenesis.

C20.2 | Open Symposium (Theory)

Investigating the biological significance of evolutionary conflict using convergent traits in plants as case studies

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The transformative shift in data collection capacity in the first quarter of the 21st century has enhanced the explanatory power of existing approaches to the study of evolution. However, traditional methods have remained limited in their ability to fully exploit the wealth of data now accessible, hindering knowledge leaps. For example, phylogenomic datasets often contain a large amount of underutilised information such as patterns in the topology of gene trees, which may conflict with the overall species tree in various ways. The recruitment of existing alleles, whether from an ancestral pool of variation or from hybridisation with closely related species, can be a major contributing factor in evolutionary adaptation. This phenomenon, which can be identified as conflict between gene tree and species tree, could underlie the emergence of phenotypic evolutionary convergence. However, the relative importance of this kind of hemiplasy compared with true molecular convergence across the Tree of Life is still unclear because tools to comprehensively investigate conflict in whole homolog trees are lacking and because studies on gene tree conflict have not historically been carried out on species amenable to functional investigation. I have developed a new computational tool, CAnDI (Conflict And Duplication Identifier) to identify evolutionary conflict comprehensively in homolog trees, and I investigate the biological relevance of this phenomenon to trait evolution using examples from two plant families displaying morphological convergence: the carnivorous Caryophyllales and the Trionum complex, a small group of Hibiscus species.

C20.3 | Open Symposium (Theory)

Learning developmental trajectories from time-lapse embryo imaging using unsupervised deep learning

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Understanding how embryonic development unfolds across species requires quantitative approaches capable of extracting information from high-dimensional imaging data. We developed an unsupervised deep-learning framework that combines convolutional autoencoders with recurrent neural networks to learn latent developmental trajectories directly from time-lapse microscopy. Applied to datasets from zebrafish, Drosophila, Xenopus and mouseembryos, the model reconstructs and predicts developmental dynamics and identifies phenotypic perturbations without labeled data, enabling comparative analysis of developmental trajectories across species.

C20.4 | Open Symposium (Theory)

Multimodal study of the convergent evolution of molar shape in rodent molars

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Repeated evolution occurs when different lineages evolve similar traits independently. If these traits are underlain by the same genetic and/or developmental changes, the basis of adaptation might be predictable. Recent genomic studies have advanced our understanding of this question but much remains unknown, when it comes to complex morphologies produced during development. The upper molar in mouse and Acomys independently acquired the same new morphology, with two supplementary cusps, while the lower molars retained the ancestral cusp number. We asked whether the evolution of this new shape was sustained by convergent developmental changes. We show it is only partially the case, by using a multi-scale approach in a strong phylogenetic context with 2 more species as controls. Based on quantitative morphology using 3D images and gene marker monitoring, we show that the last cusps form at the same time in mouse and acomys but the detail of the dynamics differs and carries a strong phylogenetic signal. Moving to an analysis of transcriptome timeseries, we observed varying degrees of convergence in expression trajectories depending on the way stage correspondences across species are established throughout molar development. Multiome data and tooth germ imaging show that convergent evolution occurred along a very ancient difference between upper and lower molar development, but development mostly evolved differently in mouse and acomys. Our work highlights how comparative work in embryos and developmental genomics benefit from each other, to reveal how ancestral developmental properties enable reaching similar morphologies with largely different genetic and developmental paths.

C20.5 | Open Symposium (Theory)

Modularity and Canalisation Shape Early Craniofacial Divergence in Arctic Charr

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Why do some craniofacial regions diversify readily while others remain remarkably stable? Developmental systems differ in how tightly they are organised into modules and how strongly they buffer variation, and these differences shape the evolutionary paths available to organisms. Arctic charr (*Salvelinus alpinus*) from Lake Thingvallavatn offer an ideal opportunity to explore this: sympatric trophic morphs show striking craniofacial contrasts, and previous work indicates that these differences arise before the onset of external feeding. We quantified lateral and ventral craniofacial shape in larvae from planktivorous (PL), small benthic (SB), and hybrid crosses reared under common garden conditions and sampled before first feeding. Using geometric morphometrics, we found clear PL–SB shape divergence in both anatomical views, demonstrating that benthic–limnetic differences are established during embryogenesis. Despite reflecting distinct developmental modules, lateral and ventral datasets showed parallel group separation, indicating that early craniofacial divergence is expressed through an integrated developmental architecture. Hybridisation revealed asymmetric robustness among modules. Ventral craniofacial elements were highly canalised, with hybrids showing largely additive shapes and shared allometric trajectories with parental morphs. In contrast, lateral morphology displayed altered allometry and increased shape variance in hybrids, suggesting reduced canalisation and greater susceptibility to developmental mismatch. Together, these findings show that ecological specialisation, modular developmental organisation, and differential robustness interact to shape early craniofacial divergence in Arctic charr. This multilevel perspective demonstrates how modularity and canalisation structure the evolvability of trophic phenotypes during the earliest stages of development.

C20.1 | Open Symposium (Artho)

A developmental mechanism producing dimorphism in ovary size between queen and worker ants

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A key innovation in ants was the evolution of a non-reproductive female caste: the workers. Worker-queen differentiation stems from a developmental polyphenism, with larvae developing caste-specific morphologies, including differences in ovariole number (the modular egg-producing units that comprise the ovaries). Across species, queen-worker dimorphism in ovariole number varies widely, with no dimorphism in some species, vast dimorphism in others. Despite the importance of ovariole number as a reproductive trait, little is known about how caste differences in ovariole number are produced, and what factors shape the variation in this trait. To address these questions, I describe the process of ovary development during larval and early pupal stages in a focal ant species (*Lasius niger*). In the model insect *Drosophila,* ovariole number is determined by the number of cell stacks, terminal filaments, that form adjacent to the germ cells by the end of larval development. However, based on my study of ovary development in *Lasius*, my further sampling of larval ovary morphology in phylogenetically diverse ant species, and on rediscovering older drawings of ant larval ovaries from the 1920s and 1950s, I suggest an unexpected mechanism that underlies ovariole number dimorphism in ants: the 'clustering' of terminal filaments in the worker to allow caste-specific regulation of ovariole number. I also provide evidence from ancestral state reconstruction that this mechanism evolved in the common ancestor to the most diverse ant clade, and discuss its potential evolutionary importance for reproductive division of labour in ants.

C20.2 | Open Symposium (Artho)

Evolutionary Flexibility of CpG Methylation and Histone Modifications in Beetles

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Insects exhibit remarkable phenotypic plasticity, potentially shaped by epigenetic mechanisms such as CpG methylation and histone modifications. Vertebrate genomes typically show medium to high levels of CpG methylation, which is closely linked to histone modifications and commonly associated with transcriptional repression. In contrast, most invertebrates display sparse or absent CpG methylation. Where present, it is predominantly restricted to gene bodies of active genes, and its regulatory role remains unclear. A conserved mechanism involves the co-transcriptional deposition of H3K36me3, which recruits the *de novo* DNA methyltransferase DNMT3 to unmethylated CpGs. However, DNMT3 has been repeatedly lost in invertebrates, raising questions about how epigenetic system interactions evolve following its loss.

Widely used invertebrate models such as *Drosophila melanogaster* and *Caenorhabditis elegans* lack CpG methylation entirely, challenging assumptions about its regulatory importance. Coleoptera exhibit interspecific variation in CpG methylation and DNMT retention, providing a natural framework to study epigenetic evolution.

We combine RNA-seq, Enzymatic Methyl-seq, and CUT&Tag to generate genome-wide profiles of CpG methylation, H3K36me3, and H3K27ac across coleopteran species. We characterize the genomic distribution of these epigenetic marks and assess their associations with transcription. Our results reveal both conserved and lineage-specific patterns, highlighting the evolutionary flexibility of epigenetic regulatory systems and cautioning against broad generalization across taxa.

C20.4 | Open Symposium (Artho)

How to build a powerful water conserving system: the cryptonephridial complex of the beetle *Tribolium*

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Insect renal systems are responsible for removing waste and maintaining ion and water homeostasis. They have evolved to meet the different environmental challenges faced by different species, and their evolutionary modification is thought to have enabled the exploitation of new ecological niches. Dramatic remodelling of the insect renal system has occurred in a major lineage of beetles, in which existing organs along with an unusual tissue, have been brought together into a novel organ system, the cryptonephridial complex (CNC). This allows the beetles to recycle water from their primary urine and gut contents back into their body, and can even act as a novel route for water uptake from humid air. It enables survival in environments where water is scarce. We are using the model beetle species *Tribolium castaneum* to understand the mechanisms by which the CNC develops (the signals shaping its morphogenesis, and the specification of its constituent cell types), as well as mechanisms underlying its mature physiological function. For this we are using single-cell transcriptomics to guide functional studies in the developing and mature beetles. Through comparison with species lacking a CNC (e.g. *Drosophila melanogaster*) we are gaining insights into the evolution of this novel organ system.

C20.5 | Open Symposium (Artho)

Macroevolution of nutritional plasticity in exaggerated dung beetle forelegs and its underlying developmental mechanism

Ebony M Argaez[1], Patrick T Rohner[1]

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Developmental plasticity in response to nutritional conditions allows organisms to differentially invest in trait growth. However, understanding the extent to which nutritional regulation underlies developmental plasticity and how it contributes to diversity within and across species is unclear. In dung beetles, the males of some species evolve exaggerated, highly nutrition-dependent forelegs that are used in reproductive behavior. This exaggeration is absent in other species, providing an opportunity to explore the mechanisms linking nutrition sensing to developmental plasticity and morphological diversity. Here, we trace the evolutionary history of nutritional plasticity and sexual dimorphism in foreleg length in 12 species that diverged more than 45 million years ago. Species not only differed strongly in overall size but also the degree to which males and females differed in relative foreleg length. Surprisingly, strong sex differences in exaggeration and nutritional plasticity have been lost multiple times independently, suggesting the convergent reduction of sexual dimorphism. Comparative functional genetics using RNA interference in two species with exaggerated or secondarily lost trait exaggeration implicate the members of the insulin signaling pathway linking nutritional variation to developmental processes. This provides insights into how the evolution of nutritional regulation of developmental processes shapes phenotypic diversity.

S21 | Advances in Theoretical Evo-Devo

Symposium S21: Friday 12 June, 09:00 - 10:40
Contributed Session C21: Friday 12 June, 11:05 – 12:30

Organisers: Mauricio González-Forero (Konrad Lorenz Institute Vienna, Austria), Tom Van Dooren (iEES Paris Sorbonne University, France and Naturalis Biodiversity Center Leiden, Netherlands)

Evo-devo has contributed concepts, theories and mathematical modelling approaches to biology. These contributions have involved a broad range of deductive and inductive approaches, including information theory, dynamical systems and their control, quantitative genetics, explainable AI, network analysis, spatially explicit simulations and philosophy. This symposium will present a state of the art of current mathematical modelling and other theoretical research in evo-devo.

The evolutionary implications of genotype-phenotype map structure – from molecular examples to general principles

Nora S. Martin

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To make evolutionary predictions, we need quantitative information on variation: at what rates specific new phenotypes are introduced through random mutations, and how these rates change during adaptive evolution. In theory, this information is contained in the relevant genotype-phenotype (GP) map. However, in practice, GP maps contain an intractable number of possible genotypes. Thus, even for genotypes limited to a short sequence length and for phenotypes that can be predicted computationally, the full map is typically intractable. Thus, a fruitful strategy focuses on coarse-grained and statistical descriptions of GP maps and their structural properties. Taking this perspective, the central question becomes, which coarse-grained structural properties are important for a particular evolutionary question. In this talk, I will illustrate this general, system-independent question with examples. For instance, assume we are given a GP map and use this information to build a fitness landscape, applying a random phenotype-fitness allocation as a baseline. Then a natural question is, how the structural properties of the initial GP maps determine the topography of the resulting fitness landscape, for example its peaks and their fitness. Using analytic calculations, we found that the answer depends only on a small set of GP map properties, referred to as neutral component sizes and evolvabilities [1]. While our computational tests rely mainly on tractable computational GP maps for molecular phenotypes, the resulting principles are expected to be more general and illustrate, which structural properties are relevant for this specific evolutionary question. [1] Srivastava, Louis, Martin, *GENETICS*, iyag026 (2026).

The Extended Life Cycle

Nayely Velez-Cruz[1], Manfred D. Laubichler[1,2,3]

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It is well-established that evolutionary change emerges from processes operating at different levels of biological organization and on different temporal and/or spatial scales. Development generates phenotypes within lifetimes; hereditary processes transmit biological information across generations; and population and ecological dynamics operate over longer time periods. Together, these processes are responsible for the construction, maintenance, and regulation of the organismal life cycle through time. However, a persistent challenge is integrating these multilevel and multiscale dynamics into a unified mathematical framework capable of identifying and modeling the mechanisms underlying evolutionary change in complex systems. Here, we present the Extended Life Cycle (ELC), a general multilevel–multiscale mathematical framework that centers the life cycle as the fundamental unit of evolutionary analysis. The ELC formalizes complex biological systems as interacting ontological levels, each potentially expressed at multiple temporal or spatial scales, within a unified probabilistic state-space formulation. Our framework enables the modeling and inference of level-specific processes while accounting for cross-level coupling and preserving distinct process rates and level-specific sources of stochasticity. As a concrete instantiation, the ELC represents development, heredity, and population/ecological dynamics as distinct but coupled levels. Because mechanisms at some levels may not admit a convenient closed-form specification, we introduce a bidirectional Gaussian process state-space modeling (BiGP-SSM) approach that learns from time series by inferring latent states and latent transition dynamics while preserving the ELC's multilevel dependency structure.

S21.3 | Advances in theoretical evo-devo

A theoretical perspective on evolvability

Thomas F. Hansen[1]

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The concept of evolvability emerged around 1990, and became part of the conceptual foundations of evodevo. A case can be made that understanding evolvability is the focal research problem that makes evodevo into a distinct research program. In this talk I will review some theoretical findings about evolvability and its evolution, and discuss how these can help operationalize and illuminate empirical research. I will also discuss how evolvability serves to link evodevo with other research programs in evolutionary biology such as theoretical population genetics and evolutionary quantitative genetics.

Constructive Internal Selection: a neglected factor in developmental evolution

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Internal selection is a form of natural selection in which fitness differences between variants of certain characters, i.e., those under internal selection, arise from the relations between traits *within* individuals, and the fitness rank of the variants is invariant across environments (Arthur 1997; Wagner and Schwenk). Internal selection has been most successfully applied to explain evolutionary conservation such as that of the vertebrate phylotypic stage (Galis and Metz, 2001). In this contribution we discuss whether internal selection can also be involved in evolutionary transformation rather than only evolutionary conservation. The basic scenario is this: a character, say body shape, is under directional natural selection. This is the focal adaptive character. Evolutionary changes in the focal character cause a disruption of the relationships between other characters, necessitating their adaptive adjustments. Those are the induced evolutionary changes of dependent characters due to constructive internal selection. An example is the evolution of body shape in the genus *Chalcides*, where body shape varies between generic tetrapodal lizard shapes (like *Ch. ocellatus*) to elongated and limb reduced shapes like *Ch. chalcides*. Correlated with the evolution of body shape is an increase in placental matrotrophy, likely a consequence of constraints imposed on egg size due to smaller body circumference (Vincenzo Caputo, U. Ancona). Another example is the evolution of extremely elongated early embryos in pigs and their relatives. We suspect that constructive internal selection plays a role in other clades and body systems as well and can be detected once it is recognized as a possibility.

Linking Developmental Dynamics and Statistical Models of Evolutionary Variation

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Phenotypic variation is the raw material for evolution, yet a persistent gap remains between developmental and statistical representations of that variation. This disconnect limits our ability to link the mechanistic processes generating variation with evolutionary predictions. In this talk, we present a formal framework that bridges developmental and statistical accounts of phenotypic variation. By modeling development as a dynamical system, we derive explicit relationships between small perturbations to developmental processes and standard quantitative-genetic parameters. This framework yields two primary results. First, we demonstrate that the full developmental trajectory contains information that improves the inference of evolutionarily relevant statistical parameters with respect to using endpoint data alone. Second, we characterize how different sources of variation, like genetic, environmental, and stochastic, shape the distribution of phenotypic variation. This reveals the conditions under which covariance matrices are expected to align, offering a developmental explanation for statistical patterns observed across different evolutionary scales. Together, these insights clarify how developmental processes structure variation, affect evolutionary dynamics, and ultimately influence evolvability. This provides a way to integrate developmental mechanisms into the predictive core of evolutionary theory.

Why are multicellular differentiation programs directional?

Somya Mani

KLI, KLI, Klosterneuburg, Austria

Multicellularity arose from unicellular ancestors multiple times independently in the history of life. During the evolution of multicellular lineages, multicellular and unicellular life-cycles have diverged significantly from each other: In particular, multicellular development is directional: Cell-types that arise early in development (eg. embryonic cells) are not seen again in later stages (eg. adult). Whereas, unicellular cell-type switching programs are cyclic and contain bidirectional 'differentiations'. Here, we theoretically explore why multicellular development might have evolved to be directional. Importantly, we do not know what the developmental programs of ancestral metazoans looked like, but evidence from studies on basal metazoans as well as unicellular holozoans provide clues. We incorporate these clues as constraints in a rule-based model — notably, during multicellular development, cellular decisions to divide, die or differentiate depend intimately on the developmental context, which is a crucial aspect of the model. We sample the huge space of possible ancestral metazoan developmental programs using Monte-Carlo sampling. Through this model, we explore how concepts of stability and robustness apply to these developmental programs. Our work indicates that organisms with stable body sizes tend to have directional development, and that selection for different kinds of robustness — to mutations, to perturbations, and to somatic mutants — have distinct effects on shapes of developmental programs.

C21.3 | Advances in theoretical evo-devo

A mathematical synthesis of genetics, development, and evolution

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Mathematically integrating genetics, development, and evolution is a longstanding challenge. I will give an overview of new general mathematical theory that integrates sexual, discrete, multilocus genetics, development, and evolution. This theory yields an exact method to describe the evolutionary dynamics of allele frequencies and linkage disequilibria in multilocus systems and the associated evolutionary dynamics of mean phenotypes constructed via arbitrarily complex developmental processes. The theory shows how development affects evolution under realistic genetics, namely by shaping the fitness landscape of allele frequencies and linkage disequilibria and by constraining adaptation to an admissible evolutionary manifold (high dimensional region on the landscape) where mean phenotypes, phenotype (co-)variances, and higher moments can be developed. I will also describe a first-order approximation of this exact method, which yields equations in gradient form describing change in allele frequency, linkage disequilibria, and mean phenotypes as constrained, sometimes-adaptive topographies. Both the exact and approximated equations describe long-term phenotypic and genetic evolution, including the evolution of mean phenotypes, phenotype covariance matrices, "mechanistic" additive genetic cross-covariance matrices, and higher moments. The theory obtained is referred to as evo-devo dynamics, which can be interpreted as an extension of population genetics, with some similarities to quantitative genetics but with fundamental differences. The theory provides tools to re-assess empirical observations that have been paradoxical under previous theory, such as the maintenance of genetic variation, the paradox of stasis, the paradox of predictability, and the rarity of stabilising selection, which appear less paradoxical in this theory.

C21.4 | Advances in theoretical evo-devo

What happens to the questions and answers of evolutionary theory when we can model the genotype-phenotype map and its evolution

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Evolutionary theory has developed without much understanding of the genotype phenotype map (GPM) and the processes responsible for it (e.g. development). Most models assume GPMs with specific characteristics (e.g. linearity) without any understanding on the underlying development. In this talk we introduce EvolutionMaker, a general model of evolution in which the GPM is not assumed but arises from networks of gene and cell interactions. The model considers a population of individuals, each with a genotype and a phenotype. The phenotypes are 3D morphologies that arise from running each genotype (i.e. a gene network) through a general model of development, EmbryoMaker. The latter model can simulate extracellular signal diffusion, cell behaviors (cell division, cell adhesion, etc.) and any arbitrary gene network. In each generation there is mutation, drift and selection on the individual phenotypes

We found that morphology, development and the GPM evolve but that, quite often, populations do not reach the optimal phenotypes. Even for relatively simple optima, the underlying GPM is so complex that populations get trapped in local optima from which further adaptive mutations are unlikely. We also found that development becomes more robust against mutations over time but not because of any adaptive advantage of robustness but because of developmental system drift. We are able to explain, from development, why trapping occurs, when it occurs and why robustness increases (and when). We also found that other classical answers to evolutionary questions do not hold, while others do, when considering realistic and evolvable GPMs, as we do.

Loss, persistence and reversal of phenotypic traits

Giobbe Forni[1], Filippo Nicolini[2], Heather Bruce[3], Andrea Luchetti[1], Giuseppe Fusco[4]

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The irreversibility of complex trait loss has long been a tenet of evolutionary biology. However, this idea is increasingly at odds with the numerous documented exceptions across the Tree of Life. By synthesizing data from the literature across a diverse array of taxa and traits, and integrating macroevolutionary, genetic, and developmental perspectives, we propose a theoretical framework for understanding the evolutionary conditions that enable trait reversal. Central to our argument is that reversal is commonly fostered by some form of persistence in the generative developmental pathway of the lost trait.We identify three overarching modes of trait reversal, supported by multiple case studies: bypleiotropy (the involvement of the same generative components in other traits and/or functions), by plasticity (environmental-dependent expression of the trait), and by hemiplasy (persistence in another lineage, followed by reticulate evolution). We also examine important affinities between trait reversal and evolutionary novelties. While these can be clearly distinguished as macroevolutionary patterns, the commonalities of their underlying mechanisms, through repurposing latent developmental resources, undermine a neat distinction between what is old and what is new in evolution. This theoretical framework can provide the basis for future explorations of these macroevolutionary dynamics, whose occurrence is increasingly documented, but whose mechanistic basis remains largely uncharted.

The evolution of tissue shape and differentiation in a computational model of plant morphogenesis

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Morphogenesis, the process by which tissues take shape, results from the combined forces generated by cells that have taken on different cell fate decisions. Previous theoretical work investigating the evolution of morphogenesis has demonstrated an interplay of different organisational levels: selection on tissue shape can result in the evolution of distinct cell-types; and conversely, selection for distinct cell-types can result in the emergence of (complex) tissue shapes. These findings relied on, among other processes, differentiation and subsequent migration of cells. Cell movement – migration or exchange of neighbours – is a major driver of animal morphogenesis, but cannot occur in plants due to the presence of cell walls. This major difference in mechanical cell properties means that the space of constraints and possibilities are very different in the evolution of plant development compared to that of animals, potentially resulting in highly divergent developmental control of morphogenesis. We developed a computational model of plant developmental evolution to investigate these different constraints in the evolution of morphogenesis in plants. In this vertex based model, an evolvable gene regulatory network governs the mechanical properties of cells and regulates cell division. We use similar fitness criteria as the previously reported models of animal developmental evolution, either selecting for complex shapes or the development of different individual cell-types. We find that between, and within, the different fitness criteria a plethora of different morphologies evolves. Our perfect ancestral record allows us to investigate the emergence of (novel) morphological traits from a gene regulatory perspective under these different selection conditions.

S22 | Forty years of molecular paleontology and beyond

Symposium S22: Wednesday 10 June, 12:30 – 15:10
Contributed Session C21: Wednesday 10 June, 15:30 – 16:40

Organisers: Philip Donoghue (University of Bristol, UK), Davide Pisani (University of Bristol, UK)

In 1986 Bruce Runnegar published an influential paper in the Journal Palaeontology where he set out a research agenda for the field of "Molecular Palaeontology". A core pillar of this approach was the integration of palaeontology and molecular biology, something that has blossomed since the original publication and now encompasses the areas of both "Molecular Palaeobiology" and "Paleo-Evo-Devo". These areas seek to leverage the ease of genome sequencing and comparative genomics, new techniques for interrogating fossils and new evolutionary model species for testing functional predictions across the tree of life. The aim of this symposium is to showcase the current state of the field of "Molecular Palaeontology" 40 years after Runnegar's initial paper and to identify research questions that should be focal points for the future. We plan for the symposium to feature talks spanning animals, plants and fungi and major transitions and debates across the tree of life, integrating "classical" and "molecular" paleontology.

The diversification of land plant body plans through the lens of molecular palaeobotany

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The Silurian–Devonian Terrestrial Revolution, which lasted for roughly 50 million years from 425-375 million-year-ago, saw an explosion in diversity of vascular plants. This time interval, akin to the Cambrian explosion of animals, saw vascular plants radiate from tiny branching stems to giant trees with deep roots and leaves. Developmentally this interval saw the origin of many key novelties including new modes of branching, tissues types, through to novel organs such as leaves and roots. A central goal in plant evo-devo is to establish the steps that occurred during this pivotal time interval. The branch of molecular paleontology targeted specifically at plants, molecular palaeobotany, offers the ideal framework for understanding this key time period. Here I will provide an overview of how my lab is taking a molecular palaeobotany approach to shed light on the evolution of land plant body plans. By combining together evidence from fossils, the diversity of form in extant species and underlying genetic networks we aim to piece together the origin of land plant innovations such as the evolution of sugar transporting tissues and leaves.

Unraveling the Deep Evolutionary History of Microbial Life

Edmund Moody

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Microbial evolution shaped the Earth long before the rise of oxygen, driving the chemistry of the oceans and atmosphere from the planet's earliest history. The last universal common ancestor (LUCA), living over 4 billion years ago, was metabolically sophisticated rather than minimal, likely thriving in anaerobic, hydrogen-rich environments. LUCA appears to have possessed energy-conserving pathways such as acetogenesis and methanogenesis, indicating that complex bioenergetic systems emerged very early. Following LUCA, the deep divergence between Bacteria and Archaea established life's primary domains. Phylogenetic analyses that account for horizontal gene transfer, paralogy, and substitutional saturation support a substantial evolutionary separation between these lineages, emphasizing the ancient roots of microbial diversity. During the first two billion years of Earth history, microbial innovation gave rise to the core metabolic pathways underlying carbon, sulfur, and hydrogen cycling. Later developments, including photosynthesis and oxygenic metabolism, transformed planetary redox conditions and expanded ecological opportunities. Exploring LUCA's physiology, early metabolic innovation, and the deepest branches of the universal tree of life provides insights into how microbial evolution shaped the biosphere, highlighting the timing and impact of the earliest biological processes on Earth's evolving environment.

Molecular Palaeobiology of Echinozoan skeletons

Jeffrey Thompson[1,2], Nicolás Mongiardino Koch[3], Mike Reich[4], Imran Rahman[5], Laurent Formery[6], Lauren Hudson[1], Timothy Ewin[5], Samuel Zamora[7]

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Echinozoans are the clade of animals that includes echinoids (sea urchins) and holothurians (sea cucumbers), a diverse clade occupying a range of ecologies and habitats in todays oceans. Echinozoans occupy a disparite range of body plans, from the soft-bodied vermiform holothurians, to the multi-plated globular echinoids. The echinozoans are first known from the fossil record in the Ordovician period, and despite their well-characterized body plans in today's oceans, little is known of the morphological characterization of their earliest representatives and the morphological transitions leading from their earliest representatives to their extant biodiversity. In order to better understand the evolution of echinozoan body plans, we combined micro-CT scanning of some of the earliest representatives with total-evidence morphological and molecular phylogenetics to characterize and quantify the evolutionary relationships of extant and extinct echinozoans, as well as the patterns of body plan evolution from the Palaeozoic to living forms. We identify clades of different echinozoan groups, and clear differences in the evolution of holothurian and echinoid body plans. To understand the developmental and molecular basis for echinozoan bodyplan evolution, we subsequently assayed the expression of key skeletogenic transcription factors, signalling molecules, and differentiation genes during the development of sea cucumber and sea urchin body plans. We find largely conserved patterns of skeletal gene expression despite the divergence of echinozoan body plans. Combining these disparate sources of data provides a holistic understanding of animal body plan evolution, and sheds light on the origin of the echinozoan body plan from paleontological, molecular and developmental angles.

Convergent genome evolution shaped the emergence of terrestrial animals

Jialin Wei[1], Davide Pisani[1], Philip Donoghe[2], Marta Álvarez-Presas[3], Jordi Paps[1]

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The challenges associated with the transition of life from water to land are profound, yet they have been met in many distinct animal lineages. These constitute a series of independent evolutionary experiments from which we can decipher the role of contingency versus convergence in the adaptation of animal genomes. Here we compare 154 genomes from 21 animal phyla and their outgroups to reconstruct the protein-coding content of the ancestral genomes linked to 11 animal terrestrialization events, and to produce a timescale of terrestrialization. We uncover distinct patterns of gene gain and loss underlying each transition to land, but similar biological functions emerged recurrently pointing to specific adaptations as key to life on land. We show that semi-terrestrial species evolved convergent functional patterns, in contrast with fully terrestrial lineages that followed different paths to land. Our timeline supports three temporal windows of land colonization by animals during the last 487 million years, each associated with specific ecological contexts. Although each lineage exhibits distinct adaptations, there is strong evidence of convergent genome evolution across the animal kingdom suggesting that, in large part, adaptation to life on land is predictable, linking genes to ecosystems.

Towards a philosophy of reproduction for evo-devo: an organismal and relational approach

David Cortés-García

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Biological reproduction is a foundational concept in the life sciences, yet it remains largely under-theorized as a phenomenon in its own right. Its relevance is typically taken for granted in terms of heredity and adaptive evolution, while its material complexity, developmental organization, and interactive character receive comparatively little attention. Evo-devo, with its focus on developmental processes, organismal integration, and the evolution of biological systems, is uniquely positioned to address this gap. This talk offers a philosophical analysis that aims to make explicit, clarify, and systematize conceptual commitments already present in evo-devo research on reproduction. I distinguish two broad ways of approaching reproduction that have structured biological thinking: one centered on the transmission of heritable information, and one centered on the developmental production of new, integrated biological individuals. Cases such as the evolution of viviparity and eutherian pregnancy, the developmental organization of holobiont reproduction, and the evolution of complex parent-offspring systems illustrate how reproductive phenomena resist adequate treatment within transmission-centered frameworks, and call for an approach that foregrounds developmental integration, organismal relationality, and the emergence of new biological individuals. Drawing on this analysis, I outline an "organismal and relational approach" in which reproduction is understood as a materially grounded, developmentally structured, and ecologically embedded process of individuation. This approach seeks to contribute to the ongoing theoretical development of evo-devo by offering conceptual tools for a more integrative and systematic account of reproductive diversity and evolution.

Developmental morphological rates and the emergence of animal body plans: insights from early metazoan evolution

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The timing and tempo of early animal diversification remain central questions in evolutionary biology. While molecular clock analyses have been widely used to infer genomic evolutionary dynamics, the relationship between molecular evolution and the emergence of developmental innovations remains poorly understood. Here we focus specifically on the evolutionary tempo of developmental traits during the early evolution of animals. Using a Bayesian morphological clock framework, we estimated evolutionary rates for a subset of developmental characters selected from a larger dataset of phenotypic traits spanning major animal lineages. These characters represent key aspects of developmental organization, including cellular differentiation, reproductive strategies, and larval stages. Our results reveal a pronounced acceleration of developmental morphological rates prior to the Cambrian period. Notably, elevated rates are concentrated along deep branches leading to Metazoa, Bilateria, Protostomia, and Deuterostomia, suggesting bursts of developmental innovation during the establishment of major animal body plans. The temporal pattern indicates that many critical developmental transformations occurred before the Cambrian radiation of animal diversity. We propose that these rapid shifts in developmental morphology may reflect underlying changes in gene regulatory networks (GRNs) that control body plan patterning. By isolating developmental traits within a broader morphological dataset, our study provides a macroevolutionary perspective on how developmental systems contributed to the early diversification of animals and the origin of complex body plans.

C22.3 | Forty years of molecular paleontology and beyond

Stepwise evolution of vertebrate median bony structures: from dorsal scutes to fin rays

Kazuhide Miyamoto[1], Tomoyuki Mikami[2], Junpei Kuroda[3], Yuki Honda[4], Ricardo Hattori[4], Satoshi Ansai[5], Koji Tamura[1], Masahiro Uesaka[1]

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Understanding the evolutionary history of morphological structures is a central goal in evolutionary biology, yet the evolutionary paths of only a few have been elucidated. In vertebrates, median bony structures, such as fin rays, scutes, and spines, have undergone extensive diversification. Particularly, fin rays in bony fish have contributed to their ecological and evolutionary success. However, the evolutionary relationships between these median bony structures have been hitherto unknown. Here, we compare the developmental mechanisms and morphology of median bony structures in extinct and extant non-tetrapod vertebrates. First, we show the similarity in the developmental mechanisms between scutes in sturgeons and fin rays in teleost fish using comparative analysis focusing on osteoblasts, the extracellular matrix (ECM), and the transcriptome. Combined with this investigation, our morphological comparison of the median bony structures in extinct and extant vertebrates belonging to various phylogenetic positions suggests that the fin rays in bony fish emerged from the dorsal scutes (scale-like structures) found in ancestral agnathans and placoderms. We thus reconstruct the evolutionary transition from ancestral scutes to the diverse fin rays of modern bony fish. By highlighting the conserved anterior-posterior domains and ECM usage in crown gnathostomes, we propose that bony fin structures first emerged as spines in the anterior domain of placoderm fins, and that these developmental mechanisms were subsequently co-opted into the posterior domain of the bony fish lineage. Together, we provide a model for the stepwise evolutionary progression of median bony structures in vertebrates.

Bayesian timetree inference using Hamiltonian dynamics

Laura Mulvey, Mario Dos Reis

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Bayesian divergence time estimation is central to reconstructing evolutionary timescales, yet current methods struggle to scale to modern phylogenomic datasets. The widely used Metropolis-Hastings (MH) sampler, as implemented in mcmcTree, is a random walk algorithm. It updates parameters without knowledge of the posterior geometry, generating proposals with high autocorrelation and low acceptance rates. Combined with likelihood calculations, that scale linearly with tree size, this renders large-scale divergence time analyses prohibitively slow, often taking months. Here we introduce HMCdiv, an R package for Bayesian divergence time estimation using Hamiltonian Monte Carlo (HMC) sampling. HMC sampling updates all branch lengths jointly via gradient-informed proposals, reducing thousands of likelihood evaluations to a single calculation per cycle. This yields a dual advantage: HMC's informed proposals produce lower autocorrelation and higher effective sample sizes (ESS) per iteration than the MH sampler, while the reduction in likelihood evaluations substantially shortens computational time.

Benchmarking against mcmcTree using four empirical phylogenomic datasets (36–329 taxa), we first confirm both programs converge on equivalent posterior distributions of divergence times and evolutionary rates, validating our approach. We then demonstrate that HMCdiv substantially outperforms mcmcTree in both ESS per iteration and ESS per second, with gains that increase with dataset size. Together, these results suggest HMCdiv can transform the scale at which Bayesian timetree inference is feasible. For example, an MCMC of 30 million iterations for a 329-taxon dataset takes 59 days in mcmcTree but just 22 hours with HMCdiv, demonstrating the significant speed of this novel method.

C22.5 | Forty years of molecular paleontology and beyond

The first quantitative evidence of ontogenetic growth in the early Cambrian ecdysozoan worm *Cricocosmia jinningensis* reveals the earliest energy reallocation

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Ontogeny is both biologically pivotal and ubiquitous among extant ecdysozoans. Ontogenetic data from the fossil record offers direct evidence for resolving questions of developmental evolution within this clade. However, such data remain exceedingly scarce especially for ecdysozoan worms. Here, our study provides the first comprehensive analysis of the growth pattern of the early Cambrian ecdysozoan worm *Cricocosmia jinningensis* from the Chengjiang biota. *Cricocosmia* exhibits isometric growth of the trunk, allometric growth of the sclerites and introvert scalids, and the number of sclerites remains constant through ontogeny. This unique growth pattern provides the earliest direct evidence for the evolution of developmental patterns of ecdysozoans. More importantly, investment in sclerites shifts from negative to positive allometry at the juvenile-adult transition, a strategy of energy reallocation that closely matches the post-maturity defense enhancement seen in modern ecdysozoans. This study is the first to quantitatively capture an ontogenetic inflection point in the fossil record, pushing back the observation of a juvenile-to-adult functional shift to 518 million years ago. Keywords: growth pattern, resource allocation, ecdysozoan worm, Chengjiang biota, early Cambrian

ABSTRACTS (POSTERS)

P002

Braiding in the developmnetal hourglass model

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The enormous disparity in animal crania is underpinned by evolutionary changes in development, our understanding of which is dependent on insights from living organisms and the fossil record. Alternative hypotheses regarding the evolution of interclade developmental divergence have been proposed, including 1) von Baerian divergence and recapitulation, along with 2) the hourglass model. We assessed some of the predictions of these hypotheses at the tissue-level by comparing skull osteogenesis in representatives of the bird clade Galloanserae (fowl). We used embryonic series of four species: chickens and quails, representing Galliformes (landfowl), and ducks and geese, representing Anseriformes (waterfowl). Our comparisons revealed that common and unique cranial features within our taxon sample arise at similar points during osteogenesis, and that most cranial osteological features shared by all galloanserans arise after hatching, contrary to predictions of von Baerian hypothesis. We extended our comparisons to post-hatching stages across thirty fowl species, covering the living diversity of Galloanserae. This comparison revealed that galliform and anseriform ontogenies further diverge from one-another at post-hatching stages, and that waterfowl cranial development shows signatures of peramorphosis relative to that of galliforms. Anseriform peramorphosis likely evolved from a galliform-like ancestor, as the skull of *Asteriornis*, hypothesised to represent a stem galloanseran, more closely resembles the skull of galliforms than anseriforms. Our results indicate that interclade similarities may wax and wane throughout development, representing an extension of the hourglass model that we dub the 'braided hourglass', highlighting the importance of broad phylogenetic sampling and consideration of fossils for understanding the evolutionary history of developmental

divergence among clades.

P003

Unravelling ancient thyroid hormone signalling roles during larval and metamorphosis stages of the marine annelid *Platynereis dumerilii*

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Thyroid hormone (TH) signalling plays salient roles in metabolism, adaptation, and metamorphosis in vertebrates, yet its presence and function in invertebrates remain poorly understood. Here, we investigate the components and developmental roles of TH signalling in the marine annelid *Platynereis dumerilii*, which undergoes an indirect life cycle involving free-swimming larval stages followed by settlement metamorphosis into a benthic juvenile. Comprehensive genomic analyses revealed that *P. dumerilii* possesses the full complement of genes required for TH synthesis, transport, and metabolism. Expression profiling showed that most TH-related genes are expressed in foregut-pharyngeal region during pre-settlement larvae, while specific genes are differentially regulated in post-metamorphic stages, suggesting stage-specific developmental functions. Tandem mass spectrometry (MS/MS) detected the presence of multiple TH compounds, including T3, rT3, and T2, deposited in eggs. Functional experiments demonstrated that exogenous TH treatment (T3 and T4) resulted in elongated body morphology in 3 days post-fertilization (dpf) larvae and induced precocious expression of gut markers in 6 dpf larvae, with T4 showing a particularly strong effect. In contrast, inhibition of TH synthesis using thiourea disrupted trunk formation during early larval development (2–3 dpf). Notably, thyroid receptor (*tr*) expression peaks during trunk formation and declines prior to metamorphosis, whereas deiodinase (*dio*) expression shows the opposite trend. Together, these findings suggest that TH signalling plays a major role in trunk formation and a more limited role during the onset of settlement metamorphosis in *P. dumerilii*, providing insights into the evolutionary origins of TH signalling systems.

P004

Cryptic persistence of truncated abdominal legs in insects and the origin of novel structures

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An iconic feature of insects is the apparent lack of legs on the abdomen, which is believed to be due to the repression of the leg-patterning gene Distalless (Dll) by abdominal Hox genes. However, in contrast to these molecular observations, it is not widely appreciated that the embryos of most insect groups do in fact form paired protuberances on most abdominal segments that appear to be homologous to the thoracic legs. However, these degenerate before hatching to form the abdominal body wall. To resolve this discordance between molecular and morphological observations, the expression patterns of pannier and araucan, genes known to distinguish proximal leg segments in all arthropods, are examined in embryos of the flour beetle Tribolium castaneum. In Tribolium embryos, all pregenital abdominal segments develop leg-like paired protuberances, and the stripes of pannier and araucan expression that delineate the proximal leg segments of the thorax are also expressed in the same configuration around these abdominal protuberances. This suggests that insect abdominal legs are homologous to only the proximal portion of the thoracic legs, which in insect adults forms the body wall (lateral tergum and pleura/subcoxa). These cryptic, truncated abdominal legs – likely inherited from their crustacean ancestors – appear to be an important wellspring for new functions in insects, such as caterpillar prolegs, mayfly gills, and structures for camouflage and aposematic warning. These and other results suggest that ancient structures can persist in a cryptic, truncated, or highly modified state in intermediate lineages, and then become elaborated again in later lineages, making the ancient structure appear as though it is novel.

P005

Mechanical induction in metazoan development and evolution: from earliest multi-cellular gut-characterized organisms to modern animal embryos

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The development and origin of animal body forms have long been intensely explored, from the analysis of morphological traits during antiquity to Newtonian mechanical conceptions of morphogenesis. Advent of molecular biology then focused most interests on the biochemical patterning and genetic regulation of embryonic development. Today, a view is arising of development of multicellular living forms as a phenomenon emerging from non-hierarchical, reciprocal mechanical and mechanotransductive interactions between biochemical patterning and biomechanical morphogenesis. 1- Here we discuss the mechanosensitive molecular processes involved and put forward findings on how early biochemical and biomechanical patterning of metazoans may have emerged from a Myosin-II and Beta-catenin dependent primitive gut formation as a behavioural mechanotransducive feeding response to marine environment, which might have initiated the development of first animal multicellular organisms (Nguyen, Merle et al. 2022, Nguyen and Farge 2024). 2- We additionally found such mechanosensitive processes as acting today, and possibly conserved from 1-, in the maintenance of stems cells physiological levels required of adult mice colon homeostasis, in response to non-peristaltic high frequency Myogenic pulsations present in many mammalians, which function was unknown. As well as in the tumorigenic over production of stems cells in response to tumour growth pressure amplifying colon cancer progression (Fernandez-Sanchez, Barbier et al. 2015, Nguyen Ho-Bouldoires, Sollier et al. 2022, Nguyen and Farge 2024). Fernandez-Sanchez, M.E., et al. (2015). *Nature* 523(7558): 92–95. Nguyen Ho-Bouldoires, T.H., et al. (2022). *Commun Biol* 5(1): 137. Nguyen, N.M. and E. Farge (2024). *Nat Commun* 15(1): 10695. Nguyen, N.M., et al. (2022). *Front Cell Dev Biol* 10: 992371.

P006

Defining multiciliated cell types in *Platynereis* larvae: Dispersed vs. Aligned ciliary arrays

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Multiciliated cells (MCCs) generate fluid flow and enable locomotion in many animals, yet how distinct MCC subtypes arise and form differences in subcellular organization and morphology remains unclear. The spiralian annelid *Platynereis dumerilii* provides a powerful system for understanding how cell type diversification shapes the evolution of larval tissues. We performed an anatomical reconstruction of MCCs in the three-day-old larva and identified 80 MCCs. By manually tracing every basal body and cilium, we generated the first complete count of cilia in an organism. This analysis revealed two distinct arrangements of MCCs. Prototroch and telotroch exhibited dispersed ciliary arrays, whereas para-, meta-, and akrotrochs exhibited aligned arrays. These two MCC subtypes form distinct larval ciliary bands in positions along the anterior to posterior axis of the larva, suggesting that regulatory inputs along this axis are linked to morphological subtypes of MCCs. To uncover the molecular basis of this diversification, we performed single-nucleus RNA sequencing at 48 hpf. Clustering identified distinct MCC populations, including two clusters strongly enriched in ciliary genes. Gene expression mapping linked these clusters to MCC types: cluster-37 markers (*pkhd1l1c*, *mmel1*) localized to prototroch and telotroch cells, corresponding to dispersed arrays MCCs, while cluster-35 markers (*foxM*, *centrin2*) to paratroch cells, identifying aligned arrays MCCs. Ciliogenesis regulators *foxJ1* was shared between clusters, indicating a common MCC core program overlaid with type-specific regulatory modules. Together, these findings provide a path to unravel how diversification of a conserved cell-type generates distinct subcellular organizations, a framework for understanding the evolution of ciliated tissues.

P007

Investigating germ cell source and sex differentiation in juvenile *P. dumerilii*

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Most organisms have reproduction-specific organs (gonads) that contain germ cells which ultimately become either eggs or sperm. However, the marine annelid *Platynereis dumerilii* lacks gonads, its germ cells float across its body, in the coelomic cavity. Juveniles have two germ cell clusters: an anterior cluster near the head and gonial clusters that float throughout the body and become sperm or eggs. The exact origin of the gonial clusters, and the factors that drive their differentiation into sperm or eggs are currently unknown. We hypothesize that the anterior cluster acts as a germline stem cell pool, continuously producing gonial clusters. As worms grow, the anterior cluster remains in early stages, even when gonial clusters are progressed in gametogenesis. In near-mature worms, the anterior cluster dissipates suggesting that the stem cell pool is depleted. These findings support that the anterior cluster acts as a germline stem cell pool, supplying gonial clusters. We are currently investigating the factors driving gonial cluster sex differentiation to determine whether the gonial clusters are subject to systemic signals (i.e.: hormones) or if they cell-autonomously become either sperm or egg. We are testing this by implementing gonial cluster transplantations where transgenic female gonial clusters are transplanted into wild-type males (and vice-versa). If the donor cells maintain their sex after transplantation, this will indicate a cell-autonomous process, if not, systemic signals are necessary for differentiation of gonial clusters. Overall, these experiments aim to answer questions about germ cell development in *Platynereis*, paving the way for further research.

P008

The origin and diversification of a cellular patterning mechanism: Lateral inhibition governs ancestral cellular specification in fossil and extant land plants

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The incompleteness of the fossil record and the absence of genetic information limit our ability to understand ancient developmental processes and reconstruct their evolution. Subtle fingerprints of development, such as gene expression patterns, physiological pathways, or cellular differentiation processes, are often lost, leaving essential gaps in our understanding of the evolution of development. Here, we combine comparative morphology and dynamical systems theory to establish an evolutionarily conserved patterning mechanism underlying the specification of liverwort oil body cells, a synapomorphy of the lineage involved in anti-herbivory defense. We demonstrate that oil body cell homologs in fossils of the oldest known liverwort (the Middle-Devonian *Metzgeriothallus sharonae*) exhibit the spatial organization characteristic of lateral inhibition patterning, including overrepresentation of isolated oil body cells and characteristic cell spacing. Through mathematical modeling, we uncover the mechanistic basis underlying these regular patterns, accounting for the diverse arrangements observed in fossil samples. We demonstrate that the same mechanism can reproduce oil body cell patterns in extant liverworts, including the distantly related *Treubia lacunosa*, *Apotreubia nana*, and *Marchantia polymorpha*, suggesting a deeply conserved mode of cell specification. Our framework explains lineage-specific phenotypic transitions—such as the complete loss of oil bodies or the shift from ubiquitous to patterned oil body cell distribution—as outcomes of a conserved patterning module being repurposed over evolutionary time. These findings shed light on the origin and evolution of oil body cell patterning, underscoring its evolutionary significance for liverwort development and survival.

P011

Using the sensory lateral line to explore enhancer evolutionary co-option between fish and mammals

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Biological novelty often arises from the redeployment of conserved developmental genes. Characterizing the regulatory mechanisms behind the emergence of new expression patterns will improve our understanding of how gene functions diversify across organisms and lead to the emergence of new traits. Invertebrate models have provided foundational examples of how modifications to existing enhancers can lead to novel gene expression patterns. However, it is still unknown whether enhancer co-option also is an important source of novelty in vertebrates. Using the zebrafish lateral line, I aim to identify instances of enhancer co-option from an aquatic ancestor to a terrestrial vertebrate. Despite the loss of this sensory system during the water-to-land transition, the persistence of some lateral line enhancers in mammals raises intriguing questions about their current roles. I analyzed chromatin accessibility data in zebrafish to identify putative lateral line enhancers conserved in the mouse. Transgenic reporter assays in zebrafish subsequently confirmed enhancer activity for 12 out of 72 candidates. I will next characterize the novel regulatory functions of these 12 enhancers in mice by transgenic reporter assays. This multispecies functional approach yields insights that comparative sequencing and single species studies alone cannot provide, ultimately helping to establish a predictive framework for how regulatory changes drive phenotypic diversity and innovation.

P012

Big Questions from a Small Nervous System. Dissecting Neuronal Cell Types in Sea Urchin Larvae

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Our brain contains about 86 billion neurons, whereas a sea urchin larva possesses only about one hundred. Yet both systems must detect environmental cues, integrate signals, and produce coordinated behavioral outputs. Whether similarities between their neurons arise from shared ancestry or convergent solutions to these fundamental demands remains unresolved. Using the Mediterranean sea urchin *Paracentrotus lividus* larva, we integrate molecular, morphological, and functional datasets to build a comparative framework for characterizing larval neuronal cell types. Using focused subclustering of a single-cell transcriptomic atlas, we identify neuronal populations defined by neurotransmitter and neuropeptide signatures, terminal selector transcription factors, and a reliance on calcium-based excitability in the absence of canonical Nav channels. By combining these transcriptomic identities with 3D geometrical models, morphometrics and the first recordings of larval neuronal activity through calcium imaging, we outline distinct neuronal classes and their functional properties. Rather than inferring homology from any single criterion, we evaluate how molecular profiles, morphology, and functional responses align or diverge across neuronal types. Together, these data position sea urchin larvae as a tractable comparative model for examining how deuterostome neuronal diversity may have arisen, and illustrate how even a 100-neuron system can illuminate principles of nervous system evolution.

P013

Cellular origins of complex and variable traits: integrating genome-wide association studies and single-cell transcriptomics to investigate the cellular basis of craniofacial variation and congenital conditions across development

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Craniofacial morphology varies extensively across vertebrates and within human populations, yet the cellular and developmental origins of this variation remain poorly defined. Although genome-wide association studies (GWAS) have identified numerous loci associated with craniofacial traits and congenital malformations, these associations are rarely resolved to specific cell populations or developmental stages. As most craniofacial traits are highly polygenic and emerge during embryogenesis, we hypothesized that genetic underpinnings of both normal-range variation and congenital conditions converge on discrete cell types and critical developmental windows during craniofacial morphogenesis.

To test this, we integrated human craniofacial GWAS datasets with a mouse single-cell transcriptomic atlas spanning embryonic day 8.5 to birth. By mapping morphology- and disorder-associated gene sets onto the cellular and temporal landscape of craniofacial development, we assessed whether polygenic signals are enriched in specific cell populations and stages.

We find that genes associated with craniofacial morphology are not uniformly distributed across development but preferentially enriched in defined cell types and developmental windows. Distinct enrichment patterns further differentiate normal-range variation from congenital craniofacial disorders, identifying candidate cell populations and periods of heightened genetic sensitivity.

By resolving polygenic associations into cell-type- and stage-specific contexts, this framework bridges human genetics and developmental biology, providing a mechanistic map of how complex traits emerge during embryogenesis. Beyond its biomedical implications, this approach offers a comparative strategy for identifying shifts in spatiotemporal gene enrichment across species, highlighting how cell populations may serve as a substrate for morphological evolution.

P014

Ready, set, glia: Single-cell RNA sequencing reveals novel populations of glia cells in larvae of the annelid worm *Owenia fusiformis*

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The idiosyncratic mitraria larva of the annelid *Owenia fusiformis* has been used as a model system to study spiralian development and nervous system evolution. We performed single-cell RNA sequencing of 48h-old *O. fusiformis* larvae and subsequent clustering analysis to investigate larval gene expression and characterize transcriptional cell types. Results were then validated by combining gene expression visualisation via hybridisation chain reaction with fluorescence confocal imaging. While confirming a range of expected cell types, our analysis also yielded a surprising result, indicating the presence of two novel populations of glial cells in the prototroch of the 48h-old larvae. The term glia describes a range of electrically passive nervous system cell types found across bilaterian species, counterparts to the electrically active neurons. Classically considered simple support cells for nervous tissue, glia have moved into the spotlight in recent years and are now considered complex and highly adaptable signalling partners to neurons, intricately involved in modulating nervous system output. Yet, very few studies into spiralian glia exist, and little is known about the development of larval glia and their roles in growing nervous systems. Interestingly, the nervous tissue these glial cells occupy is lost during metamorphosis, suggesting an active physiological function for these cells in the free-swimming larva, rather than constituting a precursor population in a developmental pathway. This novel finding of glia in *O. fusiformis* larvae therefore opens up a range of avenues for further study and opportunities to gain new insight into annelid larval physiology and the development and evolution of spiralian nervous systems.

P015

Lunar rhythms impact on cell states in marine invertebrates

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While single-cell transcriptomics has been widely used to explore cell type composition and trajectories during development, adult cell types are often assumed to be stable, particularly neurons. Yet significant changes have been documented in individual cases across seasonal cycles, in some cases leading to the loss and rebirth of neurons (e.g. in songbirds[1]) or shifts in neurotransmitters expression (e.g., in the rat hypothalamus[2]). Besides seasonal timing, monthly time (controlled by the moon) serves as an important time cue for organisms. Especially many marine species, from corals to bristle worms, echinoderms to fishes, synchronize their reproduction with the lunar cycle to coordinate gamete release and maximize fertilization success[3]. Evidence for transcriptomic changes over lunar time exists for various organisms, including corals and the bristle worm *Platynereis dumerilii*, but only from bulk sequencing approaches of mixed tissues and cell types. We hence wondered how such changes would impact on cell-type plasticity across four lunar phases in the adult brain of the bristle worm *P. dumerilii* and in adult tissues of the coral *Stylophora pistillata* using single-cell transcriptomics. Our results reveal lunar-phase–dependent plasticity in specific cell populations. Some responses appear to be similar between species, while others are species-specific. Our findings highlight that adult cell types can exhibit significant periodic plasticity and provide a framework to explore how lunar signals shape cell-type identity and tissue dynamics in animals.

1 Goldman and Nottebohm (1983) PNAS 80:2390–2394.
2 Dulcis et al. (2013) Science 340:449–453.
3 Ritter and Tessmar-Raible (2024) EMBO Rep. 25:3169–3176.

P016

Heterochrony diversifies accessibility to tissue volumetric growth across species during homologous organ morphogenesis

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Imagine shaping pizza dough—stretching and reshaping existing material without adding new material—versus building with LEGO bricks, where new material is continuously incorporated. What if you were constrained to just one strategy, yet had to arrive at the same final structure regardless of which you chose? We show that vertebrate organogenesis faces an analogous challenge. During inner ear morphogenesis, the otic vesicle, a cyst-shaped epithelial primordium, expands in size. In zebrafish, cyst growth relies on elevated luminal pressure and elastic deformation of the epithelium. In mouse, epithelial volumetric growth maintains constant luminal pressure, such that coordinated expansion of both the epithelium and the lumen drives overall cyst growth. The key distinction between these two strategies lies in the accessibility to tissue volumetric growth. In zebrafish, a cell size reduction trend decouples cell division from tissue growth, imposing an intrinsic constraint on tissue volumetric growth. In mouse, cell size is maintained, allowing cell division to directly drive tissue growth. By comparing development across 12 species representing the major vertebrate classes, we propose that species-specific accessibility to tissue volumetric growth is explained by developmental tempo and heterochrony, that is, evolutionary modifications of developmental schedules that co-vary with tempo. Our work demonstrates that organ homology does not necessarily imply conservation of the underlying morphogenetic processes, as viewed through the lens of tissue developmental mechanics. These findings further suggest that embryo type, defined in part by developmental tempo, may serve as an important criterion for animal model selection in developmental biology.

P017

Vitamin D elicits tissue-specific isoform expression in Atlantic salmon muscles

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Accounting for isoforms is likely key to understanding muscle transcriptomic divergence. Muscles offer classic examples of tissues where changes in isoforms alter function and structure in response to stimuli like increased exercise or novel nutrient regimes. To determine how an essential micronutrient alters muscle isoform production, we examined how vitamin D supplementation influences transcription at multiple hierarchical levels across four Atlantic salmon (Salmo salar) muscle tissues. Specifically, we investigated whether analyses of differential transcript expression (DTE), differential transcript usage (DTU), and alternative splicing (DAS) recovered different responses to vitamin D compared to differential gene expression (DGE) alone. Vitamin D modulates salmon muscle transcription at the level of the gene, transcript, and splice junctions in all four muscle tissues. However, the strongest effects at all levels were found in the heart. But, there was little overlap among significant genes found at the gene, transcript, and splicing levels. For example, we found that several myosin light chain kinase isoforms were particularly impacted by vitamin D in the heart, but many of the genes exhibited isoforms that were differentially expressed in opposing directions and were thus masked in DGE analysis. We also identified myofibrillar genes that are impacted by vitamin D at all levels of gene regulation, demonstrating that vitamin D impacts many structural proteins that are directly involved in striated muscle contractions. The limited overlap among significant genes found at the gene, transcript, and splicing levels suggests examining only DGE can mask important genes that show differential effects at other levels.

P018

Inter-species large synthetic replacements suggest transcriptional and developmental robustness at the Hoxd cluster

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Extreme natural morphologies provide powerful systems to study complex developmental processes. This variation is thought to be largely due to changes in genomic regulation. However, identifying and validating candidate regulatory regions remains challenging.

To dissect the molecular origin of the bat wing we generated an integrated multiomic dataset of forelimb and hindlimb development in bat (*Carollia perspicillata*) and mouse (*Mus musculus*). Comparative analyses revealed major differences within the *Hoxd* cluster, a key regulator of limb development. Notably, *Hoxd* genes that are normally restricted to proximal and early expression in mouse limbs gain distal and late expression in the bat forelimb, matching strong epigenomic changes in the locus itself.

To test the contribution of these cis-regulatory differences we first synthesized the entire bat *Hoxd* locus using a yeast-based assembly technology. We then replaced the mouse *Hoxd* cluster with the orthologous bat sequence (170 kb, ten developmental genes plus associated regulatory changes) using advanced CRISPR-based engineering. Mice generated with this replacement showed a normal phenotype and a fully functional bat allele. Our data indicate that heterozygous replacement alone does not reproduce all expected molecular differences, pointing to missing trans-factors and/or additional synergistic regulatory changes. We are now extending this framework by testing orthologous *Hoxd* clusters from more distant species (chicken, zebrafish) to investigate the robustness of this regulatory module.

Overall, this work highlights the engineering of large genomic regions as an elegant and powerful approach to functionally test complex modules and dissect their autonomy within conserved developmental gene networks.

P019

Expansion of isoform diversity in key developmental transcription factors at the origin of vertebrates

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Intercellular signalling pathways such as Wnt/β-catenin, Hedgehog, and BMP are deeply conserved across animals and repeatedly used during embryonic development. Although most pathway components show strong sequence conservation, vertebrates exhibit increased regulatory complexity that cannot be explained solely by gene number expansion. Alternative splicing provides a powerful mechanism to diversify gene function without increasing gene count. Our previous work showed that TCF/LEF transcription factors, the principal effectors of Wnt/β-catenin signalling, display elevated gene number and isoform diversity in vertebrates compared to invertebrates. We therefore asked whether this pattern extends to other developmental signalling effectors at the origin of vertebrates. To address this, we generated de novo long-read transcriptomes spanning the invertebrate–vertebrate transition, including the tunicate *Ciona intestinalis*, the cyclostome *Lampetra planeri*, and the gnathostome *Xenopus tropicalis*. This approach enabled accurate cross-species quantification of full-length transcript isoforms during embryogenesis and organogenesis. We detect a significant increase in isoform number per gene at the origin of vertebrates that disproportionately affects the transcriptional effectors of Wnt/β-catenin (TCF/LEF), Hedgehog (GLI), and BMP (SMAD) signalling, potentially altering the regulatory dynamics of the developmental networks they control. Taken together, these findings support targeted expansion of isoform diversity in central developmental regulators as a contributor to the emergence and diversification of vertebrate developmental complexity, with potential implications for the structure and behaviour of developmental gene regulatory networks. They highlight alternative splicing as an underappreciated axis of regulatory evolution and demonstrate the power of phylogenetically grounded long-read transcriptomics.

P020

How to Build a Head: Developmental Divergence in Vertebrate Embryogenesis

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The vertebrate head is a complex, multi-partite structure and a major evolutionary innovation, shaped by ecological and functional adaptations. Despite the diversity of head morphologies across vertebrates, the head is formed by broadly conserved cellular sources, such as cranial neural crest, mesoderm, and ectoderm. Additionally, the global morphogenetic processes shaping the vertebrate head, from cranial neural crest migration to its differentiation into specific structures and cell types, are largely comparable. However, the cellular, molecular, and regulatory changes that underlie the evolutionary diversification of head morphology remain incompletely understood. Here, we use single-cell RNA sequencing and single-cell ATAC sequencing to investigate head development across five stages in four vertebrate species spanning key phylogenetic distances: mouse, rat, chicken, and catshark. Focusing on the conserved phylotypic period and consecutive stages, we compare gene expression and chromatin accessibility at single-cell resolution across major tissue compartments, including neural, ectodermal/endodermal, and mesenchymal/mesodermal derivatives. Our aim is to identify when and in which cell populations developmental divergence emerges between closely related species (mouse–rat) and more distantly related vertebrates (shark–chicken–mouse). By integrating transcriptional and regulatory landscapes, we seek to define conserved cellular programs of vertebrate head formation and pinpoint lineage-specific shifts in gene regulation, timing, and cell-state trajectories. This comparative framework will allow us to disentangle deeply conserved developmental modules from evolutionary innovations, and to identify the cellular and genomic contexts in which morphological diversification of the vertebrate head arises.

P021

Convergence of Emergent Mechanical Traits

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The beauty and variation in nature is often linked to specific evolutionary traits. Many of these traits are under genetic control, where the purpose of the genes are to generate products that build the trait. In unique situations, groups of traits can form a collective and novelty can emerge. Through co-operation of component parts, a higher level patterning can emerge that is greater than the sum of its parts. Here, the activity of multiple genes need to be within certain parameters to co-operate and allow for an emergent trait to form. It remains unknown what the boundaries of such a parameter space are and how they might differ between species. A tractable example of such a complex trait is iridescence in flowers. There is no single gene sufficient for iridescence, this trait emerges from the activity of multiple genes, which are not directly linked to iridescence. The co-operation between the activity of these genes has a mechanical effect on the petal cuticle. Ordered striations on the petal surface form a diffraction grating, which interacts with light to produce structural colour. This enhances pollinator foraging through an alternative pathway to pigment-based colour. Through microscopy, mechanical measurements and computational modelling, I will generate a space that defines the parameters for floral iridescence. I aim to test this space using multiple iridescent families to identify unifying commonalities across species, or if different paths to this complex trait have been taken, and understand how a sophisticated trait emerges through the co-operation of component parts.

P022

Molecular signature, heterogeneity, and evolutionary conservation of the vertebrate perichondrium

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Most embryonic and some adult vertebrate cartilaginous elements are ensheathed by the perichondrium, a tissue layer central to cartilage growth, shaping, and regeneration. It consists of an outer fibrous layer and an inner chondrogenic layer. During embryogenesis, cells of the inner layer proliferate and generate transversely oriented columns of nascent chondrocytes that integrate into the cartilage matrix. This appositional growth mechanism drives the expansion and morphogenesis of embryonic cartilages. In adults, the perichondrium is thought to retain regenerative potential in specific locations, including the ribs and nasal septum. Despite its functional importance in development, regeneration, and skeletal evolution, the molecular identity of the perichondrium remains poorly defined, largely due to its transcriptional similarity to adjacent chondrocytes. Here, we use single-cell RNA sequencing to resolve the molecular signature and cellular heterogeneity of the embryonic perichondrium and to assess its evolutionary conservation. In addition, we leverage transcriptomic data to infer signaling interactions between perichondrial cells and chondrocytes, uncovering candidate pathways underlying coordinated cartilage growth. High-resolution analyses reveal distinct molecular stratification between the outer fibrous and inner chondrogenic layers and identify transcriptionally defined progenitor populations associated with nascent chondrocyte columns. Comparative analyses in the small-spotted catshark demonstrate conservation of key molecular features, supporting an evolutionarily conserved role for perichondrium-driven appositional growth in vertebrate skeletogenesis.

P023

Manipulation of a polyphenism-switch network reveals the molecular basis for canalization of alternative forms

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Developmental polyphenism is the production of discrete, alternative phenotypes in response to environmental inputs. Although the genetics of plasticity switches has been described, the molecular basis of how those forms are realized—that is, canalized as stereotyped forms—is still unclear. The nematode *Pristionchus pacificus* has a resource polyphenism of two adult morphs, eurystomatous (Eu) and stenostomatous (St), controlled by a known switch network. To test how this switch is implemented transcriptionally, we used staged RNA-seq across the polyphenism's window of sensitivity with mutants that are fixed for the wild-type Eu or St morphs and mutants that stably produce intermediate morphologies. We then identified the developmental stages with the strongest morph-associated transcriptional divergence and compared expression patterns across canalized and intermediate genotypes. Instead of showing a mosaic pattern of Eu-specific and St-specific genes being misexpressed in patches, intermediate morphs largely showed shifts in the level and timing of a shared morph-execution program. This supports a graded model, in which the underlying transcriptional state is continuous, while normal development collapses that continuum into discrete, stable morphs. We also asked whether genes associated with graded morph variation also show distinct evolutionary patterns across nematodes, specifically in lineages that have secondarily lost the polyphenism. Using branch-site analyses, we found elevated signatures of episodic selection in these genes in monomorphic lineages relative to those that have retained the polyphenism. Together, these results describe a molecular basis for canalization of plasticity and link this basis to repeated evolutionary change in polyphenism.

P024

Single-cell sequencing in flies and beetles reveals regulators of neural stem cell specification as novel candidates for evolutionary modification of cellular brain composition

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Evolutionary adaptations in brain structure and function are essential for animal survival and emerge during development. For example, the different sizes of the *mushroom bodies* and the varying timing of *central complex* development are likely to affect the learning and navigation abilities of different insect species. Parts of the variation may evolve through modifications to the number and identity of neural stem cells (neuroblasts, NBs). Indeed, the number of NBs has been observed to diverge evolutionarily between insect clades. However, the regulatory genes that determine NB identity remain unknown, while they are candidates for modifying the specific number and types of neural cells that an individual NB contributes to the brain. To study NB diversification, we first determined the conserved core of NB specification genes. To that end, we generated single-nuclei and single-cell data from NBs of both the fly *Drosophila melanogaster* and the beetle *Tribolium castaneum*. We determined the conserved core of transcription factors, that are expressed in NBs and are therefore likely to be involved in specifying NB identity. These genes serve as markers to study NB identity across species but they are also prime candidates for driving brain diversification.

P025

An ancient monoaminergic signaling system coordinates contractility in a nerveless sponge

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Chemical neurotransmission was a key animal innovation, enabling multicellular coordination of physiology and locomotion. Sponges are early-diverging animals that lack neurons and muscles, yet still coordinate contraction and relaxation of their filter-feeding water canals. Here, we combine behavioral imaging, isotope tracing, heterologous expression, and functional proteomics to characterize the cellular players and molecular pathways involved in regulating whole-body movements for the freshwater sponge, *Spongilla lacustris*. Unlike canonical transmitters—including glutamate, acetylcholine, and catecholamines—used by other animals to regulate homeostasis, *S. lacustris* synthesizes tryptamine, phenethylamine, and tyramine as simple monoamines derived from amino acid decarboxylation to elicit distinct canal behaviors.We further demonstrate *S. lacustris* employs previously uncharacterized members of the pyridoxal-5'-phosphate-dependent enzyme and major facilitator superfamilies as decarboxylases and vesicular transporters, respectively, in secretory neuroid and metabolic cells for monoamine biosynthesis and transport *de novo*. Phosphoproteomics and label-free 3D imaging of *S. lacustris* reveal that tryptamine activates GPCR signaling and Rho GTPases, remodeling actomyosin and adhesion complexes in contractile canal epithelia to drive localized constrictions and whole-body deflations. Our findings support an evolutionary scenario in which molecular modules responsible for unicellular chemotactic crawling were co-opted into specialized cell types, thereby establishing the first monoaminergic circuits in animals to control tissue contractility, before undergoing further elaboration to support the neuromodulation of synaptic transmission in nervous systems.

P026

From Mantle to Shell: Cellular Foundations of Structural Colour Patterning in the Blue-Rayed Limpet *Patella pellucida*

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The blue-rayed limpet, *Patella pellucida*, produces vivid, discretely bounded shell stripes through a highly ordered mineral photonic architecture – one of the most striking examples of biologically constructed structural colour. The species exhibits marked variation in shell patterning most notably across its two recognised ecomorphs, *pellucida* and *laevis*, associated with distinct kelp microhabitats. Despite the physical basis of these stripes being well characterised, the mantle epithelial organisation underpinning their formation remains unresolved. This study presents preliminary histological and ultrastructural analyses of the *P. pellucida* mantle across ecomorphs, with the aim of establishing the cellular framework through which spatially precise, periodic shell patterns are generated. Mantle tissues from both morphs have been fixed, sectioned, and analysed using light and electron microscopy to resolve epithelial cell types, regional specialisation, vesicle characteristics, and the shell–mantle interface. A particular focus is in characterising the localisation and cellular origin of pattern-associated pigmentation, and how these features differ between ecomorphs and across patterned and non-patterned mantle regions. These analyses reveal the fine-scale cellular and ultrastructural organisation of the mantle epithelium for this species. These findings establish a cellular framework for interpreting how spatial patterning information is encoded and executed within the mantle, providing the structural foundation for ongoing bulk and spatially resolved transcriptomic analyses aimed at identifying the gene-expression programs that specify these epithelial territories. Together, this work positions *P. pellucida* as a tractable model for studying how spatially organised developmental programs give rise to shell pattern formation as a fully integrated, multi-scale biological process.

P027

Evolutionary drift in biomineralization processes questions the nature of ‘osteocytes’ and ‘chondrocytes’ in early vertebrates

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Skeletal mineralized tissues evolved early in the vertebrate lineage, more than 415 Myr ago and under various forms including mineralized cartilage, dermal and perichondral bone. Endochondral bone though has evolved as a general rule in the bony fish lineage only, to which most model organisms for developmental biology belong (tetrapods or teleost fishes). In the cartilaginous fish lineage, bone is considered secondarily lost despite conspicuous mineralisation of the cartilaginous skeleton, also under several forms. The mineralizing chondrocytes in cartilaginous fish have classically been though to be comparable to hypertrophic chondrocytes of endochondral ossification. Our data obtained in the small-spotted catshark *Scyliorhinus canicula,* both by RNAseq and proteomics, informs the developmental dynamics, the cellular characteristics and the genetic actors of skeletal mineralisation in cartilaginous fishes. These data have highlighted several aspects of cartilaginous and bony fish-specific evolutionary events, including gene duplication, diverged developmental processes and cell type identity. The comparative analysis of these data highlights two controversies: (i) although considered ‘shared’ across vertebrates, mineralizing chondrocytes or perichondral bone osteocytes are not similar on a molecular level when different lineages are compared; (ii) as a consequence, and although considered ‘ancestral’ for vertebrates, their composition in fossils of early vertebrates might be rather different from extant skeletal tissues.

P028

Morphological innovation without gene co-option: the *Drosophila* sex comb evolved via changes in developmental tempo and energy metabolism

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The individualization of serially repeated homologs is one route through which novel traits are thought to evolve. Under this model, a repeated character—like a limb, digit, or sensory bristle—is individualized from its homologs by changes in the regulatory apparatus ('character identity network', ChIN) that specifies its development. Individualization then enables downstream gene networks that build the repeated character to diverge from one another in different parts of the body, ultimately allowing new phenotypic endpoints to be reached. Despite this model's intuitive appeal, the genetic mechanisms through which new ChINs rewire trait-building gene networks remain largely uncharacterized. A promising system in which to study this process is the *Drosophila* sex comb. Found in a sublineage of *Drosophila* species, the sex comb is a recently evolved, male-specific organ that evolved from a more evolutionarily ancient precursor—the mechanosensory (MS) bristle—following the gain of a novel ChIN centered on the sex determination gene *dsx* and HOX gene *Scr*. Here, we use time-series single-cell RNA-seq to show that rather than co-opting new genes, this new ChIN orchestrates quantitative and heterochronic changes in the ancestral MS bristle transcriptome. These changes affect gene modules that control energy metabolism, endoreplication, and actin dynamics. The net effect of these changes is an organ-specific shift in developmental rate, leading to accelerated growth in sex comb teeth. Collectively, our work suggests that morphological innovation can proceed without the co-option of new genes into downstream trait-building networks and instead through metabolically driven differences in developmental rate between serial homologs.

P029

Heterochronic activation of TGF-β signaling drives the diversity of the avian sterna

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Adaptive evolution has driven the diversification of vertebrate skeletal morphology, enabling a wide array of locomotor modes and lifestyles. In volant birds (carinates), the sternum develops a ventral keel that provides an attachment site for massive pectoral muscles essential for powered flight. In contrast, flightless ratites have lost both flight capability and the keel, resulting in a striking morphological contrast between their sterna. Here, we interrogated the cellular and molecular basis underlying this morphological divergence by using chicken embryos as a carinate model and emu embryos as a ratite model. Through a series of analyses including spatiotemporal transcriptomics and a spheroid culture system, we found that TGF-β signaling, which promotes proliferation of ventral sternal chondroprogenitors, is activated in both species until the stage when the left and right sternal progenitors meet; however, in chicken this activation persists beyond this stage, driving ventral extension of the keel primordium, whereas in emu it shuts off early, culminating in the absence of a protruding keel. Together, our findings suggest that skeletal morphological changes associated with behavioral transitions can arise from heterochrony in developmental signaling, thereby deepening our understanding of the evolutionary logic shaping skeletal diversification.

P030

Variability of asexual development trajectories in clonal zooids of the ascidian *Botryllus schlosseri*

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The ascidian *Botryllus schlosseri* forms modular colonies in which three generations of zooids coexist: adult zooids producing right and left primary buds, which in turn bear secondary buds. Zooids exhibit asymmetric budding, which favours the growth of the right buds, always larger than their left counterparts. Cyclically, during the takeover phase, adults undergo regression, primary buds become new filtering adults, secondary buds develop into primary buds and produce a new generation of secondary buds. To assess whether and how zooid variability shapes this complex life cycle that guarantees colony homeostasis in terms of robustness of cycling and resilience to perturbation, we analysed growth (body size), regression, and physiology (heartbeat frequency) across coexisting generations, considering zooid genealogy. We found that adult size is not stable as previously believed but increases until regression and correlates with reproductive output: adults sustaining the development of two buds are larger than those carrying only one bud. Moreover, the different size of right and left primary buds is reset at takeover, equalising new adults. The size of new adults and therefore their ability to produce buds in different numbers and sizes is independent of the size they had when they were buds. Finally, both buds and adults developing in parallel, and individuals at the same stage belonging to different generations in colony life, exhibit generation-specific differences in heartbeat frequency. Results reveal that the takeover provide to the colony a great physiological plasticity despite the zooid genetic identity and coordination development guaranteed by the shared circulatory system.

P031

Evolution of Novel Contacts in Orthologous Regulatory Elements

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Thousands of Conserved Non-coding Elements (CNEs) are shared by jawed vertebrates, some of which were shown to be important developmental enhancers. However, how these intriguing elements evolve at the functional level and why they are so conserved remain some of the biggest mysteries in regulatory genomics. Here, we build a novel view of CNE function and evolution based on recent breakthroughs on enhancer pleiotropy. This working hypothesis posits that CNEs are ancient transcription factor binding hubs that act as dynamic multi-genic regulators to which new gene targets can be added and removed during evolution. Taking advantage of the exceptional conservation of CNEs, we test this hypothesis on orthologous elements in major vertebrate clades using cutting-edge methodology combining Capture-HiC, RNA-seq and ATACseq in embryonic brain. This approach provides comparative CNE-target contact maps that reveal conserved, gained and lost interactions across species, addressing long-standing questions on the evolution of the regulatory genome.

P032

Metabolic control of multicellular transition in a close unicellular relative of metazoa

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The emergence of multicellular animals from unicellular ancestors remains a mystery. Current research has focused on key processes for multicellular life, such as cell adhesion, cell signaling and transcriptional regulation. However, the potential role of metabolism in this transition has not been studied. The filasterean *Capsaspora owczarzaki*, a close unicellular relative of animals, provides a powerful model to address this gap, as it exhibits three well-defined life stages: a quiescent, resistant cystic stage; a proliferative filopodiated stage; and a multicellular stage acquired through chemically induced aggregation (e.g., fetal bovine serum). Previous transcriptomic analyses revealed marked differences between these stages. Here, we extend this work by integrating lipidomic and metabolomic analyses to uncover metabolic remodeling across the unicellular-to-multicellular transition. Lipidomics reveals pronounced but stage-specific membrane reorganization. Cystic cells display rigid, storage-oriented membranes and reduced mitochondrial activity. Filopodiated cells initiate membrane remodelin, coupled to mitochondrial activation. In aggregates, cells undergo large-scale membrane expansion dominated, while maintaining lipid homeostasis. Metabolomic profiling further shows that the transition to multicellularity is accompanied by the upregulation of reductive metabolic pathways, including the pentose phosphate pathway and glutathione metabolism, suggesting enhanced buffering of reactive oxygen species to promote survival in the multicellular state. Finally, pharmacological inhibition experiments demonstrate that glycolysis is required for the early steps of aggregation. Together, these results highlight metabolism as an active driver of the transition to animal multicellularity, opening new avenues to explore how metabolic innovation contributed to the evolutionary origins of multicellular life.

P033

Evolutionary modulation of the peripheral nervous system gene regulatory network in the ascidian *Molgula appendiculata*

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The development of organisms relies on finely coordinated mechanisms, such as gene regulatory networks (GRNs) and inductive signals. While these networks are globally conserved, their modification can lead to the emergence of new structures, morphological losses, or phenotypic diversity. Ascidians, the sister group of vertebrates, provide a powerful framework to investigate how evolutionary changes in developmental programs generate such diversity. In ascidians, the peripheral nervous system (PNS) derives from the neural plate borders and shares evolutionary origins with vertebrate placodes and neural crest. The GRN underlying PNS specification has been extensively characterized in *Ciona intestinalis*, providing a reference framework to explore evolutionary variation. Using a comparative approach across four species (*Ascidia mentula, Phallusia mammillata, Clavelina lepadiformis, and Molgula appendiculata*), I examined how this conserved regulatory program is deployed during PNS development. While the overall morpho-molecular organization of sensory neurons is largely conserved across species, *Molgula appendiculata* displays a marked reduction in neuron number. This reduction correlates with altered spatial expression of key transcription factors and atypical BMP signaling dynamics. These findings suggest that PNS reduction in *M. appendiculata* is not due to the loss of core regulatory components, but rather to modifications in the connectivity and signaling thresholds within a conserved network architecture. More broadly, this study illustrates how evolutionary changes in GRN deployment can generate trait reduction while maintaining the overall developmental scaffold, offering new insights into how biological complexity is rewired through evolutionary time.

P034

Post-embryonic development networks underlying trait evolution in teleost fishes

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Comparative embryology has revealed a high degree of homology and conservation in early animal development. Beyond the embryo, however, animals exhibit striking variation in post-embryonic developmental strategies. How genes underlying these strategies evolve and give rise to phenotypic variation remains unknown. In this study we characterised post-embryonic development across five teleost fish families to uncover the links between developmental processes and trait evolution. Comparative gene regulatory networks revealed a conserved set of genes underlying post-embryonic development in teleosts. These genes were organised into discrete modules associated with aerobic respiration, hormone signalling, and the formation of major tissue systems, including the eye, musculature, and nervous system. Evolutionary analyses revealed that these genes exhibit extensive copy-number turnover across teleosts, with major expansions following the teleost-specific whole-genome duplication and additional lineage-specific gains and losses. Phylogenetic modelling identified significant associations between copy-number variation in these rapidly evolving genes and phenotypic diversity across teleost lineages in traits such as age of maturity, swimming performance, and body shape. Single-cell transcriptomic data from zebrafish confirmed expression of these genes in cell types relevant to the associated phenotypes, and functional perturbation experiments supported their roles in corresponding tissues. Together, these findings indicate that core post-embryonic developmental programs originated early in teleost evolution and were subsequently refined through gene copy-number dynamics, contributing to the phenotypic diversity across clades.

P035

Novel RA-Wnt-Nr2f1 Conserved Regulatory Networks in vertebrate atrial development

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Nuclear Receptor Subfamily 2 Group F (Nr2f) transcription factors (TFs) display a conserved role in the heart development of vertebrates. In zebrafish (*Danio rerio*), *3'reg1-Nr2f1*, an ultra-conserved enhancer at the 3' of *nr2f1a*, forms a regulatory cascade with Foxf1 and Wnt signals to induce atrial differentiation. Using stable transgenic lines with *3'reg1* orthologs from ghostshark (*Callorhinchus milii*) and human showed functional enhancer conservation in zebrafish atrium. Additionally, multiple signaling manipulations and epistatic analyses applied on *3'reg1* orthologs suggest that the Nfatc1 pathway forms a regulatory cross-talk with Wnt, promoting *3'reg1* atrial expression. However, *3'reg1* analysis did not clarify the network underlying the relationship between retinoic acid (RA) signaling and *Nr2f* genes in vertebrate cardiac development. Integrating distinct comparative genomics approaches (VISTA plots, manual alignments), we discovered a novel 3' enhancer, *3'regM-Nr2f1*, conserved exclusively in teleosts and mammals and expressed in atrial cardiomyocytes (CMs). Intriguingly, a comparative transcription factor binding site (TFBS) survey showed that *3'regM* is enriched in conserved nuclear hormone receptors (NHR) and Tcf binding sites, suggesting its responsiveness to both RA and Wnt pathways. Mutating each of the three conserved NHR sites reduced atrial *3'regM* expression, while a single Tcf mutation upregulated the enhancer expression. Furthermore, drug manipulations confirmed that RA and Wnt concurrently control *3'regM*, while epistasis approaches highlighted their interaction with both Nfatc1a and BMP pathways. Overall, our data evoke the existence of a conserved atrial transcriptional code orchestrated by a complex gene regulatory network, in which Nr2f1, Wnt, and RA act as conserved hub signals.

P036

Redrawing the Prosomeric Map: Hypoprethalamus as primordial ontogenetic territory in tetrapods.

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TextThe prosencephalon is a morphological, functional and ontological sophisticated brain structure, whose complexity increases throughout the evolution of vertebrates. Within it, the prethalamus is a key transitional zone since it represents the caudal limit with the zona limitans intrathalamica (zli) (involved in the telencephalic regionalization) and it mediates the thalamocortical connectivity involved in the sensory integration, motor response and in higher cognitive processes. Often overlooked, this region may be central in the evolutionary diversification of vertebrate brain. The prethalamic region is currently considered the most rostral prosomere of the diencephalon (p3), flanked by the hypothalamus rostrally and the zli followed by the thalamus (p2) caudally. Nonetheless, genoarchitectonic studies in amphioxus suggest a closer ontological link with telencephalohypothalamic territories. Our study in Xenopus laevis during neurula and early embryonic stages supports this view, revealing a nearby correlation of p3 with hypothalamo-telencephalic areas more than with other diencephalic prosomeres. These findings align with those of amphioxus and recent RNAseq studies in other vertebrates, pointed to an ontological closeness of the prethalamus region with hypothalamic and subpallial populations and less with the thalamo-pretectal complex not only in primitive chordates, but also in tetrapods.

P037

Co-option of conserved retinal cell types by a male-specific *doublesex*-related gene underlies the evolutionary origin of the mayfly Turbanate eye

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The mayfly Turbanate eye is a male-specific dorsal visual structure that develops alongside the lateral compound eyes shared by both sexes. This sexually dimorphic organ represents a striking morphological innovation within the group, offering a compelling model to study how novel structures evolve within an existing developmental context.

Using single-cell RNA-seq combined with bulk ATAC-seq, we reconstructed the gene regulatory network underlying Turbanate eye development and characterised its constituent cell types. The Turbanate eye does not deploy a novel cell type repertoire — instead, it recruits the same retinal cell types responsible for lateral eye growth. The evolutionary origin of this morphological novelty therefore operates through regulatory rewiring of a conserved cellular toolkit rather than through cell type innovation.

Central to this rewiring is *dsxy*, a male-genome-specific doublesex-related gene. Regulatory inference places *dsxy* upstream of core visual system components. Moreover, its activity in early embryos indicates that sex-specific developmental commitment is established at the onset of development, suggesting that co-option of the retinal programme into a new organ context is initiated early in embryogenesis.

Together, our findings demonstrate that co-option of a conserved character identity network, driven by *dsxy* as a sex-specific regulatory innovation, can generate a morphologically distinct organ. The Turbanate eye provides a mechanistic example of how higher-order structural novelty can emerge through regulatory rewiring alone, without requiring the origin of novel cell types.

P038

The *Hes*/*her* gene *deadpan,* a paralog of *hairy,* plays a partially redundant role in *Drosophila* segmentation

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The *Drosophila* anteroposterior patterning system is a classic model for animal segmentation, involving pair-rule genes expressed in seven stripes delineating future body segments. The pair-rule gene *hairy* (*hry*) has a paralog, *deadpan* (*dpn*) which also exhibits striped expression during segmentation. *dpn* has not previously been classified as a segmentation gene because its null mutants lack an obvious segmentation phenotype. However, the dpn^{7} allele, which has a point mutation in its DNA-binding domain, shows abnormal segmentation. This raises the question of how *dpn* is involved in segmentation and why dpn^{7} has a more severe phenotype than the null mutant. We reanalysed the phenotypes of various *hry* and *dpn* alleles using cuticle preparations and multiplexed HCR-FISH. Both dpn^{7} and *dpn* null alleles showed derepression of the Hry target genes *fushi tarazu* and *odd-skipped*, though only dpn^{7} had cuticular segmentation defects. Strikingly, for both *hry* and *dpn*, point mutations in the DNA-binding domain had more severe phenotypes than gene deletions. We propose that this is explained by protein-protein interactions between Hry and Dpn, similar to the “dimer cloud” of homologous *Hes*/*her* genes involved in vertebrate somitogenesis. The point mutants presumably produce non-functional heterodimers, additionally reducing effective levels of the other protein compared to null mutants, and thus having a more severe phenotype than deletions.

P039

The structure of tooth evo-devo: Trp63 binding targets in the embryonic face

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Vertebrate faces, teeth and jaws are amazingly diverse. Some jawed vertebrate groups have evolved selective or complete tooth loss. The developmental-genetic mechanisms viably integrating dentitions within jawbones as animals grow and species evolve remain enigmatic. Originating from previous microarray and other gene transcript analyses, here we build out the architecture of a Trp63-driven gene regulatory network (GRN) essential for odontogenesis but arguably peripheral to jaw morphogenesis. Contrasting wildtype/toothed (Trp63+/+; Trp63+/-) and toothless mutant (Trp63-/-) mouse models, ChIP-sequencing of embryonic (E10.5, E12.5) upper (MxP) and lower (MdP) facial prominences as well as second arch (PA2) tissues (n=3/stage/tissue type/genotype) identified >10,000 Trp63 primary binding targets. We filtered this list for genes specific to our previous analyses (>50 targets). Some targets were common among MdP, MxP and PA2 at E10.5 and E12.5 (Ckb, Cldn3/10, Dst, Gsc, Trp63), others were common at one stage only (e.g., E10.5: Arg1, Fermt1, Otx1/2; E12.5: Fgfr2, Ifitm3, Pitx2, Trim29). Other targets were bound specifically in just two or only one tissue type. Overrepresentation analyses implicated targets in an array of functions, notably cell adhesion and cytoskeletal dynamics. Protein expression analyses found Trp63-dose-dependent activity of E-cadherin and F-actin in E10.5 and E12.5 tooth organ epithelium. While a Trp63-driven GRN is vital to dental epithelium integrity, this GRN appears important to sustain epithelium-involved morphogenesis more broadly in the embryonic face. For a given stage, tissue-specific differences in Trp63 binding targets may reflect evolutionary tinkering of this GRN within embryonic craniofacial structures, whether they form teeth or not.

P040

Nuclear targeting enables highly efficient transgenesis in the milkweed bug *Oncopeltus fasciatus*

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Transgenesis mediated by transposable elements such as *piggyBac* is a powerful genetic tool for investigating gene function and regulatory elements. However, for many non-model arthropod species, transgenesis still remains technically challenging and available methods are limited. Here, we establish a highly efficient *piggyBac*-mediated transgenesis method in the milkweed bug *Oncopeltus fasciatus* by enhancing nuclear targeting of transposase. First, we generated a useful mutant strain with both bright eye color and whitish body color using CRISPR/Cas9, providing an easy-to-score transformation marker background. We then compared the transformation efficiencies mediated by *piggyBac* transposases containing 0x, 1x, 3x, and 5x nuclear localization signals (NLSs) from the SV40 large T-antigen and found that 1x and 3x NLS showed the highest efficiencies, reaching 30% G0 somatic transformation and 30% G1 germline transformation. Using this optimized method, we successfully established multiple transgenic strains carrying expression cassettes ranging from 1.8 to 3.6 kb in length. Our results indicate that the simple modification of adding nuclear localization sequences to a transposase improves overall transgenesis efficiency and should be readily applicable to a wide variety of animals.

P042

Mechanisms specifying invariant ovariole and testiole number across insects

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Throughout development, organisms must pattern their organs to achieve precise organization. One fundamental question in this process is how a specific number of substructures is specified within a single organ. The development of insect gonads provides a useful system in which to investigate this problem, as insect gonads are partitioned into a discrete, quantifiable number of repeated units - ovarioles and testioles ("gonadioles"). While many insect species exhibit variation in gonadiole number among individuals, multiple lineages have independently evolved an invariant state in which gonadiole number is fixed. Despite its repeated evolution, the developmental and genetic mechanisms underlying this invariance remain poorly understood. Previous work in our lab showed that in *Oncopeltus fasciatus*, invariant gonadiole number is established during embryogenesis and is associated with a defined subset of abdominal segments. Each gonadiole primordium forms within one of segments A1-A7, and manipulation of Hox gene expression alters both their number and position, suggesting a role for segmental identity in determining gonadiole number. Here, we extend this investigation to two additional species with invariant gonadiole number - *Thermobia domestica* and *Plodia interpunctella*. Using germline and somatic gonad markers, we reconstruct gonad formation from embryogenesis through adulthood. These analyses allow us to determine when during development gonadiole number is specified and whether invariance is similarly coupled to early axial patterning. By comparing distantly related insects with invariant gonadiole number, we test whether this trait reflects convergence on a shared developmental mechanism or the evolution of distinct strategies that produce the same invariant outcome.

P044

Evolution of vertebrate facial morphogenesis

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Vertebrates display an extraordinary diversity in craniofacial morphology, which reflects their adaptation to distinct ecological niches. However, this diversity arises from conserved cellular sources and developmental signaling. After a conserved mid-embryonic stage, facial prominences are formed and start to exhibit distinct morphologies. This raises the question whether differences in adult facial morphology arise primarily from modifications of growth and patterning of facial prominences. We hypothesize that evolutionary alteration of vertebrate facial architecture is driven by lineage-specific changes in patterns of positional genes, that are expressed by the individual facial prominences and their subregions. Because each prominence exhibits a distinct molecular signature, characterized by specific combinations of transcription factors and signaling activities, evolutionary shifts in spatial gene expression domains may potentially alter prominence growth and fusion, ultimately reshaping facial morphology at a macroevolutionary scale. To evaluate this hypothesis, we integrate detailed knowledge of craniofacial morphogenesis along vertebrate phylogeny with comparative mapping of positional gene expression in representative species. By aligning morphogenetic dynamics with region-specific molecular signatures in a phylogenetic framework, we assess whether divergence in developmental programs at the level of individual prominences correlate with the emergence of fundamentally distinct facial configurations. This integrative approach moves beyond descriptive comparisons and links modifications in gene expression patterns to large-scale differences in facial morphology. It seeks to provide a conceptual framework for understanding how conserved embryonic modules are repurposed to generate the remarkable diversity of vertebrate faces.

P045

Mayflies, an emerging model to investigate the origin of insect wings

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Mayflies are key organisms to study the evolution of new traits given their phylogenetic position within insects, their life cycle, ecology and innovative morphology. In recent years, our laboratory has established the mayfly *Cloeon dipterum* as a new model organism by developing (i) a continuous inbred culture and (ii) a comprehensive toolkit of developmental and genomic approaches, including genome assembly, in situ hybridization, RNA-seq, and ATAC-seq. These efforts have laid the groundwork for utilizing mayfly as a valuable model to delve into EvoDevo question such as the origin of insect wings. Together with Odonata, mayflies are believed to be among the first insects to have evolved wings. Additionally, during their aquatic life stage, they possess abdominal gills that have been proposed to be serial homologs of wings. Our previous bulk RNA-seq analyses revealed striking similarities between the gene expression profiles of wings and gills in *C. dipterum*. However, bulk transcriptomics only captures average gene expression across entire tissues, masking the diversity of cell types and developmental trajectories underlying these similarities. To truly understand the evolutionary and developmental relationship between wings and gills, we must resolve gene regulation at the level of individual cell populations. To this end, we leveraged recent advances in single-cell transcriptomic technologies and obtained preliminary results comparing transcriptomic states across cell populations throughout *C. dipterum* embryogenesis.

P046

A whole-organism single-cell perspective on larval haematopoiesis and the evolution of innate immunity in molluscs

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The immune system of molluscs remains one of the least understood aspects of their biology. It is composed of diverse tissues, with haemocytes as the primary effector cells. Haemocytes are macrophage-like cells that perform a broad range of functions, including phagocytosis, cytotoxicity and nutrient distribution, yet how they acquire such functional diversity remains unknown. Although previous efforts have attempted to describe haemocyte development in adult molluscs, canonical early progenitors have remained undetected. To address this gap, we aimed to characterize molluscan haemocyte development throughout larval ontogeny. We generated a multi-stage, whole-organism single-cell RNA-seq atlas spanning mid-organogenesis, late-organogenesis, early veliger and late veliger stages in the caenogastropod *Crepidula fornicata*. Our atlas identifies multiple haemocyte subtypes and reveals the first pan-haemocytic marker described to date, *serpin i2*. By combining HCR-ISH (Hybridization Chain Reaction) validation, we visualized larval haemocytes for the first time and confirmed the distinct subtypes inferred from the data, highlighting the high resolution of our atlas. Moreover, the atlas unveils candidate haematopoietic progenitors, suggesting sites of early haematopoiesis and revealing the putative origin of haemocytes in molluscs. In combination with interspecies comparisons, our data will help define a general developmental trajectory for haemocyte formation and offer new insights into the evolution of haematopoiesis in molluscs and more broadly across the Spiralia.

P047

An Integrated Framework for Thermosensation in *Platynereis dumerilii*

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One of the most important environmental stimuli for ectothermic animals is temperature. Understanding how animals can sense, translate, and react to temperate, is a fundamental question. To answer this, I chose the model organism *Platynereis dumerilii*, which has stereotypic development and available ultrastructural resources. Together with our workshop, I developed a thermal gradient arena to investigate animal positioning and trajectories within a stable gradient or fixed temperature regime. The setup also enables modulation of light and gravity orientation (for photo- and geotaxis). To link behavior to the underlying molecular pathways, I optimized the SHiNE protocol for *Platynereis*. I replaced toxic formamide with a urea-based buffer and adjusted dextran sulfate concentrations. These adjustments reduce toxicity and improve sensitivity, ensuring all protocol components work in harmony. The resulting light microscopy data is registered to a whole-body electron microscopy (EM) volume previously collected by the lab. Using custom software, I map the HCR signal of Transient Receptor Potential (TRP) channel expression onto the EM connectome. This work is the beginning of understanding thermal sensing in marine invertebrates. Having established baseline behavior and receptor locations, we must now test if these receptors are responsible for temperature sensing and determine their active ranges. This will be done using knockout lines and calcium imaging. This data will allow us to understand the underlying molecular and neuronal pathways from sensors to motor neurons and behavior.

P048

Transcriptomic analysis of novel kidney-related genes in lesser spotted catshark (*Scyliorhinus canicula*) embryos

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Many studies over the years have identified novel regulatory pathways that guide vertebrate kidney morphogenesis and the transitions between its different developmental forms. However, considerably less is known about pronephros (the primary vertebrate kidney) field formation and organogenesis. Here, we present the results of a transcriptomic analysis of pronephros formation in an early gnathostome organism, the catshark *Scyliorhinus canicula,* and hence we deal with evolutionary perspectives of pronephros development. By isolating embryonic tissues that give rise to the pronephros—both before and during its formation—from other anterior, non-competent tissues, we identified novel candidate pronephric genes. Using temporal and spatial differential expression analyses, we subsequently validated several candidate genes by RNA *in situ* hybridization. Ultimately, our goal is to identify and characterize novel regulators and signaling pathways responsible for establishing the vertebrate primary kidney morphogenetic field.

P049

Developmental and evolutionary origins of the tetrapod neck

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The neck is a morphological innovation that emerged congruent with limbs during the vertebrate water-to-land transition and that required profound developmental rearrangements. In vertebrates, the muscular systems of the head and trunk have different mesodermal origins with distinct gene regulatory networks. Most head muscles derive from the cardiopharyngeal mesoderm whereas trunk muscles originate from the somitic mesoderm. The neck connecting the head to the trunk has mixed mesodermal origins and its evolutionary history remained elusive. During the past decade, we have investigated the relative contribution of mesodermal populations to the emergence and adaptation of the tetrapod neck combining comparative embryology and anatomy approaches in key vertebrate organisms. By genetic lineage tracing strategies in zebrafish and mouse embryos, we showed that the muscle groups that connect the head to the trunk have conserved cardiopharyngeal or somitic mesodermal origins in jawed vertebrates, characterizing the homology of structures at the head-trunk transition. By performing CT scan analyses and 3D reconstructions of the musculoskeletal system of ray-finned fish, lobe-finned fish, salamander and lizard juveniles, we show that the tetrapod neck muscles evolved from posterior expansion of the cardiopharyngeal mesoderm into the trunk region and opposite anterior expansion of the somitic mesoderm into the head, assuring new functions including head mobility, food capture, aerial respiration and vocalization processes on land. Our data provide new insights into the emergence and evolution of morphological features that permitted the adaptation of vertebrates to terrestrial life.

P050

Development of transgenic reporters in *Parhyale hawaiensis*

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The marine amphipod crustacean *Parhyale hawaiensis* is an emerging model to study regeneration. Our lab uses a combination of transgenic reporter tools and live imaging to investigate leg regeneration in this species. My work focusses on developing diverse transgenic reporters for live imaging and to manipulate gene expression in a conditional and cell-type specific manner. We employ both bulk ATACseq data and single-nucleus ATACseq data to identify regions of open chromatin in diverse cell types, including epidermal, neuronal, muscle and blood cells. Additionally, we utilise low coverage genome sequencing of three congeneric species of *P. hawaiensis* – *P. darvishi*, *P. aquilina* and *P. plumicornis* – to identify islands of conservation across the genome. With these resources, I pursued putative *cis*-regulatory elements in *P. hawaiensis* genome that can drive robust expression of fluorescent reporters ubiquitously and in specific cell types. Resulting from this pursuit I developed two transgenic reporter tools that are expressed ubiquitously throughout the animal.

P052

Begonia, a promising evo-devo model for studying the role of duplication in the diversification of the leaf developmental pathway

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A plant's form mediates their interactions with the environment; yet, the evolutionary factors driving morphological diversity remain uncertain, highlighting the need for new evo-devo models. With over 2,200 species, and more identified every year, *Begonia* is one of the largest angiosperm genera. Although recognisable by their characteristic asymmetry, *Begonia* leaves show remarkable diversity in size and shape. A whole-genome duplication event at the base of *Begoniaceae* suggests that neofunctionalisation of duplicated leaf developmental pathways may have driven diversification. With genomic and transcriptomic resources available, *Begonia* offers an excellent model to study the impact of duplication on leaf shape evolution. We have also developed a toolbox of biotechnological techniques, including the first transient transformation of *Begonia* for co-localisation studies, to allow functional characterisation of diverged duplicates in *Begonia*. Detailed genome mining identified duplication of key developmental regulator ARP (AS1/RS2/PHAN homologues) in *Begonia*, with subsequent loss of the characteristic MYB domain in one paralog that is nonetheless retained across the genus. We also identified another duplicated pair of paralogs with high homology to AS1, previously uncharacterised as it is missing from *Arabidopsis*. Broader plant kingdom mining revealed both MYB-less ARP and the additional AS1 homologue are common throughout angiosperms. Expression data, gene network, and co-localisation analysis indicate functional divergence in all four AS1-like genes in *Begonia* following duplication. Together these results support the role of duplication in leaf developmental diversity, and the use of *Begonia* as a new leaf shape evolution and development model.

P053

Phylogenetic diversity and symbiotic associations of photosymbiotic acoels

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Acoels are marine flatworm-like invertebrates within the phylum Xenacoelmorpha, characterized by their lack of a gut and coelomic cavity. Convolutidae is known for the only family forming photosymbiotic relationships with green algae and/or dinoflagellates, inhabiting sun-exposed environments such as coral surfaces, macroalgae, and sandy substrates in tropical and subtropical regions. Recent studies have reported symbiotic acoels across the western Pacific, including southern Japan and Palau. However, acoels in Taiwan have not been well studied, with only indirect reports of *Waminoa* species. To investigate the biodiversity of symbiotic acoels in Taiwan, we targeted coral reef habitats along northern (Magang), eastern (Green Island), western (Penghu), and southern (Xiaoliuqiu Island) Taiwan, ultimately 119 acoels were collected. Using PCR, MDA, and Nanopore technology, 18S rRNA markers were extracted to identify the host worms; rbcL and 28S rRNA markers to their symbiotic algae. Phylogenetic analysis showed that most collected acoels belong to Convolutidae, forming two major clades closely related to *Heterochaerus* and *Convolutriloba* species. *Tetraselmis* and *Symbiodinium* were identified as their symbionts. Interestingly, acoels presences only at tourist sites on each island, suggesting that nutrient enrichment caused by human activities might promote their abundance. Additionally, several *Hofstenia* acoels were discovered, indicating the diversity of non-photosymbiotic acoels in Taiwan. This study provides the first comprehensive molecular studies of acoels in Taiwan, enhancing our understanding of their diversity and distribution in the western Pacific.

P054

Genomic insights into latitudinal variation in reproductive diapause in *Drosophila triauraria*

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Seasonal environments impose strong selective pressures on reproductive timing. In many insects, reproductive diapause varies gradually along latitudinal gradients, yet how such continuous variation is genetically maintained despite ongoing gene flow remains poorly understood. The fruit fly *Drosophila triauraria* is distributed across the Japanese archipelago (26°N–43°N) and exhibits clear geographic differences in female reproductive diapause. Northern strains enter diapause under short-day and low-temperature conditions, whereas southern strains continue ovarian development under the same conditions. This system provides an opportunity to investigate the molecular basis of latitudinal divergence in seasonal adaptation. We quantified diapause induction in both females and males across multiple strains spanning the species' latitudinal range. The results showed continuous geographic variation in sensitivity to photoperiod and temperature, rather than discrete northern and southern types. To identify genomic regions associated with this divergence, we analysed whole-genome sequences of 21 strains including 14 newly sequenced. In addition to conventional F_{ST} analysis, we applied a phylogeny-based "monophyletic window" approach, which is well-suited for moderate sample sizes. This analysis highlighted a candidate region containing regulatory motifs (E-box and TER-box) in the circadian clock gene *timeless*. Quantitative PCR showed that variation in *timeless* isoforms expression was partially associated with female diapause incidence. Our findings suggest that natural variation in circadian clock regulation contributes to geographic adaptation of reproductive timing and demonstrate the usefulness of tree-based genomic methods for detecting adaptive divergence.

P055

Emergence and evolution of phototransduction: lessons from early animals

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Light detection is a fundamental process in animals, and its evolutionary history has been puzzling biologists for a long time: Darwin himself called the vertebrate camerated eyes as “organs of extreme perfection and complication” that could undermine the foundations of his theory of natural selection. More than 150 years after Darwin’s reasoning, we have now gained a better perspective of the process that may have ruled the evolution of such complex organs. Yet, our understanding is still limited when digging deeper into the cellular and molecular levels: how have light-sensitive cells evolved? And what about the molecular ground plan of phototransduction? What is the degree of homology of such a system? The answers to these and other questions are now emerging, thanks to cutting-edge molecular and evolutionary analyses involving early animal clades. In this contribution, we highlight how sponges, ctenophores, and placozoans represent optimal models to investigate the emergence of the bilaterian phototransducing machine, showcasing the latest advancements in the understanding of phototransduction cell type identity and evolution. To this purpose, we produced new single-cell RNAseq and genomic data of a sponge and a ctenophore species, to be integrated in broad scale comparative analyses with other non bilaterian species to understand the early steps of light detection in animals.

P056

Comparing early embryogenesis among teleost species from a mechanical perspective

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The process of early embryogenesis is conserved across metazoa. A zygote undergoes rapid cell cleavage to generate blastomeres, followed by the mid-blastula transition, during which the zygotic genome begins to activate, accompanied by cell cycle elongation and desynchronization. Ultimately, the embryonic tissue undergoes morphogenesis and cell fate specification. Previous studies have shown that tissue mechanical properties controlled by early cell cleavage and cell fate specification are required for subsequent morphogenesis, specifically during epiboly and gastrulation. In this study, we investigate how the temporal interplay of the developmental programs (mid-blastula transition and zygotic genome activation (ZGA)) with tissue mechanical changes shapes the morphogenetic diversity of early embryogenesis across teleost species. Teleost embryos share similar embryonic structures, with a blastoderm tissue lying on top of a large yolk. However, the spreading of the blastoderm and consequent gastrulation vary among species. Unlike zebrafish embryos, in which organizer compaction, an event marking the initiation of gastrulation, occurs at 50% epiboly, organizer compaction in Medaka and killifish occurs at 30% epiboly and the completion of epiboly, respectively. Here, we investigate a scenario where a heterochronic shift in organizer compaction during gastrulation, along with differences in cell cycle elongation and ZGA timing, may underlie the morphogenetic differences among the three species. We hypothesize that these temporal shifts alter tissue mechanical transitions in the embryonic tissues and give rise to distinct modes of early embryogenesis among teleost species. This study demonstrates how different morphogenetic programs arise from the mechanistic link between heterochronic developmental programs and mechanical transitions.

P057

Developmental functions of zebrafish *isl2a* and its enhancer in relation to the evolution of paired fins

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One of the most prominent novelties of vertebrates is the evolution of paired appendages. Early vertebrates, however, only possessed median fins derived from the paraxial mesoderm (PM). Thus, it is widely held that the fin developmental program first emerged in the median position and was subsequently co-opted by the lateral plate mesoderm (LPM) to give rise to the paired fins. Interestingly, a recent study demonstrated the duplication potential of an LPM-derived median fin, the pre-anal fin fold (PAFF), in zebrafish, and proposed it as an intermediate state between unpaired and paired fins. However, it remains unclear to what extent the developmental program of the PAFF is conserved with that of the LPM-derived paired fins, and which molecular mechanisms underlie its duplication. Previously, our lab has identified an ISLET family of transcription factor gene, *isl2a*, and its associated enhancer CR2 (conserved region 2) as potentially important and conserved elements for pelvic appendages development in jawed vertebrates. However, their functions have not been characterized in zebrafish, and it remains unknown whether they are involved in PAFF development. Here, we show that CR2-knockout fish exhibit reduced *isl2a* expression in the pelvic fin bud and structural defects in pelvic fin skeleton. Moreover, *isl2a*-knockout fish display a reduction in mesenchymal cells within the PAFF. These results demonstrate that the regulatory role of CR2 is essential for the proper expression of *isl2a* during fin development. Furthermore, the recruitment of CR2-mediated *isl2a* function into the fin developmental program likely predates the duplication of the LPM-derived median fin.

P059

GRN feedback dynamics in the Tribolium embryo across maternal–zygotic and extraembryonic–embryonic spatiotemporal contexts

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Across metazoans, an early event in body plan formation is the specification and organisation of the body axes. This process is often initiated by maternal gene products deposited in the egg, which activate cascades of zygotic gene expression that drive axial patterning. Embryonic axis patterning is governed by gene regulatory networks (GRNs) that translate positional information into an organised body plan. The hierarchical gene regulatory networks, shaping embryonic axis patterning, have been well elucidated in many insect models. Although GRNs are broadly conserved across insect species, evolutionary divergence has introduced species-specific variations. In the beetle Tribolium castaneum, the GRN orchestrating anterior patterning includes, among other components, the maternally contributed genes *germ cell-less* (*gcl*) and *axin*, as well as *zerknüllt1* (*zen1*) and *homeobrain* (*hbn*), both of which are regulated by Gcl. Despite Tc-Zen1 being regarded as the master regulator for specification of the extraembryonic serosal tissue, there is a surprising level of feedback from downstream target genes in the anterior-terminal region of the embryo, with fundamental consequences for tissue identity across embryonic and extraembryonic fates. Knockdown of downstream targets of Tc-Zen1 has revealed additional components in this GRN. Our ongoing work focuses on investigating how the anterior patterning GRN integrates diverse spatiotemporal inputs to refine and stabilise anterior developmental outcomes.

P060

From insertion to integration: the impact of regulatory landscapes in transposable element co-option

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How newly emerged genes become integrated into preexisting regulatory networks remains a central unresolved question in evolutionary biology. Here, we studied how Arc, a neural gene that originated by co-option of a retrotransposon in tetrapods, initially acquired its characteristic neural-specific regulation. We hypothesized that the preexisting regulatory landscape at the place of the insertion constrains and biases the evolutionary trajectory of the retrotransposon co-option. First, we identified ARC's syntenic neighbourhood in tetrapods and fishes, enabling us to reconstruct the ancestral genomic environment in which the retrotransposon was inserted. This environment was characterised by the presence of two neural-enriched genes conserved across vertebrates: Adgrb1 and Tsnare1. We identified conserved chromatin interactions between the Arc and Adgrb1 locus across amniotes. These regions coincided with ATAC peaks in neural samples, suggesting the presence of CREs interacting with Arc within Adgrb1. We also observed equivalent ATAC peaks in zebrafish and shark, suggesting that they predate the origin of Arc. We demonstrated that these CREs drive brain expression in zebrafish, leading us to hypothesize that insertion of a retrotransposon near neural CREs could have instantly induced neural expression of its proteins, which could have subsequently been co-opted for cellular functions. Finally, we expressed Arc in zebrafish brain. This ectopic expression did not cause an obvious deleterious phenotype, but induced mild behavioural and transcriptomic alterations, demonstrating the phenotypic impact of abrupt regulatory integration prior to extensive co-evolution. Our study shows how new genes can arise from a contingent scenario in which novel elements gain specific regulation.

P061

Sauropsid embryonic brains lack the mammalian temporal transcriptional program

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Vertebrate brain neurogenesis is classically understood as being governed by spatially restricted genetic programs shaped by conserved morphogenetic gradients. More recently, studies in mammals have described a global temporal transcriptional code that sequences neuronal production, defining early, intermediate, and late progenitor and neuronal cells. However, the evolutionary conservation of this temporal program outside mammals remains unclear. Here, we investigated whether the mammalian temporal neurogenic code is conserved in sauropsids. We analyzed neurogenic regions of the embryonic chick telencephalon using*n Situ Sequencing* (ISS), enabling the simultaneous spatial and quantitative assessment of genes associated with early (e.g., *Onecut*, *Hmga1/2*), intermediate (e.g.,*Pou2f2*, *Zfhx3*), and late (e.g., *Nfia/b*, *Tcf4*) neurogenic programs. We examined multiple developmental stages spanning early, mid, and late neurogenic windows. Our results show that sauropsid transcriptional profiles do not recapitulate the temporal progression described in mammals. Specifically, we do not observe a conserved sequential activation nor a clear segregation of molecular signatures corresponding to early, intermediate, or late neurogenic states. These findings suggest that the global temporal transcriptional program identified in mammals may not be conserved in sauropsids. Together, our data support the hypothesis that the mammalian temporal transcription code represents a lineage-specific evolutionary innovation rather than a universal feature of amniote neurogenesis. This study highlights the power of spatial transcriptomics for addressing fundamental questions in evolutionary developmental neurobiology.

P062

Early regulatory repatterning underlies lizard-snake craniofacial divergence

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The vertebrate craniofacial complex is among the most evolutionarily diverse anatomical systems, yet the molecular events that initiate major lineage-specific skull trajectories remain largely unresolved. Squamates (lizards, snakes) provide a powerful system to address this gap due to their extreme cranial disparity. Building on our ontogenetic geometric morphometric analyses demonstrating that lizard-snake divergence arises unexpectedly early, already in craniofacial primordia, we investigate the gene regulatory architecture underlying these developmental shifts. We hypothesize that squamate skull evolution is driven by early rewiring of cis-regulatory enhancer landscapes that redirects transcriptional programs within cranial neural crest-derived domains. To test this hypothesis, we generated an integrated multi-omics resource combining transcriptomics and chromatin accessibility profiling from key craniofacial primordia in three morphologically divergent squamate species (two lizards and one snake). Cross-species joint analyses reveal a robust conserved core (>800 genes) alongside a substantial lizard-snake divergent component (>1,000 genes). Chromatin accessibility data independently support approximately half of the conserved gene set and ~20% of the divergent candidates, directly linking transcriptional divergence to changes in regulatory state. Conserved programs are enriched for transcription factors and developmental regulators involved in neural crest differentiation, osteogenic specification, and neurodevelopmental patterning. In contrast, divergent programs point to lineage-specific shifts in craniofacial patterning, epithelial-mesenchymal signaling, and the Wnt signaling pathway. Ongoing molecular and genomic validation will test prioritized regulatory modules. Together, these findings move beyond descriptive accounts of squamate skull diversity to define its early regulatory architecture, providing mechanistic insight into how embryonic regulatory landscapes generate evolutionary novelty in vertebrate craniofacial form.

P063

How Development Shapes Mutational Rates and Directions: An Organismal View of Genotype Space Exploration

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Mutation rates represent a central parameter of population genetics that drives the pace and potential of evolutionary change. In multicellular organisms, all mutations first arise in a single cell in a developmental context. Whether a mutation enters a population's gene pool can therefore depend on developmental events that affect the likelihood of mutant cell lineages of producing gametes. For this reason, the evolution of mutation rates in populations is governed not only by changes in the rates at which mutations occur at the molecular level, but also by changes in developmental features of organisms and how mutations impact cellular fitness during development. We present a theoretical framework that, supported by empirical data from mammals and experiments on the fruit fly, demonstrate how generational mutation rates can be shaped by changes in developmental parameters and intraorganismal selective processes even when molecular mutation rates are presumed constant. Our model highlights how the diversity of mutation rates observed across animals may be the byproduct of organismal development rather than the result of direct selection against mutator alleles. As such, development not only introduces phenotypic biases, it also shapes the rates and trajectories of genetic diversity and is thus at the core of evolutionary theory.

064

Early Tetrapod Pallial Neurogenesis Revealed in *Xenopus laevis*: Implications for the Evolutionary Origins of the Cerebral Cortex

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The pallium of amphibians represents an important evolutionary stage in the origin of the cerebral cortex and other pallial derivatives of amniotes. In *Xenopus laevis*, the organization and developmental dynamics of pallial neurogenesis remain poorly understood, particularly in comparison with the complex neurogenic patterns described in mammals. Here, using cell birthdating assays in combination with molecular markers of pallial domains and cortical hem, we analyzed the temporal and spatial dynamics of neurogenesis across the pallium of *Xenopus laevis*. Our results reveal particular neurogenic schedules among pallial subdivisions. providing a comprehensive view of pallial neurogenesis in an anuran amphibian and suggesting that prolonged and region-specific neurogenic programs were already present in early tetrapods. Understanding these developmental dynamics offers insight into the evolutionary origins of the hippocampus, cerebral cortex, and other pallial structures of amniotes.

P065

The temporal skull region is shaped by the jaw musculature through development

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Amniote evolution is characterised by drastic modifications and substantial innovation in skull morphology. A prime example of this is the outstanding disparity of the temporal skull region, reflected in the great variation in number, position and shape of bony bars and openings (fenestrae) across lineages. Traditionally, modifications of the temporal bony scaffold were thought to allow changes in jaw muscle size, attachment and orientation through development, fostering the diversification of the jaw musculoskeletal system. However, recent studies suggest that changes in the musculature drive the evolution of temporal fenestration and not vice versa. Whether the primacy of musculature development over the dermal skeleton (i.e., muscles direct bone growth through development) correlate with the sequence of skull ossification remains unclear, so the mechanistic relationship behind the diversification of temporal fenestration patterns is unknown. Here, we test the hypothesis that the jaw musculature moulds temporal skeleton through development using two species of lizards (*Pogona vitticeps* and *Lepidodactylus lugubris*) with drastically different temporal region configurations. Through contrast-enhanced micro-CT imaging of developmental series across skull ossification, we show that the jaw muscles condense much earlier than the dermal ossification of the temporal skeleton, and they later migrate towards the ossifying temporal bones in both species. In Pogona, the upper temporal and postorbital bars ossify around the external adductor muscle complex. Our study counters traditional hypotheses and demonstrates that jaw musculature shapes the temporal region structure through development. We propose this mechanistic model as the developmental basis for the diversification of fenestration patterns in amniotes.

P066

Evolution of complete metamorphosis in insects

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The origin of the complete metamorphosis (holometaboly) in insects with their larval, pupal and adult stages has been debated for over a century. We examined the expression and functions of larval specifier gene, *chronologically inappropriate morphogenesis* (*chinmo*), and the pupal specifier gene, *broad*, in an insect that undergoes incomplete metamorphosis and an insect that undergoes complete metamorphosis. Our study shows that the evolution of holometaboly was accompanied by heterochronic changes in the expression of and the evolution of a novel regulatory interaction between *chinmo* and *broad*. Our findings support the notion that complete metamorphosis evolved when embryonic characteristics were retained in the larval stage and the ancestral juvenile stages were compressed into a single pupal stage.

P067

Environmental perturbation increases and unmasks genetic regulation for gene expression variability

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A key aspect of how populations respond to environmental perturbation is the degree of environmentally-induced trait variability, inter-individual variation from the trait mean. Although canalization and robustness theory predict increased variability under stress, it remains to be tested whether such environmental responses have a genetic basis. That is, are there environmental robustness-regulating loci? While there is evidence that trait variability is under genetic control in constant environments, our knowledge of how environmental perturbation impacts trait variability and its genetic basis remains far from comprehensive. To address this, we collected nearly 2,000 matched genomes and transcriptomes from an outbred population of Drosophila melanogaster before and after exposure to a new high-sugar diet. We find that environmental perturbation induces a transcriptome-wide increase in expression variability. However, key developmental pathways showed the opposite pattern - lower variability under stress - suggesting that development is particularly robustly regulated. Stress also unmasked pervasive genetic regulation for expression variability (veQTL). Unlike mean expression variation (eQTL), these loci are mostly environment-dependent, show distinct functional and evolutionary constraints, and tend to harbour detrimental low-variability alleles. Our work adds new dimensions to the legacy of Drosophila canalization research established by Waddington and Rendel, and supports shifts in gene expression variability as a key population response to environmental change.

P068

Deciphering the ontogeny of cell types during embryonic development in the common octopus using trajectory analyses

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The common octopus, despite its very ancient evolutionary divergence from vertebrates, has a very large and complex brain with a cell number comparable to mammals. This leads to a wide variety of sophisticated behaviors such as recognizing itself, using tools or learning through observation. To better understand how this brain complexity is emerging during the development of this coleoid cephalopod species, whole embryo single-nuclei RNA was sequenced and analyzed at three different stages of organogenesis. Based on marker genes specific to cell types, the nuclei were clustered into multiple groups: retinal, glial, neuronal progenitors, differentiated neurons, mesodermal and epithelial. In order to establish the ontogeny of certain cell types, trajectory analysis was performed to track developmental progression along different embryonic stages. This analysis revealed the cell fates at the end of organogenesis and suggested roles of the different sub-types through identification of marker genes which vary along the cell type trajectories. This works brings a deeper insight in the development of coleoid cephalopod cell type diversity throughout organogenesis, and will be an important resource for future studies in this field.

P069

Did the homeotic function of *AGAMOUS* genes evolve from ancestral shoot regulation?

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The identity of reproductive organs in flowering plants (angiosperms) is famously regulated by the ABCE model of protein-protein interactions, primarily between MADS-box proteins which form floral quartets [1]. The *AGAMOUS* (*AG*) clade in particular is required to specify floral reproductive organs (stamens, the carpel and ovules). The floral homeotic subfamilies of MADS-box genes are specific to the seed plants [2] and so the question remains: how did this seemingly-unique homeotic function evolve?

The ferns are the closest extant relatives of all seed plants [2], but at present MADS-box functions in this family remain under-explored. The example with most information available is the *Ceratopteris richardii* gene *CMADS1*, which belongs to the fern sister-clade of seed plant MADS-boxes including *AG* [2] and which is expressed in developing fern reproductive frond (sporophyll) tissues, including in sporangia [3]. However, the precise native function of CMADS1, and its evolutionary relationship with that of AG-clade functions, remains unknown.

Here we present evidence that CMADS1 protein is incapable of replicating AG floral homeotic functions when expressed heterologously in the angiosperm model *Arabidopsis thaliana* but is capable of affecting floral organ growth similarly to *AG* ectopic expression, suggesting that this might represent conservation of an ancestral function. To further test this and elucidate the native function of CMADS1 in *Ceratopteris* we present analyses linking *CMADS1* to fern vegetative shoot and frond development.

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P070

Distinct mechanisms decommission redundant enhancers to facilitate phenotypic evolution

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The evolutionary loss of morphological traits is often driven by changes in gene regulation. Many developmental genes are controlled by multiple redundant enhancers, raising the question of how robust regulatory systems can be dismantled to permit phenotypic transitions. Here, we show that the loss of larval trichomes in Drosophila sechellia resulted from the independent inactivation of four embryonic enhancers of the shavenbaby gene. Each enhancer was extinguished by a distinct mechanism: (1) a large deletion that removed essential sequences, (2) the loss of activator sites and gain of repressor sites, (3) the acquisition of a long-range silencer, and (4) the unmasking of pre-existing repression. Notably, three of these mechanisms relied on repression, pointing to repression as a rapid route for the evolutionary loss of robust regulatory elements. These results show that robustness in gene regulation does not prevent morphological change but instead provides multiple opportunities for mutations to reduce enhancer activity, giving selection many paths to reshape form.

P072

Modularity, robustness and the evolvability of body plans

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The evolvability of animal body plans is limited. For example, evolutionary changes are strongly constrained during the early stages of organogenesis, also known as the phylotypic stage, when most of the body plan is established. Thus, the conservation of body plans is implicated in the conservation of this stage. Two hypotheses have been proposed to explain this strong conservation. One states that the conservation reflects a strong interactivity between developmental modules, meaning that mutations would have many pleiotropic effects, and be subject to stabilising selection. The other states that, at least in insects, the conservation is caused by the robustness of a centrally important organiser gene network. I discuss how there is little empirical or theoretical support for the robustness hypothesis, but considerable support for the pleiotropy hypothesis. Mutations affecting this developmental stage are common. These mutations have many pleiotropic effects, that are visible at the phenotypic level and are usually subject to strong stabilising selection. This highlights the importance of developmental modularity for evolvability. Finally, I discuss mechanisms that facilitate the breaking of the constraints.

P073

Protocol variation and cross-species comparisons: A conceptual discussion

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In vitro modeling enables comparing developmental processes of different species under similar, highly controlled culture conditions. A popular methodological principle in this context is to apply an identical culture protocol across species so that any differences observed between *in vitro* models can be attributed to the species difference. However, using an identical protocol is not always possible, and even not ideal. Conducting a meaningful cross-species comparison sometimes requires varying or adjusting culture protocols because the in vivo environments surrounding the embryos and/or the in vivo temporal regulations differ across species. The aim of this presentation is to clarify conceptual issues concerning protocol variation in cross-species comparisons and promote methodological discussion on this topic. I distinguish between two methodological approaches to cross-species comparisons: “equality” approach, which aims to provide biological systems with identical external conditions; and “equity” approach, which intentionally varies external conditions to make biological systems reach comparable states or stages. Based on interviews conducted with researchers working on *in vitro* cross-species comparisons, I identify advantages and disadvantages of each approach and discuss methodological, material, and institutional factors that influence the choice between the two approaches. Importantly, the conceptual distinction between the equality and equity approaches will be relevant beyond *in vitro* research and applicable broadly in fields that compare different species under experimental conditions. I discuss comparative cognition research as an example.

P074

Differential Evolution of Immunoglobulin Heavy Chain Variable Genes between Teleosts and Mammals

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Germline immunoglobulin heavy-chain variable (IGHV) genes are key determinants of antibody diversity, yet the evolutionary forces shaping their variation across vertebrates remain unclear. We performed phylogenomic analyses of IGHV genes across fifteen vertebrate species, including mammals and teleost fish, organized into nine species pairs with comparable divergence times. Mammalian IGHV genes trace to a single ancestral lineage after the teleost–mammal split and subsequently underwent rapid lineage-specific diversification and extensive birth-and-death evolution. In contrast, teleosts retain eight ancient IGHV clans and preserve substantially more cross-species gene families with limited expansion bias. Repertoire analyses revealed contrasting regulatory architectures. In humans, naïve IGHV usage is highly conserved across populations, and antigen exposure drives diversification of antigen-experienced repertoires. In zebrafish, naïve IGHV usage diverges between populations, whereas antigen stimulation induces convergence toward a naïve-like configuration through the influx of newly generated naïve clonotypes. These findings indicate distinct selection regimes: relaxed diversification in mammals versus long-term balancing and stabilizing selection in teleosts. This not only links deep evolutionary history with functional antibody repertoire dynamics but also entail practical implications for vaccine design and Ab-based therapeutics in mammals versus teleosts.

P075

Selective Hox Gene Loss and Axial Patterning in Early-Branching Flatworms

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Establishment of the anterior-posterior axis is a fundamental feature of bilaterian body plans and is typically mediated by a clustered and spatially collinear Hox gene complement. Despite its deep conservation, multiple animal lineages exhibit breakdown of the canonical Hox cluster, raising questions about how conserved patterning systems remain functional following gene loss and cluster disintegration. Flatworms represent an extreme case of Hox restructuring. In planarians, the Hox repertoire is genomically dispersed and several ancestral classes are absent. To investigate the evolutionary history of this reduction, we performed comparative genomic and transcriptomic analyses of early-branching flatworms, including Catenulida and Macrostomorpha. The results reveal recurrent loss biased toward central Hox classes, alongside lineage-specific duplications of retained paralogs, suggesting non-uniform reduction and structured reorganization of a deeply conserved developmental toolkit. We further examined Hox gene expression in the early-branching catenulid *Stenostomum brevipharyngium*, a species that develops through regeneration and asexual reproduction. Regeneration time-course transcriptomics reveal temporally dynamic, region-specific activation of retained Hox paralogs during tissue remodeling and axial re-establishment. Complementary spatial analyses define their expression along the anterior-posterior axis during homeostasis, regeneration and asexual reproduction to determine how axial identity is established within a reduced gene complement. Together, our findings show that gene loss and architectural fragmentation can alter the composition of a canonical patterning system while retained Hox genes remain actively involved in axial processes.

P076

The Evolutionary Origin of TGM1 Mediated Epidermal Cornification in Tetrapods is found in Non-Cornified Skin of Aquatic Ancestors

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The environment serves as an important driver for new evolutionary traits such as cell types. Cornification of the regular skin of terrestrial vertebrates evolved in stem-tetrapods, potentially as consequence of drought stress, and allowed them to conquer the land and become independent of aquatic environments. The cornified skin is built by keratinocytes which differentiate into corneocytes by expressing structural proteins which are crosslinked by transglutaminases (TGMs), particularly TGM1. Mutations of TGM1 can cause severe skin diseases in humans and Tgm1-deficient mice die post-natal. TGM1 homologs predate cornification as they were recently reported in primary aquatic vertebrates and cephalochordates. All of them lack a regular cornified skin and the expression sites of their TGM1 homologs remains elusive. Herein, we investigate the TGM1 homologs in lancelets, cyclostomes, sharks, ray-finned fish, and lungfish via transcriptomic analysis. Our analysis shows TGM1 mRNA expression in exposed epithelia such as skin in all tested species. In lungfish, the closest relatives to tetrapods, TGM1 expression is upregulated during aestivation, a process which is triggered by drought stress. Our results show that TGM1 was already relevant for the development of exposed epithelia before the appearance of tetrapods and thus is one of the most critical molecular co-options for the evolution of cornification.

P077

Investigating cell type-specific gene expression in *Salpingoeca rosetta*

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Developmental gene regulation is a fundamental biological process which is essential for cell differentiation. In animals, once specialized, most cells cannot revert to a more potent state. Meanwhile, in choanoflagellates, the sister group of animals, cells can be temporally differentiated and can adopt cell identities while retaining the capacity to form other cell types as part of their, often complex, life cycles. The model species *Salpingoeca rosetta* presents multiple cell types representing distinct stages in its life cycle including multicellular rosettes, sessile Thecate cells, and solitary Slow swimming cells. Thecate cells are the only diploid stage, and they show highly distinct gene expression profiles compared to their haploid counterparts. The underlying basis of these differences and how this compares to the mechanisms deployed during animal cell differentiation is unknown. We aim to investigate this by comparing gene expression and regulation in Slow swimmers and Thecate cells by sequencing a *de novo* genome using Nanopore, and profiling histone post-translational modifications with ChIP-seq. Finally, we dissect how chromatin changes may underlie the observed transcriptional differences by mapping nucleosome occupancy using MNAse-seq. Preliminary results indicate a difference in MNAse digestion among Thecate and Slow swimming cells. Cell type specific genes are marked by H3K27me3 with cases of H3K4me1/K27me3 bivalency. Our study will provide an in-depth view of cell type-specific gene expression in *S. rosetta* and lead to a better understanding of temporal differentiation in choanoflagellates and its evolutionary links to animal development.

P078

Dissecting the role of TGFβ signaling in connective tissue precursors for skeletal muscle patterning

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Skeletal muscle patterning relies on the assembly of myofibers into muscles with specific identity, shape, innervation, and skeletal attachment. Connective tissue (CT) progenitors have emerged as key regulators of this process, yet how muscle–CT interactions are coordinated along the body axis remains unclear. CT derives from distinct embryonic lineages - neural crest in the head and lateral plate mesoderm (LPM) in the ventral trunk and limb. Defects in CT-muscle progenitors communication have been suggested to underlie some human congenital disorders affecting the musculoskeletal system. Using conditional Sox10Cre and Prx1Cre;TGFβR2flox mice, we selectively inactivated TGFβ signaling in neural crest- or LPM-derived CT. Loss of TGFβ<R2 in neural crest derivatives mildly impacts cranial muscle patterning and fiber organization, whereas its inactivation in LPM derivatives causes severe hypoplasia of neck, scapular, and thoracic hypaxial muscles. These defects correlate with impaired attachment sites to associated skeletal components and to the loss of LPM-derived CT tendinous fate. Our data indicate that TGFβ signaling acts in a region-specific manner to orchestrate CT–muscle crosstalk during axial development. We will now reconstruct the TGFβ-dependent gene regulatory networks using single-cell multiome (RNA+ATAC) sequencing to identify downstream effectors mediating CT–muscle interactions. Candidate genes will be validated *in vivo* and *ex vivo*. Understanding how TGFβ coordinates musculoskeletal morphogenesis will shed light on the developmental basis of TGFβ-related congenital disorders such as Klippel–Feil, Marfan, Loeys–Dietz, and Myhre syndromes, with potential impact on prenatal/postnatal diagnosis and clinical monitoring.

P079

Phenotypic plasticity versus phenotypic determinism. Variations of colour in Ephemeroptera *Cloeon dipterum*

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Pigmentation is a trait that can be channeled by different factors, which means its regulation may differ depending on the case. Although sexually dimorphic pigmentation and pigmentation plasticity are wide-spread characteristics in animals, little is known about the genes involved in these traits in key groups of insects, such paleopterans (Ephemeroptera and Odonata). The emergent evo-devo model *Cloeon dipterum* presents an excellent opportunity to study the regulation of pigmentation. The adults of this Ephemeroptera species exhibit sexually dimorphic pigmentation, while the pigmentation of nymphs varies depending on the background. Therefore, we aim to compare the genetic control of both sexually dimorphic pigmentation and plastic pigmentation. On one hand, we first described a transcription factor involved in arthropod sexual differentiation and sexual dimorphism: *doublesex* (*dsx*). For this, we built a phylogenetic tree using orthologs, and we identified three paralogs: *Dsx*, *Dsx-like* and a novel gene, *Dsx-y*. Second, Raman spectroscopy allowed us to identify melanin in the thorax and an additional unknown pigment in the abdomen of both sexes. Finally, we found differential expression in melanin-associated genes. Different paralogs of the *yellow* gene were upregulated in both sexes, and *ebony* showed a female-biased expression. In addition, the ommochrome-associated gene *vermilion* presented a male-biased expression, suggesting that the unknown pigment may be an ommochrome. On the other hand, we subjected nymphs to different backgrounds to investigate nymphal phenotypic plasticity and found that darker backgrounds generate darker nymphs and vice versa. These results provide new insights into the regulation of pigmentation in *C. dipterum*.

P080

Deep conservation of *cis*-regulatory elements and chromatin organization in echinoderms uncover ancestral regulatory features of animal genomes

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Despite the growing abundance of sequenced animal genomes, we only have detailed knowledge of regulatory organization for a handful of lineages, particularly flies and vertebrates. These two taxa show contrasting trends in the molecular mechanisms of 3D chromatin organization and long-term evolutionary dynamics of cis-regulatory element (CREs) conservation. Here we study the evolution and organization of the regulatory genome of echinoderms, a lineage whose phylogenetic position and relatively slow molecular evolution have proven particularly useful for evolutionary studies. We generated new reference genome assemblies for two species belonging to two different echinoderm classes: the purple sea urchin *Strongylocentrotus purpuratus* and the bat sea star *Patiria miniata* using PacBio and HiC data and characterize their 3D chromatin architecture. We show that these echinoderms have TAD-like domains that, such as in flies, do not seem to be associated with CTCF motif orientation. We systematically profiled CREs during sea star and sea urchin development using ATAC-seq, comparing their regulatory logic and dynamics over multiple developmental stages. Finally, our analysis of sea urchin and sea star CRE evolution across multiple evolutionary distances and timescales showed several thousand elements conserved for hundreds of millions of years, revealing a vertebrate-like pattern of CRE evolution that probably constitutes an ancestral property of the regulatory evolution of animals.

P081

Towards a Developmental Atlas of Oncopeltus fasciatus: A Single-Cell RNA-seq Time-Course

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Holometabolous insects, such as Drosophila melanogaster, dominate the field of developmental biology but represent a derived mode of embryogenesis. While much work has been invested in expanding our knowledge base of hemimetabolous insects, we still lack an understanding of the cells that form embryos, their gene expression profiles, and their fates. Single-cell transcriptomic analyses have been revolutionary in describing early developmental landscapes of cellular genetics in a range of animals, but not so much in insects. To enhance the tools available to the study of non-model insects and provide important phylogenetic and developmental context, we generated a single-cell transcriptomic atlas ofOncopeltus fasciatus, a hemimetabolous insect. Nine embryonic time points, spanning mid-blastoderm to late germ-band stages, were sampled to capture transcriptional changes during early development and trace the differentiation process of individual developmental domains. For preliminary results, we processed an initial subset of the data to validate our experimental and bioinformatic framework. Our technical validation revealed high-complexity libraries with a median of over 1,200 unique genes per cell and an R= 0.96 correlation between total transcripts and feature detection. Initial manifold analysis resolved nine distinct clusters representing major embryonic tissues and transient progenitor states, confirming that the dataset captures robust biological signal across the time-course. This foundational dataset provides the first high-resolution view of gene-expression dynamics during hemimetabolous development, enabling future cross-species comparisons and supporting a wide range of developmental and evolutionary studies.

P082

Tooth-row patterning and heterodonty in lizards

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Squamates (lizards and snakes) display extraordinary dental diversity, making them a powerful model system for studying the evolution and development of vertebrate teeth. Yet the extent of tooth-morphology variation within and among species, and the developmental mechanisms that generate it, remain largely unresolved. Here, we combine developmental series with quantitative morphometrics to characterize tooth size and shape variation in two lizard species with heterodont dentitions, *Anolis carolinensis* and *Pogona vitticeps*, sampled under normal and/or mutant conditions. Morphometric analyses reveal a conserved anterior-posterior increase in tooth size and complexity in both species, but with fundamentally different patterns of variation across the tooth row. In *A. carolinensis*, tooth morphology shifts from anterior unicuspid teeth to wider posterior tricuspid teeth, with a variable intermediate zone where both morphotypes occur. In wild-type *P. vitticeps*, tooth morphology is comparatively conserved, with triangular teeth that widen posteriorly and bicuspid teeth at the extreme posterior. In EDA mutant *P. vitticeps*, this positional relationship is disrupted: most teeth are significantly larger and often more complex (with shape abnormalities) than wild-type teeth at equivalent jaw positions, effectively shifting the size-complexity trajectory forward along the jaw. Developmental data indicate that these interspecific and mutant differences are specified early by distinct tooth-row patterning dynamics and patterns. Together, our findings link adult dental variation to developmental patterning and demonstrate how both evolutionary divergence and perturbations to developmental signaling reshape heterodont trajectories in squamate dentitions.

P083

A Conserved BMP-Nfatc1-Wnt regulatory network controls the formation of valve interstitial cells in vertebrates

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Cardiac valves represent an evolutionary novelty of vertebrates, and their formation relies on precise gene regulatory networks (GRNs) that interpret multiple signaling inputs to establish distinct valve subpopulations. While the signals driving valve formation are well characterized in vertebrates, we do not know the conserved GRNs that orchestrate the development of valve interstitial cells (VICs), which are poorly characterized valvular structural cells. Intriguingly, we found a conserved enhancer located upstream of zebrafish *nr2f1a* (*5'reg2–Nr2f1* or valve-enh), specifically expressed in VICs, from 72hpf to juveniles. Even if the enhancer does not recapitulate the expression of the endogenous, we utilized it as a tool to shed light on VICs' regulatory networks. To identify potential conserved signals, we performed a multi-organism transcription factor binding site (TFBS) analysis, which suggested the existence of a conserved transcriptional code for Wnt, BMP and Nfatc1 pathways. Site-directed mutagenesis of conserved binding sites within suggested that Tcf, Smad and Twist control *valve-enh* expression, as sites of activation. Furthermore, signaling manipulations confirmed that Wnt, BMP and Nfatc1 activate the enhancer expression in VICs. Intriguingly, we performed multiple epistatic analyses that demonstrated a clear cross-talk between Wnt and Nfatc1 in the context of VICs development, with BMP acting as a primary activating input. Overall, these results identify the5'reg2–Nr2f1 enhancer as a conserved signaling integration hub that interprets multiple developmental pathways to establish valve-specific expression, leading to a deeper understanding of the conserved molecular mechanisms that control the VIC development in vertebrates.

P084

A single-cell approach to study axial patterning in Annelida

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In Spiralia (e.g. molluscs and annelids), the role of Bone Morphogenetic Protein (BMP) in axial patterning is still contingent, even though they share an ancestral pattern of cell division—spiral cleavage—and make use of ERK to specify an axial organising cell, the spiralian organiser. Here, we show that BMP signalling is the ancestral mechanism setting up dorsovental (DV) polarity downstream of ERK by studying four annelids representing the main branches of the annelid tree. Embryos of *Owenia fusiformis* and *Spirobranchus lamarcki* exposed to the BMP inhibitor DMH1 fail to form dorsoposterior tissue at larval stages. Unlike these two annelids, *Capitella teleta* relies on Activin/Nodal to specify its DV axis, while BMP controls DV patterning in the head of *Platynereis dumerilii*. Therefore, axial patterning, particularly the DV axis, develops through lineage-specific upstream regulators, even between species within the same phylum. This is a striking case of developmental systems drift affecting upstream signals in GRNs. Now, we are using single-cell atlases of larvae of *O. fusiformis* and *C. teleta* to assess similarities and differences in cell types that are under the control of BMP and Activin/Nodal. We aim to discern how co-option and genetic innovations drive shifts in the signals that trigger axial patterning during a critical phase of animal development.

P085

Linking Environmental and Mutational Robustness Across Mouse Phenotypes

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According to stabilizing selection theory, gene regulatory networks evolve to buffer organisms against environmental perturbations, maintaining traits within an equilibrium range despite fluctuating conditions. Such buffering mechanisms are predicted to increase mutational robustness of the same traits. We tested whether environmental robustness (ER) and mutational robustness (MR) are correlated across mouse phenotypic traits using data from the International Mouse Phenotyping Consortium (IMPC). Environmental robustness was quantified as between-laboratory variation in control mice. Mutational robustness was quantified in two complementary ways: (i) as the proportion of single-gene knockouts significantly affecting a given trait; and (ii) as a composite signal intensity score defined as $|\beta| \times (-log10p)$, where β represents the genotype effect estimate and p the associated p-value for that trait. We analysed 115 IMPC traits tested in ≥4 independent laboratories and across ≥1000 knockouts. Both biallelic (homozygous) and monoallelic (heterozygous) knockout data were examined. ER and MR were positively correlated ($r = 0.39$ for biallelic; $r = 0.38$ for monoallelic; both $p < 0.001$), indicating that traits more buffered against environmental variation are also more robust to genetic perturbations. Between 44% and 65% of significant genes overlapped between traits classified as highly or weakly robust to environmental and mutational perturbations. In over 70% of traits, signal intensity was stronger in biallelic than in monoallelic knockouts, indicating that zygosity modulates phenotypic impact. These findings provide empirical support for a positive relationship between environmental and mutational robustness across mouse traits, consistent with predictions from stabilizing selection and evolutionary developmental theory.

P086

Exploring the evolution of vertebrate telencephalon patterning: a comparative study across distantly related species

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The vertebrate telencephalon is the most evolutionarily diverse brain region. While extensive research has illuminated the diversity in its neurogenic developmental processes, little attention has been paid to the early period of telencephalic development - it's phylotypic stage - when progenitor identities are delineated. We assess, across three distantly related species, whether following anterior plate closure, early modulations may drive the telencephalon's later phenotypic diversity revealing early divergent cell fate decisions. Focusing on the GRN downstream of ventral Sonic Hedgehog (SHH) and dorsal Wingless/Int8B (WNT8B), we developed a multiplexed, whole-mount hybridization chain reaction-based imaging protocol and analysis framework to quantitatively measure, over developmental time, the gene expression patterns characterising the telencephalon's progenitor identities in mouse, chick, and shark. We detected variations in the molecular signatures of the pallial/subpallial fates identifying heterochronic differences in molecular events. Our results reveal that the spatial-temporal dynamics of the pallial/subpallial compartments correlate with the posterior-to-anterior emergence of SHH into the telencephalon, suggesting a functional role of telencephalic SHH induction in the cross-species variation in the telencephalon's 3D organisation. Finally, we developed a mathematical framework that captures the gene-regulatory interactions in the telencephalon and recruits imaging data to probe cross-species modulations in estimated model parameter values.

P087

Divergence of neural patterning programs between larval and adult stages of indirect developing hemichordates

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In most model species, development of the adult body plan is direct and nervous system development begins during embryogenesis. Even in *Drosophila*, the archetype of an animal that builds separate larval and adult bodies, the larval and adult nervous systems both descend from neuroblasts specified in the embryo. However, these models are inadequate for the large developmental diversity of marine invertebrates characterized by indirect development and extreme metamorphosis. When and how adult neural programs are established in these groups remains poorly understood. Here, we examine neural patterning across life stages in the indirect developing hemichordate *Schizocardium californicum*. Embryos of this species develop into pelagic, ciliated larvae and then metamorphose into benthic, vermiform adults. Using a single cell RNA sequencing atlas spanning development, we compared neural patterning programs between larval and adult stages. We found that neural cell populations primarily cluster by stage when considering all genes, consistent with patterns observed in other tissues. Strikingly, larval and adult neural populations remained distinct when comparing only transcription factor expression, indicating that patterning programs differ even between populations with similar neurotransmitter usage. Comparing neurons that deploy the same neurotransmitters revealed mixed trajectories: a few adult populations appear continuous with larval counterparts, while most are generated *de novo* during or after metamorphosis. Our data suggest that neural effector gene expression is largely dissociated from upstream, body plan specific patterning programs. This modularity may allow nervous system architecture to adapt to the ecological demands of each life history stage in indirect developing organisms.

P088

Does regeneration re-use embryonic gene regulatory networks?

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Many animals, such as salamanders, fish, flatworms and crustaceans, regenerate with varying capacities. To understand regeneration we question whether regeneration re-uses the gene regulatory networks from development, or if dedicated networks evolved. The crustacean, *Parhyale hawaiensis*, regenerates its limbs with high fidelity, producing regenerated legs that are indistinguishable from the original in terms of morphology and cellular content. Similar sets of genes are also transcribed in regenerating and developing embryonic legs, but with differing temporal patterns, suggesting that *Parhyale* leg regeneration may not entirely be a replay of leg development. Here, we ask how the chromatin accessibility changes genome-wide during leg development, and how this compares to leg regeneration, later focusing on genomic regions around leg patterning genes. We performed ATAC-seq across multiple stages of leg development and regeneration to observe genome-wide changes in chromatin accessibility. Differential accessibility analysis showed that the most dynamic peaks are associated with genes involved in appendage development and morphogenesis. Accessible regions with similar temporal dynamics during development were clustered. We questioned whether these dynamics are repeated during regeneration. We further explored these open chromatin regions for enrichment of transcription factor binding motifs. Lastly, we focused on the chromatin landscape surrounding genes with well-characterised roles in arthropod leg patterning to determine whether chromatin accessibility dynamics during development mirror those seen during the course of leg regeneration. While chromatin accessibility during leg development follows a mostly stereotypical pattern, dynamics during regeneration appear more complex.

P089

Reconstructing The Evolution of Neuro-Sensory-Secretory Cell Types by Cross-Species Larval Single-Cell Transcriptomics

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How did neurons evolve? Growing evidence supports an ancient sensory–secretory cell prototype predating canonical nervous systems. Larval stages of early-branching metazoans could provide a powerful window into this question: planktonic marine larvae perform similar ecological tasks despite hundreds of millions of years of divergence. Sponge larvae coordinate behaviour through sensory–secretory systems that mediate environmental sensing, settlement, and metamorphosis without neurons, whereas cnidarian larvae use neuronal cells and circuitry. These functional similarities and genetic/morphological differences make sponges and cnidarian larvae compelling models for reconstructing nervous system origins at the cell-type level. We analysed larval single-cell transcriptomes using the Self-Assembling Manifold Alignment Pipeline (SAMap) and Weighted Gene Co-expression Network Analysis (WGCNA), comparing the cnidarians *Nematostella vectensis*, *Clytia hemisphaerica*, *Stylophora pistillata*, *Astroides calycularis*, and *Pocillopora acuta* with the demosponge *Amphimedon queenslandica*. Within cnidarians, neuronal, secretory, gastrodermal, cnidocyte, and apical cell types showed clear homology, alongside transcriptional overlap among secretory–neural, secretory–cnidocyte, and neural–cnidocyte clusters. In contrast, *Amphimedon* lacked one-to-one counterparts; instead, sponge populations aligned with multiple cnidarian sensory, neural, and secretory identities, suggesting these functions were integrated within fewer ancestral cell types. We further identified conserved nervous system signalling components and sensory–neural transcription factors enriched in cnidarian sensory–secretory cells. In *Amphimedon*, these markers are predominantly expressed in flask and epithelial populations, consistent with published evidence of neurogenic and synaptic molecular signatures in subepithelial sensory cell types. Together, these findings support an ancestral epithelial sensory–secretory cell that later diversified into specialised neural and secretory lineages.

P090

Single-cell transcriptomics reveals early *Wolbachia* infection dynamics in *Drosophila melanogaster*

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Intracellular bacterial symbionts must navigate complex host cellular environments to establish persistent infections while evading immune clearance. The alphaproteobacterial endosymbiont *Wolbachia pipientis* manipulates host biology through reproductive manipulation, developmental reinforcement, and viral blocking. The *w*Mel strain, natively infecting *Drosophila melanogaster*, has proven effective for biocontrol, blocking viral transmission and suppressing reproduction when transinfected into mosquito populations. Despite these applications, the molecular mechanisms underlying infection establishment remain poorly understood. *Wolbachia* strains demonstrate cell-type preferences and induce tissue-specific phenotypes, yet bulk RNA-seq studies have yielded inconsistent differential expression signals, likely due to averaging across cells with heterogeneous infection titers and developmental stages. Single-cell resolution is therefore critical for elucidating early somatic infection biology and assessing biosafety risks of novel host range expansion by transinfected insects. We employed single-cell RNA sequencing to investigate how *w*Mel colonization shapes the host transcriptome during early infection in D. melanogaster JW18 neuroblast-derived somatic cell lines. Using 10X Genomics Chromium and the lower-cost PIPseq platforms, we developed a method to quantify symbiont titer in poly-A scRNA-seq data by leveraging rRNA mispriming, enabling per-cell infection quantification without specialized library preparation. We demonstrate that nascent *w*Mel infections induce distinct transcriptional changes, creating novel cellular states. Through unsupervised clustering, pseudotime analysis, and marker gene identification, we reveal how *Wolbachia* reprograms host somatic cells to evade immunity, establish infection, and facilitate nutrient acquisition. These findings illuminate the cryptic role *Wolbachia* plays in eukaryotic cellular biology and provide mechanistic insights from the native host system to advance cell-type-specific biocontrol strategies.

P091

Evolution of *cis*-regulatory landscapes in limb bone elongation: a comparative genomics approach focusing on bipedal rodents

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Cis-regulatory elements (CREs) play central roles in the evolution of trait development. However, substantial work remains to comprehensively catalog causal CREs and to establish a mechanistic understanding of how CRE evolution shifted developmental programs. To approach this, we focus on the evolution of the tetrapod limb skeleton. The limb skeleton has diversified through modular alterations to the lengths of individual skeletal elements, suggesting a shared set of genes is under local *cis*-regulatory control in different bones and different species. To elucidate the evolution of causal CREs and their regulatory mechanisms, we apply comparative genomics, epigenomics, and transcriptomics in quadrupedal mice and bipedal jerboas, which have extraordinarily disproportionately elongated feet. To narrow the genome to putative CREs with presumed function in the skeleton, we performed integrative analyses of chromatin accessibility and underlying sequence evolution of mouse and jerboa. Using a multiple whole-genome alignment, we systematically identified putative CREs that experienced accelerated nucleotide substitution in the jerboa lineage. Accessibility of these jerboa-accelerated CREs in metatarsals of jerboa feet is distinct from orthologous mouse sequences when compared to the forearms that elongate more similarly in both species. Furthermore, these CREs are significantly enriched in their proximity to genes that are disproportionately differential expressed between mouse and jerboa foot bones. Together, our results identify 1287 candidate causal CREs and their distinct evolutionary and regulatory profiles in mouse and jerboa, and provide a foundation for interrogating the functional link between CRE diversification and downstream molecular processes that cause macroevolutionary shifts in the limb skeletal proportion.

P093

The Role of Divergent Disordered Domains in the Developmental Activity of Bicoid

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Gene expression changes drive phenotypic variation between species and between healthy and diseased tissues/individuals. Transcriptional regulation is mediated by transcription factors (TFs) composed of conserved DNA-binding domains (DBDs) and less-conserved, disordered activator domains (ADs). While DBDs contribute mainly to DNA-binding specificity, ADs might diversify this specificity, affect its affinity, and therefore modify TF activity. Bicoid (Bcd) is a Homeodomain (HD) TF, which is essential for *Drosophila* anterior-posterior patterning. While Bcd's HD is highly conserved, its disordered ADs are significantly diverged across fly species. We hypothesize that these disordered ADs are critical for diversifying TF actions—including DNA binding, protein interactions, and localization—leading to both distinct developmental and species-specific functions of Bcd. To investigate the unique contributions of these ADs, we generated transgenic *Drosophila melanogaster* with targeted AD deletions. Preliminary results show that individual and combined deletions produce distinct developmental phenotypes demonstrating their unique roles. We are currently utilizing ChIP-seq and expression analysis to evaluate how these domains confer transcriptional specificity. This research will elucidate the role of disordered domains in Bcd's target-specific functions and provide an evolutionary perspective on how AD divergence might contribute to the speciation, robustness and developmental diversity across the *Drosophila* lineage.

P094

Genomic structural changes and the evolution of novelty in animals

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Genomic approaches have transformed evolutionary developmental biology by enabling comparisons of genes, regulatory elements and expression programmes across species. Much of this work has focused on sequence-level variation, whereas the large-scale organisation of genomes has received less attention. Chromosome rearrangements, fusions and other structural changes represent an additional layer of genomic evolution that may influence both lineage diversification and the emergence of novel traits. Here we examine how comparative analyses of chromosome-scale genomes can inform animal evolution and developmental innovation. Using examples from lophotrochozoans and cnidarians, we show that rare genomic structural changes clarify deep evolutionary relationships and indicate when key biological traits emerged. Comparisons between corallimorpharians and stony corals, for instance, indicate that coral skeletons originated within scleractinian lineages rather than being secondarily lost. Integrating chromosome-scale genomics with single-cell transcriptomic data further shows that specialised calcifying cells, the calicoblasts, occur only in stony corals. This observation links genome evolution with the origin of a cell type underlying skeletal formation. Together, these examples illustrate how combining comparative genomics with cell-type–resolved transcriptomics can connect genome evolution with developmental processes. Integrative approaches of this kind provide a path towards understanding how large-scale genomic changes contribute to the origin of biological novelty across animal lineages.

P095

Large Scale Mouse Mutagenesis Reveals High modularity and plasticity in the mammalian sternum

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Little is known about genetic underpinnings of variation in the mammalian ribcage, nor its integration with other body systems. This study asks what genes are driving morphological variation and how they correlate with other anatomical systems using the International Mouse Phenotyping Consortium (IMPC). Of 3633 mutant knock-out mice phenotyped by the IMPC, 3.3% produced an abnormal thoracic cage morphology. The majority of these are associated with abnormal sternum morphology (68%) while the remaining genes are associated with abnormal rib (13%) and vertebrae (3%) morphology. Further, changes to the ribs are strongly pleiotropic; they are associated with many abnormal phenotypes including multiple changes to the whole skeletal system. Whereas changes to the sternum tend to appear in isolation. Sternal and rib phenotypes were never associated with one another, suggesting that morphological variation is independently patterned in the two ribcage elements. Taken together this indicates increased plasticity of the mammalian sternum associated with reduced pleiotropy. Sternum plasticity may be associated with innovations such as powered flight in bats, facilitated by a fused sternum with a keel, and the land to water transition, associated with fewer sternebrae. The IMPC is a valuable big data resource for identifying candidate genes and measuring gene pleiotropy that may be relevant for understanding evolutionary variation in ribcages across mammals.

P096

Studying the shark embryo at the single cell level to understand the cellular origin and evolution of the vertebrate telencephalon

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The emergence of predation and associated complex behaviors in jawed vertebrates (gnathostomes) were major drivers of brain evolution. To gain insight into the neuronal organization of the last common gnathostome ancestor, we investigated the development of the shark telencephalon using single-cell and spatial transcriptomics. Our findings indicate that many features previously considered specific to amniotes—or even to mammals—likely have a more ancient origin, present in the common ancestor of all jawed vertebrates. We provide evidence for the presence of Cajal-Retzius cells in the developing shark telencephalon, showing a conserved molecular profile and spatial distribution comparable to those in mammals, coinciding with the appearance of early pallial layering. We propose that these conserved features emerged during the origin of jawed vertebrates, contributing to the increasing complexity of early vertebrate brains.

P097

Photic ecology and the evolution of octopod rhodopsin

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Octopods possess camera-type eye and use image-forming vision for prey detection, predator avoidance, communication, and orientation. Yet, unlike vertebrates and arthropods, they mainly depend on a single visual pigment, rhodopsin (r-opsin1), to mediate visual tasks. How this “single-molecule” visual system adapts to the contrasting light environments of shallow coastal waters and the deep sea remains poorly understood. Here, we investigated the molecular evolution of the r-opsin1 in seven octopod species inhabiting the Gulf of Naples (Italy) spanning littoral and deep-mesopelagic habitats. Transcriptomic analyses of retina and optic lobe confirmed that r-opsin1 is the most consistently expressed opsin across the different species. Positive selection analyses did not detect a general signal associated with deep-sea versus littoral habitats. Instead, signatures of positive selection were only restricted to a single deep-mesopelagic species *Pteroctopus tetracirrhus*. Structural modelling revealed that three residues under selection are located in proximity to the retinal binding pocket. Functional *in vitro* assays demonstrated that two of these mutations strongly affect spectral properties in a context-dependent manner. Single substitutions induced pronounced bathochromatic shifts, whereas the double mutant restored a wild-type–like absorption spectrum, suggesting stabilizing interactions between these sites. Notably, wild-type rhodopsins of *P. tetracirrhus* and *Octopus vulgaris* share a similar λmax (~481 nm), indicating conserved peak sensitivity despite ecological divergence. Homologous mutations were identified in other deep-sea octopods from different oceanic regions, supporting convergent molecular trajectories associated with low-light and bioluminescent environments. This work highlights how visual adaptation in extreme environments can occur through targeted molecular changes without opsin diversification.

P098

On the stability of dental complexity

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Developmental systems must buffer perturbations while still permitting morphological change. How regulatory networks balance stability and variation can be directly tested in structures in which patterning is well understood, such as the mammalian molar tooth. Here, we use graded perturbations of *Sostdc1* - a WNT and BMP pathway modulator that regulates enamel knot dynamics - to examine how changes in signaling influence both tooth complexity and developmental stability. Using CRISPR-Cas9, we generated *Sostdc1* null mutants and mice lacking an enhancer region of *Sostdc1* and intercrossed these lines to establish a quantitative gradient of SOSTDC1 expression *in vivo*. High-resolution three-dimensional dental topographic analysis was used to quantify molar crown morphology across genotypes. To assess developmental robustness, we measured fluctuating asymmetry between paired molars within individuals. Because left and right teeth share the same genotype and environment, asymmetry provides a sensitive readout of buffering capacity within the developing tooth. We observe that decreasing the regulatory input is linked to changes in asymmetry, suggesting an alteration in developmental stability. Together, these findings suggest that altering regulatory input affects both tooth complexity and developmental robustness and provide an experimental approach for understanding how changes in developmental buffering shape morphological variation.

P099

Nectar Spurs: on the development of an evolutionary key innovation in flowering plants.

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Spurs are a striking example of the impact of plant-pollinator interactions on the evolution of floral morphology and diversity. These tubular outgrowths of the perianth, often containing nectar, position the pollinators in a way that optimises pollen delivery. They evolved independently in many distant taxa of the *Angiospermae* and in a wide variety of developmental contexts: in corollas (as in *Aquilegia*) or calyces (as in *Tropaleum*), in actinomorphic (as in *Centranthus*) or in zygomorphic (as in *Viola*) flowers, suggesting cases of convergent evolution rather than parallelism. Moreover, evidence suggests that spurs are "key innovations", i.e. that evolution of this trait in a taxon has a positive effect on its diversification rate. The present research proposes to build on previous work to decipher the molecular basis of spur development in the species *Linaria vulgaris* (*Plantaginaceae*) which has a yellow, zygomorphic flower carrying a long (~2cm) spur on its ventral petal. We use different approaches (including Virus Induced Gene Silencing and whole-mount mRNA *in situ* hybridisation) to characterise the role of previously proposed candidate genes. We focus mostly on a possible mechanism involving a localised increase of cell proliferation in early stages of spur genesis under the control of genes of the *KNOX* family *HIRZINA* and *INVAGINATA* (orthologs of *Arabidopsis thaliana SHOOT MERISTEMLESS*), which are involved in sustaining cell indeterminacy, and of genes of the cytokinin pathway, including a putative cytokinin response transcription factor and the ortholog of *A. thaliana LONELY GUY* which codes for a phosphoribohydrolase producing active cytokinins.

P100

Identification of melanogenesis-disrupting variants in squamate reptiles reveals species-specific effects of conserved pigmentation genes

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Skin coloration is crucial for the survival of animals and ranges from spectacular colourful displays used to attract a mate to cryptic camouflage used to avoid predators. Among the three main types of chromatophores, melanophores are the most widespread and well-studied in vertebrates and can set the skin tone by the amount of melanin they produce and store in dedicated vesicles, the melanosomes. Mutations associated with melanophore differentiation and maturation result in hypermelanistic, hypomelanistic and amelanistic phenotypes, all extensively studied in mammals but less so in snakes and lizards. We characterized at the genomic, transcriptomic, and histological level, two corn snake (*Pantherophis guttatus*) and two leopard gecko (*Eublepharis macularius*) colour morphs with disrupted melanogenesis. Thanks to the chromosome-level genome assembly we produced, we could identify candidate mutations in the same two melanogenesis factors for both species: tyrosinase (*TYR*), an essential enzyme for melanin synthesis, and the P protein (*OCA2*), a transmembrane transporter for tyrosine. Through our extended bulk RNA sequencing analyses and electron microscopy imaging results, we show how the disruption of these genes has species-specific impact on known pigmentation pathways, melanin production, melanosome motility and subcellular structure in the skin of the two species studied. These findings demonstrate that conserved pigmentation genes can produce species-specific effects, underscoring the modular nature of skin coloration in vertebrates. Our work establishes reptiles as pigment cell biology models and reveals evolutionary flexibility in the genetic regulation of melanogenesis.

P101

Blurring the line: blade-sheath boundaries and the *extended auricle1* mutant

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The maize leaf is neatly separated into distinct tissues along its proximodistal (base-to-tip, PD) axis: the proximal sheath wraps around the stem and provides structural support, and the distal blade bends away from the stem to intercept light. At the blade-sheath boundary, the specialised structures of the ligule and auricles arise, which regulate leaf angle. Leaf angle and shape are key agronomic traits, but how such a boundary is specified, and how these specialised structures form, is not well understood.The recessive *extended auricle1 (eta1)* mutant has a diffuse, shifted blade-sheath boundary and waves of excess auricle tissue along the blade margins. The eta1 phenotype suggests that boundary specification is disrupted, indicating that ETA1 may be a component of the gene regulatory network that defines the blade-sheath boundary. Here, I detail my work in characterising and mapping the mutation, as well as recent findings in novel patterning components and their implications for our understanding of maize architecture.

P102

Microbes Support Neural Circuit Resilience under Prolonged Starvation in Hydra

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Nerve nets are considered the evolutionarily oldest form of nervous systems. How such networks develop into functionality and how resilient they are under extreme stress is largely unknown. In the phylogenetically ancient model organism Hydra, we demonstrate that a nerve net composed of distinct neuronal subpopulations is precisely replicated during asexual proliferation by budding. During this process, precursor cells first accumulate at the site of the emerging neural network, followed by a stepwise formation of functional neural circuits composed of distinct neuronal subpopulations. The newly formed nerve net in an adult Hydra is stable throughout its life and exhibits remarkable scalability. We found that neuronal architecture in the adult is well maintained, even under extreme conditions such as prolonged starvation. The endodermal neural circuit associated with the feeding response maintains its neuronal densities under starvation and preserves its mouth-opening function. However, removal of the microbes affects mouth-opening in starved animals, while recolonisation with the native microbiota restores this function. Although the neuronal architecture is still maintained in germ-free starved animals, we suspect a metabolic regulation of neuronal functions.

P103

Quantitative pleiotropy drove the convergent emergence of anterior fin spines

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Anterior fin spines have evolved independently as a defence mechanism across the radiation of fishes and provide a striking example of convergent evolution. It remains unclear whether biased genetic mechanisms have contributed to the repeated channelling of evolution towards spines as an adaptive solution. Here, we demonstrate that across broad evolutionary distances, the developing anterior fin spines are consistently characterised by the expression of the highly pleiotropic master control transcription factor alx4. In alx4 CRISPR/Cas9 knockout mutants of the cichlid Astatotilapia burtoni, we observe severe defects in both fin spine mineralization and the formation of the craniofacial opercular complex. Mechanistically, both defects arise from altered downstream Hedgehog signalling. Furthermore, we reveal that spine hypermineralization and the transcription of downstream targets are alx4 dosage-dependent. We propose that the recurrent evolution of fin spines was driven by the pleiotropic role of alx4 as a default regulator of the anterior fin and driver of craniofacial mineralization via Hedgehog signalling—allowing the repeated integration of the craniofacial pathway into the fins. Furthermore, the dosage-sensitive regulation of the downstream mineralization network by alx4 has the potential to generate continuous—rather than on/off—phenotypic variation. We propose that such a quantitative mechanism increases evolvability, providing a further genetic bias driving the convergent evolution of fin spines.

P104

Establishing CRISPR/Cas9 mutagenesis in annelids with Vitellotag

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Being able to edit and mutate the zygotic genome is fundamental to gaining a functional understanding of the genetic control of animal embryogenesis. Microinjection is the most common method for delivering molecular tools into oocytes and has been successfully applied to many organisms over the past few decades. However, it often requires complex and expensive equipment, fine skills, and heavy labour. Vitellotag is a novel cargo delivery method that is based on the natural vitellogenesis process. By incubating oocytes with protein tagged with the vitellogenin receptor binding domain (Vtg), the protein is recognised and taken into the oocyte via endocytosis, before being released into the cytoplasm with the help of lysosome inhibitors. This technique has shown to be effective in echinoderms and hemichordates. However, as the process of vitellogenesis is conserved in many animal phyla, Vitellotag has the potential to become a more high-throughput, less expensive, and less skill-demanding alternative for microinjection, especially for non-model organisms. To investigate this, we tested Vitellotag in two distantly related annelids, *Owenia fusiformis* and *Capitella teleta*. After incubating the oocytes with Vtg-GFP, both species showed GFP signals. When incubating oocytes with Vtg-Cas9, we obtained stable gene knockout phenotypes, and the mutation was further validated by PCR and molecular cloning. Together, we developed a Vitellotag protocol for two annelids, demonstrating the utility of this new delivery method in non-model animals outside the Ambulacraria and opening an exciting new path for functional research in emerging annelid models.

P105

Characterising the cuticle striation patterns in *Hibiscus trionum* petals

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The colours in flowers are produced by the presence and distribution of cells containing pigments; additionally, modifications on the surface of epidermal cells can interfere with light, creating additional optical effects. For example, the petals in the *Hibiscus trionum* flower develop a purple region with nano ridges in the cuticle of the adaxial cells, which affects light diffraction and produces iridescence. The latest model for striation patterning in the petal epidermal cell cuticles predicts the contribution of the stiffness of the cuticle layers, cell wall curvature, cell in-plane expansion, and cell growth rates in the formation of the observed patterns at the single cell level. This work aims to identify the specific mechanisms underlying the fine-tuning modification of the striation patterns in the petal cuticles. We are studying the formation of ridges at the micro- and macroscale level. At the whole petal level, we have observed differences in the striation patterns (ridge density, direction, amplitude, wavelength), which are probably related to whole-organ features such as cell curvature, size and shape across the petal, and differences in cuticle properties. Additionally, we are studying how the dynamics of cuticle proper growth affect the initiation and features of the striation patterns.

P106

Heritable plasticity in Arabidopsis flowers promotes a pollination mode switch

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Flowers are genetically and environmentally robust in development, which promotes accumulation of standing variation. Upon environmental change, accumulated mutations sometimes translate into phenotypic changes in a form of heritable plasticity and become exposed to natural selection. In our work, we aim to elucidate the release of robustness in flowers of Arabidopsis thaliana in response to foliar shade and high ambient temperature. A phenotypic screen of 18 A. thaliana accessions of a broad geographical origin under four combinations of conditions, two temperature regimes, 22 and 27 °C, and two light regimes, full white light and simulated shade, has revealed that elevated temperature increases the variance of floral traits, such as sepal, petal, stamen and pistil length and promotes anther-stigma separation. High temperature enables shade-mediated plasticity in organ lengths in some accessions. We carried out a mutant analysis and established a candidate for the factor enabling developmental buffering under named conditions. Analysis of bulk RNA-seq data from inflorescences of 38 ecotypes grown under three temperature regimes, 17, 22 and 27 °C, revealed a group of universal temperature-responsive transcripts. Some of them encode floral regulators, and reconstruction of their regulatory network through a combination of co-expression analysis and publicly available ChIP-seq results revealed significant genotype-by-environment interaction in regulon expression, suggesting that phenotypic plasticity is enabled through network-level changes.

P107

The influence of developmental mechanisms on phenotypic variation and evolutionary outcomes

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Evolution is driven by natural selection, but also depends on phenotypic variation that originates from genetic mutations, and that is shaped by the mechanisms of development. Quantitative genetics models so far rarely incorporate these mechanisms, and often assume characteristics of heritable phenotypic variation to be constant. We here show how evolution on fitness landscapes of varying complexity is mediated by phenotypic variation and the underlying gene regulatory networks (GRN), changing evolutionary trajectories and domains of attraction. Even in the minimal GRNs we considered, the G-matrix can change radically over phenotype space. Knowledge of the GRN or certain elements of it can help us determine the phenotypic variation supplied by mutation. While on single-peaked and very rugged fitness landscapes, evolutionary outcomes are largely independent of the underlying gene regulatory architecture, on fitness landscapes of intermediate complexity, evolutionary outcomes are determined to a considerable degree by GRN topology. We foresee that advances in the identification of these mechanisms will enable quantitative genetics to in the future increasingly integrate the genotype-phenotype map in its model framework.

P110

And growth on form? How tissue expansion disrupts Turing-like patterns and enables eukaryotic species to be more and/or less visible by generating novel shapes and colours

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Many Turing-like color patterns are perceived by con- and hetero-specifics, and are known to be under selection for signaling and/or camouflage. We show that PGTCPs (Putative-Growth Turing-like Color Patterns), produced by the interaction of a Turing-like system with tissue growth, like the well-known leopard rosettes, are adorned by thousands of diverse species of Eukaryotes, suggesting specific biological functions. We therefore wondered whether their specific geometric and color features could improve signaling or camouflage compared to classical Turing patterns.

First, the novel mixed colorations caused by growth allow species to retain the camouflage capacities of Turing-like patterns periodicities, while reducing the very contrasting color segregations which are typical of these patterns.
On the other side of the spectrum, some characteristics of PGTCPs could enhance their visibility and their attractiveness or repulsion to conspecifics or other species. We show that growth is a source of novel and striking geometries (e.g. ring patterns and line-and-dot alternations), absent in abiotic environments, which might then confer them a higher detectability by vision systems compared to classic Turing-like patterns. Finally, by exhibiting supplemental periodicities than classic ones, we hypothesize that PGTCPs could be more detectable by con- and hetero-specifics.
With this eco-evo-devo approach at the scale of Eukaryotes, we show how PGTCPs could be an evolutionary source of improved biological functions for Turing-like patterns, more than only mere by-products of developing tissue growth. We will therefore discuss possible avenues for behavioural studies that could be conducted to validate or invalidate all these hypotheses.

P111

Time-gated patterning in the *Drosophila* blastoderm establishes distinct expression boundaries downstream of moving stripes

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Over the first three hours of *Drosophila* development, segmentation genes are patterned down to single cell resolution. For example, single cell rows of *engrailed* expression are defined by precise single cell offsets between the posterior borders of overlapping *paired* and *sloppy-paired* stripes. Both borders are patterned by the transcription factor Even-skipped (Eve), raising the question of how a single input factor produces two distinct spatial outputs. Classic models propose that the Eve stripes act as concentration dependent morphogen gradients with distinct thresholds for *paired and sloppy-paired*; more recently, we have proposed that the offsets reflect an anterior shift of the Eve stripes combined with distinct temporal windows for patterning *paired* and *sloppy-paired*. Using MS2-MCP live imaging, multiplexed fixed imaging, computational image analysis, and genetic perturbations, we find strong support for the temporal model. *paired* becomes indifferent to Eve levels soon after its stripes are established, thus "locking in" their initial boundaries, which eventually overlap high levels of Eve. This regulatory switch is mediated by the pioneer factor Odd-paired, while maintenance of the *paired* boundary requires the transcriptional repressor Hairy. As Hairy expression spans the *paired* boundary, we suspect that the mechanism by which *paired* "remembers" its original boundary is epigenetic.

P112

The molecular origins of phloem tissue in plants

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The acquisition of vasculature was a key innovation in the evolution of land plants. In plant vasculature, specialised phloem tissue is responsible for sugar and nutrient transport. However, this tissue did not arise *de novo*, and instead evolved by the co-opting and diversification of existing pathways of growth and development. From genomic evidence, we can infer that the freshwater algal ancestor of plants had some capacity for dealing with stress, such as nutrient starvation, light response, water status, and biotic stress. We hypothesise that transcriptional regulators controlling responses such as these were co-opted for complex adaptations for life on land, such as vascular development. Through a phylogenetic approach of regulators previously identified to have conserved expression domains in phloem tissue, we will probe the evolution of these families in order to determine the molecular origins of phloem tissue. We will pay particular attention to regulators conserved in even non-vascular plants, which may have functions more reminiscent of the pre-vascular ancestor of land plants. We will also look for putative regulators of phloem development which are conserved across vascular plants. This study will provide insights into the ancestral origins of genetic regulators of phloem, and fundamental evolutionary principles guiding gene regulatory network evolution.

P114

Single-cell evidence for neural crest hyper-function in the evolution of a phenotypic syndrome

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Developmental bias can arise when multiple traits covary because they share a common origin in the same stem cell population. We test this idea by investigating the developmental basis of the recently evolved, sexually selected "nigriventris syndrome" in the common wall lizard (*Podarcis muralis*), in which coloration, morphology, and behavior are exaggerated relative to the ancestral phenotype. These traits share developmental origins in neural crest cells (NCCs), making NCC modulation a plausible mechanism of their coordinated evolution. Inspired by the hypothesis that NCC hypo-function underlies the domestication syndrome, we ask whether altered NCC activity explains the evolution of the nigriventris syndrome. We generated the first single-cell transcriptomic atlas of NCCs in a non-avian reptile, including NCCs from both nigriventris and ancestral embryos. Comparative analyses reveal that NCCs in nigriventris embryos exhibit elevated proliferative and migratory signatures, and show increased transcriptional activity. Flow cytometry independently confirms higher total NCC numbers. Together, these results support NCC hyper-function as a developmental mechanism underlying coordinated trait exaggeration. More broadly, they demonstrate how modulation of a shared stem cell population can generate phenotypic integration and bias evolutionary trajectories.

P115

Mapping the evolution of phyllotaxy in lycophytes

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Phyllotactic patterns in land plants – the position where organs develop on an shoots - present a surprisingly low level of diversity considering the endless physically and developmentally possible arrangements. Fibonacci spirals have long been shown to be the predominant pattern in euphyllophytes (angiosperms, gymnosperms and ferns). Recent evidence shows lycophytes (early diverging vascular plants sister to euphyllophytes) may deviate from this rule, but a clear analysis spanning the lycophyte phylogeny is missing. This is the first large-scale analysis of phyllotaxy in lycophytes, sampling species globally by using the living and herbarium collections at Royal Botanic Garden Edinburgh, as well as fieldwork carried out in the UK and collaborators based in North America. The dataset so far spans 19 species of all extant orders (Isoetales, Lycopodiales and Selaginellales).

We combine classical histology with new imaging approaches, including 3D reconstructions of shoot apices, to resolve long-standing ambiguities in phyllotactic development in lycophytes. Preliminary results suggest that several taxa historically described as spiral in fact exhibit whorled phyllotaxy, and that non-Fibonacci spirals occur at elevated frequencies relative to euphyllophytes. Selaginellales is an order where striking discoveries are made.

By mapping phyllotactic states across a taxonomically critical lineage, our work reframes our understanding of spiral patterns in plant evolution. Through this research we hope to gain insight into the origin and evolution of phyllotactic patterns in vascular plants.

P116

Gene expression variability, polymorphism, and evolvability in chordates

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A major goal of evolutionary biology is to understand patterns of clade diversity. Species-poor lineages that exhibit plesiomorphic characteristics and appear to evolve slowly provide opportunities to examine questions of evolvability. Historically, scientists used morphological, developmental, and fossil evidence to determine that a taxon was slow evolving. However, genomic evidence reveals that the releationship between morphological and developmental stasis and molecular evolution is not simple. Understanding the degree to which changes at one level of biological organization impact other levels uncovers the mechanisms shaping patterns of diversity. Here, we compared gene expression variability and genetic polymorphism across chordates that vary in their morphological and genetic diversity. We chose to examine gene expression because it is an important link between genotype and phenotype and its variability is shaped by developmental and evolutionary constraints. We analyzed genome and transcriptome profiles from multiple organs of three ray-finned fish, zebrafish (*Danio rerio*), Northern pike (*Esox lucius*), and spotted gar (*Lepisosteus oculatus*), and a cephalochordate that exhibits morphological and developmental stasis, amphioxus (*Branchiostoma lanceolatum*). We compared per-gene expression variability across organs with measures of nucleotide diversity to understand the relationship between heterozygosity, gene regulation, and organ function. Linking DNA sequence variation with gene expression across species that vary in morphological diversity contributes to our understanding of the genotype-phenotype map and the origins of the genetic and developmental variability that selection acts upon to produce diversity.

P117

Assessing the Identifiability of Complexity-Generating Regulatory Networks from Time-Series Expression Data

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Many biologically relevant regulatory networks (RN) are expected to exhibit complexity-generating features, such as high levels of non-linearity, feedback, and redundancy. One key challenge in Systems Biology is to assess to what extent such features theoretically obstruct the reconstruction of the underlying RNs, even in the presence of abundant and accurate time-series data. Here, we show that a theoretical toy model for transcriptional regulation of gene expression allows us to quantitatively investigate this question in the framework of developmental biology. Concretely, we study a next-to-minimal, non-linear, ordinary differential equation system representing a large class of RNs, where effective competitivity and self-cooperativity are modeled with the fewest possible number of extra parameters. Given synthetic time-series data simulated by this mathematical model from a known RN, we assess the possibility of inference of the RN. More specifically, we estimate the distribution of data-compatible network topologies by evaluating the Bayesian posterior distribution on parameter space using a Metropolis coupled Markov chain Monte-Carlo algorithm. We apply this testing environment to RNs that have been artificially evolved to generate gradient-driven "French-flag" pattern (i.e., patterns exhibiting the spontaneous coexistence of multiple stable states). We study the amount and nature (native and/or perturbed) of input data that allows us to completely and uniquely reconstruct various types of RNs.

P118

The evolution of the polar lobe in Spiralia

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Spiral cleavage is unique among animals in specifying the first embryonic fates through three distinct cell division patterns. In “equal” spiral cleavage, the first two zygotic divisions are symmetric, producing four equipotent blastomeres of equal size, with cell fates specified later through inductive interactions. This equal development is ancestral, occurring in all phyla with spiral cleavage and showing high molecular conservation. In contrast, many molluscan (e.g., snails and clams) and annelid (e.g., lugworms) embryos undergo “unequal” spiral cleavage, where post-fertilization divisions are asymmetric, segregating maternal cytoplasmic mRNAs into a larger cell. These asymmetric divisions involve the mitotic spindle's asymmetric positioning, or a unique cortical protrusion called the polar lobe. The transition to an unequal development, including polar lobe evolution, has occurred independently multiple times, becoming a remarkable case of parallel evolution. In clams and snails, the actin cytoskeleton is involved in the early stages of polar lobe formation, and myosin and microtubules contribute to its resorption to the larger cell. However, whether similar mechanisms act in other molluscs and annelids, as well as the exact molecular and mechanical mechanisms triggering polar lobe formation are largely unknown. To fill this knowledge gap, we are investigating a range of molluscs and annelids, exploring first the conservation of such cytoskeletal mechanisms, and profiling the transcriptomes and proteomes of spiralian oocytes with different cleavage modes. Together our work can identify cytoskeletal mechanisms and genes involved in the parallel evolution of polar lobes and asymmetric divisions.

P119

Interspecies Differences in Developmental Mechanisms Shaping the Mammalian Ovarian Reserve

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The size of the ovarian reserve is a key factor determining the female reproductive span in mammals. It is established during embryonic and early postnatal development through the proliferation and attrition of germ cells in the gonads. Although this process is broadly conserved among mammals, the final size of the reserve differs among species, and the factors underlying these differences remain little understood. Here, we investigate the establishment of the ovarian reserve in mammals with contrasting lifespans, specifically in the naked mole-rat (*Heterocephalus glaber*) as a long-lived rodent model and the laboratory mouse (*Mus musculus*) as a representative short-lived species. To characterize interspecific differences in the developmental dynamics that shape ovarian reserve size, we examined the proliferative activity of post-migratory germ cells and used flow cytometry to quantify their subpopulations across several developmental stages. We further compared chromatin accessibility in regulatory regions of ovarian cells to identify potential species-specific mechanisms influencing germ cell development. Together, these findings provide insight into how species-specific developmental mechanisms may contribute to differences in ovarian reserve size in mammals.

P120

Smed-cbp-3 is essential for progenitor cells lineage progression and gene regulation in planarians

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Characterizing how stem cells self-renew and differentiate in different organisms is pivotal to understand essential questions in evolution and developmental biology, such as tissue renewal and regeneration. Freshwater planarians are able to regenerate any part of their body based upon the presence of adult pluripotent stem cells. CBP (CREB-binding protein)/p300 proteins have an acetyltransferase activity and belong to a conserved gene family which functions as transcriptional co-activators regulating gene expression. Several CBP homologues have been identified in planarians and functional analyses have uncovered an important role for *Smed-cbp-3* in stem cell differentiation. On-going bulk and single-cell RNA-sequencing analyses have identified specific biological processes and cell populations affected after silencing *cbp-3*. Thus, different gut and secretory cell populations, among others, are affected, many neural genes are downregulated and, remarkably, many genes expressed in parenchymal cells appear up-regulated after *cbp-3* RNAi. Also, most of the identified transcription factors miss-regulated after silencing *cbp-3* are expressed in neoblasts or progenitor cells. In addition, our recent work has shown that stem cells and progenitors accumulate in the absence of *cbp-3* due to its inability to pursue differentiation, while mature differentiated cells of several cellular lineages are reduced, both under regeneration and homeostatic conditions. This inability might be caused by cell cycle alterations, as cycling cells were found to be accumulated. All this data should allow to better characterize the role of *cbp-3* in the regulation of stem cell differentiation.

P121

A morphospace of fly ageing

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Phenotypic variation within populations is dynamic, emerging not only from genetic and environmental differences, but also from intrinsic developmental and life-history trajectories. Ageing represents a major yet underexplored source of such variation, generating progressive phenotypic divergence among individuals as a consequence of developmental timing, physiological decline, and cumulative environmental exposure. We investigate how ageing structures phenotypic variance in a single population of Drosophila melanogaster exposed to controlled environmental stressors (temperature, humidity, and diet). Using high-throughput optical coherence microscopy combined with self-supervised deep learning–based phenotyping, we quantify subtle morphological differences across individuals of different ages at high resolution. Our ongoing analyses aim to disentangle age-specific variance and to assess the extent to which age interacts with environmental conditions to contribute to the pool of potentially selectable phenotypic variation.Such results can position ageing as a central mechanism in micro-evo-devo, linking lifetime developmental dynamics to population-level evolutionary potential. More broadly, our approach illustrates how high-throughput phenomics can inform about developmental processes and evolutionary change within populations.

P122

“Heads or tails?”: The evolution of trunk growth heterochronies in animal larvae

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Intermediate larval stages are widespread across animals and play central roles in life-cycle evolution, yet the developmental mechanisms underlying their origin and diversification remain poorly understood. Heterochrony in trunk development has been proposed as a key mechanism shaping larval and life-cycle evolution in bilaterian animals. In annelids, the timing of head and trunk development varies across life cycles: non-feeding larvae and direct developers form the trunk during embryogenesis, whereas feeding larvae delay trunk formation until late larval stages, effectively decoupling head and trunk development. This project aims to identify the cellular and regulatory mechanisms underlying heterochrony in trunk formation by comparing annelids with contrasting developmental modes and evolutionary histories: the planktotrophic larva of the early branching oweniid *Owenia fusiformis* and the lecithotrophic larva of the sedentarian annelid *Capitella teleta*. Separated by more than 500 million years of evolution, these lineages provide a powerful framework to identify deeply conserved mechanisms of trunk development. To resolve the cellular programmes underlying trunk formation, we generated time courses of single-nucleus transcriptomic profiling across four key developmental stages in both species. Using a metacell-based pseudobulk strategy, we reveal cell-type transcriptional signatures enriched for transcription factors that define tissue and trunk patterning dynamics. These analyses are uncovering unexpected homologies in cell types and developmental programmes despite the deep evolutionary divergence between the two species, providing new insight into how conserved regulatory mechanisms generate heterochrony in trunk formation and shape the evolution of bilaterian life cycles.

P123

Splicing regulates gene dosage during limb development

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Gene regulatory networks (GRNs) governing limb development are highly conserved throughout tetrapods; however, the resulting morphology is incredibly diverse. Differences in the amount and temporospatial dynamics of gene expression are thought to underlie this variation. Previous work has shown that differences in regulatory sequences and epigenetic modification contribute to evolutionary differences in gene expression. We hypothesize that alternative splicing (AS) is an additional mechanism contributing to limb GRN differences. AS produces multiple protein isoforms from a single precursor mRNA. However, the impact of these isoforms on downstream targets is less understood and may alter effective GRN levels. To test this hypothesis, we used NIH3T3 cells incubated with either of two isoforms of key developmental gene *Fgf8*: *Fgf8a* and *Fgf8b*. These variants have been shown to play different roles during the development of tissues such as the brain and have differing binding affinities to their receptor. However, their impact on downstream *Fgf8* targets is less well known. We show that Fgf8b has a higher impact on the activation of the downstream signaling pathway pERK, target gene expression, and proliferation compared to Fgf8a. These results suggest that AS of *Fgf8* can act as a mechanism of modifying gene dosage. In order to test for potential environmental effects during development, we treated developing chick limbs with perflurooctanesulfate (PFOS), a toxic chemical used in a variety of consumer products. This resulted in limb malformations. We are currently evaluating changes in gene expression and splicing.

P124

The genetic basis of convergent placenta evolution

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How novel organs emerge during evolution remains a central, unresolved question in evolutionary developmental biology. These complex structures can arise from cells and tissues that gradually interact and transform into integrated, functional units. However, empirical evidence on how new organs originate is still limited. Here, we studied the molecular and cellular drivers of the repeated evolution of placentas in the fish family Poeciliidae. In this family, the placentas evolved from the interaction between maternal and embryonic tissues within the ovary, enabling nutrient transfer from the mother to the embryo. Notably, the Poeciliid placentas evolved at least nine times independently and in a relatively short period of time, making them an exceptional model for dissecting the evolutionary and developmental mechanisms underlying the repeated origins of a new organ. Using single-cell RNA sequencing, we have characterised the cellular composition of the ovary in nine species, covering five independent origins of a placenta. We discovered that a novel ovarian cell type evolved at the base of the family and was independently repurposed five times to make the placenta. In each of the five placental species, the novel cell type deployed genetic programs associated with nutrient transport, enabling the evolution of a placenta. We found strong evidence for convergence at the gene expression and protein levels, with specific genes and pathways being repeatedly recruited to enable placentation. This work provides an empirical example of how the emergence and subsequent modification of a novel cell type enabled the evolution of an entire new organ repeatedly.

P125

An intrinsically disordered glycoprotein and a glycan-binding protein enable strong underwater adhesion in *Hydra vulgaris*

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Hydra, a freshwater cnidarian, achieves reversible attachment via a specialized adhesive secreted from its basal disc. While previous studies have identified proteins and carbohydrates as key components of this adhesive, its molecular composition and mechanisms remain poorly understood. Here, we identify glycans, including N-acetylglucosamine and chitin, as critical structural components of the adhesive matrix and functionally assess the role of 15 secreted adhesive-specific proteins. Two proteins emerge as essential: an arabinose-binding domain-containing protein (Ab2) and a large, intrinsically disordered glycoprotein lacking conserved domains, which we named *Hydra vulgaris* adhesive protein 1 (HvAP1). Ab2 knockdown disrupts the adhesive structure and severely impairs attachment, consistent with a role in glycan-mediated crosslinking within the adhesive matrix. In contrast, HvAP1 knockdown abolishes attachment and depletes most adhesive material, identifying HvAP1 as the principal structural scaffold. HvAP1 is highly glycosylated, and we propose that Ab2 interacts with its sugar moieties to organize and stabilize the adhesive. Our findings reveal a glycan-protein architecture in Hydra's reversible adhesive and establish HvAP1 and Ab2 as core components. Additionally, our results highlight its potential as a model for developing innovative, scalable adhesive technologies inspired by nature.

P126

Investigating the ancestral function of the known phloem-developmental regulator, BREVIS RADIX gene, in non-vascular plants

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The evolution of vasculature was a key innovation in the plant colonisation of land. This includes the phloem which transports organic molecules, and xylem which transports water. Despite the essential role of vasculature allowing for increased size and complexity of land plants, little is known about the evolution of its genetic underpinnings. Understanding the evolution of vascular development can provide insights into key conserved regulatory elements and mechanisms, uncovering broader rules which can be extended across tracheophytes. In *Arabidopsis thaliana*, the land plant-specific BREVIS RADIX (BRX) gene encodes a nuclear, polar membrane-associated transcription factor for protophloem specification. We hypothesise that the ancestral function of BRX is maintained in non-vascular plants, and that the family diversified in vascular plants. I carried out a phylogenetic analysis of the BRX gene family, revealing angiosperms have evolved the BRX family genes, comprising the sister clades BRX genes and BRX-LIKE (BRXL) genes. This also highlighted the presence of homologs of the BRX gene in non-vascular plants, including *Marchantia polymorpha* (MpBRX). My structural analysis of the BRX protein revealed disproportionally shorter domains in MpBRX compared to AtBRX and AtBRXL1-4. To characterise the function of MpBRX, we will generate null mutants by CRISPR-induced mutagenesis. We have generated transgenic lines with fluorescently tagged MpBRX to investigate if the polar localisation of BRX is conserved. This study will characterise the origins of a vascular development gene by understanding its function in a non-vascular lineage, revealing its ancestral function before it was involved in phloem development.

P127

Gene loss and the evolution of the open pangenome in the *Mytilus* species complex

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Marine mussels of the *Mytilus* edulis species complexprovide a compelling example of massive gene family expansion followed by divergence and gene loss. These speciespossess an “open” pangenome characterised by extensive structural variation and gene presence–absence variation (PAV). Many gene families in *Mytilus*, particularly those associated with immunity and environmental responses, are highly expanded and show remarkable variability among individuals and species. Comparative genomic studies suggest that the ancestor of the *Mytilus* complex possessed large repertoires of these expanded gene families; as lineages diverged, many genes underwent differential loss, pseudogenisation, or lineage-specific retention. These processes have generated extensive presence–absence variation (PAV) across genomes, producing substantial differences in gene content both within and between species. Here we explore patterns of gene presence and absence across species of the complex, including both Northern and Southern Hemisphere lineages. We examine how lineage-specific gene loss contributes to the structure of mussel genomes and shapes patterns of genomic divergence.

P128

Characterisation of neural progenitors and their fate potency in the postnatal shark retina

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In the mammalian retina, neurogenic activity is restricted to embryonic stages, while retinal neurogenesis persists into postnatal life in other vertebrates. In the postnatal fish retina, new neurons derive from two neurogenic niches: the ciliary marginal zone (CMZ) and Müller glia. In teleosts, the CMZ generates all retinal cell types except for rod photoreceptors, which derive from Müller glia. In sharks, although neurogenesis is known to persist in the postnatal retina, the fate potency of retinal progenitors is unknown. To address this, we used 5-ethynyl-2'-deoxyuridine (EdU) pulse-chase labelling in combination with the immunohistochemical detection of markers of differentiated retinal cells in juvenile small-spotted catsharks (*Scyliorhinus canicula*), which showed newly generated rod photoreceptors scattered throughout the central retina. Furthermore, we observed both rhodopsin-immunoreactive cells and proliferating Müller glia in the inner nuclear layer. Taken together, these findings suggest that new rod photoreceptors are generated from Müller glia in the postnatal catshark retina. Analysis of single-nucleus RNA sequencing data of the juvenile retina showed several populations of retinal progenitors, photoreceptor precursors and rod photoreceptors. Pseudotime analyses allowed us to reconstruct the developmental trajectories of rod generation in the postnatal shark retina, identifying putative regulatory genes. Our results suggest a potentially conserved mode of postnatal rod photoreceptor generation from Müller glia between teleosts and sharks, which would constitute an ancestral trait of jawed vertebrates. Further comparisons with other species will allow us to better understand the evolution of postnatal neurogenesis in the vertebrate retina.

P129

Uncoupling regeneration: Gut cell reprogramming via morphallactic tissue remodeling can progress even when epimorphic blastema-based regeneration fails in a freshwater annelid

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Regenerating the whole-body axis upon bisection often leaves organisms with incorrect body proportions that need to be remodeled via morphallaxis. While there are many studies that focused on epimorphic regeneration via blastema formation, the morphallactic tissue remodeling that takes place during or after regeneration to achieve accurate body composition remains understudied. Here we investigate tissue remodeling in a highly regenerative segmented worm (annelid) *Pristina leidyi*, and test the hypothesis that tissue remodeling can be uncoupled from blastema-based regeneration. We specifically focus on the gut tissue, which is highly regionalized, and undergoes extensive remodeling during regeneration and asexual fission. For example, bisected worms reprogram their gut by turning their intestine cells into stomach cells without losing gut integrity. Using gut cell specific molecular markers identified via scRNAseq, we characterized gut remodeling after amputation and during asexual reproduction by fission, as both processes trigger morphallactic tissue remodeling in the gut. We found that the anterior intestine cells first dedifferentiate, and then turn into stomach cells only in 3 days. We next asked if this process requires cell division and normal progression of epimorphic regeneration, or whether reprogramming can happen without these two processes. We inhibited cell proliferation and epimorphic blastema formation (via small molecule treatments or via specific amputations that result in failed blastema formation). We found that these headless worms were able to reprogram their intestine cells into stomach cells, concluding that these two regenerative processes can be uncoupled. Future research will focus on transcriptomic changes and live imaging gut reprogramming.

P130

Revealing the genetic innovations during the evolution of holometaboly

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Holometaboly is one of the most successful evolutionary innovations and is characterized by a complete reorganization of body plans across larval, pupal, and adult stages. However, the genetic changes underlying the evolution of holometaboly remain only partially understood. To address this, we combine comparative genomics and functional genetics to identify gene families whose evolutionary history suggest a contribution to the origin of holometaboly and test them for their developmental roles. First, we used both previously published data on gene family evolution in Arthropoda and our own large-scale orthogroup inference based on 200 newly annotated insect genomes to identify candidate genes that emerged at the basis of holometabola and could therefore have a role in holometabolous development. To test their role in vivo, we perform RNA interference (RNAi) knockdowns of selected candidate genes in the red flour beetle *Tribolium castaneum* and systematically analyze the effects on metamorphosis. This approach allows us to distinguish gene families that are not only holometabolous-specific but also necessary for the proper execution of holometabolous development. One of our candidate genes, the SP factor *buttonhead* (*btd*), is not present in hemimetabolous insects but can be found in Holometabola. We saw a function in moulting during metamorphosis in *T. castaneum*. However, the function seems minor and redundant with another SP factor. Overall, this work highlights both the potential and the challenges of using genome-wide analysis of gene family evolution to analyze the origin of complex life history traits.

P131

Inferring the ancestral wing developmental program across major insect lineages

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Insect wings originated more than 350 million years ago and were key to the ecological success of winged insects. However, the structure of the ancestral wing developmental program remains unresolved. Most previous studies have relied on candidate genes or single model systems, limiting comparative and phylogenetic inference. To address this, we profiled dorsal tissues across insect lineages representing major evolutionary branches: the paleopteran Cloeon dipterum; the polyneopterans Blattella germanica and Gryllus bimaculatus; the holometabolous beetle Tribolium castaneum; and the wingless zygentoman Thermobia domestica, an outgroup to Pterygota. In each species, we isolated wing pads or their morphological equivalents (including lateral terga in T. domestica) at two developmental stages, together with tergal epithelium and legs as internal controls. We generated single-cell transcriptomic datasets across species together with bulk ATAC-seq data to characterise chromatin accessibility in these tissues. Current analyses focus on defining corresponding cell populations across lineages and exploring regulatory modules associated with wing-related territories. The integration of transcriptional and chromatin accessibility data will allow us to investigate the regulatory landscape underlying wing development in a comparative framework. I will present this multi-species dataset, the cross-species analytical strategy, and how this approach can be used to explore the evolution of the wing developmental program.

P133

Genomic resources to unravel sex determination and regenerative plasticity in *Syllis prolifera*

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Regeneration and reproductive specialization represent key innovations underlying the evolutionary success of many marine invertebrates. Among them, syllid polychaetes exhibit one of the most remarkable reproductive strategies in Metazoa: stolonization, a process in which posterior reproductive units (stolons) differentiate, detach, and release gametes, followed by rapid regeneration of the parental body. However, despite their biological relevance, genomic resources for Syllidae remain extremely limited. Here, we present the first chromosome-level reference genome of *Syllis prolifera*, assembled from PacBio HiFi long reads (~37× coverage) and scaffolded using Hi-C data. The final assembly has 751.6 Mb and comprises 152 scaffolds, with an N50 of ~42.2 Mb. Notably, 17 scaffolds account for 90% of the total assembly length (L90 = 17), and the longest scaffold reaches ~75 Mb, indicating high contiguity. BUSCO completeness is high (~95–98%), supporting the overall structural integrity of the assembly. We annotated 23,540 protein-coding genes. Approximately 40.82% of the genome is repetitive, dominated by LTR retrotransposons, highlighting the substantial contribution of transposable elements to genome architecture. This genome provides a powerful resource to investigate the basis of sex determination in syllids, an unresolved question that may involve polygenic, chromosomal, hormonal, or environmentally influenced mechanisms. It also enables exploration of the molecular and regulatory pathways underlying regeneration and stolonization. Furthermore, the abundance of transposable elements offers a framework to assess how genome architecture contributes to regulatory flexibility, genome evolution, and morphological innovation in annelids.

P134

Do larval and juvenile skeletons share a common gene regulatory network in sea urchins?

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The gene regulatory network (GRN) of skeletal development from embryos to pluteus larvae has been extensively studied in sea urchins, making them a compelling model for studying GRN evolution in metazoans. Among the genes identified as central to this developmental programme is the Vascular Endothelial Growth Factor (VEGF) signalling pathway, which regulates primary mesenchymal cell position and syncytium formation required for skeletal rod development. During growth, sea urchin larvae undergo a spectacular metamorphosis, leading to the formation of juveniles characterised by pentaradial symmetry and a high diversity of skeletal elements. However, it remains unclear whether the GRN underlying the formation of the complex juvenile skeleton relies on the same pathways as larval skeletogenesis. To fill this gap of knowledge, we investigated the expression of genes known to play crucial roles in early larval development throughout the growth of juveniles, from rudiment formation to the first stages of post-metamorphic growth in the species *Mespilia globulus*, using hybridisation chain reaction (HCR) RNA-FISH. In addition, we conducted a perturbation experiment using a pharmacological VEGF pathway inhibitor to assess whether this pathway is also essential for juvenile skeletal development. Our results show that larvae and juvenile skeletal GRNs exhibit conserved gene expression in skeletal cells. In addition, perturbing the VEGF pathway impacts the growth of the skeleton in late larvae and early juveniles, highlighting the importance of this pathway later in development. Overall, this study shed light on GRN conservation across development and transition from bilateral to pentaradial symmetry in echinoderms.

P135

The contribution of transposable elements to mammalian organ development and evolution

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Mammalian genomes are replete with transposable elements (TEs), so much so that they outnumber protein-coding sequences by well over an order of magnitude. Nonetheless, TEs are often omitted from genomic studies due to the challenges inherent to their repetitive nature and because most of these sequences are expected to be non-functional. However, several studies have demonstrated that TEs have the capacity to contribute to the concerted regulation of genes, effect phenotypic change, and engender novel proteins, as is the case of syncytins. Here, we performed genomic and transcriptomic analyses to probe the TE repertoire of human, rhesus macaque, mouse, rat, rabbit, guineapig, opossum, and chicken as an outgroup. By looking at the genomes of these species, we found that, even though the complement of TEs varies considerably across species, there are common patterns to the way they contribute to the proteome. Further, we have assessed the transcriptional output of these elements throughout the development of seven organs (cerebrum, cerebellum, heart, kidney, liver, ovary and testis) from early organogenesis to adulthood across the 8 species. We found the brain and ovary to be consistently amongst the organs with the largest TE load on the transcriptome, with liver showing the smallest TE output across all species analysed. We also observed consistent patterns of transcriptional permissibility of these elements depending on context across all species and organs studied. Altogether, our results shed light on the logic governing TE contribution to organ development and evolution in mammals.

P136

Birth and death of monoterpene synthases underlie chemodiversity evolution in *Tanacetum vulgare* (Asteraceae)

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Tanacetum vulgare (tansy, Asteraceae) populations are highly chemodiverse, particularly with regard to their monoterpenoid composition. However, the molecular mechanisms underpinning this diversity have not been elucidated. Field sampling and GC–MS profiling of 39 parent plants and 849 descendants identified a total of 32 monoterpenoids. Of these, 493 plants were dominated by a single terpenoid, whereas 411 plants contained at least 25 different terpenoids. Chemotypes were constitutive. Despite the high chemodiversity in the population, structural analyses and compound distributions suggested that only a limited set of monoterpene synthases (MTSs) underlies chemotype differentiation. Correlation-guided selection, followed by functional analyses, identified both specific and multi-product MTSs. Genome and quantitative transcriptome analyses indicate that the major cause of chemodiversity is expression loss - essentially a regulatory “death” - with minor contributions from presence-absence variation and changes in enzyme specificity. At least 14 unlinked MTS loci enable extensive variation by recombination in the obligate outcrosser tansy. Phylogenetic analysis and ancestral reconstruction revealed “birth” through duplication and neofunctionalization, leading to the de novo evolution of specialized sabinene and alpha-pinene synthases. These results demonstrate that chemotype diversification in *T. vulgare* is driven by the evolutionary birth and regulatory death of MTS genes, providing a mechanistic explanation for adaptive chemodiversity in natural populations.

P137

The highly heterozygous European amphioxus (*Branchiostoma lanceolatum*) at the edge of panmixia

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Amphioxus are small marine chordates that have broad ecological ranges, yet as adults form local settlements and exhibit limited mobility. Genomic surveys of two amphioxus species have suggested that they rank among the most genetically diverse metazoans. Here, we present the first accurate assessment of genomic diversity in the European amphioxus (*Branchiostoma lanceolatum*) and investigate the processes underlying this diversity. We leverage high-coverage whole-genome sequencing data from multiple individuals sampled at two geographically distant Atlantic and Mediterranean locations. Consistent with previous estimates in other amphioxus species, we measure exceptionally high genomic diversity, with an average heterozygosity of 2.73% in *B. lanceolatum*. Despite the large geographic separation between sampling sites, population differentiation is minimal, indicating extensive gene flow among distant adult settlements. Phylogenetic analyses combined with population genetic simulations confirm that this elevated genomic diversity is primarily driven by a large effective population size. Although adult amphioxus have limited mobility, our results indicate that long-distance larval dispersal mediated by ocean currents is sufficient to generate a near-panmictic population structure across their broad ecological range.

P138

Rab repertoire as a tool to understand critical evolutionary steps in intracellular trafficking

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Intracellular trafficking represents a fundamental feature for the evolution of eukaryotic cell. Rab proteins are the main players in the regulation of the transportation among diverse cellular compartments and their evolution is considered a pivotal event underlying the emergence and evolutionary success of multicellularity. Although it has already been established that Rabs dramatically expanded in gnathostomes, their function and critical early steps of metazoan evolution remain still obscure. Here, we adopted a comparative evolutionary genomics approach to detect how *Rab* toolkit changed in metazoans and correlate the multicellularity and *Rab* occurrence, using unicellular eukaryotes as outgroup. We designed a script to detect the presence of *Rab* genes in all the available genomes, which we called RAB-HIT. Subsequently, we performed a maximum-likelihood phylogenetic approach utilizing key unicellular and animal Rab datasets, demonstrating that the main *Rab* clades (A, B, C) were already present in the ancestor of all metazoans. Furthermore, additional evolutionary analyses highlighted the emergence of multiple ancestral duplications in early-branching metazoans. Intriguingly, we identified *Rab12* as a novelty of chordates, whose expression during zebrafish (*Danio rerio*) embryonic development is cardiac-specific. Henceforth, we depicted the evolutionary landscape of *Rabs* in metazoans, leading to discover multiple novelties alongside the main evolutionary transitions in the context of eukaryotic membrane trafficking.

P139

Early Developmental Scaling Drives Axial Diversification in Lake Malawi Cichlids

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Vertebral number is a highly evolvable trait linked to ecological adaptations in vertebrates. Changes in vertebral count arise through modulation of somitogenesis, which depends on the segmentation clock and the size and growth dynamics of the presomitic mesoderm (PSM). However, most comparative studies span deeply divergent taxa, limiting inference about the sequence and developmental basis of evolutionary change. Lake Malawi cichlids represent a remarkable example of adaptive radiation, having evolved from a common ancestor into hundreds of species inhabiting a wide range of ecological niches and exhibiting diverse morphologies, including a variable number of vertebrae. Previous work in our lab compared the process of somitogenesis between the generalist putative ancestor of the radiation *Astatotilapia calliptera* (30 vertebrae) and the predatory *Rhamphochromis* sp. “chilingali” (36 vertebrae). Differences in the rate of somitogenesis are not enough to explain these differing vertebral numbers. Instead, *Rhamphochromis* has a larger PSM at the onset of somitogenesis, sufficient to prolong somitogenesis and generate additional somites. These findings indicate that vertebral evolution can occur through modification of tissue size without altering core segmentation dynamics, an example of morphogenetic evolution via altered initial conditions. Building on this work, I investigate the developmental basis of PSM size divergence. Because the PSM is established during gastrulation, I compare gene expression during early mesoderm specification and patterning between *Astatotilapia calliptera* and *Rhamphochromis* sp. 'chilingali' using Hybridisation Chain Reaction (HCR). Ongoing analyses, planned hybrid crosses, and Tol2 reporter experiments aim to further elucidate the regulatory mechanisms underlying PSM expansion.

P140

Evolution of neuronal diversity in the insect visual system

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How does neuronal diversity evolve? How diverse are the neuronal types across species? And how do developmental changes lead to new cell types? We use the insect optic lobes as a powerful model system to tackle these questions. In *Drosophila melanogaster,* the optic lobes contain 120,000 neurons, with nearly 200 identified neuronal types that differ in morphology, physiology and molecular identity. Recently, the Drosophila optic lobes were characterized at high resolution through the generation of a scRNA-seq atlas, profiling their molecular identity and linking many adult cell types to their developmental birth order.

This extensive characterization allows us to investigate how neuronal diversity evolves. We have generated scRNA-seq data from optic lobes of 8 adult insects spanning over 400 million years of divergence, in order to identify homologous and new cell types by constructing cell type atlases, address whether neurons with different properties evolve at different rates by comparing the transcriptomes of the homologous cell types, and understand how new cell types emerge by using scRNA-seq in developing brains and comparing gene expression changes of neural stem cells.

We identified a conserved ancestral core of homologous cell types, as well as differentially enriched cell types in either *D. melanogaster* or the other species. Moreover, building up on previous findings in vertebrates, we confirmed that inhibitory neuronal types have a tendency of being more conserved than excitatory ones, and we are also currently aligning the presence/absence of adult neuronal types with their developmental origin.

P141

Regrowing the growth zone: metamorphosis kickstarts regeneration in the annelid, *Capitella teleta*

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The ability to regenerate can vary across an animal's life history. Annelid regeneration has been widely surveyed, but few studies examine the variation across developmental stages. We previously showed that *Capitella teleta*, an annelid worm, gains regenerative ability with age and developmental maturity. Although larvae do not replace lost structures, juveniles and adults regenerate posteriorly following metamorphosis. To determine whether metamorphosis enables juveniles to regrow structures lost as larvae, we amputated *C. teleta* larvae, removing posterior segments, hindgut, posterior growth zone, anus, and pygidium. Metamorphosis was then induced in these amputated larvae and reared as juveniles for 3, 7, or 14 days. New growth in juveniles was assessed for regeneration using confocal microscopy, EdU incorporation, immunohistochemistry, and nuclear staining. A pgz and new segments were observed by 7 days post-metamorphosis. Remodeling of the digestive system in preexisting tissue was also observed. Additionally, re-amputation of juveniles resulted in regeneration of the pgz, segments, and hindgut. Our results demonstrate that amputated *C. teleta* larvae can metamorphose into functional juveniles capable of growing new segments, suggesting that metamorphosis acts as a switch to enable regeneration of structures essential for growth. This study highlights the impact of metamorphosis on changes in developmental plasticity.

P142

Mapping Divergence in a Sexually Selected Ornament in Lake Malawi Cichlids

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The role of sexual selection in driving adaptive radiation, and speciation more generally, remains contentious. The vast array of male nuptial patterns and courtship behaviours, and the importance of interspecific gene flow in driving divergence, make East African cichlids an excellent model for studying this question. Egg-spots, a sexually selected and evolutionarily innovative trait, are found across the breadth of the Lake Malawi radiation, exhibiting remarkable phenotypic diversity both within and between species. Here, we present the findings of an interspecific QTL cross between Astatotilapia calliptera, a generalist 'ancestral-like' species with a stereotyped egg-spot pattern, and Aulonocara stuartgranti, a specialist benthic invertivore with egg-spots that are highly disrupted. We describe a polygenic basis for egg-spot divergence between the two parental species, with different egg-spot pattern phenotypes mapping to candidate regions across the genome. To validate putatively causal loci, we also present the results of bulk RNAseq analysis and genome-wide selection scans, alongside developmental imaging of the parental species. Finally, we examine instances in which multiple phenotypes map to common regions of the genome, including an example of lateral line expansion, an ecologically selected trait, being significantly correlated with egg-spot number and associated to a shared genomic region. We conclude that relaxation of sexual selection in response to the colonisation of a new ecological niche plays a role in driving divergence between these two species.

P143

Evolution and development of grass organs

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Grasses evolved ~70mya and today dominate many terrestrial ecosystems. There are ~12,000 grass species and 50% of global calories directly depend upon a handful of species from different lineages, collectively called the cereals. A clear understanding of the gene regulatory networks (GRNs) that control grass development is of critical importance to the development of cereal crops for more resilient food production. We currently do not have this understanding. In part this gap is because grasses are distinct: Their leaves have a semi-3D structure consisting of a wrapped leaf base and a strap-like upper leaf domain which bends away from the main axis of the plant. Their vegetative phyllotaxy is distichous, differing from the dominant spiral phyllotaxy observed in angiosperms. And their floral structures differ from the more familiar sepal-petal-stamen-carpel structure common in other angiosperms. To better understand how grasses evolved, what genetic changes set them apart, and how these influence developmental mechanisms and morphology we use comparative genetics and computational modelling to determine homology relationships, and identify core GRNs that are common across organs and species. We recently used this approach to resolve the homology of grass and eudicot leaves. This revealed a shared GRN underpinning the specification of leaf domains. Extending our analysis tested the homology between the grass leaf and the awned lemma, a specialized grass floral organ. This work revealed that the core GRN is conserved between leaves and awned lemmas. We are now investigating how diversity in awn shape may arise despite sharing the leaf GRN.

P144

Impact of Gene Regulatory Network Architecture on Developmental Robustness : Insights from Ascidian Gastrulation

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Across the tree of life, many examples illustrate that similar phenotypes can be produced by different genetic contexts. The relationship between genotype and phenotype is thus degenerate. Ancestrality can produce conserved phenotypes despite genetic divergence, while convergence can produce similar phenotypes in distinct phylogenetic branches. The developmental mechanisms that allow different genetic configurations to produce the same phenotype remain poorly understood. The architecture of gene regulatory networks could be a major control step in buffering molecular variation between and within species. Our project aims to describe the role of a gene regulatory network in producing a reproducible complex morphogenetic process, gastrulation. We are using ascidian embryos, which develop with invariant cell lineages, show high robustness to gene expression variation and are perfectly suited for quantitative approaches. We focus on the gene regulatory network controlling the invagination of endodermal cells. We use light imaging to quantify both gene expression and phenotype in the same embryo. Using a MERFISH-like approach, we are setting up methods to measure the inter-individual (co)variations in the expression of all genes of the regulatory network. This will allow us to determine how the architecture of the gene regulatory network buffers or amplifies transcriptional noise as it propagates through the network. To move beyond these correlative observations, we will test the effect of transcriptional modulations by targeting specific enhancers using a dead-Cas9 coupled to activators or repressors. This work will provide a mechanistic framework to better understand how organisms produce highly reproducible phenotypes despite inter-individual molecular variation.

P145

Homologies of gastrulation initiation across vertebrates

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According to the “hourglass model” of vertebrate development, early and late development are more divergent than the conserved pharyngula stage appearing in mid-development. This work aims to reassess conservation in early development by identifying homologies in the induction of the leading edge prior to gastrulation. We define the leading edge as a population of cells that is marked by the expression of Chordin, and that initiates the first gastrulation movements. Furthermore, we propose that a population of cells lying vegetal to the leading edge, the “hypoblast”, can be traced in pre-gastrula embryos across the vertebrate phylogeny. Regardless of its origin through different modes of cleavage, or its status as embryonic or extraembryonic tissue, this population shows a consistent transcriptomic profile and spatial position relative to the leading edge in the early embryo, along with a conserved inductive function. We present a spatially resolved single-nucleus gene expression atlas, coupled with chromatin accessibility data from the pre-gastrula stages of the chicken and frog embryos, which provides evidence for the homology of the hypoblast and confirmation of the homology of the leading edge in vertebrates.

P146

Imaging neurogenesis during the pelagic-to-benthic transition in *Platynereis dumerilii* using a modular Flamingo light sheet microscope

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Life-cycle transitions can involve profound changes in tissue organization and behavior. In lophotrochozoans with biphasic development, a swimming trochophore larva transforms into a crawling nectochaete larva. The shift from cilia-driven to muscle-driven movement reflects not only a behavioral change, but also a reorganization of neural circuitry. A central question is whether this transformation depends mainly on remodeling of existing larval neurons or on the addition of newly generated neuronal populations.

To explore this phenomenon, we study neurogenesis during the pelagic to benthic transition in the marine larva of the annelid *Platynereis dumerilii*. Given that the nervous system present in both larval stages supports distinct modes of locomotion, this organism provides an ideal framework to investigate developmental continuity and change. We use a combination of genetic fluorescence labelling techniques to label the nuclei, the cell membranes and neurons and a modular Flamingo X-SPIM light sheet microscope to image neuronal dynamics. This multiview configuration, comprising two opposing detection lenses, enables high-resolution *in vivo, in toto* imaging while minimizing phototoxicity, making it well suited for long-term recordings of larval development. The resulting datasets are analyzed using Mastodon in Fiji to perform cell lineage tracing and reconstruct lineage trees, thereby uncovering the cellular mechanisms underlying neurogenesis. Preliminary data suggest that neurons controlling the two distinct locomotion strategies originate from separate lineages, raising the possibility that the larval transition involves heterogeneous developmental programs.

Together, these approaches allow us to uncover how neural development accommodates ecological and functional transitions during development.

P147

Distinct cellular routes to budding in tunicates: epithelial reprogramming and lineage-restricted progenitors

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Colonial tunicates are the only chordates capable of generating complete functional bodies from somatic tissues through different forms of budding. Yet the cellular origin of bud founder cells remains debated, and different tunicate lineages employ non-homologous cells and tissues and distinct budding strategies to eventually generate similar bodies. In the stolidobrach *Botryllus schlosseri*, peribranchial budding produces new zooids cyclically from the a differentiated epithelium. We generated a multi-stage single-cell transcriptomic atlas spanning early bud initiation to near-mature zooids. The data capture the diversity and dynamics of cell states during budding. While circulating mesenchymal populations display transcriptional signatures associated with stem-like states, they show no clear contribution to early bud morphogenesis. Instead, we identify a founder population arising from the peribranchial epithelium that seems to progressively acquires developmental potency through transcriptional reprogramming, consistent with dedifferentiation of committed epithelial cells. A different picture emerges from stolonial budding in the pelagic thaliacean *Thalia democratica*. Anatomical re-examination of the budding stolon combined with pulse–chase experiments using thymidine analogues reveals several spatially distinct populations of proliferative cells at the stolon base. These populations appear lineage-restricted and contribute to specific regions of the developing zooid. Single-cell RNA-seq across the stolonial budding cycle supports the presence of these discrete progenitor populations. Together, these datasets suggest that tunicate budding does not rely on a single common cellular strategy. Instead, phylogenetically distant species appear to employ different routes, epithelial reprogramming in *Botryllus* versus spatially organized progenitor pools in salps, while still sharing elements of developmental regulation.

P148

Investigating postnatal oocyte formation in the naked mole-rat ovary

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In mammals, oocytes originate from primordial germ cells (PGCs) specified during embryogenesis. PGCs migrate to the fetal gonad, proliferate, enter meiosis, and become enclosed by pre-granulosa cells to form primordial follicles, thereby establishing the finite ovarian reserve during the perinatal period. Unlike insects and teleost fish, which maintain continuous oocyte production, mammals are widely believed to lack postnatal germline renewal. Although *in vitro* studies in mice suggest germline stem cell potential, *in vivo* lineage-tracing and nucleotide analogue labelling have not demonstrated physiologically relevant adult neo-oogenesis. The long-lived African naked mole-rat (*Heterocephalus glaber*) exhibits unusual reproductive traits, including an expanded follicle pool and proliferative PGC-like cells in breeding females, suggesting possible postnatal oocyte formation. We hypothesized that adult breeding females may replenish their ovarian reserve at a biologically meaningful rate. To test this hypothesis *in vivo*, breeding females were administered the low-toxicity thymidine analogue (2'S)-2'-deoxy-2'-fluoro-5-ethynyluridine (F-ara-EdU). Short cumulative exposure labelled proliferating granulosa cells as positive control confirming analogue bioavailability at minimal dosage. In long pulse–chase experiments, F-ara-EdU-positive cells were observed within the ovarian cortex, with partial signal overlap with Ddx4 in some sections; definitive co-localization will require high-resolution imaging. These preliminary results indicate, but do not yet conclusively demonstrate, adult neo-oogenesis in breeding female naked mole-rats. Ongoing studies aim to determine whether this phenomenon, if confirmed, represents a rare event or occurs at a rate sufficient to replenish the ovarian reserve. This work confronts a long-standing question in mammalian reproduction and contributes to insights into ovarian longevity and regenerative potential.

P149

Mechanisms of Cell Division Orientation in the brown alga *Sphacelaria*

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The developmental mechanisms governing brown algae remain poorly understood. Unlike most multicellular organisms, brown algae exhibit a distinctive pattern of three-dimensional tissue formation, involving sequential plateaus of specific cell division orientation. *Sphacelaria*, with its simple polystichous filamentous morphology, serves as a valuable model for investigating the functional relationship between cell shape, cell–cell communication, and cell division orientation. During filament elongation, the apical cell divides transversely, generating a sub-apical cell while self-renewing. Following several transverse divisions in both the apical and sub-apical cells, the division plane reorients longitudinally, parallel to the filament's main axis. This transition marks the shift from one-dimensional to two-dimensional growth. Time-lapse confocal microscopy of *Sphacelaria* filaments, cultured in microfluidic chips and labelled with a cell-contour-specific fluorescent probe, revealed that the transition from transverse to longitudinal division occurs within approximately 40 hours and after four transverse divisions in a cell positioned fifth from the filament tip. Pharmacological reduction of growth rates demonstrated that the timing and location of division plane reorientation are influenced by cell shape and size. Laser ablation experiments further indicated that the apical cell exerts minimal control over the reorientation of sub-apical cells, suggesting that cell shape acts as a primary trigger for division plane tilting, independent of apical dominance. These findings contribute to the broader understanding of cell division plane reorientation in tissue development, with potential parallels to processes such as lateral root emergence in land plants.

P150

Developmental insights into distal skeletal transformation during the fin-to-limb transition through Hoxa13 regulatory changes

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The evolution of tetrapod limbs from fish fins represents one of the most consequential innovations in vertebrate history, enabling the transition to terrestrial life approximately 380 million years ago. This transformation involved distal expansion of the endochondral skeleton that ultimately produced the autopod (wrist and digits), accompanied by a reduction of dermal fin rays. Despite the evolutionary significance, the mechanisms that generated these changes in distal skeletal patterning remain poorly understood. Hox genes regulate proximal–distal appendage patterning and have long been implicated in appendage evolution. Notably, Hox13 genes play key roles in distal appendage patterning in both fish and tetrapods. In tetrapods, Hox13 regulates formation of the autopod, whereas in fish it regulates distal fin structures, including dermal fin rays. This suggests that evolutionary changes in Hox13 regulation contributed to expansion of the endochondral skeleton during the fin-to-limb transition. To test whether changes in Hoxa13 expression levels affect distal skeletal patterning, we generated transgenic zebrafish that overexpress Hoxa13 during pectoral fin development. Cartilage staining of juvenile pectoral fins in transgenic fish shows expansion of distal radial elements during early endochondral development. This pattern persists into adulthood, with elongation of distal radials and reduction or fusion of proximal radials. These findings demonstrate that modulation of Hoxa13 expression alters distal skeletal patterning in fish fins and provides developmental insight into genetic mechanisms that may have contributed to the fin-to-limb transition.

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Keep an eye on the gland: size variation and comparative anatomy of the Harderian gland across squamates.

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The Harderian gland is an orbital gland present in most vertebrates, located anteromedially to the eye and sometimes extending posteriorly within the orbit. Although initially described as a lubricating gland of the nictitating membrane (third eyelid), it is now recognized in some species to also contribute to pheromonal communication, vomeronasal function, and local immune defense. In squamates, the gland is not consistently associated with the nictitating membrane. Instead, histological and functional evidence indicate a close relationship with the vomeronasal organ (VNO), which detects predominantly non-volatile chemical cues. In snakes, highly specialized vomerolfactory taxa, the gland can be exceptionally developed, particularly through postorbital expansion, yet its morphology has not been assessed across Squamata within a broad comparative framework. Here, we test the hypotheses that snakes have relatively larger Harderian glands than those of other squamates, and that variation in gland size covaries with ecological context. Using contrast-enhanced micro-computed tomography and our SmARTR cinematic rendering pipeline, we generated high-resolution 3D models and applied semi-automated segmentation to quantify gland volume and characterize topology. We sampled 127 species spanning 97% of squamate families and used phylogenetically informed analyses to evaluate scaling relative to head size while incorporating ecological parameters. Across Squamata, gland volume scales with head size, suggesting strong allometric constraint or functional integration. However, we find no overall enlargement in snakes. Instead, both snakes and lizards exhibit substantial variation in gland size and topology, indicating that Harderian gland evolution reflects both sensory specialization and broader ecological diversification.

P152

From local cell interactions to pigment pattern diversity: Insights from an agent-based model of egg-spot development in cichlids

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Understanding how development shapes macroevolutionary diversification remains a central challenge in evolutionary developmental biology. The egg-spots of eastern African haplochromine cichlids provide a powerful system for linking cellular behaviours to radiation-level phenotypic diversity. We present an agent-based model in which local pigment cell interaction rules generate egg-spot patterns on a growing fin domain. Model parameters are informed by empirical measurements of pigment cell distribution and fin morphology in *Astatotilapia calliptera,* which approximates the ancestral bauplan of the Lake Malawi radiation. The model reproduces aspects of pattern variation observed within *A. calliptera* and can generate patterns resembling those found in other lineages of the radiation. Systematic exploration of parameter space suggests that a range of egg-spot phenotypes may emerge through quantitative modulation of a relatively small number of pigment cell properties, without requiring qualitative changes to the underlying generative rules. These results are consistent with the hypothesis that diversification in egg-spot patterning reflects evolutionary exploration of a constrained generative parameter space.

P153

Redefining the development of the freshwater sponge canal system using 4D optical coherence microscopy

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Branching tubular networks have evolved throughout animals to distribute nutrients, oxygen, and remove waste. The cellular and molecular machinery underlying biological tube formation has been studied extensively in bilaterian model organisms, and yet the evolutionary origins of this process remain unknown. Sponges (phylum Porifera) are one of our most distant animal relatives; they have only a handful of distinct cell types, yet possess a surprisingly complex canal system with largely uncharacterized development. While it has many of the homologous genes and pathways utilized in tubulogenesis, it also lacks certain features considered necessary in other animals, which include a basal lamina, tight junction proteins, and the Hedgehog signaling pathway. Here, I address this by characterizing the developmental stages of the canal system in the freshwater sponge *Spongilla lacustris* using 4D optical coherence microscopy to perform label-free time-lapse imaging of the entire sponge canal system across development. We observe that *Spongilla* reproducibly develops its incurrent and excurrent canals, passing through 5 distinct developmental stages in a temporally regular manner. We also find the canonical Wnt signaling pathway is implicated in regulating this process—inhibition of the Wnt pathway severely delays incurrent subdermal lacunae and canal formation, whereas activation of the Wnt pathway results in ectopic lumens forming below the outer tent. These findings highlight a remarkable conservation in developmental staging of sponge tubulogenesis despite high plasticity in the adult animal. Furthermore, the role of Wnt signaling suggests this fundamental metazoan pathway was essential in directing early animal tubulogenesis and development.

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The Role of Thyroid Hormone Signalling in the Divergence of Pigmentation Patterns in East African Cichlid Fishes

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Changes in hormonal signalling represent an important yet understudied mechanism for generating novel phenotypes. Although hormonally mediated phenotypic plasticity has been proposed to facilitate evolutionary change, empirical studies directly testing the role of hormonal signalling in adaptive trait evolution remain scarce. Here, we investigate how variation in thyroid hormone (TH) signalling contributes to divergence in egg-spot pigmentation patterns in East African cichlid fishes. The East African cichlid radiation provides a powerful system for studying the evolution of novel phenotypes due to its remarkable morphological diversity despite low genetic divergence. One of the best-studied traits in this system is the egg-spot phenotype, a diverse pigmentation pattern located on the anal fins of hundreds of cichlid species that plays a key role in reproductive behaviour. To examine the role of TH signalling in egg-spot pattern divergence, we studied *Astatotilapia calliptera* and *Tropheops mauve*, two Lake Malawi species with distinct egg-spot phenotypes. Comparative RNA-seq analysis of anal fin tissue revealed substantially lower local TH signalling in *A. calliptera* than in *T. mauve*. Moreover, experimentally inducing hyperthyroid conditions in *A. calliptera* through exogenous triiodothyronine treatment recapitulated the *T. mauve* egg-spot phenotype. We propose that TH signalling regulates egg-spot phenotypes by controlling the density of iridophores, silvery pigment cells. While iridophores appear to be necessary for egg-spot initiation, high iridophore density may inhibit egg-spot development, restricting egg-spots to the iridophore-poor posterior edge of the anal fin. We are currently testing this model by generating CRISPR/Cas9 mutants with varying iridophore densities.

P155

Cell type diversities underlying body plan evolution in sponges

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The diversity of animal life arises from the evolution of distinct cell types and the adaptations that organize them within tissues. Yet, how these evolutionary innovations give rise to structural complexities remains poorly understood. This project investigates how the evolution of novel cell types underlies transitions in the morphological complexity of the sponge's aquiferous canal system—a branching network that regulates feeding and water flow. Different sponges are thought to have independently evolved complex canal architectures multiple times. With only written morphological descriptions of their canal systems available, the cellular basis of this transition remains unknown. To address this, I use single-cell RNA sequencing to compare cell type diversity across sponges representing simple, intermediate, and complex canal grades. I will reconstruct a cross-species cell type phylogeny and apply evolutionary models to test whether morphologically similar canal cell types are homologous or have evolved convergently. By inferring ligand-receptor signaling networks and validating spatial gene expression patterns, I will assess whether complex canal structures are associated with the emergence of novel signaling centers and redeployment of developmental pathways. Finally, I will use functional perturbation and transplantation experiments to determine whether putative signaling centers are active in the development and maintenance of canal structures in the mature sponge. his study integrates molecular, cellular, and functional data, and assesses whether complex canal systems arise through shared or distinct innovations. Upon completion, this project will provide new insights into how signaling and cell type evolution jointly shape morphological transitions in an ancient animal body plan.

P156

Evolutionary and developmental origin of pancreatic endocrine cells through neural gene co-option

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The origin of the pancreas is a prime example of the stepwise evolution of complex organs. Pancreatic endocrine cells (PECs) make up 2–5% of the pancreas and comprise five specialized cell types organized in the islets of Langerhans within the exocrine tissue. Among them, insulin-secreting beta cells are central to glucose homeostasis. Yet the evolutionary origin of PECs remains unclear. Although they arise from the endoderm, PECs share many molecular and functional features with neurons, leading to the hypothesis that they evolved through co-option of neuronal regulatory programs. To investigate neural gene co-option in PECs, we analyzed bulk RNA-seq data from mouse tissues. We identified PEC-enriched genes and compared them with genes enriched in neurons defining a pancreas-neural (PN) module. Gene Ontology analysis of these shared genes highlighted functions including synaptic organization, exocytosis, and ion transport, consistent with common properties of these cell types. We next reanalyzed scRNA-seq datasets of mouse endocrine pancreas development to trace the temporal expression of the PN module. Most PN genes were sharply upregulated at the endocrine progenitor stage, coinciding with activation of key neural transcription factors. Analysis of available ChIP-seq datasets identified candidate regulators of the co-opted neuronal module. Across vertebrates, PN orthologs showed high levels of conserved enriched expression in both neural and PEC samples. In contrast, amphioxus and fruitfly lacked this pattern and their orthologs of the mouse PN displayed enriched expression only in neural samples. Thus, here we define and characterize the neural-specific gene modules co-opted by PECs in vertebrates.

P157

Mobile elements drive episodic genome expansion and functional novelty in obligate chemosynthetic symbionts of marine bivalves

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Intracellular symbionts enable novel bacterial functions within invertebrate cells, driving evolutionary innovation and adaptation. Chemosynthetic symbioses have evolved multiple times in marine bivalves, facilitating colonization of extreme environments such as hydrothermal vents and cold seeps. As hosts become nutritionally dependent on their symbionts, they often evolve drastic morphological changes, including enlarged gills and severe digestive system reduction. Symbionts themselves often experience reductive genome evolution, particularly under strict vertical transmission, losing genes unnecessary for intracellular life. Vesicomyid clam symbionts are one such example of these associations, as they are obligate, maternally inherited, and possess compact genomes consistent with long-term host restriction. However, several lineages within this clade have unexpectedly large genomes, previously attributed to retention of ancestral gene content. In contrast, thyasirid bivalves harbor phylogenetically and functionally diverse chemosynthetic symbionts varying in transmission mode and degree of host integration. The placement of a thyasirid symbiont within the vesicomyid clade represents a rare host-switching event, raising questions about how genomic flexibility arises within a reduced-genome lineage. Using comparative genomics, we show that genome expansions in multiple vesicomyid-clade symbionts, including the thyasirid symbiont, is driven by recent invasions and proliferation of mobile genetic elements. These expansions occurred after divergence from a reduced-genome ancestor and are associated with horizontally acquired genes of potential functional relevance. Contrary to an ancestral-retention model, our results demonstrate that mobile elements can facilitate episodic genome expansion within obligate symbioses, generating genetic novelty that may enable ecological transitions, host switching, and renewed evolutionary potential in otherwise streamlined lineages.

P158

Does signaling network interconnectivity explain the phylotypic conservation?

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The mid-embryonic organogenesis phase shows striking molecular and morphological conservation across vertebrates. Intrinsic developmental properties of this phase have been suggested to contribute to its lower evolvability and evolutionary conservation, yet the underlying mechanisms remain poorly understood. One of the classical hypotheses proposed that, compared to other developmental stages, highly interconnected signaling networks among embryonic tissues during this phase constrain evolutionary change (Raff, 1996). Although this idea has been discussed for decades, direct empirical tests have been limited, largely due to the lack of effective approaches for defining comparable organ primordia and quantitatively comparing their signaling network properties across developmental stages. Here, we leveraged spatially resolved transcriptomics with near cell-level resolution to overcome this limitation. Using spatial transcriptomic data from mouse embryos, we tested this hypothesis by comparing the signaling network interconnectivity among developing organ primordia between the highly conserved stage (E9.5) and a less conserved stage (E13.5). Unexpectedly, comparison of reconstructed inductive signaling networks revealed no significant difference in network interconnectivity between E9.5 and E13.5, while organ primordia in E9.5 expressed a higher proportion of pleiotropically expressed genes. These findings suggest that while the original hypothesis was not supported here, enrichment of pleiotropic expression may be a future direction for understanding how development may have biased the phenotypic evolution of the phylotypic phase.

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How acoels evolved to live with algae: a single-cell transcriptomic perspective

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Photosymbiosis is an association in which an organism lives with a photosynthetic partner, such as algae. This association is widely found in animals, including corals, giant clams, and acoel worms, enabling them to evolve specialized functions such as the symbiosome in corals and zooxanthella tubes in giant clams. In this study, we focus on acoels, flatworm-like marine bilaterians whose algal symbionts reside in the intercellular spaces of host tissues. This unique association may have driven acoel evolution; however, the mechanisms underlying acoel–algal photosymbiosis remain largely unknown. Here, we first performed single-cell RNA-seq and identified 13 cell types. Photosymbiotic acoel lineage-specific genes were enriched in digestive cells, suggesting that this cell type may have evolved in association with photosymbiosis. Together with bulk RNA-seq data obtained under photosynthetically inhibited conditions, we found that digestive and endodermal cells are associated with the maintenance of symbionts. Additionally, the differentially expressed genes were predominantly conserved across other animals, indicating that acoel–algal photosymbiosis relies on the co-option of existing molecular machinery. Endodermal cells appear to play a central role in many animal–algal photosymbioses. For example, cnidarians such as corals and green hydra maintain their symbionts in gastrodermal (endodermal) cells, and giant clams possess zooxanthella tubes derived from the digestive system. The consistent involvement of endodermal cells across distantly related animals points to a conserved cellular strategy for establishing and maintaining photosymbiosis, and acoels offer a unique opportunity to dissect the evolutionary origins of this strategy.

P160

Homogeneous cell lines concentrate the signal of wMel infection, revealing evidence of nutritional complementation and thwarted reproductive manipulation

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Host-associated bacteria influence eukaryotic cellular differentiation, but the underlying mechanisms are difficult to disentangle, likely because of cell-type-specific tropisms and phenotypes. The association between *Wolbachia pipientis* and *Drosophila melanogaster* offers a tractable system for gaining high-resolution insight into how host and microbe gene expression interact. Using *Wolbachia*-infected *D. melanogaster* cell lines, we show that the native *w*Mel strain alters host phenotypic, transcriptomic, and chromatin conformation states. High-throughput imaging reveals that *w*Mel infection differentially alters host cellular phenotypes in a titer-independent manner in some cell lines. These phenotypically altered host cells exhibit differential gene expression complementary to the expression patterns observed in their *w*Mel symbionts. Infection was also negatively associated with chromatin organization and histone modification pathways, prompting us to investigate differences in chromatin conformation between infection states. Micro-C chromatin contact data suggest that some infection-induced changes are epigenetically encoded: *w*Mel infection conferred reduced *D. melanogaster* chromatin contacts coinciding with transcriptional derepression. The X chromosome exhibited reduced contact probabilities across its entire length in infected cells, corresponding with increased H4K16 acetylation but no disruption of the X:autosome transcript ratio, consistent with a dosage compensation-mediated male-killing mechanism encoded by *w*Mel that has been suppressed in *D. melanogaster*. These findings suggest that the character of *Wolbachia* symbiosis, cooperative or antagonistic, emerges in response to the host cellular environment. Overall, these results demonstrate that in vitro cell lines offer promising avenues for uncovering the functional genetic basis of microbe-induced host phenotypes.

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Distinct functions of splice variants of *Ultrabithorax* in *Camponotus floridanus*

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Hox genes encode conserved transcription factors defining segmental identity during embryogenesis in bilaterian animals, including insects. In the carpenter ants *Camponotus floridanus*, however, the Hox genes of the Bithorax complex (BX-C) have acquired novel roles. *Ultrabithorax (Ubx)*, one of the genes in BX-C, shows maternal expression, and its loss-of-function causes severe abdominal truncation rather than classical homeotic transformations. To better understand the function of *Ubx* in this species, we performed stage-specific transcriptomic analyses. We report the discovery of a hitherto unknown transcript variant uniquely present during the embryonic blastoderm stage, with coverage mostly in the homeodomain and markedly reduced over the 5' untranslated region (UTR) and the first exon. Targeting *Ubx* 5'UTR and the first exon, excluding the homeodomain, by RNA interference (RNAi) produced a striking split phenotype in which the germband developed into two separate units putatively split at the thoracic-abdominal boundary. We further performed transcriptomic analyses of embryos where different parts of the gene were targeted using RNAi to identify the downstream targets of the alternative *Ubx* isoforms associated with the truncation or split phenotypes. These results suggest that an alternative isoform of *Ubx* is necessary for development of the thoracic-abdominal boundary region. Keywords: Hox genes, Bithorax complex, *Ultrabithorax*, Carpenter ants, *Camponotus floridanus*, RNA interference.

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Neuronal Differentiation in *Oncopeltus fasciatus*: Insights from Late-Embryonic RNAi and Single-Cell Transcriptomics

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The molecular cascades driving neuronal cell fate and the regulatory systems governing neural differentiation are central to understanding insect development and evolution. However, many key neurogenic genes are highly pleiotropic, possessing essential roles in early embryogenesis that complicate the experimental isolation of their specific functions during later nervous system development. This pleiotropy may stem from the evolutionary co-option of gene regulatory networks between segmentation and neurogenesis, obscuring the distinct dynamics of each process. To address these challenges, I will present two complementary methods: alate-embryonic RNA interference (RNAi) protocol, and a single-cell RNA sequencing (scRNA-seq) atlas collected at specific time points relevant to the development of the nervous system in the hemimetabolous insect *O. fasciatus*. By integrating targeted late-stage gene knockdown with our scRNA-seq dataset, we aim to map the spatial and temporal expression dynamics that drive neural complexity, separating early and late gene roles in the neurogenesis process. These combined tools establish a robust framework for investigating the molecular basis of neurogenesis in *O. fasciatus* and offer broader insights ancestral and derived mechanisms of the arthropod GRNS involved on both neurogenesis and segmentation.

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Linking micro- and macroevolution through a developmental genotype-phenotype map

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The relationship between micro- and macroevolutionary dynamics has long been considered paradoxical. Trait variance and covariance within populations, used in quantitative genetics to calculate “evolvability”, were not expected to reliably predict phenotypic divergence across macroevolutionary timescales, yet many empirical studies show a positive correlation between the two. Two broad explanatory classes have been proposed: "constraint" hypotheses, where genetic variation limits evolutionary rates; and "selection-shapes-variation" hypotheses, where selective forces driving long-term divergence also determine the pattern of phenotypic variation. However, neither class offers a mechanistic explanation, particularly given that evolvability is known to change over time.

Here we argue that development provides a missing mechanistic link. Using a computational model of tooth development embedded in an evolutionary algorithm, we simulate populations evolving under different selective regimes: directional, functional constraint, oscillatory and neutral. We show that while evolvability changes over time across all regimes, these changes are not arbitrary, and follow certain rules determined by the underlying developmental mechanisms. In particular, we find that the conserved reaction-diffusion system governing tooth morphogenesis creates a consistent lateral-central cusp height correlation. Although this pattern can be transiently altered, statistical signatures remain conserved over long evolutionary periods. Because this pattern is deeply integrated in the logic of the developmental process, it helps explain the divergence-variance correlation even when assumptions of quantitative genetics are violated. These results reconcile two perspectives often treated independently: the evo-devo emphasis on developmental constraint as a driver of covariation stability, and the quantitative genetics framework that links variation to long-term evolutionary change.

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How insects hacked their BMP toolkit to build a better wing

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The range over which a morphogen gradient provides reliable positional information is limited by intrinsic noise. We identify a regulatory circuit that counteracts this constraint for Dpp, a BMP that organizes the anterior/ posterior axis of *Drosophila* wings. The transcriptional repressor Brinker (Brk), a Dpp target, enhances positional precision by repressing Dad, an inhibitory Smad, thereby extending Dpp's effective range. Thus, Brk mediates a feedback circuit that selectively amplifies low-level Dpp signals as would a logarithmic amplifier. This circuit also achieves temporal integration, mitigating the inevitable noise penalty associated with amplification. Although a core component of BMP signaling in flies, Brk is found exclusively in insects. Phylogenetic and expression analyses in the apterygote insect *Thermobia domestica* suggest that Brk originated in insects and was incorporated into the BMP network in pterygotes, possibly to permit long-range signaling in wing primordia. Brk exemplifies how gene regulatory network (GRN) evolution can enhance developmental precision, thus opening the door to increased morphological complexity.

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Genomic and Transcriptomic Mechanisms Underlying the Evolution of Feeding Ecology in Phytophagous True Bugs

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Insects differ widely in feeding breadth, from being monophagous to highly polyphagous generalists. Explaining the molecular basis of this variation is essential for understanding how ecological diversification and adaptation arise. Yet, it remains unclear whether feeding transitions are primarily driven by changes in gene content or by shifts in how genes are regulated. We are investigating how genomic architecture and gene regulation contribute to transitions in feeding ecology in Hemiptera. We focus on phytophagous true bugs with contrasting dietary strategies, including *Oncopeltus fasciatus, Caenocoris nerii, Lygaeus creticus, Spilostethus pandurus,* and *Halyomorpha halys*. As a first step, we are improving the genome assembly and gene set of the milkweed bug *Oncopeltus* to a chromosome-scale reference for this key model species. Controlled diet-switch experiments will be combined with transcriptomic analyses to examine transcriptional responses to dietary change, investigating quantitative differences in expression levels as well as potential alternative splicing patterns. Comparative analyses will test whether dietary specialization is associated with gene family expansion, differential gene regulation, isoform diversification, or a combination of these mechanisms in hemipteran genomes, which exhibit notable intron gain and intron turnover. By linking dietary shifts to gene expression, transcript isoform usage, and phenotypic outcomes, this study aims to clarify the genetic mechanisms underlying feeding adaptation and diversification in Hemiptera.

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Single-cell RNA-seq analysis of ovarian reserve in mammalian species

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Ovarian reserve is the storage of primordial follicles inside the female ovary, which has the potential to produce mature oocytes for ovulation. The potential for female fertilization can be determined through ovarian reserve. In the early development, the number of primordial follicles has been established as a finite pool and is recruited for folliculogenesis until reaching menopause. The naked mole-rat, a mouse-sized rodent with more than 40 years maximum lifespan, also has a long female fertility span. Recently, a study found that ovarian reserve in the naked mole-rat is unusually large, which is established postnatally, potentially with new oocytes generated in adulthood. Pluripotency genes such as Blimp1, Tfap2c, Pou5f1, and Prdm14 are also expressed in primordial germ cells (PGCs), precursors of oocytes, while Stra8 marks early oocytes entering meiosis. These genes were previously shown to be expressed in the germline of juvenile and even adult naked mole-rat females, alongside more developmentally advanced oocytes, suggesting asynchrony in the germline development. Using publicly available ovarian single-cell RNA-seq datasets across multiple embryonic and postnatal developmental stages of mouse and human, we mapped the expression of these markers to determine the timing and the level of asynchrony of their expression in the germ cells. In both species, the expression of listed markers did not persist until adulthood, however, it was considerably more asynchronous in humans than in mice. We aim to generate equivalent RNA-seq libraries from multiple unconventional species in order to understand the conservation of the persistence of early germ cells into adulthood.

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Peptidergic integration of behavior in Placozoa

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Placozoa have an extremely simple morphology with only two layers of epithelial cells populated by various secretory cells and interconnected by a loose network of fiber cells. Despite this apparent sim-plicity, these animals display a wide range of behaviors that require highly coordinated activity from their different cell types. In the absence of a nervous system, the coordination of behavior in these animals relies on the transmission of chemical signals in the form of peptides that mediate intercellular communication. In our research, have identified and described a variety of peptides that induce specific behaviors in two species: Trichoplax adhaerens and Hoilungia honkongensis. Additionally, we are using light and electron microscopy to locate these peptides and their corresponding receptors to identify which cells are involved in specific behaviors. With these experiments, we aim to map cell-cell interactions in Placozoa, therefore providing meaningful insights into the evolution of animal behavior and into the mechanisms of intercellular signaling in animals without neurons.

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The patterning mechanisms underlying shoot development in lycophytes

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Variation in shoot and leaf morphology are key aspects of vascular plant biodiversity. As such, understanding the evolution of shoot architecture can provide important insights into the evolutionary processes that enabled this diversity and the success of vascular plants. Lycophytes are a sister group to euphyllophytes, so investigating patterning mechanisms within lycophytes can provide crucial evolutionary understanding of developmental processes within vascular plants. The role of auxin and cytokinin in shoot development is well characterised within angiosperms, but less well understood in lycophytes. Previous investigations in the lycophyte genus *Selaginella* have demonstrated that auxin and auxin transport regulate several shoot developmental processes including branching, shoot indeterminacy, and leaf vascular formation. We aim to further explore the role of these hormones in determining *Selaginella* shoot architecture, with a particular focus on the developmental mechanisms underlying phyllotaxis, the regular arrangement of leaves around plant stems. Along with leaves, phyllotaxis evolved independently in lycophytes and euphyllophytes, and the developmental mechanisms involved in lycophyte phyllotaxis are unknown. We are using a combination of microscopy, surgical manipulations, hormone treatments, and genetic analyses to determine the nature of the phyllotaxis patterning mechanism. Through comparisons within lycophytes, and between lycophytes and euphyllophytes, we hope to gain key evolutionary insight into the drivers of biological diversification over time.

P170

A novel progenitor cell type in the embryonic mouse hippocampus suggests an ancient origin for cortical folding mechanisms

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The mammalian neocortex varies strikingly in surface folding, from highly convoluted (gyrencephalic) to entirely smooth (lissencephalic) brains. The evolutionary origins and functional significance of cortical folds remain actively debated. A key cellular driver of folding is the outer radial glia (oRG), initially characterised in the developing neocortex of humans and other gyrencephalic mammals. During cortical development, radial glia lining the ventricle generate most neurones. oRG, a distinct progenitor population located further from the ventricle, amplify this process by expanding the progenitor pool and driving tangential growth that underlies fold formation. Small numbers of oRG have been found in the lissencephalic mouse neocortex, but all reports to date have been confined to the neocortex. The hippocampal dentate gyrus presents an intriguing contrast. A phylogenetically ancient cortical structure, the dentate gyrus is folded in virtually all mammals, including monotremes and marsupials, making it one of the most conserved cortical convolutions. Yet the progenitors underlying this fold remain unexplored. We developed a novel *in utero* electroporation method targeting the embryonic mouse dentate gyrus and identified a previously undescribed progenitor population. These cells divide away from the ventricular surface, extend a process towards the hippocampal fissure, and exhibit somal translocation-like behaviour before mitosis. Molecular analysis revealed multiple features shared with neocortical oRG, leading us to designate them oRG-like cells. These cells displayed a more undifferentiated state than oRG anywhere in the mouse neocortex. The discovery of oRG-like cells in a conserved cortical fold outside the neocortex suggests that the cellular machinery for folding may be an ancient mechanism retained in the hippocampus of even lissencephalic species.

P171

The large ovarian reserve in the naked mole-rat – a unique adaptation or a general characteristic?

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In mammals, body size positively correlates with the size of the ovarian reserve – the number of oocytes, female germ cells, in the ovary. One of the exceptions to this rule is the naked mole-rat, a mouse-sized African subterranean rodent from hystricognath lineage with lifespan > 40 years. Unusually large ovarian reserve likely supports its protracted female fertility, with no decline in pup number or quality as female ages. In addition, ovaries of adult females surprisingly contain considerable number of germ cell nests composed of mammalian oocyte precursors, potentially generating new oocytes later in life. It remains to be elucidated whether these traits are unique adaptations of the naked mole-rat for its long lifespan, or rather more common features of a wider phylogenetic lineage. Our research has shown that while naked mole-rat has substantially higher number of oocytes compared to mouse, this is shared with other long-lived rodents, both in hystricognath and myomorph lineages, and to some extent also with short-lived hystricognaths. In addition, we showed that germ cell nests are present in adult ovaries of both short-lived and long-lived hystricognaths, suggesting that it is a conserved feature in whole lineage. We are now studying the genetic and regulatory basis of these differences between mouse and other rodents, as the ovarian biology of mouse, a classical mammalian model, appears to be the outlier across rodents or potentially all mammals.

P172

Regulation of the delayed meristemoid-to-GMC transition and cell division patterns during stomatal development in succulents

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Stomata on the leaf epidermis act as a gas exchange interface between the plant's interior and its environment, enabling CO_2 uptake for photosynthesis while water vapor and oxygen exit. To avoid excessive water loss, succulents have evolved several innovations including Crassulacean Acid Metabolism (CAM) photosynthesis, in which stomata open mostly at night. In CAM plants, stomata are usually surrounded by subsidiary cells (SCs). The leaf succulent model *Kalanchoë laxiflora* features three circularly arranged SCs around the paired guard cells (GCs). Time-lapse microscopy showed that the stem cell-like meristemoids first generate pavement cells and then SCs in two distinct phases of asymmetric cell divisions (ACDs). Analysis of mutants, overexpression and reporter lines indicated that the bHLH transcription factors *KlaxMUTE1* and *KlaxMUTE2* drive SC-producing ACDs. How this program is ended and guard mother cell (GMC) fate is established remains unknown. Here, I investigate the regulators and cellular aspects of the meristemoid-to-GMC transition through time-lapse imaging, transcriptomics and functional genomics of MUTE's putative heterodimerization partners *KlaxSCRM1* and *KlaxSCRM2*. Mutant analysis suggests a role for *KlaxSCRM2* in ending ACDs and guiding the transition to GMC. We propose that KlaxMUTEs can pair with different heterodimerization partners to execute distinct functions, driving ACDs to make SCs and transitioning to GMC, respectively. Furthermore, quantitative 4D image analysis of developing stomatal complexes showed that decreasing cell size and division asymmetry might also contribute to this transition. This project will give insights into the regulation of the delayed meristemoid-to-GMC transition in succulents and whether early and late MUTE function depends on distinct heterodimerization partners.

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Deciphering the emergence of a novel olfactory neuron population via gene module co-option in tetrapods

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In vertebrates, neurons involved in sensory perception possess specialized ciliated structures whose molecular determinants remain poorly understood. A multi-omics factor analysis of the mouse sensory ciliome revealed that olfactory sensory neurons (OSNs) uniquely express motile cilia genes, whose specific expression is shared with spermatozoa/testes. Since OSN cilia are non-motile in mammals, the functional relevance of this Gene Expression Module (GEM) is a mystery. By performing transcription factor binding motif and ChIP-seq analyses, we identified Rfx3 as the key transcription factor regulating this module in mammals, consistent with its known role in motile ciliogenesis. Expression analyses of Rfx3 and its paralogs in testes revealed Rfx3 dominance in vertebrates but a shift to Rfx2 dominance in mammals. This transition coexists with an opposite one in OSNs, suggesting that Rfx3 has been co-opted to OSNs to drive motile cilia gene expression. Interestingly, mouse immunostaining and in-house single-cell transcriptomics show that not all OSNs express the same levels of the GEM and of Rfx3, suggesting potential cell subtype functional diversification. To trace GEM evolution, we performed additional single-cell RNA-seq of the olfactory epithelium of chicken, frogs, zebrafish, and shark. Our analyses indicate that the GEM is conserved in tetrapods, but not in zebrafish or shark, whose OSNs do not express the GEM orthologs nor share restricted genes with testis. These results indicate a stepwise GEM co-option to OSNs in tetrapods from spermatozoa and other cells with motile cilia via recruitment of Rfx3, potentially contributing to OSN specialization during the transition from water- to air-based olfaction.

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Not all noise is bad: Selection for high developmental noise depends on population size

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The degree of developmental robustness is under genetic control. That is, some genotypes are less robust to microenvironmental perturbations during development resulting in higher phenotypic variability andwithin-population variation. Although high variability is often considered to be deleterious, it can be favored when populations are displaced from a fitness optimum and beneficial phenotypes are accessible. Using a simple modelling framework, we find that the evolution of higher variability (i.e., low developmental robustness) depends on the interaction between developmental bias, fitness function, and population size in complex and surprising ways. On convex regions of the fitness landscape (e.g., far from a peak), higher-variability genotypes have greater expected fitness because high-fitness phenotypes are disproportionately rewarded. However, when phenotypic variation is biased away from the direction of selection, selection depends non-monotonically on population size. In large populations, higher-variability genotypes are disfavoured, but in small populations rare beneficial events can dominate evolutionary dynamics, allowing them to win. Interestingly, the opposite pattern arises on concave regions of the fitness landscape (e.g., near a peak) when variation is biased toward the direction of selection. Here, high variability is favored in large populations but disfavored in small ones, where occasional extreme low-fitness phenotypes cause extinction.

Generally, we propose that non-monotonic population size effects will emerge whenever rare extreme fitness effects oppose mean fitness differences. Together, these results suggest that biased phenotypic variability could evolve opposite to expectation in small populations, which future research should consider particularly in predicting evolutionary outcomes after rapid environmental change.

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The Study of Phylogeny and Morphological Character Evolution in the Genus *Zingiber*

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The genus *Zingiber* (Zingiberaceae) comprises many species distributed throughout tropical Asia and shows high morphological diversity, holding important roles in medicine and economy. The evolution of this genus remains poorly studied, and the diversity of its morphological traits has not been fully understood yet. This study aims to investigate the character evolution in *Zingiber* by integrating evolutionary tree analyses with morphological data. The phylogenetic trees were reconstructed with the Maximum Likelihood method in IQ-TREE2 and Bayesian inference in MrBayes though CIPRES, using nuclear ITS region and chloroplast region (matK, rbcL) sequences from 26 field-collected samples and additional data from GenBank to increase species coverage in this genus. Seven morphological characters were analyzed, including inflorescence origin, peduncle direction, labellum main color, dorsal corolla main color, bract main color, inflorescence length, and ligule type. Ancestral state reconstruction was conducted in Mesquite and RStudio (package phytools) to infer ancestral character states and evolutionary changes. The topology indicates that section *Cryptanthium* is a monophyletic group and section *Dymczewiczia* is still nested within section *Zingiber* like previous phylogenetic research. As a result of character evolution, the rhizome-originated inflorescence, vertical peduncle, short inflorescence, and bilobed ligule are the ancestral state characters of genus Zingiber. However, the ancestral state of labellum main color, dorsal corolla main color, and bract main color is still unclear. It is hypothesized that morphological changes in *Zingiber* may be influenced by pollination mechanisms or by adaptive responses to environmental conditions.

P176

Quantitative characterization of heart development in *Tribolium*

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Heart development is an essential aspect of organogenesis, and both the genetic specification and aspects of structure of the heart are conserved between animal species. Yet, the geometric and mechanical conditions of the early heart differ within invertebrates. For example, the *Drosophila* heart is initially comprised of two discrete rows of cardioblasts within a small, thin embryo. In contrast, in the beetle *Tribolium*, cardioblasts form a connected ring within a larger, fatter embryo (~150% greater egg width/length aspect ratio), and additional extraembryonic tissues (a separate amnion and serosa) contribute contractile forces for cardioblast migration to the midline. Are different mechanisms required to achieve robust heart development in these different morphological contexts, or are conserved mechanisms deployed in differing ways? To tackle this question, we use quantitative live imaging of transgenic lines marking the cardioblasts, extraembryonic tissues, and cytoskeletal components in Tribolium. Preliminary data suggest striking diversity between species in heart development: *Tribolium* has 3-4 times more cardioblasts than *Drosophila*, and, unlike *Drosophila*, the *Tribolium* cardioblast rows increase in length by around 20% egg length as development progresses. This implies that different mechanisms may control heart tube formation in each species. Future work will utilize genetic (pRNAi) and mechanical (laser ablation) perturbations to understand which tissues and mechanical forces are involved in normal development, with quantitative assays for robustness relative to the scale of inter-embryo variability. Altogether, this will help us understand how a mechanically robust and functional heart tube is formed in the differing morphological contexts between species.

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Starvation induces an anaerobic metabolic program in the sea anemone*Nematostella vectensis*

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Starvation is a recurring challenge for most animals, yet our understanding of the physiological strategies that promote survival in the absence of food largely stems from bilaterians. Here we investigate the metabolic and transcriptomic adaptations underlying starvation tolerance in the sea anemone *Nematostella vectensis*, which survives months without food while undergoing reversible whole-body shrinkage. We analysed the transcriptome of juvenile polyps during a starvation and refeeding timecourse and found a gene expression pattern of progressive suppression of mitochondrial oxidative metabolism, accompanied by upregulation of glycolytic enzymes, the hypoxia-inducible factor (HIF) pathway, and genes associated with anaerobic energy metabolism. Physiological measurements confirmed a metabolic shift: after 20 days of starvation, oxygen consumption was reduced by approximately 50% compared to fed animals. We hypothesized that during starvation, *Nematostella* switches to anaerobic energy production, using fumarate rather than oxygen as terminal electron acceptor in the mitochondrial electron transport chain. This reaction requires a specific electron carrier, rhodoquinone, which targeted metabolomics revealed to accumulate during starvation. This indicates that this pathway is not only present in *Nematostella* but actively induced by nutrient deprivation rather than hypoxia. Refeeding reverses both the metabolic and transcriptomic changes, confirming that this represents a regulated and reversible adaptive program. Together, our findings suggest that *Nematostella* uses an anaerobic metabolic program during starvation despite normal oxygen levels, offering new insights into the evolution of metabolic flexibility in animals.

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A continuum-mechanical framework for morphogenesis

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Here we provide NewtonBioMorphX, a user-friendly, high-performance, GPU-accelerated, continuum-mechanical framework for simulating morphogenesis across multiple tissue layers. The framework incorporates not only differential growth and differential material properties —elasticity, plasticity, and viscosity— but also the presence of passive fibres (e.g., collagen) and active tensile filaments (e.g., actin). NewtonBioMorphX relies on tetrahedral meshes in which the equations of motion and nematic field dynamics are discretised by the finite element method. Beyond its computational engine, NewtonBioMorphX provides a control panel for defining model parameters and generating or importing geometries, as well as an interactive rendering window for real-time visualisation of developing geometries and nematic fields. Initial geometries can be imported from external sources or generated directly within the framework as primitive shapes including a box, disk, tube, cone, sphere, and torus. All mechanical parameters, active forces, and growth rates can be specified as constants or analytical functions of space and time, enabling highly flexible model design. The simulation results can be exported as sequences of mesh files or recorded directly as a rendered movie. This framework has already been successfully used to recapitulate the morphogenesis of multiple skin features such as (i) crocodile and tortoise head scales (Nature 2025, iScience 2025), (ii) the polygonal network of creases in the skin of mammalian noses (Current Biology 2024), (iii) chicken reticulate scales (Open Biology 2025), and (iv) snake overlapping asymmetrical scales (in review).

P179

Syncytins: Shaping placentation? Identifying endogenous retroviral envelopes in 103 bat genomes

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Endogenous retroviruses (ERVs) have long been implicated in the evolution and development of mammalian placentation. An example is the emergence of syncytins, retroviral envelope derived proteins that mediate cell-cell fusion during formation of the syncytiotrophoblast. They have also been indicated to potentially facilitate the evolution of differently invasive trophoblast morphologies. However, it remains unclear to what extent interspecies variation in syncytin activity during development contributes to the divergence of trophoblast morphologies. Bats exhibit a high level of diversity in the morphology, development and organisation of their trophoblasts, some even unique among mammals. The Bat1K project has worked to create 103 high-quality chromosome level genomic assemblies for representative bat species. Using these assemblies, we identify syncytin-like integrations and other potentially functional ERVs in bats using SERVE, a pipeline constructed for the detection of retroviral envelope-derived genes. We identify multiple conserved envelope candidates, recover associated long terminal repeats, and estimate their relative ages of integration. To link these candidates to placental development, we integrate transcriptomic data to identify envelopes expressed in placental tissue. We map the projected integration time of candidates to the bat species tree and investigate the relationship between envelope integrations and the evolution of different trophoblast morphologies. Together, these analyses test whether there is evidence that ERV-derived envelopes contribute to or facilitate the evolutionary divergence of placental morphologies. Our results expand the understanding of how ancient viral genes are repeatedly recruited into placental developmental programs that shape mammalian reproductive biology.

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Geographic variation in the microbiome of benthic phase Platynereis worms

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Host-microbe interactions are critical sources of phenotypic variation during development and evolution. Recent studies have highlighted the need to study wild microbiomes of model organisms to capture the full range of symbiotic interactions in a system. To this end, we profiled the microbiome of wild Platynereis dumerilii, a marine annelid model organism for development and evolution, across its European range. We found that wild P. dumerilii microbiomes differ greatly between sites, and are often dominated by bacterial taxa such as Endozoicomonas and Arcobacter that are known symbionts in other marine hosts. Despite the strong geographic variation in these dominant taxa, we also find evidence of a core microbiome: Aliivibrio are highly prevalent and lowly abundant in P. dumerilii across sites. Curiously, we do not find these bacteria in P. dumerilii from our lab culture, suggesting that these are not obligate symbioses. We sought to further explore the function and lifestyle of these dominant microbial taxa and were able to generate Endozoicomonas metagenome-assembled-genomes (MAGs). We found several genes in these MAGs like nutrient transporters and secretion systems that suggest Endozoicomonas adopts a facultative symbiosis with wild P. dumerilii. Altogether, our findings reveal environmental and host genotypic associations with microbiome composition, as well as potentially novel symbioses in a tractable model for developmental and evolutionary studies. We therefore propose that P. dumerilii represents a valuable and fruitful model system to merge host-microbe interactions with the fields of ecology, evolution, and development at both laboratory and planetary scales.

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Pre-bilaterian roots of the chordate body plan

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Similarity of body-plan features across distant phyla provides striking testimony to the Darwinian concept of common descent throughout Metazoa. In both the cnidarian Nematostella and chordates, gastrula organizers direct the initial establishment of embryonic axes. Whether these domains share a common evolutionary origin is a fundamental question for understanding the origin of the chordate body plan. To address this question, we combine trans-species transgenesis, perturbations of signaling pathways, cross-phylum single-cell transcriptomic comparisons, and transplantation assays. We show that cis-regulatory elements that recapitulate NvBmp2/4 and NvChordin expression in Nematostella—and therefore report the endogenous cnidarian organizer regulatory environment—also drive organizer-restricted activity in vertebrate embryos, consistent with vertebrate organizer gene-regulatory logic. Moreover, we show that, as in vertebrates, Smad2/3-mediated signaling is active in the Nematostella organizer and is required for organizer-specific expression of NvBmp2/4 and NvChordin. Together, our data support remarkable homology between cnidarian and chordate gastrula organizers and are consistent with the idea that the cnidarian–bilaterian ancestor possessed a blastopore organizer with the potential to generate dorsal–anterior chordate fates. On this basis, we propose a “skewed evo-schistostomy” scenario for the evolution of the chordate body plan from a cnidarian–bilaterian ancestor.

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Developmental modularity facilitates the evolution of vertebral number over long, but not short, evolutionary timescales

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Vertebrates show enormous diversity in the number of vertebrae in their skeleton. This diversity has evolved via changes to two developmental variables: the frequency at which the somites (embryonic precursors of vertebrae) are formed, and the duration of body axis elongation. In a previous computational study, we showed that the dynamics of the gene-regulatory 'segmentation clock' controlling this frequency and the morphogenetic processes controlling elongation can vary independently of one another, suggesting that developmental modularity underpins the evolvability of vertebral number. However, modularity is contingent on notch-delta signalling being sufficiently strong and of the correct timescale, something we could only verify for Zebrafish *Danio rerio*. It was therefore unclear if this modularity is a widespread feature of vertebrate development, and if it has facilitated the evolution of vertebral number. To address these questions, we used chemical inhibitors to assay notch-delta signalling in the cichlids *Astatotilapia calliptera* and *Rhamphochromis* sp. 'chilingali', which despite diverging 500,000 years ago possess 30 and 36 vertebrae, respectively. We find that strength and timescale are conserved with Zebrafish, implying conservation of modularity across 90% of ray-finned fishes, or 34% of extant vertebrates. However, modularity has not facilitated the evolution of diverse vertebral number here, as only the duration of elongation differs between these two species. Indeed, comparing intraspecific variation in vertebral number across vertebrates suggests that elongation is generally more evolvable than the clock. These results suggest that modularity is a source of evolvability in vertebral number over long timescales but not for short-scale evolution.

P183

Environmentally dependent developmental induction as a potential driver of heart evolution

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Developmental biology and evolutionary theory have traditionally emphasized gene mutations as the primary drivers of new traits, with natural selection shaping the resulting variation. However, recent insights highlight the role of environmental factors during development in shaping trait evolution. In this Commentary, I introduce the 'environmentally dependent developmental induction' (EDDI) model, which proposes that phenotypic evolution is driven not only by genetic changes but also by environmentally induced modifications to the core developmental program. Using cardiogenesis as an example, I argue that environmental triggers such as oxygen levels and mechanical forces expand the genotypic toolkit available to heart development, activating new pathways that lead to the emergence of novel cardiac structures. These lineage-specific environmental changes might thus influence the differentiation of cardiac progenitor cells, resulting in modifications to the cardiac building plan. The EDDI model provides a novel explanation for how the basic cardiac plan was expanded during evolution while simultaneously explaining why cardiogenesis is vulnerable to malformations, even in the absence of genetic defects.

P184

Hox gene evolution and patterning of the miniaturized annelid *Ophryotrocha labronica*

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While some microscopic animal lineages are ancestrally small, many others have become small secondarily, a process particularly common within Annelida. Miniaturization refers to this evolutionary reduction in body size, often accompanied by reduced morphological complexity. It has been hypothesized that miniaturization may result from gene loss, heterochrony, and temporal collinearity. Hox genes complements and expressions may be key to understand miniaturization with their fundamental role in anterior-posterior body patterning during early development. *Ophryotrocha labronica* La Greca & Bacci, 1962 is a miniaturized annelid easily kept in aquarium lab cultures, making it highly suitable for experimental studies. With its development recently characterized and chromosome level genome assembly underway, *O. labronica* is emerging as a model organism among marine invertebrates. To search for genomic signatures of miniaturization, we sequenced transcriptomes from ten developmental stages, spanning early embryos to juveniles and adults. Spatial and temporal expression patterns of Hox genes were analyzed using differential gene expression analyses and in situ Hybridization Chain Reaction. The data are being examined for heterochronic changes in expression that can be related to miniaturization. The Hox gene complement was identified using transcriptome and genomic data. Our findings indicate that *O. labronica* possesses an incomplete Hox gene set, with apparent losses of *Antp* and *Lox2*. Interestingly, *Lox2* is also absent in the meiofaunal annelid *Dimorphilus gyrociliatus*, suggesting that the loss of this gene may represent a recurrent evolutionary feature associated with body miniaturization in annelids.

P185

Unveiling the genes underlying molecular patterns of development conservation across the tree of life

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Since the time of Aristotle, the close resemblance of vertebrate embryos has puzzled biologists. In the last two centuries, many Evo-Devo theories have sought to explain such patterns of morphological conservation, including the recapitulation theory of Haeckel or Von Baer's laws of development. Among the current zeitgeist features the "developmental hourglass model", where the middle period of embryogenesis is posited to define a phylum's body plan, being more evolutionarily conserved. Molecular methods have emerged to probe these theories, such as the Transcriptomic Age Index (TAI), which combines macro-scale protein evolution information with developmental gene expression data. This approach has uncovered patterns of development conservation across the tree of life, despite multicellularity having evolved independently in each Eukaryotic kingdom. This hints towards structural constraints in the architecture of already established regulatory systems, inhibiting the integration of new genes in critical stages of development, and hence the evolvability of body plans. Alternatively, an adaptationist might argue simply that selection acts differentially across developmental stages. There is thus much debate on what causes these constraints, which molecular markers to focus on, and whether there are universal constraints to the evolution of multicellular systems. In this work, we attempt to ground the discussion towards functional interpretability. As such, we have developed GATAI, a tool for the extraction of key genes underlying the TAI patterns. We use this to analyse developmental evolutionary transcriptomics datasets from over one hundred species, providing a systematic investigation into the genetic origins of developmental constraints across the tree of life.

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With a *Hand* in your heart. Functional analysis of *Hand1/2* knockdown in the evolutionary deconstructed cardiopharyngeal gene regulatory network of *Oikopleura dioica*

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Cardiopharyngeal development is regulated by a highly conserved set of orthologous transcription factors across chordates. The appendicularian *Oikopleura dioica*, however,has experienced massive gene losses through evolution, making it an evolutionary knockout (eKO) model with a deconstructed cardiopharyngeal gene regulatory network (GRN), ideal for studying the impact of gene loss on the evolution of developmental mechanisms. This deconstruction has resulted in a minimalistic heart, made up of very few cells organized in two layers of mesodermal tissues (myocardium and pericardium) forming a contractile “bag” wedged between the stomach wall and the intestine that facilitates haemolymph circulation in the open circulatory system. To dissect the evolutionary implications of this extreme GRN simplification and to identify new candidate genes involved in chordate heart development, we performed RNA-seq and differential gene expression analysis of animals depleted of the early transcription factor *Hand1/2* using a novel silencing technique currently being developed in our laboratory. We describe here, for the first time, the *Hand1/2* knockdown phenotype of *O. dioica*, characterized by the formation of an aberrant heart displaying disorganized myocardial overgrowth and loss of peristaltic activity. Transcriptomic analyses revealed differential expression of genes involved in heart development. Altogether, our results identify new candidate regulators that may represent core players in chordate heart development, revealed through the evolutionary simplification of the *O. dioica* cardiopharyngeal GRN.

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Developmental shifts shape the evolution of color vision in phyllostomid bats

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Understanding how complex traits are repeatedly gained or lost during evolution remains a central challenge in evolutionary developmental biology. Color vision in mammals is a striking example of phenotypic lability, yet the developmental mechanisms underlying its diversification remain poorly understood. Previous work in noctilionoid bats showed that the loss of short-wave-sensitive (S) opsins occurs through changes at different levels, including DNA, transcript, and protein, highlighting that changes extend beyond gene sequence evolution. Here, we investigate how color vision phenotypes emerge during development using a comparative framework combining transcriptomics and 3D morphology. We generated whole-eye transcriptomes for five phyllostomid bat species spanning distinct dietary niches, including insectivory, frugivory, and nectarivory, and complemented these data with high-resolution µCT-based 3D reconstructions of eye morphology. Our preliminary results reveal substantial diversity in eye structure across species, alongside variation in opsin expression and associated developmental pathways. Rather than being solely determined by the presence or absence of opsin genes, these patterns suggest that the evolutionary trajectories of color vision may be established early during eye development. We observe parallel changes in gene expression profiles consistent with shifts in photoreceptor differentiation and retinal organization across dietary lineages. Together, these findings suggest that the evolution of color vision in phyllostomid bats involves early developmental changes at the molecular levels. This integrative approach highlights the importance of developmental timing and regulatory variation in shaping sensory diversification.

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Looking for the origin of neurons by dissecting the peptidergic nervous system of ctenophores

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The evolutionary origin of neurons has been heavily debated. Recent research of typical neuronal features in early diverging animal lineages (Ctenophora, Porifera, Placozoa, Cnidaria, and Bilateria) suggested that neuropeptides were probably key components of the first neurons. Ctenophora is the earliest evolving lineage equipped with the nervous system, which may have evolved independently. We recently showed that the ctenophore nerve net is highly peptidergic and it combines both unique (e.g. ctenophore-specific neuropeptides) and common molecular features (e.g. highly conserved RNA-binding protein Elav and ion channels). The subepithelial nerve net, the aboral sensory organ and many sensory cells probably act through extrasynaptic neuropeptide secretion. However, neuropeptide receptors remain unknown. In other animals, neuropeptides mostly bind to G-protein coupled receptors (GPCRs), and ctenophores have hundreds of GPCRs, however peptidergic GPCRs have not been identified so far. We are applying a comprehensive bioinformatics approach to identify candidate peptidergic GPCRs in ctenophores. Using putative ctenophore GPCR sequences, HMM profiles were built to search for homologues in annotated reference genomes/transcriptomes with known peptidergic GPCRs. This dataset was used to build phylogenies on both protein sequence and 3D structure levels (using Alphafold-predicted structures). Our preliminary data suggest that peptidergic GPCRs from the reference genomes group together more often in the phylogeny constructed from 3D structures, compared to the sequence phylogeny. In the 3D phylogeny, ctenophore GPCRs form several monophyletic clades with some isolated GPCRs grouping together with peptidergic clades from other animals. These candidates will be tested in vitro to confirm their peptidergic nature.

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10-plex imaging of segmentation gene expression in *Drosophila* and *Tribolium* embryos

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Segmentation in *Drosophila melanogaster* and other arthropods has been studied for decades, based largely on visualising expression patterns of segmentation gene products and how they are perturbed in mutant or RNAi embryos. Gene expression patterns were first studied with radioactively labelled probes, followed by colorimetric stains with 1-2 colours, and later by fluorescence confocal imaging with 2-4 channels. Although imaging methods have improved and become more quantitative, technical constraints on the number of gene products that can be visualised simultaneously still limit the information provided by each embryo. This bottleneck is more severe in less established model species, where colorimetric methods remain common and embryos may be more difficult to collect and prepare. Here we combine multiplexed HCR in situ hybridisation with white light laser confocal microscopy to collect quantitative imaging datasets containing up to 10 gene expression channels per embryo, with minimal crosstalk between channels. In *Drosophila*, we use a custom computational pipeline to process image stacks of whole mount embryos in 3D. We extract intensity traces for each gene along the anteroposterior axis, controlling for dorsoventral orientation and precisely determining embryo age. We have begun systematically characterising patterning dynamics across mutant genotypes and have already identified new inputs and cross-regulatory interactions that influence gap gene boundaries. We are also extending multiplexed imaging to other insects including the flour beetle *Tribolium castaneum*. This will enable a more detailed and comprehensive comparison of segmentation network dynamics across species, accelerating the study of how different modes of segmentation have evolved.

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Flexibility of cell fates and functions across sex determination systems revealed by comparative single-cell analyses

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Sex determination in vertebrates can be initiated by a wide range of genetic or environmental triggers. Yet, the degree to which gonadal cell types and genetic programs are conserved remains unresolved. Here we employed single-cell transcriptomics to characterize the temperature-dependent sex determination (TSD) program in gonads from the turtle species *Trachemys scripta*. Comparative analyses against species with genetic sex determination, like mouse (XY) and chicken (ZW), revealed a marked divergence in cell type repertoires and functions during vertebrate evolution. Unlike mammals, fetal Leydig cells are absent from the early gonads of *T. scripta*, where the supporting lineage expresses genes required for androgen synthesis. Evolutionary reconstructions show that this lineage derives from a *Pax2*-positive mesenchymal population, suggesting an ancestral condition in Archelosauria that differs from the primarily coelomic epithelium origin in the mammalian clade. Transcriptional dynamics and co-expression analyses revealed the recruitment of lineage-specific transcription factors, including Twist1 or Runx1, into the genetic programs of vertebrate clades. Our findings reveal extensive plasticity of the cellular and genetic mechanisms of vertebrate sex determination and suggest that this flexibility is a key feature of gonadal evolution.

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Exploring the role of Mohawk homeobox A in the zebrafish musculoskeletal system

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Locomotion in vertebrates relies on the coordinated function of muscles, bones, and connective tissues, including tendons and ligaments that transmit force across the musculoskeletal system. While the transcription factor Scleraxis is well established as a regulator of tendon development, the functional role of Mohawk homeobox (Mkx) in the musculoskeletal system is much less well characterized. Although Mkx has been associated with connective tissues in mammals, its broader developmental and physiological contributions have not been extensively characterized in fish. To investigate mkxa function in zebrafish, we generated a reporter knock-in line that enables visualization of mkxa expression while simultaneously disrupting gene activity. Endogenous activity of mkxa was characterized in the craniofacial and axial musculoskeletal systems and the loss of mkxa was assessed through analyses of growth and viability in comparison to wild-type siblings. Locomotor performance was further evaluated under controlled environmental conditions to determine potential functional consequences of mkxa disruption. In parallel, we are performing musculoskeletal tissue characterization, differential expression analysis and non-destructive chemical analysis studies to determine how mkxa inactivation affects surrounding tissues and co-localized tissue-specific markers. Together, these findings indicate that mkxa may play a broader developmental role beyond tendon regulation, potentially influencing musculoskeletal integrity, that is essential for normal locomotion and viability. Together, this study seeks to clarify the developmental role of mkxa and to determine whether its functions extend beyond classical tendon regulation and whether they are conserved between mammals and teleost fish.

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Dynamic roles of embryonic glial cells in octopus brain development

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Coleoid cephalopods have evolved large nervous systems with cell numbers similar to small mammals. How these nervous systems develop and what transcription factors drive neurogenesis and neural diversification is not yet fully understood. Here we profiled gene expression and accessibility using single cell multiome sequencing during embryogenesis of *Octopus vulgaris* and reveal conserved and novel key regulons for neuronal and embryonic glial cells. We discovered that embryonic glial cells are populating the initial brain cords before neurons are generated. They are different from previously described *ascl1*+ neuronal progenitors and are defined by a strong orphan nuclear receptor *nr4a* regulon. Using non-negative matrix factorization and pseudotime inference we described their dynamic transcriptional profile which indicates that these cells might orchestrate cell migration, axon guidance and patterning of the brain before differentiating into typical glial cells, making them a unique population of cells in octopus development. Here, we shed light on octopus embryonic glial cells that seem to be different than the predominantly neuro- and gliogenic radial glial cells in vertebrates. However, whether this cell type has uniquely evolved in coleoid cephalopods remains to be determined. This work highlights a novel set of transcription factors involved in glial differentiation and yet their dynamic profiles indicate structural, instructive, and functional roles in the developing nervous system of *Octopus vulgaris*.

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Evolution of FYVE_2 exocytic transport regulators at the transition to animal multicellularity

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RAB GTPases are key regulators of cellular organelle transport. In response to the increased demand for signaling molecule transport to facilitate cell communication in multicellular organisms, the transition from single-celled relatives to multicellular animals is marked by a significant expansion of the secretory RAB8 small GTPase gene. The resulting RAB8, RAB3, and RAB27 proteins interact with effectors that share a common structural feature: the FYVE_2 zinc finger domain. Mammals possess four FYVE_2 domain protein families: RIM1/2, rabphilin-3A/NOC2, SYTL3/4/5, and MYRIP/MLPH, all of which are involved in exocytic and secretory transport processes. Through TBLASTN nucleotide database searches, we demonstrate that these FYVE_2 domain effector proteins co-evolved with the expansion of secretory RAB GTPases. Notably, no FYVE_2 domain proteins have been identified in unicellular holozoa. Three of the four mammalian FYVE_2 protein families—RIM, rabphilin-3A, and SYTL—are already present in early-branching animals, such as sponges and sea anemones. The MYRIP/MLPH family, however, emerged later in the evolution of bilaterians. Phylogenetic analysis, structure predictions, and interaction studies suggest that MYRIP evolved through a domain-shuffling event, which combined the basal metazoan SYTL-RAB binding domain with a myosin-7 actin motor protein binding domain. The evolution of FYVE_2 proteins in metazoans, particularly the bilaterian refinement of MYRIP/MLPH-mediated secretory actomyosin organelle transport, represents a crucial adaptation to meet the increased demands for cell communication in animals.

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Centromeres in the nematode *Meloidogyne* species share conserved epigenetic and genetic determinants

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Root-knot nematodes of the genus *Meloidogyne* are parthenogenetic organisms and are globally important economic pests. They exhibit a unique clustered centromeric organization, in which centromeric activity is distributed across multiple clusters along the chromosome length. These species vary in their ploidy levels and modes of reproduction, ranging from mitotic to meiotic. This diversity makes them an excellent model for studying evolution of centromere under different genomic conditions. Generally, centromeres are determined by the highly evolved centromere-specific histone H3 variant (CenH3) and the sequences and organization of centromeres also vary greatly among species. In our study, contrasting with general knowledge, we identified the presence of conserved CenH3 (CENP-A) candidates in four mitotic and two distant meiotic *Meloidogyne* species. Using Primed-In Situ Hybridization (PRINS), we confirmed that a 19-bp box is present within highly variable centromeric tandem repeats across all mitotic and polyploid species. Additionally, we demonstrate that more distantly related meiotic and diploid species also possess highly similar 19-bp box sequences associated with divergent tandem repeats in the centromere. The detection of the highly conserved αCenH3 centromeric protein, alongside the conserved 19-bp box within centromeric tandem repeats across all *Meloidogyne* species, including distant meiotic species, suggests a functional role for these sequences in centromere activity. Our results indicate the preservation of both genetic and epigenetic components in the centromeres of these species.

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Centromere organization in *Meloidogyne* shaped by reproductive strategy and ploidy

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Plant-parasitic nematodes of the genus *Meloidogyne* are an excellent model for studying centromere evolution. Centromeres are chromosomal domains essential for accurate segregation during cell division and are epigenetically determined by the centromere-specific histone H3 variant, CenH3. *Meloidogyne* possess unique clustered centromeres, variation in ploidy levels, and contrasting reproductive modes ranging from mitotic parthenogenesis to meiosis. These features enable the exploration of how centromere and chromosome organisation evolve across distinct genomic and reproductive contexts. In *Meloidogyne*, centromeres correspond with CenH3-associated regions as well as different short tandem repeats carrying a conserved 19-bp motif. Using comparative genomics and extensive bioinformatic analyses of high-quality genome assemblies based on long-read sequencing from six *Meloidogyne* species, encompassing polyploid mitotic and diploid meiotic lineages, we characterised the long-range organisation of the centromere. Our analyses confirm the presence of a cluster-type holocentromere in all species, in which centromeric function is partitioned into discrete repeat arrays distributed along the chromosomes. The centromere structure is generally conserved in mitotic polyploid species, with short tandem arrays forming holo-units located within larger repetitive regions called holo-clusters. This complex higher-order organization of tandem arrays is frequently associated with autonomous and non-autonomous Helitron transposons in mitotic polyploidy species. In contrast, meiotic diploid species display simpler, predominantly monomeric arrays lacking Helitron enrichment, marking a clear difference between species with different reproductive modes. Together, these results link reproductive strategy and genome composition with distinct modes of centromere expansion and organisation, offering new insight into the evolutionary dynamics of centromeres.

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New genome assemblies from neglected phyla illuminate the evolution of Ecdysozoa

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Ecdysozoa is the second largest clade within Metazoa and includes eight phyla. Little is known about the early evolution of the clade. Morphological features would group Priapulida, Kinorhyncha, and Loricifera into the clade Scalidophora. Moreover, the organization of the scalidophoran nervous system closely resembles that of Nematoda and Nematomorpha, leading to the proposal of the clade Cycloneuralia. Both hypotheses have been questioned by phylogenomic studies, opening a discussion on the evolution of ecdysozoan traits in relation to the inter-phyla relationships. Previous phylogenies have mostly relied on transcriptomic data, particularly for phyla that are exclusively microscopic (i.e., Loricifera, Kinorhyncha and Tardigrada). For specimens smaller than 1mm, the DNA yields obtained after the extraction cannot meet the minimum requirements for long-read sequencing. Therefore, long-reads high quality assemblies can only be achieved through amplification of the extracted DNA. In this project we produced genome assemblies of six microscopic ecdysozoan: one Tardigrada (*Florarctus antillensis*), two Priapulida (*Tubiluchus corallicora* and *Meiopriapulus fijiensis*), two Kinorhyncha (*Setaphyes dentatus* and *Echinoderes intermedius*) and two Loricifera (*Armorloricus elegans* and *Nanaloricus valdemari*), with the latter four being the first assemblies representing their phylum. For each specimen, the DNA extract has been amplified using two approaches, the long-range PCR and the multiple displacement amplifications. Combining the two methods, we achieved contiguous (N50 > 1 Mb) and complete (BUSCO > 90%) genomes from single microscopic individuals. With the inclusion of the new genomes, we will assess hypotheses such as Scalidophora and Cycloneuralia and characterize the evolution of pathways underlying key ecdysozoan traits.

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Contrasting histories of gene and genome duplications shape biosynthetic and flower developmental pathways in *Ranunculales*

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Benzylisoquinoline alkaloids (BIAs) are a diverse class of specialized plant metabolites with important functions for plants and humans. They are synthesized through the coordinated activity of members from diverse gene families. However, the evolutionary history and development-specific regulation of BIA biosynthetic genes remain poorly understood. Here, we show specific mode of molecular evolution of BIA biosynthetic genes and suggest that their expression pattern is orchestrated by developmental control genes. We analyze a haplotype-resolved genome assembly together with a high-density expression atlas of California poppy, an emerging Ranunculales model organism. Reconstructing the duplication history of BIA biosynthetic genes across Ranunculales, we reveal strong lineage specificity of BIA gene family members. In California poppy, BIA genes expanded primarily through small-scale segmental duplications. BIA genes are expressed in an organ-specific manner and that is correlated with phylogenetic relatedness, suggesting shifts in function following duplication. In contrast, carotenoid biosynthesis genes, which are responsible for the striking orange petal pigmentation, show little phylogenetic clustering. Furthermore, floral developmental regulators exhibit high duplicate retention associated with ancient polyploidy events, indicating distinct evolutionary trajectories among metabolic and developmental gene families. By analysing gene co-expression networks, we aim to expand our knowledge how development influences specialized metabolism. Together, these findings highlight alternative roles of gene- and genome-level duplications in shaping metabolic pathways and developmental programs. Given the phylogenetic position of California poppy, the data generated here provide a valuable foundation for comparative genomic and transcriptomic studies in poppies and flowering plants.

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Building a cell-type atlas of the larval apical sensory organ in the model sea anemone, *Nematostella vectensis*

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Many marine invertebrates have elaborate life cycles containing a free-swimming larval stage which relies on its sensory systems to navigate within a complex environment. Successful navigation of larvae is crucial for locating and assessing recruitment sites and can influence adult population structure. Many larvae, including some cnidarian larvae, are thought to navigate using a specialized sensory structure known as the larval apical sensory organ (ASO). Despite its potential role in integrating multiple sensory inputs, the specific cellular functions of the cnidarian ASO remain poorly understood. Using the model species *Nematostella vectensis* (the starlet sea anemone) we aim to characterize specific ASO sensory cell-types and their modes of signalling to better understand ASO function. *N. vectensis* larvae have a distinctive ASO containing multiple sensory-neurosecretory cells. Without high-resolution ultrastructural information it is difficult to distinguish such cell-types from one another. To characterize the specific ASO sensory-neurosecretory cell-types, and to map cell connectivity and links to the underlying nervous system, we generated a 3D reconstruction based on serial blockface scanning electron microscopy data. We also tracked ASO cell composition throughout larval development using transmission electron microscopy to generate a time-series of ASO cross-sections from early- to late-planula stages. This morphological cell-type atlas will provide an important foundation for our future goal of mapping ASO chemical signalling at single-cell resolution. Furthermore, our atlas will serve as a comprehensive guide for identifying and comparing apical sensory cell-types across Cnidarian species.

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Comparison of single nucleus transcriptomics between sea urchin juvenile and larval mesodermal corresponding cell types reveals transcriptional similarities but also novel cell clusters

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Indirectly developing sea urchins go through a brief larva phase followed by a dramatic metamorphosis, during which, a rudiment becomes the juvenile organism, while the remaining larva is absorbed. Although both forms consist of similar organ systems, it is not yet understood how similar these organs are from a molecular and ontogenetic point of view. Gene regulatory networks controlling muscle and coelomocyte formation in sea urchin larvae are well studied. These networks are comprised by a number of conserved myogenic and hematopoietic genes, respectively. However, little is known regarding the muscle and coelomocyte regulatory signatures of the juveniles. Moreover, it is not clear whether these juvenile cell types arise de novo from unspecified cell populations or appear as already differentiated cells transferred via the rudiment. In order to understand whether there is a regulatory continuity of these cell types between larvae and juvelines we used single nucleus transcriptomics and compared the juvenile cell types to their larval counterparts. We found juvenile clusters with high similarity both for the muscle and the coelomocyte larval developmental programs. Interestingly, we also found cell clusters that have novel regulatory signatures and don't correspond to the larval types. Expression pattern analysis and lineage tracing will allow us to decipher the developmental origin and inter-relationship of the identified cell types. The ultimate goal is to perform an echinoderm cross-species comparison in order to reconstruct a cell-type phylogeny and determine the evolutionary origin of the diverse muscle and coelomocyte cell populations.

P201

Dpp-dependent gene regulation in early embryogenesis of the carpenter ant *Camponotus floridanus*

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Gene regulatory networks (GRNs) underlie developmental patterning and morphogenetic processes and are drivers of evolutionary change. Understanding how conserved signaling pathways function within GRNs across taxa requires detailed characterization of developmental systems with distinct embryonic features. The carpenter ant *C. floridanus* provides such a system, exhibiting a highly diverged embryonic architecture. Here, we investigate Bone Morphogenetic Protein signaling by knocking down the BMP ligand *decapentaplegic* (*dpp*) using RNA interference and examining the resulting developmental phenotypes. To explore molecular mechanisms associated with these phenotypes, we performed RNA-seq on blastoderm-stage embryos following *dpp* knockdown and conducted differential expression analyses to identify genes whose expression changes in response to reduced BMP signaling. We report developmental defects following *dpp* knockdown and identify candidate target genes associated with BMP-dependent regulation during early embryogenesis.

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Sustained ectopic neurogenesis as a mechanism of evolutionary brain expansion in Octopus vulgaris

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Coleoid cephalopods, including octopuses, squids, and cuttlefish, are invertebrates renowned for their remarkable cognitive abilities, such as tool use, play, observational learning, self-recognition, and long-term memory. These behaviors are supported by large, complex brains that evolved independently from those of vertebrates. In our lab, we previously characterized a major neurogenic region in *Octopus vulgaris* embryos: the lateral lips (LL), a structure surrounding the eyes that generates neurons destined for the brain. By the end of embryogenesis, the LL seem exhausted. Nevertheless, *O. vulgaris* undergoes an almost 100-fold increase in brain cell number from hatchling to adult, raising important questions about the location and mechanisms of neurogenesis during early post-hatching stages. To address this, we developed a rearing system capable of maintaining paralarvae for up to 30 days post-hatching (dph), alongside wild-caught juveniles, enabling tissue collection and in vivo experiments. Using immunostaining and hybridization chain reaction (HCR), we observed that the LL regrow during the paralarval stage and continue growing throughout juvenile development. To assess migration of newborn neurons, we optimized CFDA and EdU cell-tracking approaches to compare migration patterns with those observed during embryonic stages. To investigate the molecular mechanisms underlying brain growth, we performed pharmacological treatments targeting key signalling pathways that were active in the LL. Our findings challenge previous assumptions by demonstrating that LL cells undergo proliferative regrowth during post-hatching development, rather than neurogenesis occurring primarily in the brain. The presence of an active neurogenic zone outside the brain provides a means of continuous brain expansion throughout post-hatching life.

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Developmental evolution of feeding morphology in cave-dwelling Eurycea

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Organisms can adapt to environmental change either by evolving novel structures that arise during development or by altering the timing and sequence of developmental events. Groundwater salamanders (genus Eurycea) provide a model system for investigating how habitat shifts influence morphological evolution because multiple lineages have independently colonized subterranean environments. One demand likely altered during these transitions is feeding ecology, as both abiotic conditions (e.g., light availability) and biotic conditions (e.g., prey communities) differ substantially between surface and cave habitats. Here, we examine how repeated transitions to cave environments influence the development of feeding morphology in Eurycea. We compare musculoskeletal anatomy across species representing independent cave invasions and across developmental stages to test for shifts in the timing and pattern of cranial muscle formation. Using contrast-enhanced microCT scans, we reconstruct cranial elements and associated musculature in three dimensions and quantify developmental variation in the feeding apparatus.

P204

Evolution of Coloniality and Invertebrate Allorecognition

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Allorecognition, the ability to distinguish self from conspecific non-self, has been extensively studied in marine colonial animals such as the ascidian *Botryllus* and the cnidarian *Hydractinia*. Allorecognition is considered beneficial for colonial organisms by allowing genetically close kin to fuse, thus enhancing body size and survival, while selectively rejecting non-kin to avoid germ line parasitism. However, there is a notable lack of evidence regarding the evolution of allorecognition across different phyla, particularly in those with solitary life forms. In our research, we demonstrated that *Cladonema radiatum*, a hydrozoan cnidarian with both colonial and solitary jellyfish forms, exhibits allorecognition in both life stages, although the degree of recognition is less pronounced in the jellyfish form. Additionally, our findings indicate that *C. radiatum* has a conserved but relatively simple allorecognition complex compared to that of *Hydractinia*. To expand our understanding of invertebrate allorecognition, we are also investigating whether molluscan bivalves possess an allorecognition system. Bivalves are solitary filter-feeding organisms that can inadvertently ingest cells discharged from their surroundings. Notably, transmissible neoplasia has been reported in more than ten bivalve species, suggesting a potential inefficiency in allorecognition among these animals. Our findings reveal that tissue fusion occurs in grafts between both autogenic and allogenic tissue explants of the Pacific oyster (*Magallana gigas*), indicating the lack of an efficient allorecognition system.

P205

Haplotype-resolved genomes reveal pervasive structural polymorphism in the planktonic chordate *Oikopleura dioica*

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Haplotype-resolved diploid assemblies expose within-individual genomic variations that classical consensus genomes mask, making them particularly powerful for understanding the magnitude and dynamics of genomic diversity at the population level. Our previous work on *Oikopleura dioica*, an abundant planktonic chordate with an exceptionally compact genome (~60 Mb) and accelerated evolution, revealed that geographically distant populations are cryptic species with extremely "scrambled" genomes despite near-identical morphology. This apparent decoupling between genome architecture and phenotype raised a key question: how much genomic variability exists within single populations? Here, using long-read sequencing with trio binning and haplotype-aware assembly approaches, we generated multiple haplotype-resolved assemblies of *O. dioica* from distinct geographical locations. Comparative analyses of paired haplotypes revealed extreme intra-individual divergence: ~3.5% mean nucleotide heterozygosity, alongside abundant indels and structural variants (including inversions, translocations, and copy-number variations) that collectively affect ~25% of the genome. These magnitudes far exceed those typically reported for vertebrates and other classical model organisms, establishing an extreme case of heterozygosity and structural polymorphism within the chordate phylum. Ongoing work investigates the molecular mechanisms generating these haplotype rearrangements, their effects on the variation of coding and regulatory elements between haplotypes, and their implications for cryptic speciation and evolvability in this lineage. Overall, our results show that *O. dioica* harbors exceptionally high within-individual and within-population genomic variability. As this species continues to gain relevance as a model in developmental biology and marine ecology, these findings also underscore the value of haplotype-resolved assemblies for revealing hidden genomic complexity in non-traditional model organisms.

P206

A reaction-diffusion mechanism patterns the wing vein branching of Bicyclus anynana butterflies.

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Two major theories of biological pattern formation—positional information and reaction-diffusion—are increasingly viewed as interconnected. However, direct empirical evidence for their interdependence remains scarce in most systems. In this study, using Bicyclus anynana wing venation, we investigate how these two mechanisms act together to pattern vein formation. Previous research work has hinted at positional information involved in the positioning of primary longitudinal veins. This mechanism, however, can't explain most of the branching veins observed in the forewings and crossvein patterns of B. anynana. In the present work, using spatial mRNA profiling, drug inhibitors, CRISPR-Cas9, and mathematical simulations, we showed the presence of a reaction-diffusion mechanism playing a role in the secondary branching pattern formation. This work provides the first detailed empirical evidence of such a reaction-diffusion mechanism in play in insect wing veins.

P207

Evolutionary conserved transcriptional regulators control monoaminergic neuron development

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To what extent conserved developmental programs specify homologous cell types is a central question in biology. To address this, we first reconstruct the developmental program underlying monoaminergic neuron specification in the *Drosophila melanogaster* embryo using time-resolved single-cell genomics, spatial transcript mapping with hybridisation chain reaction, and targeted metabolomics. We identify a defined set of transcription factors that are activated prior to monoaminergic biosynthetic enzymes expression, revealing a temporal hierarchy underlying monoaminergic fate commitment. We then test the evolutionary conservation of this regulatory program by comparing monoaminergic developmental trajectories across *Drosophila*, zebrafish, and sea urchin single-cell atlases. These analyses reveal conservation in the temporal deployment of key orthologous transcription factors across ~550 million years of bilaterian evolution. At the same time, our results indicate that lineage-specific regulatory innovations contributed to the diversification of monoaminergic subtypes across species.

P208

Brain regeneration in the marine chordate: insights from sulfation dynamics of dermatan and chondroitin sulfate

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Neuroregeneration refers to the ability of nervous tissue to renew and rebuild itself after injury, a process that is highly restricted in mammals. In contrast, marine chordates such as ascidians display a remarkable regenerative capacity, making them valuable models for understanding neural regeneration. This study investigated the profiles of dermatan sulfate (DS) and chondroitin sulfate (CS) during brain regeneration in the ascidian Styela plicata. Neurodegeneration was induced by systemic administration of 3-acetylpyridine (65 mg/kg), and neural complex (NC) analyses were conducted at 1, 5, and 10 days post-injection using histology, immunofluorescence, real-time PCR, strong anion exchange chromatography,behavioral test and phylogenetic methods. One day after treatment, the cerebral ganglion exhibited significant degeneration, followed by morphological and molecular recovery at 10 days, when neuronal and synaptic markers returned to control levels. Gene expression analyses revealed early upregulation of C-6, C-4 and C-2 sulfotransferases in the latest stage. For the first time, the presence of dermatan 2,6 sulfate (D2,6S) and chondroitin 4 sulfate (C4S) in thecortex of cerebral ganglion was described and 2,6 sulfate disaccharides make up the majority of the NC,suggesting their relationship with the regeneration.Therefore, a behavioral test revealed that the co-injection of 3AP and D2,6S rescued the compromised movements of syphon induced by the injection of the 3AP only. Finally, ascidianDS epimerase (DSE) was closest related to vertebrate DSE type 2, found in the brain of mammals. Together, these results indicate that specific glycosaminoglycan sulfation patterns are dynamically regulated during neural regeneration in ascidians, especially D2,6S, offering insights into molecules and strategies to promote neuroregeneration in vertebrates and advance the field of regenerative medicine.

P209

The Impact of Vertebrate Whole Genome Duplications on RNA Binding Protein Evolution

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The evolution of transcriptional gene regulatory networks is thought to mainly be affected by mutations in transcription factors and their downstream targets. Gene duplication events such as whole genome duplications (WGDs) are thought to be particularly relevant due to the subsequent relaxation of selection on duplicated gene copies. Less information is known about the impact of WGD on the evolution of post-transcriptional gene expression regulation, which controls whether specific mRNA transcripts are translated into proteins and fine-tunes final temporal and physical protein expression in cells. The process of post-transcriptional gene expression regulation is performed primarily by a diverse group of proteins called RNA binding proteins (RBPs). Whole genome duplications occurred at the base of the vertebrate lineage, and it is therefore hypothesised that gene family expansions will have occurred, increasing the complexity of transcriptional and post-transcriptional regulation and contributing to the evolution of novel vertebrate characters. It is therefore hypothesised that, analogous to the changes to transcriptional gene regulatory networks due to gene duplications of transcription factors, there is an increase in regulatory complexity of post-transcriptional gene regulation due to the vertebrate whole-genome duplication. This could be due to simple expansion of RBP families, or by the alteration of RBP domain architecture leading to new regulatory interactions. This study aims to investigate the gene family evolution of RBPs by looking at vertebrate-specific protein family duplications, expansions of specific RNA binding domain-containing protein families in vertebrates compared to chordate ancestors, and changes in protein domain architectures between chordates and vertebrates.

P210

Stepwise cellular and tissue-level remodeling underlies the evolution of viviparity in a reptile

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A central question in evolutionary developmental biology is how complex traits arise. The transition from egg-laying (oviparous) to live-bearing (viviparous) reproduction offers a powerful model to study this process, as it requires extensive remodeling of uterine tissues and developmental reprogramming of maternal–embryonic interactions. The common lizard, *Zootoca vivipara*, represents one of the most recent transitions from oviparity to viviparity and includes a genetically distinct ancestral oviparous lineage, a derived oviparous lineage (a later-diverging oviparous lineage phylogenetically closer to viviparous forms), and derived viviparous lineages. We examined uterine tissues from the two oviparous lineages and one viviparous lineage at vitellogenesis and mid-pregnancy using histology, bulk RNA sequencing (76 samples), and single-cell RNA sequencing (56,214 cells from 12 samples). Histological analyses revealed clear morphological divergence between viviparous and oviparous uteri. Viviparous uteri exhibited reduced glandular tissue, expanded muscular layers, and increased vascularization, whereas the two oviparous lineages were highly similar in overall architecture. At the single-cell level, however, the derived oviparous lineage displayed pronounced intermediate regulatory features. Substantially more genes were differentially expressed between the oviparous lineages at mid-pregnancy than during vitellogenesis. At mid-pregnancy, the derived oviparous lineage showed reduced expression of glandular function genes, suppressed immune pathways, increased tissue remodeling signatures, and enhanced angiogenesis relative to the ancestral lineage, mirroring key molecular differences between viviparous and ancestral oviparous uteri. Together, these results demonstrate that the transition to viviparity involves coordinated changes in cell-type-specific regulatory programs and uterine tissue architecture, supporting a stepwise evolutionary transformation of maternal reproductive function.

P212

Reconstructing the habit, development, and ecology of the 407-million-year-old species *Rhynia gwynne-vaughanii*

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The Early Devonian Rhynie chert contains the oldest *in-situ* preserved terrestrial ecosystem. Here, plants and the ecosystem are exceptionally preserved, and cells, rooting structures, and growing substrate can all be seen. *Rhynia gwynne-vaughanii* is an early polysporangiophyte from Rhynie defined by branched aerial axes with hemispherical projections, and vascular tissue with tracheids. However, more work is needed to characterise its growth form and development to better understand its ecological context. By creating a clearer picture of *Rhynia* and its role at Rhynie, we can gain insights into key innovations that led to the diversification of plants during the Devonian. Notably, bryophytes are absent from Rhynie despite being important as pioneer species across many modern ecosystems including those analogous to Rhynie. Due to their absence, I hypothesise that vascular plants at Rhynie, specifically *Rhynia*, were filling the ecological role of bryophytes and acting like mosses from similar environments today. To investigate this hypothesis, I aim to generate the first large-scale, evidence-based reconstruction of *Rhynia* which will allow me to evaluate its growth form and wider habit in the Rhynie chert. Serial peels will be digitally reconstructed using the computer software SPIERS and thin sections of extant mosses selected based on ecology and growth form will be created for comparisons along with fossil blocks containing *Rhynia* axes. Overall, this study will shed more light on the ecological role of *Rhynia*, contribute to our understanding of some of the earliest known land plants and ecosystems, and provide an eco-evo-devo perspective on the absence of bryophytes at Rhynie.

P213

Decoupling phenotypic from genetic pleiotropy during evolution on a complex genotype-phenotype-fitness map

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Pleiotropy is the fact that a gene or mutation can affect several biological functions. It can occur at several scales: ranging from expression in different tissues, to phenotypes during development, up to adult phenotypes. As a result, mutations in pleiotropic genes do not always result in pleiotropic changes at the phenotypes of interest.

This is usually explained by gene modularity, in which mutations in modular enhancers enable tissue-specific variation in gene activity. But another underestimated possibility is that this decoupling also takes place at higher scales of organization. In this work, we tackle this problem using an _in silico_ individual-based model of tooth development and evolution.

In our model, individuals have a realistic genetic architecture based on the regulation of pleiotropic genes by both modular and pleiotropic enhancers. We compute tooth shapes based on gene expression using a classic developmental model, and fitness based on the quality of tooth occlusion. Our model therefore presents a complex genotype-phenotype-fitness map, in which we can evaluate pleiotropy at several scales independently. Using this model, we show that the _effective pleiotropy_ of mutations in pleiotropic genes, i.e. the phenotypic impact of mutations on both teeth, decreases during evolution, and that modular enhancers accumulate comparatively more mutations than pleiotropic ones. This shows how a decoupling between different levels of pleiotropy can evolve through the interaction between the modular and pleiotropic parts of the genome.

Overall, our results show that genetic architecture itself can evolve to buffer the phenotypic effect of mutations in pleiotropic genes.

P214

Developmental variability in serial homologues: dissociability, developmental noise, and standing phenotypic variation

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Understanding how variation is generated and structured is a central goal of evolutionary developmental biology. In quantitative genetics, evolutionary change depends on genetic variance and selection. Research on phenotypic integration and modularity has long attempted to connect quantitative-genetic theory with developmental and functional processes organizing morphological variation. Recent comparative studies show that phenotypic variance can predict macroevolutionary divergence patterns as effectively as genetic variance does, suggesting that developmental mechanisms generating phenotypic variation may also structure genetic variation. This pattern may be explained if development act as an *"imitative epigenotype"*, such that both non-genetic and genetic perturbations produce similar patterns of variation along historically favored directions shaped by past selection. Here, we test this idea using repeated morphological structures. Serially homologous traits reveal how shared developmental programs generate covariation and how their partial dissociation ("dissociability") reflects the partitioning of developmental effects. Bilateral symmetry provides a complementary perspective: differences between left and right homologs primarily reflect stochastic perturbations during development, quantified as fluctuating asymmetry and interpreted as developmental noise. Using damselfly wings (Odonata) as a model system, we combine phenomic approaches with quantitative-genetic theory to estimate phenotypic covariance, fluctuating asymmetry, and dissociability among serial homologues, providing an empirical framework to investigate how developmental variability contributes to covariance structure, modularity and evolvability.

P215

Shedding Light on Beetle Circadian Rhythms

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Biological mechanisms have evolved to enable organisms to keep time and anticipate predictable environmental fluctuations, such as the diurnal light-dark cycles. In bilaterian animals these circadian clocks are encoded as transcription-translation feedback loops that temporally regulate various processes including behavior, development, and physiology. Given their immense diversity, insects serve as an ideal group to study how diverse clock machineries can achieve a ~ 24-hour timekeeping function. We have recently started investigating circadian molecular and behavioral rhythms in Coleoptera. Despite their remarkable diversification, the composition and regulation of beetle clock systems remain understudied.

Using Tribolium castaneum as a model, our work aims to delineate the genetic basis as well as the neural substrates of circadian rhythms (e.g., locomotor behavior). To this end, we create loss-of-function mutations in conserved clock and clock-related genes, whose products may be involved in the generation or synchronization of oscillatory, self-sustained biological processes. In addition, we rely on the power of CRISPR-Cas9 to obtain transgenic lines expressing luciferase reporters that have greatly facilitated the in vivo characterization of circadian mechanisms in flies, mammals, plants and bacteria. Furthermore, together with our collaborators, we have set out to describe the clock neuronal network in the T. castaneum brain using HCR RNA-FISH.

We anticipate that our research could further contribute to our understanding of how insect clock systems have evolved at the molecular, cellular and circuit level, potentially offering adaptive benefits to different species occupying distinct ecological niches.

P216

Ordered Chaos: Photoreceptor Mosaics in the Turquoise Killifish (*Nothobranchius furzeri*) Retina Across Life Stages

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An organism's vision is dependent on the types and organisation of their light-detecting photoreceptor cells. There are two broad photoreceptor types: cones, which are responsible for daytime, colour, and high-acuity vision; and rods, which are responsible for low light vision. Appreciating the photoreceptor types present and how they are arranged – the mosaic – provides key information about the nature of vision in that animal. This work investigates the photoreceptor subtypes and mosaic organisation of the turquoise killifish (*Nothobranchius furzeri*), a diurnal annual killifish that lives in turbid ephemeral pools, across life stages. I first investigated the turquoise killifish genome for rod and cone opsin genes. I identified four classes of cone opsins with varying numbers of orthologues – one *sws1* (ultraviolet; UV), one *sws2* (blue), two *rh2* (green), and three *lws* (red) opsin genes, and one rhodopsin (*rho*) gene. I utilised multiplexed *in situ* hybridisation to determine that that each class of opsin was expressed in the turquoise killifish retina. Young adult (6 weeks post hatching) and larval (newly hatched, 21 days post fertilisation) retinas were both cone-dominant, with red cones as the predominant cone subtype. Larval killifish retinas had regionalisation with a central blue cone-sparse area and a dorsonasal region of significantly increased blue and UV cone density. Adult retinas had an ordered square mosaic, wherein red/green double cones form squares around central red/blue/UV single cones across the retina. This is the first investigation into turquoise killifish photoreceptors and will contribute to our understanding of visual system diversity, including how an animal's retina adapts to life in turbid waters.

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Developmental origin of an evolutionary innovation in a water strider

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Evolutionary innovations have been central to the diversification of Life on Earth and understanding how they originate remains a major question in evolutionary biology. Here, we use the water strider genus *Rhagovelia*, one of the few taxa that has been able to access, specialise, and diversify on a constraining habitat: fast-flowing water. This has been possible thanks to a striking key innovation; a propelling fan at the tip of their mid-leg that acts as a paddle, drastically improving locomotion. To retrace the developmental origin of this fan, we first used *de novo* genome sequencing and showed that *Rhagovelia*-restricted paralogs *gsha1*/*gsha2* emerged from a successive retrotransposition and duplication of *mogsha*. RNA-interference showed that *gsha1*/*gsha2* are necessary for fan development. Then, we used HCR-FISH to show that these genes are exclusively expressed in a single giant cell located only at the tip of fan-bearing legs that seems to orchestrate fan development throughout embryogenesis. We also used RNA-interference to show that the Hox genes *Ubx* and *Scr* block the giant cell and fan development in fore- and rear-legs, restricting it to mid-legs. We have now produced a single nuclei RNA-seq dataset to characterise the transcriptomic profile of this giant cell and to retrace its developmental origin. This study highlights that taxon-restricted genes and cells, and their interactions with pre-existing developmental programs, can play a fundamental role in the emergence of evolutionary innovations. It exemplifies well that morphological innovations are often accompanied by molecular and cellular novelties.

P218

Developmental single-cell transcriptomics of the lamprey: insights into ancestral vertebrate cell types

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The origin of vertebrates represents a major evolutionary transition within the animal kingdom, and it was underlied by the occurrence of key complex phenotypic novelties defining the group, such as a tripartite brain, neural crest cells and an endothelium among others. These innovations required the rewiring of complex gene regulatory networks that ultimately gave rise to a vast diversity of new cell types in the vertebrate ancestors. Further elaboration of these complex traits probably had a profound impact in the diversification of the group, and it was also accompanied by the generation of specific cell types and tissues. However, despite its importance and the deep research aiming at elucidating the vertebrate origins, what cell types were present in the last common ancestor of vertebrates, and how their origin sparked the appearance of vertebrate innovations, remains a mystery.Here, after generating a new high-quality, chromosome-level genome assembly with an improved and accurate gene annotation, we present the first developmental single-cell transcriptomics analysis of a representative of cyclostomes (jawless vertebrates), the Arctic lamprey, Lethenteron camtschaticum. The understanding of the developmental process of the lamprey at the cellular level enables us to draw an unprecedented picture of the last common ancestor of crown vertebrates and the cellular hallmarks behind the origin of vertebrate traits.

P219

Patterns of odour and colour biosynthesis in sexually deceptive *Ophrys* orchids

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The adaptation of *Ophrys* species to their specific pollinators facilitates an extraordinary pollination strategy. *Ophrys* orchids use sexual deception, whereby the flowers mimic the females of the pollinator species in terms of odour, colour and tactile traits to induce pseudocopulation of the male pollinator without providing any reward. Floral odour plays a key role in this pollinator interaction, because odour chemically mimics the virgin female bee sex pheromone. This pseudo-pheromone mainly consists of saturated and monounsaturated hydrocarbons (C21-C31), i.e. alkanes and alkenes, derived from very long chain fatty acids (VLCFA). In addition to olfactory mimicry, floral pigmentation by anthocyanins and carotenoids contributes to the visual resemblance of the bee. We present data suggesting that the production of odour and pigments is regulated during flower development and varies between species and different floral tissues. Moreover, we identify and evaluate candidate genes underlying these biochemical processes mediating specific plant-pollinator interactions using chemical, molecular and transcriptomic methods.

P220

Developmental basis of the diversification of an evolutionary innovation

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A major aim in evolutionary biology is to understand the emergence of evolutionary innovations, new structures allowing access to novel niches. The propelling fan in Rhagoveliinae water striders, required for locomotion in fast-flowing streams, is one example of such innovation.This cuticular structure located at the tip of legs increases the surface of contact with water and improves rowing efficiency. In this lineage, the fan is associated with access of the Rhagoveliinae to fast-flowing streams, a previously inaccessible niche. In the hyperdiverse genus *Rhagovelia*, fan-specific paralogous genes (*mogsha*/*gsha*) expressed in a single giant cell mediate the development of this novelty. Species of the sister genus *Tetraripis* alsopossess fans, albeit in both the mid- and hind-legs. *Tetraripis* and *Rhagovelia* fans are thought to have a common origin, although the two have dissimilar morphologies. We sequenced the genome of *Tetraripis* to reconstruct the genomic architecture of the *mogsha*/*gsha* gene family. Surprisingly, we reconstructed *mogsha* but we did not find any *gsha* gene. Moreover, using HCR-FISH, we found that *mogsha* retained its ancestral expression domain (similar to other water strider lineages), made of 4-6 normal-sized cells. The giant cell observed in *Rhagovelia* was absent in *Tetraripis*. Our results reveal that although the propelling fan is conserved within the Rhagoveliinae lineage, the genetic and cellular bases of fan development diverged between the two sister genera along with its phenotypic structure and organization.

P221

Testing for the origin of seed gene networks in the fern archegonium

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The evolution of seeds from ancestral seedless plants is a long-standing question in evolutionary biology, with the Monilophytes (ferns and relatives) the closest living seedless relatives to seed bearing plants [1]. Recent RNA-seq analysis of reproductive organ development in *Ceratopteris richardii*, a fern genetic model, identified potentially conserved gene networks in the gametophyte archegonium (egg chamber) that may have been important in the evolution of seeds [2]. The aim of this project is to test whether seed gene networks are conserved with the fern archegonium, potentially elucidating how the seed evolved from the seedless plant. Firstly, we will test if new seed genes can be predicted from gene networks in the fern archegonium. To do this we will look for unannotated *Arabidopsis thaliana* genes with orthologs in *the C. richardii* archegonium. These will be explored through enrichment testing, gene expression profiling and functional analysis to determine whether these genes are truly novel seed regulators. Secondly, we will test for reproductive roles of candidate *C. richardii* genes with orthologs in *A. thaliana* seed/ovule formation. The accuracy of these predicted fern genes will be validated with PCR and subsequent sequencing. Gene expression profiling will be used to determine tissue specificity, and functional analysis used to test if these genes function in *C. richardii* reproductive development as predicted. References 1. Leebens-Mack *et al.* 2019, https://doi.org/10.1038/s41586-019-1693-2

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P224

Shared logic between learning and evolution: new approaches toward predictive evo-devo

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Organismal evolution resembles learning processes in many respects: for example, both search for better solutions or better-fitted phenotypes through iterative trial and error. Although this analogy has been noted for decades, it has largely remained conceptual and underexplored as a source of developing practical research approaches. However, recent advances in machine learning are beginning to change this situation by being able to replace abstract concepts of learning with formalizable implementations. Here, we revisit the analogous relationship between learning and evolution using examples from modern machine learning. One exciting implication from the analogy is that models learning the evolution of biological organisms or phenotypes may be able to predict their evolutionary outcomes. For example, we propose approaches inspired by this analogy may open new avenues for studying general relationships between development and evolution, such as to identify intrinsic factors from developmental processes that could bias phenotypic evolution using predictive models. Importantly, we emphasize the importance of moving beyond case-by-case predictions using machine-learning models, and discuss how white-box, interpretable machine-learning approaches may help overcome these limitations. More broadly, this perspective motivates a shift from case-specific predictions toward identifying common biological factors or candidate laws for predicting phenotypic evolution and expanding the extended modern synthesis.

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Mayfly Model for Studying the Origin of Insect Wings

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The origin of insect wings remains one of the central questions in Evo-Devo. Mayflies (Ephemeroptera) represent one of the earliest diverging lineages of winged insects and retain ancestral wing features that have remained largely unchanged since the Carboniferous. Therefore, they provide a key comparative system for understanding the developmental and evolutionary mechanisms underlying wing emergence. Despite their significance, mayflies have long remained inaccessible for developmental genetics due to the lack of laboratory rearing systems and genomic resources. To address these limitations, we are establishing *Ephemera japonica* as a new experimental model for investigating the early evolution of winged insects. We developed a stable laboratory rearing system that enables continuous acquisition of embryos and nymphs, and we generated extensive genomic resources, including a high-quality whole-genome assembly and transcriptome dataset. To facilitate functional studies, we optimized RNA interference (RNAi) for gene knockdown and attempted CRISPR/Cas9-mediated gene knockout. These approaches represent some of the first functional genetic tools available for Ephemeroptera and open the door to dissecting the developmental mechanisms of ancestral winged insects. Furthermore, we initiated comparative analyses with the wingless order Zygentoma to directly test hypotheses regarding the origin and evolution of insect wings. By integrating genomic, developmental, and functional approaches, this work provides new opportunities for revisiting longstanding questions about how wings first arose in insects.

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Beyond model species: a collection of living planarians for the comparative analysis of regeneration

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Planarians exhibit extraordinary regenerative abilities and have become a powerful system for studying animal regeneration. Yet regenerative capacity varies widely across species—from whole-body regeneration in *Schmidtea mediterranea* to limited regeneration in *Dendrocoelum lacteum*. This diversity provides a natural framework to investigate the molecular and evolutionary bases of regenerative traits. However, most mechanistic insights currently derive from only two highly regenerative models, *S. mediterranea* and *Dugesia japonica*. Understanding why regeneration is robust in some species but restricted in others requires incorporating poorly regenerating species into experimental research. At the Max Planck Institute for Multidisciplinary Science, we established a living collection of planarian species spanning a wide spectrum of regenerative capacities. Through targeted sampling in biodiversity hotspots worldwide and systematic optimisation of husbandry conditions (temperature, diet, and water parameters), we currently maintain more than 50 strains. Integrative taxonomic analyses—combining classical morphology, DNA barcoding, and phylogenetics—with standardised head-regeneration assays revealed multiple head-regeneration-deficient species and resolved their phylogenetic relationships. We further assessed experimental tractability by testing RNA interference and measuring ploidy levels and genome sizes to prioritise species for genome sequencing. These analyses identified a regeneration-deficient flagship species suitable for in-depth mechanistic investigation. Systematic phenotyping also uncovered biologically informative cases, including a *Schmidtea* polychroa subcollection to investigate the consequences of polyploidy and *Dugesia subtentaculata* strains with distinct reproductive modes to explore how life cycles shape genetic diversity and somatic mosaicism. Together, this living collection establishes a long-term, open resource for the planarian community, enabling comparative regeneration research beyond established model systems.

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Decoding regulatory evolution across developmental lineages of skeletogenic cells

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The evolution of skeletogenic cells has been fundamental to the success and diversification of vertebrates. Depending on their anatomical location, these cells originate from distinct embryonic lineages and have acquired skeletogenic identity at different evolutionary time points, presenting a rare case of cell-type convergence. While skeletogenic cells share a core transcriptional program, they retain lineage-specific transcription factors and cis-regulatory landscapes inherited from their progenitors. As a result, the same cell type located in different anatomical regions, possesses distinct trans- and cis-regulatory signatures. Such an arrangement raises the question of whether these divergent regulatory signatures make different parts of the skeleton independent targets of evolutionary selection.

To address this question, we investigate the evolution of regulatory sequences in skeletogenic cells derived from different embryonic origins and test whether these sequences evolve at similar rates. We use integrated single-nucleus RNA-seq and ATAC-seq to characterize cell type-specific regulatory landscapes in skeletogenic cells from three anatomical regions across three bird species. Using these multimodal datasets, we identify putative origin-specific enhancers and compare their structure and evolutionary rates across lineages. The combination of multimodal single-nucleus technology and the unique model of convergent skeletogenic cells allows us to quantify evolutionary changes in regulatory sequences across phylogeny and explore how distinct developmental histories shape regulatory architectures.

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From Cells to Structure: Spatial gene expression during plug formation in medaka wild relatives

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In evolutionary biology, one of the central questions is how novel traits arise that enable species to exploit new ecological niches. One example of such a trait is the transient “plug” structure in pelvic brooding ricefishes from Sulawesi, Indonesia—wild relatives of medaka (Beloniformes; Adrianichthyiedae). The plug forms in the female gonoduct after spawning and anchors egg-attaching filaments, thereby facilitating external egg retention. Recent research on *Oryzias eversi* suggests that plug formation may involve a modified inflammatory response. To investigate the developmental and evolutionary basis of the plug, we profiled the spatial gene expression landscape of the female reproductive system of *O. eversi* across four time points. We identified distinct gene expression clusters that correspond to the morphological organization of the reproductive system, including clusters specific to later brooding stages. Notably, key immune-related marker genes (e.g., il1b, tgfb1, SMS, VMP1, and MRC1) are specifically expressed in the developing plug and surrounding tissue. Our results highlight the molecular complexity underlying pelvic brooding and support the hypothesis that inflammatory pathways have been co-opted for the evolution of a novel reproductive structure.

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Conserved roles of nitric oxide in regulating cnidarian larval behaviour and developmental progression

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Nitric oxide (NO) is a highly diffusible signalling molecule that regulates diverse physiological and developmental processes across Metazoa, yet its mechanistic roles in cnidarian larvae remain poorly defined. Because settlement and metamorphosis constitute pivotal developmental transitions, understanding how NO modulates these processes is essential. Using DAF FM fluorescence, we mapped NO production across developmental stages in *Nematostella vectensis*, uncovering a predominantly non neuronal, gland associated expression pattern with additional localisation in the nerve net, suggesting a multifunctionality. To assess the functional significance of this distribution, we combined pathway targeted pharmacology with quantitative behavioural and developmental analyses to examine how NO signalling regulates larval locomotion and the transition to settlement. Across planula stage, we manipulated the pathway using NOS inhibitors, an sGC blocker, NO donors, and an NO scavenger. These manipulations yielded clear, node specific and dose dependent effects: NOS inhibition consistently reduced settlement, while NO donors accelerated settlement with distinct dose response profiles. Video tracking revealed corresponding shifts in swimming speed, path length, displacement, and straightness, directly linking behavioural changes to altered ciliary structure. Together, our findings identify NO signalling as a tunable regulator of cnidarian larval behaviour, ciliary physiology, and developmental competence, suggesting that NOs regulatory roles are deeply conserved across Metazoa and shedding new light on the evolution of developmental signalling systems.

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From head to tail: How an unusual annelid builds its trunk

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Phylum Annelida —the segmented worms— are a group of spiralians that display impressive diversity in both morphology and life cycle. *Owenia fusiformis* is an early branching annelid, sister to the rest of the annelids, which exhibits 'maximal' indirect development —delaying the onset of trunk growth and forming morphologically divergent 'head–larvae'. This delay is an example of a heterochronic shift, in which changes to the timing and tempo of developmental trajectories can facilitate diversification of both life cycles and body plans. *Owenia's* divergent morphology and early branching phylogenetic position make it an informative model system for investigating evolutionary–developmental questions. One such long–standing question is that of segmentation: annelids provide the strictest examples of a metameric body plan, but the genetic toolkit underpinning their segment formation remains enigmatic. We have yet to identify a complete shared suite of ancestral annelid segmentation genes, a finding which would give useful insights into the origins of segmentation throughout Bilateria. To this end, I have generated the first high-resolution 'morphological atlas' of 16 time points across *O. fusiformis* development, enabling accurate developmental staging. This has provided a detailed view of tissue and trunk rudiment development and fundamental morphological context to ground comparative developmental studies. We have also explored gene expression dynamics during trunk segmentation, guided by bulk RNA-seq datasets, to illustrate segment formation in an early–diverging annelid. Characterising larval morphology and trunk rudiment development in *O. fusiformis* will further clarify the role of developmental heterochrony as an evolutionary mechanism and help reconstruct ancestral annelid segmentation programs.

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How did the mouse upper molar evolve a new shape?

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Molars are complex teeth with multiple cusps that are unique to mammals. Their evolutionary history is well known, making them an ideal system for studying the evolution of development. Their shape varies widely depending on diet. The rapid and extensive expansion of molar forms suggests that the developmental mechanisms are highly modular. During their evolution, mice acquired a very specific morphology of the upper molars, with the appearance of two additional cusps on the lingual surface. In contrast, changes in the lower molar were minimal: the number of cusps was retained and the modifications were limited to the connections between the cusps in order to allow for the new occlusion. In the lab we are interested to understand how the upper molar changed its form independently from the lower one. The upper and lower molars of hamsters, which represent the dental morphology of mouse ancestors, are used as controls to study this evolutionary novelty. In a previous study, we discovered that this new morphology resulted from a combination of changes in the dynamics and size of signalling centres, in addition to changes in the regulation of the bucco-lingual axis. We found that changes in signalling centres are common to both molars of a species, while regulation of the bucco-lingual axis is more specific to upper molars. As part of my PHD I am characterizing further these changes and try to understand how they combine using an integrative and comparative systems biology approach.

P234

Deciphering unique bat wing development with comparative genomics and transcriptomics at the cell population level

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Self-powered flight has evolved only four times, three times in vertebrates by modifying the development of forelimbs into wings. Bats are the latest group of animals, and the only mammals, in which flight has evolved. The bat wing is characterized by an extreme elongation of the autopodial (hand) bones II to V and a membrane connecting them, altogether a unique form of vertebrate wing. By contrast, the bat hindlimbs are not very different from those of mouse. Recently, we showed the cellular and molecular origin of the bat wing membrane. Here, comparing developmental stages, fore- and hindlimbs, as well as cross-species data, we study the developmental changes giving rise to this particular anatomy, specifically the elongation of bat wing autopodial bones. Using comparative single-cell and genomics data, we investigate the genomic changes, as well as regulatory modifications that give rise to cell-type- and cell-population-specific transcriptomic changes. We have found a few candidate regulatory regions and genes which seem to be responsible for the specific elongation of modular groups of autopodial bones. Another example of evolutionary repurposing, replicating proximal specific factors in order to produce a radically different distal morphology. Our results clarify molecular mechanisms of modular limb development and illustrate relatively simple molecular evolutionary processes giving rise to novel, extreme morphologies.

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Comparative single-cell transcriptomics across deeply divergent sponge species

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Calcareous sponges represent one of the four major classes of Porifera. They have one of the simplest animal body plans while producing distinctive extracellular calcitic spicules that form their skeleton. Despite their importance for comparative studies of early metazoan evolution, genomic resources for Calcarea remain scarce and rarely reach chromosome-level completeness, and transcriptomic data are limited. Here, we present a chromosome-level genome assembly for the calcareous sponge *Clathrina clathrus*. We identify extensive genome reorganization, including the loss of most ancestral poriferan linkage groups, likely driven by transposable element (TE) accumulation, which may also have contributed to genome expansion (~800 Mbp). The protein-coding repertoire comprises ~30,000 genes, many organized in tandemly duplicated clusters. In addition, we generated single-cell RNA-seq atlases for representatives of three major sponge classes (Calcarea, Homoscleromorpha, and Demospongiae): *C. clathrus*, *Oscarella lobularis*, and *Axinella damicornis*. Across 100,000 profiled cells, we recover major sponge cell types and class-specific specializations. Comparative transcriptomic analyses reveal both striking similarities and divergences in cell-type identity programs across these lineages. Additionally, we uncover numerous lineage-specific innovations, including enrichment of calcarean-specific genes, biomineralization-related specialization of the calcarean toolkit, and the presence of endosymbiotic bacteria within specialized cell types. Together, these resources provide a foundation for comparative genomics of cell types in early metazoans and highlight the power of integrating chromosome-scale genomics with single-cell transcriptomics to illuminate the evolution of cellular diversity within metazoans.

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Deep Evolutionary Conservation of *Hmx* Transcription Factors and their Role in Vertebrate Sensory Ganglia

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The evolutionary origin of vertebrates included innovations in sensory processing crucial for the development of a predatory lifestyle. Central to these innovations are cranial sensory ganglia (CSG), which arise predominantly from cranial placodes. The homeobox transcription factors *Hmx* are key regulators of vertebrate sensory ganglia development and play a conserved role in neural differentiation. In the tunicate *Ciona intestinalis*, *Hmx* drives the differentiation of bipolar tail neurons, suggesting homology of these cells with vertebrate CSG. Comparative genomics has revealed two conserved non-coding elements (CNEs), associated with the *Hmx3-Hmx2* locus. These CNEs are exceptional for their size, their deep evolutionary conservation across jawed and jawless vertebrates, and tandem duplication in the stem-vertebrate lineage. Remarkably, these enhancers exhibit robust regulatory function in both vertebrates and invertebrate chordate, indicating that the upstream regulatory network governing Hmx predates vertebrates.

By comparing downstream *Hmx* targets in the central and peripheral nervous system of both *Ciona* and chicken embryos, we are trying to uncover the mechanisms underlying the conserved regulation of *Hmx*. This cross-species approach will allow us to distinguish ancestral regulatory functions from lineage-specific adaptations, and to reconstruct the core gene network controlled by *Hmx* in early chordates. These findings will also provide critical insights into how deeply conserved regulatory systems contributed to vertebrate sensory innovations.

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Vertebrate-like *Hox* gene regulation in annelid worms

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The temporally and spatially collinear expression of *Hox* genes along the anteroposterior axis is a hallmark of animal development and a crucial evolutionary milestone linked to the diversification of animal body plans and life cycles. However, how their exquisite, coordinated expression originated remains unknown because we lack an understanding of the molecular mechanisms and genomic regulatory principles controlling gene expression in most animals. Here, we use high-resolution Micro-C datasets spanning the life cycle of two segmented worms (Annelida) to demonstrate that the formation of a Topologically Associating Domain (TAD) correlates with distinct timings of *Hox* gene activation. In both species, an architectural stripe along the posterior TAD border marks the presence of a 'global control region' that interacts with all *Hox* genes. Moreover, the formation of nested sub-TADs correlates with the temporal collinearity of *Hox* genes in one species. Histone post-translational modification profiling reveals opposing dynamics of polycomb-mediated repressive (H3K27me3) and permissive (H3K4me3; H3K4me1) marks, linked to activation of the 'global control region'. Together, this project shifts current thinking about the evolution of genome regulation and *Hox* gene expression in animals, proposing a new model that mechanistically links chromatin conformation dynamics to the unfolding of *Hox* collinearity in a non-vertebrate animal.

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Integration and evolution of the cichlid pharyngeal skeleton: Tinkering with a palimpsest

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The evolutionary success of animals has been facilitated by the expansion and diversification of serially homologous elements. The vertebrate pharyngeal skeleton exemplifies this process. Evolved from a uniform series of cartilaginous rods, different elements have evolved distinct shapes and functions over time. This process implies that the evolution of the pharyngeal skeleton involved the decoupling of its constituent elements; however, to ensure functionality a level of connectivity must be maintained. Thus, the evolution of the pharyngeal skeleton is likely influenced by a balance of diversifying and unifying forces. We probe these ideas using East African cichlid fishes. Shape analyses in a sample of 75 ecologically diverse species and a large F 5 hybrid population, showed a conspicuous lack of independence. Nearly all elements were correlated, a pattern reflected in the genotype-phenotype map and associated with co- expression of the candidate gene, smad7. Results are discussed in the context of the palimpsest model, whereby trait covariation is the result of numerous, hierarchical forces of covariation. Given its broad regulatory roles, we suggest that smad7 may be involved in several layers of the palimpsest, providing molecular insights into how serially homologous elements may diversify while also maintaining functionality.

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Modulation of *Hydra vulgaris* microbiota through Targeted Phage-Based Photodynamic Therapy

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The interaction between host and microbiota is fundamental for maintaining biological homeostasis and health. As the need for precise microbial control grows, the development of sophisticated tools for microbiome manipulation has become essential. We have developed an innovative platform that merges the precision of the M13 bacteriophage with photodynamic activity. By genetically engineering the phage to recognize specific bacterial components and conjugating it with light-responsive thiophenes, we developed a system capable of localized bacterial depletion and evaluated the safety and efficacy of this platform using *Hydra vulgaris* as a model (1). Since the adult *Hydra vulgaris* microbiota consists almost exclusively of Gram-negative bacteria, this platform was initially tested using phages specifically capable of recognizing this bacterial class. Our multifunctional platform, engineered to target bacterial lipopolysaccharides present on the Gram-negative cell wall, demonstrated exceptional biocompatibility at nanomolar concentrations in absence of light stimulation. Remarkably, in a time and dose dependent fashion, it showed the capability to release reactive oxygen species upon light stimulation, and to significantly deplete the Hydra microbiota. These promising results establish a foundation for future research, where we plan to produce phages tailored to specific bacterial populations to investigate how their specific absence impacts the host's physiology.

Reference: 1. Costantini PE, Saporetti R, Iencharelli M, Flammini S, Montrone M, Sanità G, et al. Phage Templated Synthesis of Targeted Photoactive 1D Thiophene Nanoparticles. Small. 2025 Jan 5;21(1).

P240

Reproduction emerges from ecological interactions at the onset of multicellularity

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At the origin of multicellularity, genetic programs used by single-celled organisms to interact with their environment were organised into a coherent developmental program. While previous work has clarified the selective advantages of simple multicellularity, the origin of development remains unclear. We investigate the ecological origins of a fundamental developmental process — multicellular reproduction — using a computational model where cells forage in a structured environment, evolve adhesion, and regulate environmental responses through a GRN. Although not pre-specified or explicitly selected for, diverse multicellular life cycles emerge in the model across different ecological settings due to their ability to regulate collective migration. Among these are life cycles in which the multicellular groups reproduce through single-celled propagules — the most prevalent reproductive strategy in multicellular life — which evolve for dispersal. These propagules are formed through the activation of a regulatory program previously used by the unicellular ancestors of the lineage to interact with neighbours in their ecological context. During the evolution of multicellularity, this program was co-opted from the ancestral GRN and repurposed to regulate reproduction. Furthermore, we show that the evolution of collective migration can itself be emergent in the model. Despite the fact individual cells cannot migrate on their own, by mechanically interacting with their neighbors they can integrate spatial information at the level of the multicellular cluster, leading to the rise of collective directional migration.

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Non-linearities, cell signalling and the paradox between robustness and evolvability: A mechanism for safely exploring novel directions of shape change during development

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Robustness is a common feature of many developmental systems. It helps prevent the bombardment of small perturbations an organism is exposed to from causing deleterious effects, a feature that is critical for species survivability. However, genetic or environmental perturbations that lead to change are the cornerstone of evolution, so while a system must retain a certain level of robustness it must also allow for change to avoid catastrophic canalisation. Critical cell signalling pathways that are reused throughout development, such as Hedgehog (Hh) signalling may tend to exhibit high degrees of robustness to variation in output due to selection against the widespread effects of their functional perturbation. We investigated the robustness of Hh signalling response in zebrafish by inhibiting Hh signalling during a specific timepoint of craniofacial development with varying concentrations of the small molecule inhibitor cyclopamine. Using 3D geometric morphometrics, we uncovered a clear threshold amount of Hh inhibition that can generate severe phenotypes and further defined a non-linear relationship between Hh signalling output and craniofacial morphology. Interestingly, this non-linear relationship also appears to allow for new directions of shape change that differ from the major “Hh loss” axis. This is particularly noticeable at below-threshold levels of inhibition, where the phenotypic changes are subtle and non-severe, but the direction is distinctly different. This change in covariance structure along the non-linear response curve suggests that the probing of limits of robustness for developmental processes may be a mechanism for overcoming developmentally constrained covariation structure to establish novel directions of evolutionary change.

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Developing a Visually Detectable Biosensor in the Fern *Pteris vittata* to monitor soil arsenic contamination

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Arsenic contamination of the environment is a widespread issue threatening ecosystems, food safety, and human health. Current detection methods are time-consuming, costly, and highly technical, thus limiting large-scale screening. Self-reporting biosensors potentially represent a cost-effective alternative that would allow *in vivo* environmental monitoring. Plants offer suitable biosensing platforms, but most species cannot survive the arsenic concentrations in contaminated areas. However, the fern *Pteris vittata* can, featuring naturally evolved hyperaccumulation mechanisms for arsenic uptake, translocation, and sequestration in its fronds, that confer tolerance at concentrations lethal to other species [1]. We aim to leverage *P. vittata*'s unique traits and the YAP8-based arsenic-sensing system from *Saccharomyces cerevisiae* to develop a visually detectable arsenic biosensor, producing a green-to-red colour change via arsenic-induced activation of the live-tissue betalain reporter *RUBY*. Since laboratory cultivation of *P. vittata* and genetic transformation both remain challenging [2], developing the necessary methodologies for these are essential first steps. We have established protocols for near full life-cycle propagation under sterile *in vitro* conditions, including spore germination, gametophyte cultivation, and callus-derived sporophyte differentiation. Although we could not replicate *Agrobacterium tumefaciens*-mediated spore transformation, successful induction of callus provides the foundation for microparticle bombardment instead, adapting the efficient transformation strategy established for the fern model *Ceratopteris richardii* [3]. Future work is focused on optimizing transformation, sensor component testing and selection strategies to successfully establish the arsenic biosensor in *P. vittata*.

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Boundary issues: investigating the role of LIGULELESS 2 in maize leaf development

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Plant organ shape is intrinsically linked to its function. As these functions differ from organ-to-organ and species-to-species, there is a huge diversity of plant organ forms, but each deriving from amorphous groups of undifferentiated cells. To achieve diverse organ shapes, developmental programs are directed by patterning mechanisms and signals which influence tissue identity and growth. In grass species, like maize, proximal-distal (P-D) patterning in leaves gives rise to distinct sheath and blade regions, bounded by specialised auricle and ligule structures. Our research focuses on understanding the gene networks that control the blade-sheath boundary formation across grass species, with a focus on the BZIP transcription factor LIGULELESS2 (LG2). In maize, mutants of *LG2* have upright leaves due to a diffuse boundary region and defective auricle and ligule development. While LG2 is known to control the expression of the key ligule development gene, *LIGULELESS1* (*LG1*), less is known about how LG2 is itself regulated. Interestingly, despite the general expression of *LG2* leaf tissues, LG2 protein localises specifically to the boundary region during early ligule development, which we hypothesise is driven by post-translational modification (PTM). To study the players involved in this potential PTM, we investigated the protein-protein interactions of LG2 *in planta* and identified candidate proteins including a number of E3 ligases and the TASSELS ON UPPER EARS1 (TRU1), a maize BOP homolog which is known to act as a sheath identity marker. These data could present a possible link between known P-D patterning factors in maize leaves and components of the ubiquitination pathway which could define LG2 protein localization.

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Dating the growth hormone gene duplication event in passerine birds

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Passeriformes (passerines), a clade of birds comprising over half of extant avian diversity, share a unique and unreversed duplication of the growth hormone (GH) gene. Although this feature sets them apart from all other birds, its potential role in the remarkable diversification of passerines remains underexplored. Here, we estimated the timing of this gene duplication event in order to assess its potential relevance to passerine diversification by dating a fossil-calibrated gene tree using a Bayesian phylogenetic approach (MCMCTree). Our results suggest that this event occurred in the early Eocene, coinciding with recent estimates for the origin of Passeriformes, preceding the geographic expansion and radiation of major passerine lineages. We also discuss challenges and approaches to improving the precision of the time line of gene duplication events.

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Molecular Evolution of Floral Diffraction Gratings in Hibiscus species

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Morphology and colour of petal surface play key roles in mediating interaction between pollinators and flowers. Diffraction gratings made of cuticular striations, which produce iridescent optical effects that are salient to model pollinator bumblebees, have been found on petal epidermal cells of diverse species. Previous studies in *Hibiscus trionum* have suggested that changes in mechanical properties of cuticle proper and cell wall during development contribute substantially to formation of diffraction gratings. However, how genes cooperate to regulate material properties during petal development remains to be elucidated. Here we analysed gene expression across different developmental stages of *H. trionum* petals to identify candidates whose expression profiles correlate with changes in material property. Floral diffraction grating formation was influenced in *H. trionum* plants with candidate genes manipulated. Phylogenetic and syntenic analyses of the candidate genes revealed duplication in Hibiscus species, and expression analyses of homologous genes in Hibiscus species without floral diffraction gratings further suggested alterations in cis-regulatory elements or upstream regulators of these genes. Together, our work sheds light on understanding molecular evolution of the floral diffraction trait in Hibiscus species.

P249

Nuclear envelope breakdown in the ichthyosporean *Chromosphaera perkinsii*

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All eukaryotes house their genetic material within a double-membrane structure, the nuclear envelope. In some organisms, including animals and plants, breakdown of the nuclear envelope is required during mitosis to allow access of the mitotic spindle to chromosomes (open mitosis). In fungi, the nuclear envelope is retained during mitosis and an intranuclear spindle is built instead (closed mitosis). Within holozoa, the clade including animals and their closest relatives, both of these mitotic strategies are employed as well as intermediate forms that are neither fully open nor closed. Even within highly related groups of organisms, mitotic strategies can vary wildly. An example of this is the ichthyosporea, a holozoan group that have multicellular stages as part of their lifecycles. Species in this group such as *Creolimax fragrantissima* and *Sphaeroforma arctica* undergo coenocytic development producing multinucleated cells have a multinucleated lifecycle and have been shown to favour closed mitosis. By contrast, *Chromosphaera perkinsii*, undergoes palintomic division and breaks down its nuclear envelope during mitosis. Using an expansion microscopy-based approach, which allows for labelling of microtubules, DNA and lipids, we explore the temporal order of cleavage division across the development of *Chromosphaera*. To enable visualisation of nuclear envelope breakdown, which has previously been challenging, we identify a novel marker for the nuclear envelope. Using this approach, we find that *Chromosphaera* switches from a fully open mitosis under unicellular conditions to an intermediate strategy as multicellular colonies are formed, indicating that mitotic strategies can be augmented depending on the developmental stage of an organism.

P250

Evolution of developmental gene regulatory networks with an emphasis on flowering plant carpels

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Developmental gene regulatory networks (GRNs) are succumbing evolutionary processes and dynamics. Studying these GRNs provides insights into how plants evolved and adapted to different environments. The spatio-temporal regulation of gene expression during the development of plant organs underlies interaction network dynamics, which are yet to be fully understood. Carpels, the female reproductive organs of angiosperms, are integral for fruit development, marking an evolutionary innovation in flowering plants that grounds biodiversity. Prior studies of TF interactions in carpels in rice, tomato and Arabidopsis have shown networks of protein-protein interactions (PPIs), but due to dataset size and missing *in vitro* research are insufficient for fully understanding the dynamics in the gene regulatory networks. We have detailed transciptome data of *Eschscholzia californica* (Californian poppy). Californian poppy belongs to the evolutionary early diverging Ranunculales and can the TF interaction landscape can help proposing the GRNs in angiosperm most recent common ancestor. To test the long-known hypotheses on the evolution of protein-protein interactions and perform PPI network analysis, a large-scale semiautomatic Yeast-2-Hybrid assay is used. The interaction data is used to identify highly connected hub gene proteins. The development of sexual reproduction via flowers was groundbreaking for the success of land plants. Studying development-stage-specific interactions can help understand the molecular principle of carpel development. With this project, we are a step closer to fully understanding the protein-protein interaction dynamics in the gene regulatory network in flowers, thereby solving questions surrounding the evolution of land plants.

P251

Cell type atlas of the Mnemiopsis apical organ

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The ctenophore apical organ is a domed structure with bundles of cilia supporting statocytes. In addition to sensing gravity, it also contains putative light and pressure sensors. However, functions of most cells are still unknown. Here we use a serial section scanning electron microscopy dataset to describe cell types in the apical organ of the 5-day-old Mnemiopsis leidyi cydippid larva. We describe 17 cell types, and compare the shape and number of various organelles, such as mitochondria and cillia. The longest cilia were found in balancer cells which form bundles to hold statocysts and are thus involved in sensing gravity. Interestingly, very long cilia were also found in the newly described intracellular multiciliated cells with multiple sunken cilia which do not exit the cell. The function of these cells remains unclear. We describe two neuronal cell types - the subepithelial nerve net, and the syncytial synaptic neurons. The syncytial synaptic neuron cells have 10 times more mitochondria than most other cell types, most of which are associated with synapses. The only other synapse bearing cell type - bridge cells - contain far fewer mitochondria and synapses. Furthermore, synapses in bridge cells are concentrated in the proximal parts of the cell, while being spread throughout syncytial synaptic neurons. Interestingly, the syncytial synaptic neurons do not receive any synaptic input from putative sensory cells.

P252

Does the tail bud contribute to posterior gut formation in chordates? Evidence from amphioxus

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Following gastrulation, vertebrate embryos elongate their body axis through a combination of tissue rearrangements (mainly convergent extension) and the generation of new cells from a posterior proliferation centre located within the tail bud, whose activity is regulated by multiple signalling pathways, most notably Wnt and BMP. The vertebrate tail bud has been extensively studied. However, most work has focused on the gene regulatory network underlying the differentiation of a subset of bipotent cells, the neuromesodermal progenitors. In contrast, the generation of endoderm from tail bud cells has received comparatively little attention, although it has been reported across all vertebrate classes. Moreover, the structure and function of the tail bud in invertebrate chordates remain poorly characterised, limiting our understanding of its evolutionary origins in vertebrates. Here, we investigate the tail bud of developing amphioxus, an early-branching chordate that, owing to its relatively slow morphological and genomic evolution, represents a useful proxy for the ancestral chordate condition. Using a combination of bulk and single-cell RNA sequencing, high-resolution hybridisation chain reaction, EdU labelling, and pharmacological perturbations, we provide a morpho-molecular characterisation of posterior cell populations in amphioxus embryos and present evidence that tail bud cells contribute to the formation of posterior endoderm. This suggests that endodermal contribution from posterior growth zones may represent an ancestral feature of chordate axis elongation, a feature that has so far largely been ignored in vertebrates.

P253

Tracing the roots of Neural Crest in Amphioxus

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Neural crest(NC) has been proposed as a vertebrate innovation that had its main impact on remodelling the "New head". This transformation involved the emergence and development of the cranium, which protects the newly evolved brain. It is linked to a major evolutionary shift from filter feeding to active predation. Recently, a new cell population has been identified in the head region of amphioxus Branchiostoma floridae, a basal chordate, which has features similar to that of the NC in vertebrates. With that comes the intriguing questions of where do these cells come from and what do they lead to, in the animal. In this project, we aim to answer these questions using single-cell sequencing, comparative gene expression analysis, lineage tracing techniques and trans-species transgenesis.

P254

Cell autonomy, plasticity and patterning evolution: fish ionocytes as an experimental paradigm

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Cell type identity is broadly conserved across many animal phylogenies, yet the developmental programs governing traits such as cell number, spatial patterning and environmental responsiveness can diverge substantially even among closely related species. However, whether such variation is driven by shifts in the degree of cell autonomy involved in such programs is yet a poorly understood concept.

We address this question by studying skin ionocytes in different fish species of the *Oryzias* group as a comparative model. Ionocytes are evolutionary conserved osmoregulatory cells whose density, morphology, and response to salinity vary markedly even across *Oryzias* species. This interspecific variation, spanning multiple phenotypic dimensions within a well-defined cell type homolog, offers a tractable system to dissect the developmental origins of cell type divergence.

Through cell lineage tracing experiments, cell morphology analysis and comparison of inter-species chimeras, we aimed to investigate the embryonic origin of these cells and their degree of cell autonomy underlying species-specific traits in density, spatial distribution and osmotic response. Our results reveal a striking evolutionary transition: in some *Oryzias* species, ionocyte traits are governed by cell-intrinsic programs, while in others the same traits are plastic and dependent on the host physiological context. These findings suggest that the extent at which a cell type executes an autonomous program is itself an evolvable property, even within highly conserved, functionally specialized cell types. We propose that shifts between cell-autonomous and environmentally responsive developmental modes might represent a potentially remarkable axis for cell type evolution in vertebrates.

P255

Cataloguing diversity in transcriptional processing mechanisms across diverse animal species and cell types

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Single-cell genomics techniques have been extensively applied throughout the past decade towards the investigation of cellular diversity and development. Comparative efforts such as the Biodiversity Cell Atlas project are now employing these methods across diverse species to understand how genes and regulatory networks are remodeled to evolve new cell types. mRNA isoform diversity, however, has remained largely out of reach due to dependence on short-read RNA-seq protocols. Here, we explore the utility and feasibility of long-read single-cell RNA-seq for comparative transcriptomics in a panel of bilaterian and non-bilaterian animals. In principle, such data provide a direct view of alternative RNA processing outcomes, namely transcription start and end sites and the combination of splice junctions, all within the context of cellular identities. However, this depends on the degree to which these processes actually exhibit regulated variation across cell types, especially uncertain in non-bilaterians, and on the quality of the resulting data. We encounter various familiar experimental and bioinformatic challenges, most critically the identification of valid unannotated transcript boundaries. Nevertheless, we demonstrate a high rate of complete transcript-spanning reads from most species, with robust detection of trans-spliced leader exons in ctenophora serving as validation. We also observe crude taxonomic trends in the rate and intensity of certain aspects of RNA processing in non-bilaterians, such as enrichment of alternative polyadenylation in cnidaria. With more data pending from additional developmental stages and species, we foresee exciting new insights into the evolutionary interplay between cellular diversity, RNA processing mechanisms, and transcriptional complexity.

P256

Testing the link between DNA methylation and phenotypic variation in the water strider *Microvelia longipes*

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Epigenetics plays a key role in phenotypic response to environmental cues through different mechanisms. To better understand the interaction between phenotypic plasticity and epigenetics, we used whole-bisulfite sequencing in a new insect model system, *Microvelia longipes*, where males show a remarkably exaggerated secondary sexual trait, the third pair of legs. To assess whether DNA methylation is linked to trait variation, we used whole individuals from inbred lines with differences in third leg length. The global methylation level at CpG sites was 12%, one of the highest reported in insects. The majority of methylation occurred in gene bodies, and throughout the genome. CpG methylation clustering was inbred-line dependent, and sex-independent. The highest difference in methylation sites was seen between the longest- and shortest-legged lines, suggesting that differential DNA methylation might play a role in third leg length. To further investigate this in a tissue-specific manner, we subjected individuals of the longest-legged line to nutritional treatment. The individuals fed with rich diet developed significantly longer legs than those fed with poor diet, providing a controlled system to study leg length in the same genetic background. Of the 3,367,541 CpG sites, only 24 sites were differentially methylated. Twenty-two of these sites were located on chromosome 1914, and 8 of which on msx2-interacting protein-like, a hormone-inducible transcriptional repressor. There was no clustering between samples for either nutrition or sex. These results suggest that nutritional input has limited but specific effect on DNA methylation pattern of a highly plastic exaggerated sexually-selected phenotype in *M. longipes*.

P257

Auxin, ARFs, and leaf architecture: The role of *ZmARF28* in leaf initiation and development in maize

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All grasses have a distinct conserved leaf shape, orientation and angle. The developmental mechanisms that control leaf traits are, in part, controlled by auxin pathways. Auxin is a key regulator of development underpinning cell fate, organ initiation, and architecture; and does so through a family of functionally distinct DNA-binding Auxin Response Factors (ARFs). *ZmARF28* is a class-B ARF (classified as a repressor) in maize that, when mutated, leads to changes in leaf number, phyllotaxy and midrib formation. The mutation results in a Ser-to-Asn amino acid substitution in a loop region in the DBD resulting in an over-accumulation of the ZmARF28 protein. A transcriptomic dataset comparing *Trf* seedlings treated with auxin versus control, revealed both up and down regulated genes within 30min of treatment. This suggests that ZmARF28 may have a more complex role, potentially acting as both an activator and repressor of gene expression. By mining published tissue-specific and single-cell RNA-seq datasets coupled with dual-luciferase reporter assays we have started to elucidate the gene regulatory network that ZmARF28 acts in during maize leaf initiation and development. Identifying key genes interacting with ZmARF28 to regulate the precise balance between auxin, spatial gene expression, and leaf development in maize will further our understanding of how auxin pathways and class-B ARFs function in maize.

P258

Temporal modularity and evolvability of postembryonic development in Orthoptera

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Postembryonic development in insects occurs in three forms: ametabolous (direct development), hemimetabolous (incomplete metamorphosis), and holometabolous (complete metamorphosis). Hemimetabolous insects hatch from eggs as immature nymphs and undergo a gradual transformation into adults through a series of postembryonic moults. The period between successive moults is termed an instar. However, how instar series compare among species remains unclear: how evolvable is instar number, how evolvable are individual instars, and how is morphological progression related to the sequence of moults? Similarly, how intraspecific plasticity in instar number and morphology relate to their evolvability and evolutionary patterns remains poorly understood. Among hemimetabolous insects, orthopterans constitute the second most species-rich order with more than 30,000 species, providing a useful model for studying the evolution of postembryonic development. Here, we systematically surveyed the fragmented entomological literature to compile and synthesise a dataset of instar numbers and morphological descriptions in Orthoptera. Our data reveal that taxa have been variably sampled, with more postembryonic studies in Caelifera than in Ensifera. Overall, instar number is higher in Ensifera than in Caelifera, and evolutionary transitions in instar number are frequent. In terms of morphology, the most prominent postembryonic changes occur in wings and external genitalia. Amid variable instar numbers, the reversal of wing buds appears constrained to the moult preceding the penultimate instar across Orthoptera. Taken together, our results suggest that instars function as temporal developmental modules, with evolvable and plastic instar number but constrained timing of moults at major morphological transitions.

P259

Sensory organ adaptation in polychaete larvae with vastly different developmental modes

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As the first frontier between an organism and its environment, sensory organs are subject to strong adaptational pressures. How the sensory systems of diverse marine larvae are adapted for successful navigation within the sea is currently poorly understood. Annelids are well-suited for studying sensory system adaptation, especially the genus *Platynereis*, one species of which, *P. dumerilii*, is a well-studied experimental model. While *P. dumerilii* has a free-swimming planktonic larva, the larva of its closely related sister-species, *P. massiliensis*, is brooded in its mother's tube on the seafloor. Using scanning electron microscopy (SEM) and immunohistochemistry, we generated a spatial map of ciliated sensory organs for each *Platynereis* larval type. Comparison of these maps reveals similar sensory organ complements and conserved underlying gross nervous system structure in *P. massiliensis* and *P. dumerilii*. We identified mechanosensory receptors, ciliary photoreceptors, and dorsal sensory organs positioned similarly in both species. However, in agreement with previous studies, *P. massiliensis* larvae lack a central ciliated apical tuft that sits above the anterior sensory-neurosecretory organ (ASN). To further investigate these sensory organs and confirm the presence/absence of *P. massiliensis* ASN, we also aim to compare sensory organ cellular and molecular compositions. This will be achieved using existing serial section EM data from *P. dumerilii* and new data we are generating for *P. massiliensis*, combined with cellular resolution immunolabelling of sensory cell signalling components. Results from our comparative study will contribute to identification of sensory cells and organs uniquely adapted to pelagic versus benthic larval lifestyles.

P260

Identifying the mechanisms of phenotypic robustness using color pattern asymmetry in cichlid fish

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Phenotypic robustness, the ability of organisms to buffer development against internal and external perturbations, is a key mediator of interactions among genomes, phenotypes, and environments. Here, we use fluctuating asymmetry in cichlid fish color patterns as an indicator of developmental robustness. To generate fish differing in fluctuating asymmetry, we crossed two closely related cichlid species with similar vertically barred color patterns but different rates of asymmetry, and tested the heritability of fluctuating asymmetry through artificial selection for increased or decreased asymmetry. Using automated quantitative methods based on a pipeline of custom computer vision models, we show that fluctuating asymmetry has moderate narrow-sense heritability in our cross. Genetic mapping of fluctuating asymmetry in F_2 individuals further supports a largely polygenic basis. Quantitative analyses also suggest that the degree of asymmetry varies across body regions, potentially reflecting developmental constraints. Finally, we measured color pattern asymmetry in wild individuals from Lake Malawi and found that asymmetric phenotypes are also common in nature, with frequencies varying among species. This supports our laboratory findings of species-specific rates of fluctuating asymmetry. Overall, our results indicate that fluctuating asymmetry has a significant heritable, polygenic component and provide a promising basis for investigating how phenotypic robustness evolves and how it may be shaped by the developmental characteristics of color pattern formation.

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Exploring chemodiversity in natural populations of *Solanum dulcamara*

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Plant chemodiversity, the extensive diversity of plant secondary metabolites, is observed across plant species but also within plant species. Spatial and temporal intraspecific phytochemical variation is often assumed to reflect diverse ecological conditions under which different combinations of metabolites are beneficial. However, empirical data on the relationship between chemodiversity and ecological interactors in native plant population remains limited. We investigated seasonal variation in leaf chemodiversity within and between native *Solanum dulcamara* populations growing in contrasting habitats in relation to herbivore pressure and abiotic factors. At three timepoints during a growth season, we analysed phenolic and glycoalkaloid composition by HPLC-DAD/-MS and quantified and leaf area damage for the same leaves of at least 12 plant individuals in each of nine populations. We also determined at the growth site of the same plants the overall damage by and the assembly of herbivores and assessed abiotic factors using indicator values of plant species in vegetation assessments for at least five focal plants per populations. We found a strong seasonal variation in chemodiversity across all populations with opposing pattern for phenolic and glycoalkaloid compounds. While phenolic chemodiversity was highest early in the season, glycoalkaloid chemodiversity was highest in late-season. Both, the decrease in phenolic and the increase in glycoalkaloid chemodiversity varied within and between populations resulting significant differences between populations at mid- and late-season respectively. Overall, we established the patterns of intraspecific variation in chemodiversity of natural plant populations across seasonal and environmental scales.

P262

Deciphering the role of cytoskeletal forces and mechanical buckling in developing butterfly scale architecture

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Biological systems fabricate complex and highly ordered nanostructures, yet the physical mechanisms that drive their formation remain poorly understood. In butterflies, wing scales arise from a single epithelial cell and configure an elaborate plan of ridges, crossribs and multilayered photonic nanostructures capable of generating vivid structural colour. Recent super-resolution imaging in Heliconius sara - a butterfly displaying both structurally coloured (blue) and non-structurally coloured (black) scales - demonstrates that the actin cytoskeleton prefigures ridge position, however its mechanical role in shaping nanostructure morphology remains largely hypothetical. Previous hypotheses propose ridge layering arises through actin-generated compressive forces that induce buckling of the plasma membrane. Morphoelastic modelling and live imaging in Painted Lady butterflies show that ridge formation begins via growth-induced membrane buckling constrained by regularly spaced actin bundles, however these predictions have yet to be functionally tested in vivo. Building upon these findings, we will investigate the role of the actin cytoskeleton in generating time-varying mechanical stresses, thereby contributing to ridge layering and nanostructure formation. Using Heliconius sara as a model, we will test this by (1) quantifying the magnitude and spatial distribution of forces during scale development; (2) determining whether these forces are sufficient to induce ridge buckling, and (3) assessing causality through targeted perturbation of the actin cytoskeleton. We will harness fluorescence lifetime membrane tension microscopy and in vivo optical microscopy to parameterise finite element models of the developing scale. Pharmacological perturbation experiments will test these models and elucidate the role of the actin cytoskeleton in structure formation throughout scale development.

P263

Black flies as emerging Eco-Evo-Devo models: linking river ecology, morphological innovation, and genomics

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Understanding how ecological pressures shape developmental innovation requires expanding genomic and experimental resources beyond traditional model organisms. Aquatic Diptera provide striking examples of morphological adaptation to extreme environments, yet several lineages remain poorly represented in evolutionary developmental biology. Black flies (Diptera: Simuliidae), positioned within Culicomorpha alongside mosquitoes and other medically relevant dipterans, display remarkable morphological specializations associated with life in fast-flowing freshwater habitats. Here we integrate ecological surveys, experimental rearing, and genomics to establish new resources for Neotropical black flies. Field surveys conducted along river systems in the Macaé region (Rio de Janeiro, Brazil) identified seven Simuliidae species. Species distribution patterns were analyzed in relation to abiotic environmental variables characteristic of lotic ecosystems, revealing distinct ecological associations among taxa. Based on these surveys, three species—Simulium pertinax, Simulium nigrimanum, and Simulium subpallidum were selected for laboratory assays. We developed a controlled rearing system capable of maintaining aquatic developmental stages under laboratory conditions, enabling experimental investigation of their life cycle and morphological trait development. Focusing on S. pertinax, a widespread and ecologically dominant species, we generated genomic and transcriptomic resources using long-read Nanopore sequencing combined with Hi-C scaffolding to produce a chromosome-scale genome assembly. Together, these results establish black flies as promising Eco-Evo-Devo systems linking ecological distribution, morphological innovation, and genomic architecture in an understudied lineage of Diptera.

P264

An active role of yolk in *Tribolium castaneum* morphogenesis

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Morphogenesis integrates genetic programs with mechanical forces that generate, transmit and interpret tissue deformations (Sutlive et al., 2021). Developing embryos are in close contact with their surroundings, including eggshell, extra-embryonic tissues and yolk, yet such influences remain underexplored in evo-devo. Yolk membrane compartmentalization is reported in Coleoptera, Orthoptera and Lepidoptera (Chapman, 1998), despite major differences in embryo–yolk geometry. In the beetle *Tribolium*, the yolk is cleaved into compartments during germband elongation; rounds of yolk cleavage correlate with germband "bouncing," and loss of yolk cleavage has been linked to germband buckling (Benton, 2018). However, the mechanistic origin of these cleavages remains unknown.

Here we show that transient, long-range actomyosin pulses in the yolk during germband elongation coincide with rhythmic yolk deformations visible in transmitted light. Recurrent pulses generate local constrictions in the yolk sac membrane that sequentially partition the yolk mass into discrete compartments of roughly equal size. These pulses have a particular spatial progression over time: early rounds occur on the yolk surface between the head lobes and posterior germband tip, spanning from one lateral germband edge to the other across the dorsal yolk; later pulses shift outward toward the anterior and posterior poles. Mechanical interaction between cleaving yolk and the elongating germband influences embryo position and shape. Together, these observations suggest that yolk is not merely a nutrient store but may act as a force-generating and force-transmitting element that influences germband elongation dynamics and embryo geometry, providing a potential axis for MechanoEvoDevo comparisons across yolk-rich embryos.

P265

The role of evolvability and modularity in shaping sexual dimorphism in cranial morphology of simiiform monkeys (Primates, Haplorrhini)

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Evolvability, defined as the amount of heritable phenotypic variation available for selection to act upon, determines the potential of populations to respond to directional or sexual selection. However, the evolutionary response of complex traits is also influenced by the structure of trait covariation. Patterns of morphological integration and modularity can either facilitate or constrain evolutionary change by channeling variation along particular directions in multivariate trait space. Here, we investigate how evolvability relates to the evolution of sexual dimorphism in the cranial morphology of simiiform primates. Using empirical phenotypic covariance matrices (P-matrices) derived from cranial measurements across multiple monkey species, we estimate evolvability in the morphospace direction of sexual dimorphism. These estimates are then compared to observed levels of sexual dimorphism across species. Because integration and modular organization influence the distribution of phenotypic variation, they may also shape how dimorphism evolves. Highly integrated systems may bias evolutionary responses along particular trait combinations, potentially affecting the magnitude and direction of divergence between males and females. Preliminary results suggest that the relationship between evolvability and sexual dimorphism is not linear. Contrary to our expectation that higher evolvability would lead to higher levels of sexual dimorphism, we found the opposite pattern. This pattern highlights how evolvability and patterns of morphological integration and modularity may relate to the evolution of sexual dimorphism in non-intuitive ways.

P266

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

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The origin of the tetrapod limb remains an enigma in evolutionary biology. The elaboration of skeletal elements along the proximo-distal axis is a hallmark of the fin-to-limb transition, marking the emergence of the tetrapod limb. While fish fins consist of proximal radials and distal fin rays, tetrapod limbs are made up of three distinct domains proximo-distally: stylopod, zeugopod, and autopod. Despite recent progress in paleontology and embryology, the genetic mechanisms governing skeletal elaboration along the proximo-distal axis remain mostly unknown. We address this gap by analyzing the functions of Hox genes, homeobox transcription factors, that provide the proximo-distal positional information during appendage development. In *hoxa13* mutant zebrafish, we observe loss of fin rays, but also an increased size and segmentation of radials. To investigate the mechanism underlying this radial phenotype, we genetically labeled and traced migration of *hoxa13*-positive cells in wild type and *hoxa13 mutant* fish. While *hoxa13*-positive cells migrate into prospective fin ray region early in development of wild type, they fail to migrate into the prospective rays and instead accumulate in radial region of mutants. These cells in the mutants display a rounder morphology and exhibit fewer filopodia, consistent with impaired migratory behavior. Transcriptomic analyses further reveal dysregulation of genes involved in extracellular matrix maintenance in the absence of *hoxa13* genes. Together, these results suggest that changes in distal cell migration during fin development may underlie the expansion and segmentation of proximal bones, providing a potential explanation for skeletal elaboration during the fin-to-limb transition.

P267

Comparative Cell Type Atlases of Sea Anemones

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Cells are the basic unit of life; yet we lack a comprehensive understanding of their evolution. Advances in methods for single-cell sequencing newly enable the production of whole-organism cell atlases, allowing the application of gene expression evolution models at a single cell level. Generating single-cell atlases of diverse species will enable a systematic characterisation and comparison of cell-type specific gene expression within and between species.

Cnidarians, such as sea anemones, are optimal systems to begin applying these methods to gain insights into the mechanisms of cell type evolution, due to their restricted number of cell types and ancestral phylogenetic position.

Here, we present single-cell atlases of three anemone species: *Diadumene lineata* , *Metridium senile* , and *Exaiptasia pallida*, with each species represented by four specimens. Their phylogenetic proximity to *Nematostella vectensis*, which has a well-annotated cell atlas with reconstructed gene-regulatory-networks, enables the identification of homologous cell types and of the evolution of gene programmes within the cnidarian lineage. Applying phylogenetic tools to these atlases will allow us to infer ancestral cell-type transcriptional states and detect gene expression evolution underlying cell-type diversification in Cnidaria.

We plan to expand this project by investigating within-species variation captured by single-cell gene expression data in *Nematostella*. Investigating cell-type-specific gene expression changes associated with genetic variants will enable the construction of genotype-phenotype maps and will inform us of the microevolutionary processes underlying cell-type evolution.

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The *hoja loca1* mutation disrupts mediolateral patterning in vegetative phytomer development

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Plant architecture is determined by the iterative building of phytomer units, each containing a portion of stem, an organ (typically a leaf or flower) and an axillary meristem. Each phytomer can follow different developmental paths underpinning the complexity, and plasticity, of plant form. Auxin is a phytohormone that plays a central role in the coordination of phytomer development, regulating organ initiation and patterning across all axes. This diversity in auxin function results from changes in the activities and expression of auxin signaling components, including the AUX/IAA transcriptional repressors. Higher land plants have multigene AUX/IAA families, which leads to functional redundancy. Dominant mutations, which prevent AUX/IAA degradation in response to auxin, have highlighted the importance of these proteins in fine-tuning organ development. Here we report a new dominant AUX/IAA mutant in maize, *hoja loca1* (*oja1*). *Oja1* has a mutation in the degron motif of ZmIAA38, specifically affecting aerial organ initiation and leaf medio-lateral patterning, while phytomer initiation remains unchanged. Interestingly, mutant phenotypes are variable both between individuals and between leaves within a mutant, suggesting the *oja1* mutation significantly disregulates developmental plasticity. Transcriptional profiling of individual meristems identified clusters of genes that may underpin the phenotypic differences and provides evidence to suggest that perturbed auxin signalling triggers disfunctional leaf development. The unique phenotype of *oja1* provides evidence of species-specific sub-functionalization of the AUX/IAAs, and illustrates the crucial role of auxin signaling in the tight coordination of phytomer unit development.

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Towards understanding the mechanisms behind petal epidermal cell shape patterning and its diversity across angiosperm species

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Angiosperm flowers exhibit various patterns on their petals. These patterns allow entomophilous plants to attract pollinators and help flowers deal with abiotic stress such as UV damage and water loss. While previous research largely focused on pigmentation patterns, structural patterns created by the combination of different cell shapes across the petal epidermis have also been observed. However, why and how plants create these structural patterns remains largely unknown. We surveyed the living collections at Cambridge University Botanic Garden and discovered that cell shape patterning across the petal epidermis is common among different angiosperm families, and a specific combination of cell shapes appeared prevalent among many sampled species. Bumblebee experiments were then conducted and confirmed that pollinators are able to distinguish between a structurally bullseye-patterned petal surface and a petal uniformly covered a single cell shape. Besides, we started to investigate the possible regulators of different petal epidermal cells in *Hibiscus trionum*, an emerging model system that displays a transition between conical cells and tabular polygonal cells on its petal surface. We examined the role of some known transcription factors such as *MIXTA-like* genes, while also identifying several genes that may influence cell shape specification during petal development.

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How well does phenotypic covariation predict evoltuionary potential? A mammal-wide comparison of G- and P-matrices

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A central goal in evolutionary developmental biology is to understand how modularity and robustness shape the evolvability of complex phenotypes. Quantitative genetic theory provides a complementary framework for linking patterns of trait covariation to evolutionary dynamics. In this framework, the structure of variation is captured by variance–covariance matrices, particularly the genetic (G) and phenotypic (P) matrices, which describe how trait covariation influences evolutionary responses to selection. However, estimating G remains difficult for most species, motivating the widespread use of P as a proxy for G and, by extension, for evolutionary potential, particularly for morphological traits. Here we use cranial traits that are functionally and developmentally related to ask when P reliably captures evolutionarily relevant properties of G across mammals. Using Bayesian mixed models that account for repeated measurements, and measurement error, we estimate G-matrices for cranial traits in rodents, primates, and a marsupial, and P-matrices for more than 400 species spanning major mammalian clades, including marsupials, bats, rodents, primates, xenarthrans, and carnivores. These covariance estimates allow evaluation of evolvability statistics - including evolvability, conditional evolvability, autonomy, and integration - and enable assessment of how well P recovers the structure, orientation, and evolvability properties of G across clades. By embedding this comparative quantitative-genetic analysis within an evo-devo framework, this study explores how patterns of phenotypic covariation reflect underlying genetic constraints and shape evolutionary potential across mammals.

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Genomic adaptations of self-domestication across the populations of the yellow fever mosquito *Aedes aegypti*

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In the arboviral vector *Aedes aegypti*, adaptation to anthropogenic environments has led to a major evolutionary shift separating the domestic *Aedes aegypti aegypti* (*Aaa*) ecotype from the wild *Aedes aegypti formosus* (*Aaf*) ecotype. '*Aaa*' mosquitoes are distributed globally and have higher vectorial capacity to viruses (e.g., Dengue, Zika, Chikungunya, Yellow Fever) than '*Aaf*' mosquitoes, which remained in Africa. A deep-rooted hypothesis among vector biologists is that the '*Aaa*' ecotype emerged through 'self-domestication', in which species evolve in response to concerted selection pressures that mimic domestication, but without humans serving as a domesticator. However, inconsistent morphological data and a complex population structure have hindered the identification of genomic signals distinguishing the two ecotypes. Here we assessed the correspondence among the geographic distribution, population structure and genome-wide selection of 511 '*Aaf*' and 123 '*Aaa*' specimens collected worldwide. We found robust signals of genomic adaptation to self-domestication in '*Aaa*' mosquitoes across one long noncoding RNA and 185 protein-coding genes that we call '*Aaa* molecular signatures'. Our results, recently published in *Nature Ecology and Evolution* 9:652-671 (2025), strongly suggest that '*Aaa* molecular signatures' arose from standing genetic variation associated with extensive ancestral polymorphisms in '*Aaf*' populations, which have been co-opted for self-domestication through genomic and functional redundancy and local adaptation. Overall, we show that the behavioral shift of *Ae. aegypti* mosquitoes to live in association with humans relied on the fine regulation of chemosensory, neuronal and metabolic functions, as also remarkably seen in the human domestication processes of rabbits, chickens, cattle and silkworms.

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Evolution of inhibitory interneurons contributes to vertebrate forebrain diversity

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The dorsal telencephalon or pallium is the brain region that shows the widest diversity in cell type identity, tissue architecture, and connectivity across vertebrates. All vertebrate pallia contain both locally-born excitatory neurons, and inhibitory GABAergic interneurons that migrate from the ventral telencephalon. While recent studies have demonstrated the extensive diversification of excitatory neurons, a detailed analysis of interneuron evolution and its contribution to changes in pallial organization is still missing. Here, we investigate the evolution of GABAergic neuron diversity across vertebrates by combining comparative single-cell transcriptomics and in situ hybridization in mammals, sauropsids, amphibians, ray-finned fishes, and cartilaginous fishes. We show that while the major pallial interneuron classes stabilized in terrestrial vertebrates, comparisons with fish lineages reveal a high diversification in interneuron identity, distribution, and developmental origin. Thus, each early vertebrate lineage has a markedly different repertoire of pallial interneurons. Even within tetrapods, we identify lineage-specific interneuron types and differences in the spatial organization of many conserved types. To understand the molecular mechanisms underlying the origin of novel interneuron types, we further analyze developmental trajectories and chromatin accessibility of interneurons across tetrapod species. Our findings reveal a surprising degree of interneuron divergence in vertebrates, indicating that changes in inhibitory repertoire have shaped the structure and connectivity of the telencephalon. The combination of class conservation and cell type divergence makes interneurons ideal systems to study mechanisms underpinning the evolution of neuron types. In particular, we show that inhibitory neurons diversified through changes in differentiation trajectories stemming from conserved progenitor domains.

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A Balanced Inversion Polymorphism Exhibits a Dominance Reversal at the Gene Expression Level that Depends on Developmental Context

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How genetic variance for fitness is maintained is incompletely understood. Antagonistic balancing selection, favoring different alleles in different contexts and involving beneficial dominance reversals, might contribute to maintaining fitness variance. Here, we show how *In(3R)Payne*, a balanced inversion polymorphism in *Drosophila melanogaster*, affects gene expression and chromatin accessibility. In embryos, alleles in the inverted (INV) arrangement tends to have dominant effects, while the standard (STD) arrangement behaves like a recessive Mendelian allele. Yet, in wing discs, this pattern is reversed: STD has mostly dominant effects, whereas INV behaves recessively. Since this shift in the dominance of the INV "allele" between developmental contexts affects the expression of suites of genes in a concerted manner, it might be mediated by a dominance modifier, for example, a transcription factor. In favor of this idea, 25% of the differentially expressed genes between INV and STD encode transcription factors. Interestingly, while only four differentially expressed genes are shared between embryos and wing discs, one of them is *HP1c*, a chromatin-binding protein and major transcriptional regulator, and thus a promising candidate for mediating the context-dependent change in dominance. Although the relationship between these patterns and fitness is presently unknown, our observations are consistent with a potential role of reversals (or, more generally, shifts) of dominance in maintaining inversion polymorphisms.

P275

Comparative insect embryogenesis under changing climate

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Insects constitute the most speciose and ecologically dominant terrestrial animal group. The earliest insects left the Silurian seas ~440 Ma, a transition that required the evolution of insect egg features such as extra-embryonic membranes (chorion, serosa) to prevent desiccation. How inter-order variation in these embryonic adaptations has shaped insect diversification, however, remains poorly understood. Here we report the first steps toward a high-resolution 3-D live-imaging pipeline for the short-germband coleopteran *Dermestes maculatus*, whose eggs are moderately drought-resistant. Pre-blastoderm co-injection of mRNA encoding fluorescent-protein-tagged histone nanobodies (nuclear marker) together with either an RNA encoding membrane-targeted fluorescent protein or a fluorescent actin marker permits simultaneous tracking of nuclear dynamics, membrane remodeling and cytoskeletal organization under the confocal fluorescence microscope. This imaging platform will enable comparative analyses with the better-understood model coleopteran *Tribolium castaneum* and, potentially, other more distantly related insects such as the lepidopteran *Plodia interpunctella* and the most-studied dipteran *Drosophila melanogaster*, testing whether certain embryonic features such as yolk volume, timing of the serosal window closure, chorion strength and overall development time covary with egg desiccation resistance and oviposition ecology. Establishing *D. maculatus* as a live-imaging model therefore provides a mechanistic framework for predicting insect resilience in a warming, drier climate.

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Incubation temperature alters stripe formation and head colouration in American alligator hatchlings and is unaffected by estradiol-induced sex reversal

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Considerations of the impact climate change has on reptiles are typically focused on habitat change or loss, range shifts and skewed sex ratios in species with temperature-dependent sex determination. Here, we show that incubation temperature alters stripe number and head colouration of hatchling American alligators (Alligator mississippiensis). Animals incubated at higher temperatures (33.5°C) had, on average, one more stripe than those at lower temperatures (29.5°C), and also had significantly lighter heads. These patterns were not affected by estradiol-induced sex reversal, suggesting independence from hatchling sex. Therefore, increases in nest temperatures as a result of climate change have the potential to alter pigmentation patterning, which may have implications for offspring fitness.

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Exploring the Molecular Principles of Heterochrony between *C.elegans* and *C. briggsae*

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Heterochrony, or changes in ontogenetic developmental time, is an important phenomenon in describing the phenotypic variations during evolution. These natural polymorphic changes have been speculated to be able to give rise to differences in body plans, but mis-timing can lead to developmental diseases. While morphological heterochrony has been extensively documented in evo-devo studies, the principles underlying molecular heterochrony remain poorly understood. The robustness and intricacy of developmental systems can pose challenges for molecular studies; systemically characterising and perturbing temporal variations of developmental regulators requires tedious optimisation, highlighting a lack of sufficient experimental designs to unveil molecular heterochrony outcomes. Here we propose an evo-devo inspired *in silico* experimental approach to study the molecular basis of heterochrony systemically. We harness DIAMOND DeepClust, a tool designed to cluster billions of proteins across the tree-of-life, and the Emerald algorithm, an alignment method that identifies suboptimal alignments which can reveal diversity in protein comparison, to infer the diversity and evolution of genetic developmental toolkit genes. We utilise a phylostratigraphy-inspired screening pipeline that applies GenERA to rapidly compute gene ages in *C. elegans* and *C. briggsae* genomes, myTAI to profile transcriptomes with a weighted average of all gene ages across developmental stages, and gaTAI to capture differentially expressed genes in embryo development with ancient emergence. With cross-referencing available single-cell embryo developmental dataset, this can identify candidate key developmental regulatory genes that govern molecular heterochrony for wet-lab hypothesis testing.

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Developmental and genomic insights into umbel evolution in Apiaceae

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The traditional name of the carrot family, Umbelliferae ("umbel-bearing plants"), refers to the distinctive blossoms shared by all its members. Within the order Apiales, early-diverging families produce relatively simple inflorescences, whereas the emergence of the umbel as a fundamental reproductive module coincides with the radiation of the clade uniting Apiaceae and Araliaceae. Although often overlooked, this architecture represents a key morphological innovation that facilitates efficient pollination and seed dispersal. Historically, umbels have been interpreted as condensed racemes or thyrses based on their mature appearance. Developmental observations, however, suggest a different scenario: umbels arise from expanding, determinate meristems with distinctive histological and morphological properties, indicating substantial rewiring of developmental programs controlling meristem maintenance, determinacy, and inflorescence patterning. To investigate the genetic basis of umbel evolution, we combined comparative genomics with developmental transcriptomics. We assembled new genome resources for selected Apiales taxa and integrated them with publicly available datasets to examine patterns of gene family expansion and contraction among regulators of meristem identity and phase transition. In parallel, we generated stage-specific transcriptomes capturing key stages of carrot umbel development, including vegetative meristem, primary umbel meristem formation, initiation of umbellet meristems, and floral meristem establishment. Comparative analyses with racemose and cymose inflorescences revealed that while many developmental regulators are conserved, umbel ontogeny is characterized by a pronounced heterochronic shift in gene expression, with transcriptional programs associated with floral meristem patterning and cell proliferation deployed earlier during umbel development.

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Understanding the Mechanism of Floral Trichome Patterning in *Hibiscus trionum*

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The colorful patterns on the petals of flowering plants are key to attracting pollinators and directly contribute to their reproductive success. These patterns combine differences in pigmentation and cell shape across the petal epidermis. Petal patterning also includes the production of 'hairs' known as trichomes. Trichomes can be unicellular or multicellular and display an impressive diversity in morphology, distribution, and function. Trichomes appear when cells acquire 'trichome fates', but the mechanisms controlling this process are not well understood. In this study, the evolutionary and developmental (evo-devo) aspects of trichome diversity in *Hibiscus trionum* were explored by characterising trichome morphology, spatial patterning, and genetic regulation. Two main types of glandular trichomes and five main non-glandular types were identified, distributed in a spatially and timely coordinated manner. Trichomes emerged and matured asymmetrically along proximo-distal and medio-lateral axes, and most trichomes fully developed before the differentiation of other epidermal cell types. Notably, a distinct 'bald zone' without trichomes at the petal bullseye was identified; these observations altogether highlight the role of spatially restricted signals in shaping trichome patterns. Comparisons with natural variants, *Hibiscus richardsonii*, revealed clear differences in trichome density and morphology. Hybrid populations derived from crosses exhibited broad variation in trichome counts, supporting a polygenic basis. Using CRISPR-Cas9 genome editing approach to target candidate trichome regulators, non-glandular trichomes were removed and glandular trichomes were thinned, generating genome-edited lines that provide a powerful tool to investigate trichome function. Integrating chemical analyses with pollinator behaviour assays will further elucidate the ecological functions and evolutionary significance of trichomes.

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Comparative Analysis of the Genomic Signatures of Miniaturised Annelids

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Aquatic biodiversity is dominated by meiofauna – ecologically vital invertebrates less than a millimetre long. Multiple meiofaunal lineages have evolved independently through miniaturisation within at least 12 of the macroscopic animal phyla, making miniaturisation a common phenomenon. These transitions from macroscopic to microscopic body plans involve dramatic morphological changes, which can include reduction of appendages, simplification of circulatory and sensory systems, and loss of adult-associated morphology. But how are these changes reflected in the genome? In Annelida (containing bristleworms and earthworms), one well-studied miniaturised species, *Dimorphilus gyrociliatus*, displays a simplified annelid segmental body plan of only six segments, reduced body cavity, and no appendages. On a genomic level, *D. gyrociliatus* has a greatly reduced genome, with lower TE repeats and evidence of intron loss. Crucially, it is not known whether these genomic signatures are shared by other, independently miniaturised lineages. By leveraging recent advances in genomic-techniques, we are now able to generate full, chromosome-scale genomes for these ultra low-input specimens. We generated a chromosome-level genome for the miniaturised annelids *Diurodrilus subterraneus*, and an unclassified species of *Trochonerilla,* and explored their genomic landscapes, assessing genome size, *Hox* gene repertoire, differential gene expression, and structural elements. We then compared these to *Dimorphilus gyrociliatus* to determine genomic similarities between these three independently miniaturised annelids. This comparison marks a new foray into the previously unexplorable realm of meiofaunal comparative genomics, and establishes the groundwork for future explorations into the genomic mechanisms underpinning miniaturisation - one of the most widespread evolutionary transitions across the animal tree of life.

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Molecular mechanisms and phenotypic consequences of paralog divergence in insects and chelicerates

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Gene duplication events have been widely observed across species and are believed to contribute to genome evolution and emergence of phenotypic novelties. Retaining multiple gene copies provides genetic material that can accumulate mutations and undergo sub- or neofunctionalization. In arthropods, both small-scale duplications and whole-genome duplications (WGDs) occur frequently. In chelicerates, in addition to recent small-scale lineage-specific duplications, ancestral WGDs were revealed in horseshoe crabs and arachnopulmonates. In insects, evidence for WGDs remains limited and debated, while tandem duplications are very common. Arthropods, one of the most species-rich animal groups characterized by phenotypic diversity and evolutionary innovations, thus represent a unique system to reveal how gene duplications contribute to phenotypic diversification. Our project aims to uncover the molecular mechanisms underlying paralog expression divergence and to identify how novel gene functions evolve after duplication events. To identify ancient and lineage specific gene duplicates and their expression divergence we integrate chromosome level genomes with homogenous annotations for phylogenetically crucial arthropod species and combine these with multi-omics data including single-cell RNAseq, tissue-specific bulk RNAseq and ATACseq data from selected model species. We will classify strict single-copy genes, ancestral and tandem duplications and analyse their role in evolutionary innovation through different bioinformatic and experimental approaches. Potential temporal and spatial expression divergence between duplicated genes will be verified by in situ hybridization or hybridization chain reaction. Selected candidate genes will be functionally assessed by RNAi-mediated gene knockdown or CRISPR-based knockout. Together these results will provide further insights into how gene duplication events shaped arthropod evolution.

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Neuronal Dynamics in the Trochophore larva of *Platynereis dumerilii*

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Ciliary-based locomotion is widely observed in micrometer-scale marine organisms. These include the spherical free-swimming developmental stage (Trochophore) of the marine annelid *Platynereis dumerilii*. Only ~24 hours post fertilisation (hpf) its equatorial ciliary ring transitions from constant ciliary beating to an alternation between neuronal-controlled ciliary arrests and resumption of ciliary beating. This rhythmic biphasic neuronal circuit was first described by Veraszto et al. eLife, 2017. The circuit includes only a handful of stereotypic ciliomotor neurons that are directly connected to ciliated cells and are expressing markers indicating acetylcholine, serotonin, dopamine and noradrenaline signalling. Now, this project aims to characterise how these neurons establish and modulate the ciliary beating swimming behaviour. For this we use zygote injection to introduce genetically encoded fluorescent sensors (e.g. GCaMP8) to measure neuronal signal dynamics (e.g. calcium, voltage, noradrenaline secretion). By imaging the whole animal using light-sheet microscopy we cannot only correlate signals between neurons but also how neuronal signals affect ciliary beating. First calcium imaging experiments support previous findings on the rhythmicity of prominent neurons, such as the master control neuron, whose activity is correlated with ciliary arrest. They also indicate that some neurons show synchronous signalling and even frequencies that are multiples of the MC neuron frequency. To further reveal the signal dynamics of individual neurons in relation to each other we will perturb the system by laser ablating single neurons or axons one-by-one. Finally, we will likely be able to provide a detailed description on the neuronal ciliomotor control system of the early developmental stage of *Platynereis dumerilii*.

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Temporal Alignment in the Omics Age: Goal-Relative Comparability Across Developmental Time

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With rapidly advancing transcriptomic techniques, evo-devo is becoming increasingly multi-dimensional. Spatial and time-resolved transcriptomics now permit fine-grained comparative studies of a broad range of organisms beyond the narrow repertoire of classic model systems. Yet these gains sharpen a conceptual and methodological bottleneck: in high-resolution, cross-species developmental datasets, "comparability" is genuinely hard to determine. The problem concerns not only the homology of traits, but also the homology of spatiotemporal contexts, including developmental stages, cell-niche microenvironment, etc.

This paper focuses on the problem of temporal alignment in the omics age. It argues that there is no straightforward homology of developmental stages and that traditional morphological and landmark-based staging (e.g., somite count, gastrulation) can systematically misalign underlying developmental progression. In cross-species omics, heterochrony and tissue-specific asynchrony mean that the "same" morphologically defined stage may correspond to different gene-regulatory states in the focal tissue. Consequently, landmark staging is increasingly supplemented by transcriptome-informed alignment of developmental trajectories. Such approaches infer progression from expression dynamics (e.g., pseudotime/velocity-style ordering) and align trajectories across datasets (cross-sample trajectory matching) to identify candidate correspondences.

I argue that this methodological shift motivates a goal-relative pluralism about developmental time in evo-devo. Morphological time, lineage/differentiation time, and regulatory-program time constitute distinct reference frames that may be suited to different explanatory aims. The payoff is a set of explicit criteria for temporal comparability in the omics era: alignments should be justified relative to the explanandum and constrained by non-circular validation (e.g., morphology, lineage information, or orthogonal modalities), rather than treated as implicit consequences of staging conventions.

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The role of developmental plasticity in the patterning and modularity of fins

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Across fishes, fins vary dramatically in their size, shape, and position. While much is known of early fin morphogenesis, it remains unclear how developmental plasticity contributes to this variation. Here we show that juvenile foraging behavior can alter fin skeletal patterning and fin modularity in the cichlid *Satanoperca daemon*. Wild-caught juveniles were raised under three feeding regimes promoting pelagic feeding, sand-winnowing, or rock-winnowing. After 20 weeks, pectoral fins were µCT-scanned and analyzed using linear measurements and geometric morphometrics. Plasticity was pervasive across the pectoral fin skeleton, with the anteroposterior patterning of both radials and fin rays affected by the feeding treatments. To test whether treatments impacted the covariance structure of fins, we analyzed their modularity and integration using maximum-likelihood inference of modularity (EMMLi), covariance-ratio analyses, and tests of integration. Pelagic and sand-winnowing fish showed support for a five-module fin architecture, whereas rock-winnowing fish showed no clear pattern of modularity, with models equally favoring three-, four- and five-module hypotheses. Covariance-ratio tests confirmed significant modular structure across treatments but revealed subtle differences in modular signal, and integration analyses supported treatment-specific associations among dorsal and anal fins. These results show that foraging behaviors in juvenile fishes can have wide-ranging impacts on the post-cranial skeleton. Both the skeletal patterning of individual fins and the modularity of fins across the body are developmentally plastic, with overlapping developmental and functional inputs contributing to adult patterns. We propose that this plasticity contributes to the evolutionary responsiveness of fins as fishes adapt to new ecological niches.

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A conserved gene regulatory circuit underlies sensory neuron identity across chordates

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Vertebrate sensory ganglia contain diverse neuron types specialised for distinct sensory modalities. In vertebrates, epibranchial (viscerosensory) and auditory neurons arise from the posterior placodal domain and are specified by distinct transcription factor programs, including *Pax2/5/8*, *Phox2* and *Err*. However, the evolutionary origin of this regulatory architecture remains unclear.

We hypothesise that *Pax2/5/8* controls the specification of posterior placode-like sensory neurons through an ancestral gene regulatory module that predates vertebrates, giving rise to distinct sensory neuron subtypes via downstream *Phox2*- and *Err*-dependent programs.

To test this, we combine comparative single-cell transcriptomics, gene expression analyses, and CRISPR-mediated perturbations across amphioxus, *Ciona intestinalis*, chicken embryos, and mice. We assess whether *Pax2/5/8* regulates *Phox2*- and *Err*-expressing sensory neuron populations across chordates and evaluate the extent to which this regulatory hierarchy is conserved.

By reconstructing conserved and lineage-specific features of this gene regulatory network, we aim to determine whether modification and expansion of an ancestral posterior placode neuron-like module contributed to vertebrate sensory neuron diversity. This work provides insight into how regulatory rewiring and gene duplication facilitated the evolution of vertebrate sensory neuron diversity.

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Insights from *Suncus murinus* for mammalian tooth evolution

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Through long-term adaptation to diverse diets and ecological environments, mammals have evolved a wide variety of dentition patterns. The ancestor of placental mammals is thought to have possessed 44 teeth, with a dental formula of 3.1.4.3 in both the upper and lower jaws. However, most major extant mammalian taxa have lost several teeth, or even entire tooth classes, relative to this ancestral condition, likely through the involvement of multiple genes. This study aims to clarify the genetic basis of tooth loss using *Suncus murinus*, a species that retains many ancestral mammalian characteristics while also exhibiting substantial variation in tooth number. An F2 population was generated through a cross between a continental lineage, characterized by large body size and minimal tooth loss, and an island lineage, characterized by small body size and a high frequency of loss of the upper third incisor (UI3) and third premolar (UP3). Genome-wide association analysis (GWAS) and quantitative trait locus (QTL) analysis revealed significant associations with several genes involved in key signaling pathways essential for tooth morphogenesis. By comparing candidate genes associated with tooth loss and asymmetry across mammals, we discuss the genetic basis underlying evolutionary changes in the dental formula.

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From Polyp to Jellyfish. Transcriptomic profiling of the chemically induced strobilation in the jellyfish *Aurelia sp*

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The origin and evolution of the bilaterian body plan represent a central question in animal biology, particularly regarding the complexity of their last common ancestor. Cnidarians (corals, sea anemones, and jellyfish) diverged before the emergence of bilaterians and are considered their sister group, sharing important developmental and genetic features. Understanding developmental processes in cnidarians can therefore provide key insights into early animal evolution. The scyphozoan jellyfish *Aurelia* sp. exhibits a life cycle transition from a benthic polyp to a free-swimming medusa through a process known as strobilation, typically triggered by environmental cues such as temperature changes. However, the compound 5-methoxy-2-methyl-indole (5Me2MI) induces strobilation up to five times faster than temperature shifts, even at concentrations as low as 1 nM. Here, we investigated 5Me2MI-induced strobilation in *Aurelia* sp. to characterize the early molecular signaling and cellular events preceding visible morphological transformation into the ephyra stage. Transcriptomic analysis during the first 24–48 hours after induction—prior to detectable morphological changes—revealed differential expression of approximately 250 genes. Nearly 100 genes were downregulated, while over 150 were upregulated. Among them were genes associated with bilaterian morphogenesis, including Wnt signaling components and homeobox transcription factors, suggesting conserved developmental mechanisms. Additional differentially expressed genes were related to signal transduction, cell proliferation, oxidative stress, epigenetic regulation, and metabolic processes. These results highlight rapid transcriptional reprogramming during chemically induced strobilation and provide insight into conserved pathways underlying early developmental transitions in cnidarians.

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Multiplexed Single-Cell Long-Read Transcriptomics Using SPLiT-seq and Oxford Nanopore Sequencing in to Study Planarian Regeneration

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Single-cell transcriptomics has revolutionised biology by enabling the systematic dissection of cellular diversity and state. However, the vast majority of single-cell RNA-sequencing platforms rely on short-read sequencing and nanodroplet-based approaches, which limit the recovery of full-length transcripts and constrain the analysis of alternative splicing and isoform diversity. In addition, these approaches offer limited multiplexing capacity, making biological replication costly and statistically challenging. Here, we describe the development of a single-cell long-read transcriptomics (SCT-LRS) workflow using Oxford Nanopore Technologies, designed to be scalable, accessible, and information-rich. Using planarians, a model system with exceptional regenerative capacity, we adapt SPLiT-seq, a highly multiplexed, combinatorial barcoding strategy that requires no specialised equipment. This enables the inclusion of multiple samples and biological replicates within a single experiment, which is essential for knockdown studies. By coupling SPLiT-seq to Nanopore cDNA sequencing, our approach enables single-cell, full-length transcript profiling, capturing isoform usage and alternative splicing events that are largely inaccessible to short-read methods. We benchmark this protocol against established single-cell and short-read datasets and apply it to replicated perturbations of splicing regulators to assess reproducibility and biological sensitivity. This work establishes a practical and cost-effective framework for long-read single-cell transcriptomics and highlights the unique strengths of Oxford Nanopore sequencing for resolving transcript diversity and splicing regulation at single-cell resolution in complex biological systems. The protocol will be used to study the impact of splicing regulators on regeneration, by incorporating it in knockdown experiments.

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Investigating developmental genetics of panicle architecture and pulvinus-controlled branch angle in *Eragrostis tef*

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This research aims to investigate how genetic variation across *Eragrostis tef* (*E.tef*) genotypes correlates with plant architecture. Using histological analysis, phenotyping of cell size and shape, and lignin quantification, this project will build on identifying structural features linked to genes that influence panicle architecture. Gene function will be explored using RNA sequencing and CRISPR-Cas9 knockouts to establish the relationship between gene expression and plant traits. The aim will be to identify how specific genes contribute to variation in *E.tef* morphology, traits and resilience.

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Translation control of developmental tempo: Evidence for a gastrulation-stage checkpoint in the chick embryo

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What controls developmental tempo and is it fixed? Using the chick embryo as a model, we have uncovered evidence for a previously uncharacterised developmental checkpoint at Hamburger-Hamilton stage 4 (HH4), the transition stage from primary gastrulation to axis elongation, that controls the shift from variable to canalised developmental trajectories. Fixed-timepoint staging of large embryo cohorts revealed that developmental stage distributions widen progressively with incubation time, with a consistent excess of embryos stalled at HH4/4+. To dissect whether this reflects individual tempo variation or a true checkpoint, we performed live-imaging experiments tracking continuous developmental progression. Inter-embryo variance in stage drops sharply upon entry to HH4+ and time of HH4+ entry is strongly predictive of final developmental stage, thereby suggesting that the tempo is "locked in" at this transition. Node-ablated embryos accumulated delay specifically before HH4+, with no additional delay thereafter, pointing to HH4+ as a functional integration point. Complementary resilience assays showed that embryos around HH4 are uniquely resistant to temperature-induced diapause, and are disproportionately likely to arrest rather than die when challenged. We further show that the HH4 to HH5 transition coincides with a sharp onset of global embryonic translation activity, as measured by OPP incorporation, suggesting that translational activation may gate checkpoint exit. Together, these findings support a model in which gastrulation triggers a translational switch that both canalises developmental tempo and licenses progression past a stress-sensitive checkpoint. Ongoing work is characterising the molecular regulators of this transition and its conservation across amniotes.

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Evolution of the cell-surface protein Tartan reshapes genital morphogenesis and reproductive interactions during Drosophilaspeciation

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The rapid diversification of sexual traits is a hallmark of animal speciation, yet the genetic and developmental mechanisms linking morphological divergence to reproductive isolation remain poorly understood. Recent work on female genitalia in *Drosophila* suggests that shared developmental regulators may generate correlated morphological evolution between the sexes and facilitate co-evolution.

We found that the leucine-rich repeat protein Tartan (Trn) contributes to male and female genital divergence between *Drosophila mauritiana* and its sister species *D. simulans*. Differences in *trn* expression contribute to the larger male claspers and shorter female vaginal plates characteristic of *D. mauritiana*. In males, these size differences arise through interactions between Trn and other cell-surface molecules, including Ten-m, which together shift the developmental boundary between the clasper and lateral plate. To test the functional consequences of this morphogenetic shift, we used CRISPR/Cas9 to introduce a *D. mauritiana trn* allele (trn^{mau}) into *D. simulans*. These flies developed larger claspers and shorter vaginal plates in males and females, respectively. Behavioural assays revealed reduced pre-fertilization success, including increased mating latency, reduced mating frequency, and shorter copulations. Surprisingly, these same morphological changes produced a post-copulatory advantage by significantly increasing fertilization efficiency, consistent with strong cryptic female choice. Some fitness deficits in trn^{mau} males were partially attenuated when paired with matched trn^{mau} females, suggesting phenotypic co-evolution between the sexes. Together, our results indicate that subtle shifts in *trn* activity can reconfigure morphogenetic boundaries, providing a developmental mechanism for the rapid co-evolution of male and female reproductive traits during speciation.

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Extreme intermittent fasting: cellular and microbial mechanisms of starvation tolerance in cavefish

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Access to nutrition is fundamental for life, yet in many environments food availability is unpredictable or chronically limited, imposing strong selective pressure on digestive systems. The Mexican cavefish *Astyanax mexicanus*, which evolved repeatedly in nutrient-poor cave ecosystems, provides a powerful evolutionary model to investigate how digestive systems adapt to extreme starvation. We previously showed that cavefish display increased starvation resistance compared to their surface-dwelling relatives. Here we show that this capacity is far more extreme than previously appreciated. In controlled experiments, cavefish survived up to 20 months without food, losing less body weight than surface fish after only two months of starvation. Remarkably, cavefish rapidly regain weight upon refeeding, indicating that digestive capacity is preserved during prolonged starvation. A key feature of this tolerance is the preservation of gut integrity. While surface fish rapidly develop mucosal disruption and compromised intestinal barrier function during starvation, cavefish maintain epithelial organization even after extended fasting. To uncover the underlying mechanisms, we generated single-cell RNA-seq maps of intestinal tissues from fed and starved fish across multiple time points. These analyses reveal enhanced activity of absorptive epithelial cell types and a finely tuned regulation of lymphoid and myeloid immune populations that preserves intestinal homeostasis. We further tested host–microbiome interactions using fecal microbiota transplantation, which reshaped starvation responses and microbial metabolite profiles in both ecotypes. Together, these findings reveal how chronic nutritional stress drives evolutionary rewiring of gut cellular states, immune regulation, and host–microbiome interactions to maintain digestive function during extreme starvation.

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The lamprey habenula provides an extreme example for the temporal regulation of asymmetric development

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By their phylogenetic position and their marked epithalamic asymmetries, lampreys are relevant models for understanding the formation and evolution of this trait across vertebrates. In this study, we use a transcriptomic approach to identify novel signature markers to characterize the highly asymmetric, bipartite organization of habenulae in lampreys. Lamprey habenulae are subdivided into two complementary subdomains related, respectively, to the lateral/ventral and the medial/dorsal habenulae of jawed vertebrates: a dorsal, right-restricted subdomain and a bilateral subdomain that includes the left habenula as well as its ventral right counterpart. Analysis of the formation of the lamprey habenula at prolarval and larval stages using a combination of morphological, immunohistochemical, and *in situ* hybridization approaches highlights a marked asymmetric temporal regulation. The dorsal right subdomain forms and already expresses all identified signature markers in prolarval stages. In contrast, the left and ventral right subdomain appears significantly later, with the first indication of neuronal identity elaboration in these territories being observed in larval stages. As in gnathostomes, Wnt signaling may be involved in the regulation of this unique, asymmetric mode of development, since b-catenin shows asymmetric and highly dynamic nuclear distributions both in neural progenitors and differentiated neuronal precursors of the two habenular subdomains. These data confirm the importance of lampreys to unravel the developmental logic underlying the recurrence and variation of habenular asymmetries in vertebrates and pave the way for future functional analyses.

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The genomic mechanisms of melanism in a jawless vertebrate

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Understanding how phenotypic divergence arises at the genetic and cellular level is a central question in evolutionary developmental biology. Colouration, especially melanism, has long fascinated evolutionary and developmental biologists. Although the genetic basis of colour variation has been extensively studied in many vertebrate and invertebrate lineages, nothing is known about colour variation in jawless vertebrates. Here, we take advantage of the evolution of melanistic ecotypes in European lampreys (Lampetra spp) to elucidate the genomic mechanisms of colour variation in a jawless vertebrate. European lamprey displays up to three ecotypes: the common ancestral anadromous-parasitic and derived non-parasitic ecotypes, both of which display olive-brown adult colouration, and the rarer lake-migratory parasitic ecotype. In several cases, this lake-parasitic ecotype exhibits a conspicuous melanistic colouration, yet the genetic and cellular basis of this dark colouration remains uncharacterised. These three ecotypes co-occur and interbreed in Loch Lomond, Scotland, providing an excellent system to examine whether the conserved melanocortin signalling pathway that typically regulates melanism in gnathostomes also operates in lampreys, one of the most basal extant vertebrate lineages. This ongoing study employs an integrative approach combining skin transcriptomics, population genomics, histological examination of melanophore density, and image-based quantification of skin darkness to identify the genetic and cellular drivers of melanism in lake-parasitic lampreys. Differential gene expression and genomic mapping were used to identify pigmentation-related genes in the melanistic ecotype, while microscopy and image analysis characterised differences in cellular composition and melanism between ecotypes.

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Cephalo-pelvic covariation and sexual dimorphism are disrupted in hybrid mice: implications for the human obstetrical dilemma

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Cephalo-pelvic disproportion and obstructed labour in humans have traditionally been interpreted through the obstetrical dilemma framework, assuming a trade-off between bipedal locomotion and childbirth. However, cephalo-pelvic covariation and pelvic sexual dimorphism might be more common adaptations to parturition among mammals. Here, we use a controlled hybridization model in mice to test whether cephalo-pelvic covariation and pelvic sexual dimorphism are population-specific, genetically structured, and sensitive to hybridisation. We analyzed skull-pelvis variation and covariation, as well as sexual dimorphism of pelvic morphology across four divergent wild-derived mouse strains and their hybrids. Hybridization induced consistent cranial and pelvic size enlargement. Only females exhibited significant cephalo-pelvic shape covariation, characterized by an association between rounder, wider birth canals and larger, rounder neurocrania, consistent with functional integration under obstetric selection. Hybrid generations showed disrupted size covariation, increased pelvis shape variance, and reduced cephalo-pelvic integration. Pelvic sexual dimorphism was systematically reduced in hybrids compared to parental populations. Our results demonstrate cephalo-pelvic covariation and pelvic sexual dimorphism are not exclusive to bipedal or highly encephalized species. They likely reflect widespread selection on birth canal morphology in mammals and have a complex genetic basis sensitive to hybridization. These findings weaken a human-exclusive interpretation of the obstetrical dilemma and highlight genetic introgression as an understudied factor shaping cephalo-pelvic integration and cephalo-pelvic disproportion risk in mammals, including the human lineage.

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Orientation of metachronal waves in prototroch multiciliated cells of *Platynereis dumerilii*

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Multicilated cells are found across the tree of life where they are present in a wide variety of tissues, fulfilling diverse functions ranging from mucosal clearance in the human respiratory tract to locomotion in the *Schmidtea spp* epithelium or *Platynereis* larvae.

Coordinated beating of cilia on multicilated cells generates metachronal wave patterns that are essential for both fluid transport and locomotion.

To study mechanisms of multiciliary coordination on a molecular level, the marine annelid *Platynereis dumerilii* has proven to be an excellent model organism. Specifically, its two day old larva has a multiciliated array (prototroch) consisting of 23 cells in an almost one-dimensional ring around the animal, simplifying modelling of waves and microscopic analysis of beating patterns.

Metachronal waves in the prototroch have been studied and a tilt in the ciliary beating plane was detected. *In silico* modelling revealed that the tilt determines the directionality of the metachronal wave within the array. The proteins that determine the basal body polarity that establishes this tilt remain unknown. We propose to identify components of this orienting network and test their function experimentally.

Candidates will be chosen based on previous work performed in *Schmidtea mediterranea*, where ODF2, VFL1 and VFL3 were shown to be essential for basal body orientation. Morpholinos will be used to stop translation of the homologues in *Platynereis dumerilii* and larvae will be imaged to extract properties of the metachronal waves in the prototroch. Additionally, expansion microscopy will be performed to confirm basal body orientation within multiciliated cells.

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Does regeneration re-use developmental gene regulatory networks?

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Regeneration restores complex structures after injury, yet it remains unclear whether the genetic regulatory programs guiding regeneration are the same as those used during embryonic development. My work investigates this question in the crustacean *Parhyale hawaiensis*, a genetically tractable model capable of regenerating its limbs with remarkable fidelity. I have generated paired single-nuclei RNA-seq and ATAC-seq (snMultiome) datasets from uninjured (control) and regenerating legs across multiple time points following amputation. These data capture transcriptional and chromatin accessibility profiles at single-cell resolution, enabling the characterization of regulatory changes during regeneration. The analysis revealed dynamic changes in both transcriptional and chromatin accessibility landscapes across regenerative stages. In uninjured legs, cell types defined by gene expression correspond closely to distinct ATAC clusters, indicating well-resolved regulatory identities. In contrast, early stages of regeneration show reduced cell-type-specific chromatin signatures, suggesting widespread regulatory reorganization after injury. At later stages, distinct chromatin accessibility profiles gradually re-emerge as cell identities are re-established. These results suggest that regeneration involves a transient reorganization of chromatin accessibility before the re-establishment of stable regulatory identities. Ongoing analyses aim to reconstruct cell-type specific GRNs and compare the regulatory logic underlying leg regeneration to that of leg development, from similar data collected from embryos.

P301

A Comparative Study of Abdominal Development in Drosophila

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The insect abdomen exhibits extensive variation in size, shape, and segmental proportions across species. Yet, the developmental and evolutionary mechanisms underlying this diversity remain poorly understood. In holometabolous insects such as *Drosophila*, the adult abdomen forms during metamorphosis through the proliferation and fusion of histoblast nests, which replace the larval epidermis. While this process is well characterized in *Drosophila melanogaster*, its variation across species and its role in insect morphological diversification remains largely unexplored. In this project, we adopt a comparative framework to investigate how differences in abdominal morphology arise during development across a phylogenetically broad panel of over 100 *Drosophila* species. Our approach combines micro-computed tomography (microCT) and developmental timing measurements, alongside live imaging of growth dynamics in selected species, to characterize interspecific variation in adult abdominal size, segmental organization, growth rates, and the duration of key pupal transitions. We are generating detailed measurements of the abdominal sclerite segments, as well as histoblast progenitor number and growth parameters. In parallel, we will record the time to pupariation and adult ecdysis under controlled conditions to examine how species differ in the tempo and duration of metamorphosis. Our goal is to understand how variation in developmental timing and tissue growth dynamics contributes to the diversification of body forms. More broadly, we aim to elucidate which developmental modules align with the trajectories of morphological evolution.

P302

A reaction-diffusion process patterning the veins in a BMP-signalling competent section of early *Bicylus anyana* wings

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The positional-information model of vein formation in *Drosophila* is likely insufficient to explain how veins are set up in insects with more complex venation. Reaction-diffusion has been previously suggested as a mechanism for generating the more complex branching observed in insects with less derived wing development. Using spatial mRNA profiling of early wing development in *Bicyclus anynana* butterflies, we illustrate key differences in early vein/ intervein marker gene expression from *Drosophila* and present evidence that the emergence of the early intervein marker *blistered* is consistent with a reaction-diffusion model. By examining transcriptional readouts, we show that the BMP and EGFR signaling pathways are likely involved in the early specification of vein/ intervein territories, while Notch signaling appears to only play a role in later vein refinement and maintenance. Our results suggest that a medial, BMP-signalling competent section of the wing acts as a permissive domain for reaction-diffusion patterning of the primary veins in *B.anynana*, while veins/ interveins outside of this domain might be specified by a positional-information mechanism similar to that in *Drosophila*. Modifying the size of this permissive domain for reaction-diffusion could provide a modular source of complexity to pattern veins across Insecta.

P303

Shell-ular Biology: Single nuclei-resolved epithelial gene expression changes during moulting in *Litopenaeus vannamei*

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The periodic shedding and replacement of the crustacean exoskeleton, known as moulting (ecdysis), integrates physiological, environmental, and energetic cues through tightly regulated hormonal and molecular cascades. Modern molecular and transcriptomic approaches have identified many of the regulatory mechanisms and molecular changes that occur during well-defined moult transitions. While the epithelial tissue displays a seemingly uniform histomorphology, current knowledge lacks the resolution of epithelial cell types and cell-specific molecular expression patterns driving these phenotypic changes. In the present study, we performed an integrated analysis that combined histology, single-nucleus RNA sequencing (snRNA-seq), and *in situ* hybridisation chain reaction (HCR) to identify epithelial cell types and quantify their gene expression changes across key stages of the *Litopenaeus vannamei* moult cycle. We identified 14 distinct cell clusters that corresponded to stromal, epithelial, muscle, and two specialised clusters of cuticle- and chitin-depositing cell types. The most pronounced gene expression changes occurred immediately following moulting (post-moult) and during the late premoult phase (D3), with the cuticle-associated cell types contributing the majority of differentially expressed genes. Combined histological and *in situ* analyses revealed that these cuticle-depositing cell types form a dense and complex single-cell layer directly beneath the exoskeleton. Furthermore, moult stage-dependent transcriptomic shifts in these cells alternated between expression programmes associated with deposition of structural cuticular proteins, followed by chitin synthesis and cuticular mineralisation. These findings define specialised epidermal cells that act as a complex functional regulatory platform for endocrine-driven remodelling of the exoskeleton during moulting, coordinating the reconstruction and regeneration of the entire exoskeleton.

P304

Tracing the cellular basis of chordate evolution using single cell approaches

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Chordates comprise a group containing vertebrates and their closest relatives. Among these, cephalochordates such as amphioxus (lancelet) stand out due to their numerous morphological and developmental similarities with vertebrates. Comparative studies between amphioxus and other chordates have the potential to inform our understanding of vertebrate evolution, by reconstructing the last common ancestor of chordates (LCA). To this end, previous efforts have focused inprofiling and comparing the temporal regulation of the genome throughout development across chordates, albeit lacking the cellular resolution granted by single-cell technologies. In this project we will profile the cellular diversity of the lancelet *Branchiostoma lanceolatum* throughout development from a molecular, genomic perspective, using single cell transcriptomics and comparative genomics. We will reconstruct differentiation trajectories throughout development for all the major cell types in amphioxus, and compare these with those of a comprehensive set of chordate species. Comparing the molecular machinery (effector genes, Transcription Factors) deployed throughout these trajectories will help uncover the degree of homology of certain structures and cellular fates in chordate development. Our approach will provide a comprehensive view of cellular diversity throughout chordate development with unprecedented resolution, and will help in the reconstruction of the developmental programme of the Chordate LCA.

P305

Thyroid hormone coordinates rotation with musculoskeletal development of the pectoral fins

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Appendage positioning is fundamental for locomotion and ecological adaptation. Numerous vertebrate lineages rotate their appendages during development; nonetheless, the mechanisms underlying appendage rotation remain poorly understood. In many ray-finned fishes, including zebrafish, pectoral fins initially develop along a vertical plane perpendicular to the body axis; during postembryonic development fins rotate 90° anteriorly to adopt a horizontal orientation. Previous studies show that thyroid hormone (TH) regulates zebrafish pectoral fin growth and ray morphogenesis. Here, we show that TH levels regulate the timing and extent of pectoral fin ray growth. Further, TH coordinates musculoskeletal transformations of the pectoral fin, leading to the rotation of the appendage relative to its pectoral girdle. Rotation angle showed a dependence on TH: excess TH stimulated precocious rotation, while absence of TH delayed and prevented rotation. Exogenous TH resulted in fewer endoskeletal elements and larger muscles, whereas TH deficiency resulted in supernumerary skeletal elements and thinner muscles attached more ventrally to the girdle. Strikingly, our experimentally-induced phenotypes resembled pectoral fin morphologies observed in other ray-finned fishes with varying degrees of rotation. To test longstanding assumptions about evolutionary trends in pectoral fin rotation, we quantified rotation angles across a broad representation of ray-finned fishes, comparing endoskeletal morphology variation with phenotypes produced by our TH manipulations. Together, our results suggest that shifts in TH metabolism or its downstream targets may have provided developmental substrate for teleost evolutionary diversification. More broadly, our findings suggest that heterochrony, mediated by hormone signaling, may represent a key developmental mechanism underlying vertebrate appendage diversity.

P306

Characterisation of a deeply conserved prohormone convertase identifies neuroendocrine cells regulating lipid metabolism in larval development

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Neuropeptide signalling is a conserved cell–to-cell communication system in bilaterian animals. The release of bioactive peptides from prohormones requires processing by deeply conserved prohormone convertases (PCs). Among their many functions, neuropeptides regulate metabolic processes essential for animal growth and survival. In holometabolous insects, larval development involves substantial weight gain, yet the mechanisms coordinating lipid storage in the larval fat body and the peptidergic cells involved remain poorly understood. Moreover, most current knowledge derives from flies, which may not represent typical neuropeptidergic regulation in insects. Using the beetle *Tribolium* as a model, we investigated the expression and function of prohormone convertase PC1/3, a gene lost from dipteran genomes but conserved in most other animals. RNAi-mediated knockdown caused a metabolic syndrome marked by a complete arrest of larval weight gain, and the animals did not reach the critical weight for metamorphosis. Metabolic analyses revealed a shift from lipid storage towards increased glycogen and trehalose, the principal circulating sugar in insects. Transcriptomic profiling further showed altered expression of key metabolic enzymes, and suggests an interaction with insulin signalling. To identify the cells involved, we generated a GFP reporter line using CRISPR–Cas9/HDR that faithfully reflects PC1/3 expression. PC1/3 is expressed in gut enteroendocrine cells and in a small set of secretory cells in the suboesophageal ganglion that link the nervous system, gut, and endocrine glands. These findings reveal a central role for these cell types in regulating lipid storage and larval growth.

P307

Interrogating intra-genomic coevolution to identify loci linked to phenotypic shifts

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The phenotype develops through the coordinated deployment of webs of genes. Many of these genes affect the development or function of multiple traits. Such pleiotropy entwines selective forces, which can constrain or enhance future evolutionary pathways. Genomics has provided a trove of new data to interrogate, and, in parallel, new mathematical models for evolution and selection have emerged which take into account conflicting and collaborating selective forces acting on individual phenotypic traits. We still do not have established methodologies for defining how genome evolution is affected by such constraints, but integrating genomic data and phylogenetics in an innovative way is a promising avenue. Here I present a new sliding-window algorithm that identifies and maps co-evolutionary genomic 'rafts' and I apply it to identify shifts in co-evolutionary architecture during major phenotypic transitions in animals. I use tree topology as a first indicator of co-evolution among sites, and then Euclidean vectorial distances of normalised 'homologous' edge (i.e. edges connecting the same nodes in different trees) lengths as a finer-grained metric which can be used to compare between identical topologies. Here I showcase this algorithm, which leverages genomic data to gain evolutionary insights through the lens of evo-devo, and outline how approaches inspired by systems biology can lead us to much deeper insights on evolution.

P308

Divergent visual-system adaptations in two sturgeon species revealed by single-nucleus analysis

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Sturgeons represent an ancient ray-finned fish lineage and thus provide an informative comparative system for studying evolution of vision in vertebrates. This lineage also experienced an independent whole-genome duplication, offering a useful parallel to the more commonly studied teleost fish. We present a comparative analysis of the visual systems of two sturgeon species, the sterlet and the starry sturgeon, using single-nuclei RNA-seq retina data processed with a newly developed pipeline tailored for non-model organisms. Among the two, sterlet represents a classic visual model with a duplex retina including both rods and cones. In contrast, starry sturgeon have lost rods, as well as the expression of the middle-wavelength opsin, and therefore the green-sensitive cones. We annotated retina cell types of both species and found that clusters corresponding to rod is absent in starry sturgeon as well as a cone cell type expressing middle wavelength green opsin. We have discovered strong species specificity in cone cell type clustering and observed increased expression of long-wavelength-sensitive red opsin in starry sturgeon, but whether this represents compensation for the loss of green cones and rods remains uncertain. In contrast both species share the expression profile of UV cone type. The highly modified visual system of starry sturgeon is hard to connect to a specific adaptation, yet it represents one of the few vertebrates without rods, which does not specifically compensate for the loss.

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Investigating the MADS-box gene CRM3 of the fern Ceratopteris richardii and its association to reproductive functions by overexpression and CRISPR-Cas9-mediated knockout

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The emergence of seeds has allowed seed plants (spermatophytes) to colonize habitats that are free of external liquid water and are therefore inaccessible to non-seed plants. The genetic basis underlying the origin of the seed, however, remains largely unknown. In recent years, rapid technological advances and renewed scientific interest have driven investigations into the evolution and development of the closest extant relatives of seed plants—ferns and their allies (monilophytes). Multiple high-quality genomes, including that of Ceratopteris richardii, have been published, and several important laboratory protocols, e.g. genetic transformation, have been established. Phylogenetic reconstruction in a previous study suggested that the MIKC^C-type MADS-box gene CRM3 of Ceratopteris is closely related to the floral developmental B and B-sister genes in seed plants. Therefore, CRM3 may be associated with the evolutionary origin of seeds. Here, we present an easy-to-implement CRISPR–Cas9 system for Ceratopteris, along with an overexpression system. We applied these systems to the functional analysis of CRM3. Our CRISPR system shows a gene editing rate of >80% of the regenerated sporophytes. Phenotyping of the mutants shows an effect on the antheridia and archegonia of the gametophyte as well as the spore development. Those findings are also consistent with the expression pattern of CRM3. Our CRISPR system can be readily adapted to knock out any gene in Ceratopteris, and simultaneous knockout of multiple genes is currently being tested.

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SEM characterization of mechanoreceptors in the skin of adult Elasmobranchs

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Mechanoreceptors are sensory receptors that are located in the dermis of the skin and respond to pressure or distortion and send those signals to the central nervous system through the dorsal root ganglia. The skin is the largest organ, comprised of two main layers, the epidermis and the dermis. Elasmobranchs, a subclass of cartilaginous fish that appeared 420 million years ago, are characterized by their dermal denticles located above the epidermis. While the epidermis and dermis of elasmobranchs is well studied, the presence or abundance of the mechanoreceptors in their skin is not well understood. Previous findings in the lab had provided evidence that elasmobranch mechanoreceptors are morphologically similar to those of mammalian and reptilian mechanoreceptors. Our project was to corroborate these findings by using scanning electron microscopy (SEM). This is the first time that elasmobranch skin has been studied using SEM. We developed a novel protocol and technique to image the skin layers of Elasmobranchs. Our findings show that: 1) SEM can be used to identify lateral lines, Meissner-like structures, Pacinian-like structures, and the ampullae de Lorenzini. 2) Confirmation of the data first observed using hematoxylin and eosin (H&E) staining. SEM has the advantage of providing a richer 3-dimensional view of the mechanoreceptors in Elasmobranchs compared to the 2-dimensional analysis from H&E staining. 3) We identified that the shark structures are similar to the structures in other vertebrates. This work was supported by the CSUN Geology Department's SEM technician Gillian Rhea, NSF 2128646, and U-RISE GM136450.

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An NKX2-5 homolog is required downstream of BMP signaling to pattern the sensory-adhesive organ of a tunicate larva

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Tunicates are the sister group to vertebrates within the chordate phylum, yet unlike other chordate groups, they evolved a biphasic lifecycle alternating between motile larvae and sessile adults. The papillae of most tunicate larvae are the key sensory-adhesive organ regulating their settlement and metamorphosis. The papillae are nearly always arranged as a group of three morphologically identical organs that arise from an anterior neural plate border region nested between ventral epidermis and more dorsal/posterior neural tube progenitors. Due to their embryonic origin and molecular signatures, this anterior border has been evolutionarily linked to vertebrate placode regions. It was previously shown that the specification, patterning, and morphogenesis of the embryonic papilla region all depend on BMP signaling, though downstream mechanisms remain poorly understood. Here we show that the NKX2-3/5/6 ortholog NK4 is a key transcription factor that acts downstream of BMP signaling to pattern the papillae in the *Ciona* embryo. We present evidence that *NK4* is activated by BMP signaling and encodes a transcriptional repressor that is required to restrict the expression of the papilla regulatory gene *Foxg* to three cell clusters that give rise to the three papillae. Loss of NK4 function results in the formation of a single large papilla. In contrast, overexpression of NK4 represses *Foxg,* eliminating the papillae. We also show that the expression of *NK4* is restricted dorsally by the BMP antagonist Chordin, while the ventrally-expressed transcription factor Msx alleviates the repressive effect of NK4, potentially allowing for the characteristic tripartite patterning of the papillae.

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Lamprey Single-Cell Transcriptomics and Spinal Cord Evolution

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The vertebrate nervous system is arguably the most complex and enigmatic biological structure. With the possible exception of cephalopods, it is far more sophisticated than that of invertebrates in terms of size, organisation, and cellular diversity. Yet when and how this complexity evolved remains unclear. Research on nervous system evolution has focused primarily on the brain, while the spinal cord has received comparatively little attention. To fill this critical gap, we used single-cell mRNA sequencing to profile the spinal cord of the developing lamprey, a jawless vertebrate whose phylogenetic position provides unique insights into ancestral vertebrate traits.

We identified dozens of transcriptionally distinct progenitor and neuronal cell populations, revealing that the cellular complexity of the lamprey spinal cord is comparable to that of jawed vertebrates. Comparison of this atlas with a mouse dataset revealed that most neural clusters mapped to corresponding cell-type families and were characterised by conserved transcription factor codes. Interestingly, cross-species mapping was weaker among neural progenitor populations, with some lamprey progenitors exhibiting less defined and overlapping transcriptional signatures. This suggests that lamprey neural progenitor populations may be organised into fewer domains than those of jawed vertebrates, or that fate determination occurs later during development. Together, these findings suggest that vertebrates share a common cellular and molecular organisation of the spinal cord, defined by deeply conserved transcriptional programmes established early during vertebrate evolution.

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Cucurbitaceae: A novel model system for pigment biosynthesis

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Anthocyanins, part of the flavonoid biosynthesis, are responsible for red, purple, and blue coloration and are nearly ubiquitous across angiosperms. Exceptions are rare and have largely been restricted to betalain-producing lineages within Caryophyllales. Here, we report a previously unrecognized, family-wide loss of anthocyanin biosynthesis in the Cucurbitaceae, one of the most economically important plant families, characterized by predominantly white or yellow flowers and carotenoid-based pigmentation. Using a phylogenomics framework encompassing 258 genomic and transcriptomic datasets spanning all 15 Cucurbitaceae tribes, we systematically surveyed the presence, synteny, and evolutionary history of genes involved in anthocyanin and proanthocyanidin biosynthesis. We detected the consistent absence of key structural genes (DFR, ANS, arGST, LAR, and ANR) as well as anthocyanin-related MYB transcription factors across the family. Synteny and phylogenetic analyses support that these absences reflect true gene loss rather than annotation or assembly artifacts. Our results support an evolutionary scenario involving a stepwise loss of anthocyanin pigmentation early in the evolution of Cucurbitaceae, likely accompanied by partial functional replacement through carotenoid-based pigmentation. This discovery challenges the long-held assumption that anthocyanin biosynthesis is universally conserved in flowering plants and identifies Cucurbitaceae as a powerful natural system to identify novel anthocyanin-biosynthesis genes by contrasting Cucurbitaceae (anthocyanin-deficient) and anthocyanin-producing lineages.

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Cellular and molecular dynamics of anterior regeneration in *Pristina leidyi*

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Regeneration is a well-studied process in developmental biology that provides key insights into the evolution of Metazoa. In annelids, or segmented worms, this ability is widespread but highly variable across species, ranging from complete anterior and posterior regeneration to limited tissue repair. A central question concerns the cellular origin of newly formed tissues, specifically, whether regeneration relies on pluripotent stem cells, dedifferentiated mature cells, or lineage-restricted progenitors.

In recent years, several studies have characterized cell populations in adult annelids, both in resting state and during (posterior and parapodial) regeneration; however, little is known about anterior regeneration and the specific cell types involved in the process. To address this gap, we used single-cell transcriptomics during anterior regeneration in the annelid *Pristina leidyi*. By sampling key regenerative stages from wound healing to blastema differentiation, we define cell types, trace differentiation trajectories, and reconstruct gene regulatory networks underlying regeneration.

Our analyses recovered a rich cellular diversity and revealed distinct proliferative stem-like populations, transient progenitors and differentiating lineages contributing to tissue restoration. Lineage reconstruction analyses uncovered dynamic transcriptional transitions and activation of stem cell–associated programs across multiple cell populations.

Altogether, these findings highlight the complex and dynamic nature of anterior regeneration in *P. leidyi* and suggest that conserved gene expression programs and stem-like cell populations underlie different regenerative modes in annelids. This work provides a cellular and molecular framework for understanding regeneration and offers new insights into the evolution of regenerative mechanisms in segmented worms.

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