



ICIRD 2025

15th International Congress on
Invertebrate Reproduction and Development

June 2- 6, 2025

American University • Washington, DC, USA

CONFERENCE PROGRAM





ICIRD 2025

June 2-6, 2025 • Washington DC

The Organizing Committee of the 15th International Congress on Invertebrate Reproduction and Development gratefully acknowledges the generous financial support of the following organizations:



U.S. National
Science
Foundation

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Happy Birthday ISIRD and ICIRD!

Yes, your math is correct, 2025 is the 50th anniversary since the society was formed and of the first ICIRD congress.
Congratulations to everyone involved for these amazing 50 years.



The ICIRD 2025 congress logo!

The 15th ICIRD meeting logo was designed by American University biology graduate – TreVaughn Ellis (BS Biology 2024). TreVaughn is currently enrolled in an MS in Marine Microbiology at the University of Delaware, USA. TreVaughn is not only a biologist, but also a talented artist. He loves to combine his skill sets from both in work such as this. In his own words – “TreVaughn is a lifelong lover of invertebrates and the logo was designed for fellow invertophile and Mentor, Dr Tudge”.

Congress website: burkclients.com/ICIRD/2025/site/index.html

International Society for Invertebrate Reproduction and Development

A BRIEF HISTORY

The International Society for Invertebrate Reproduction (ISIR) was founded in 1975 when it held an inaugural meeting in India. This meeting was organized by Ken and Rita Adiyodi, who also organized the first publisher for the society's journal which is now called [Invertebrate Reproduction and Development](#). The society was later renamed to the International Society of Invertebrate Reproduction & Development (ISIRD).



Past Presidents of the Society

- 1975-1979: Wallis H. Clark (U.S.A.)
- 1980-1989: Wolf Engels (Germany)
- 1990-1992: Motonori Hoshi (Japan)
- 1993-1995: Ian Howie (Ireland)
- 1996-1998: John Pearse (U.S.A.)
- 1999-2001: Marijke de Jong-Brink (The Netherlands)
- 2002-2004: Alan Hodgson (South Africa)
- 2005-2007: Matt Bentley (U.K.)
- 2008-2010: Rachel Collin (Panama)
- 2011-2013: Amir Sagi (Israel)
- 2014-2017: Jeffrey Ram (U.S.A.)
- 2018-2025: Valerio Zupo (Italy)

Conferences

(International Congress of Invertebrate Reproduction & Development – ICIRD)

- 1975: Founding conference organized by Ken and Rita Adiyodi, Calicut, India
- 1979: Davis, California, U.S.A.
- 1981: “In-between congress” Newcastle upon Tyne, England
- 1983: Tübingen, Germany
- 1986: Lille, France
- 1989: Nagoya, Japan
- 1992: Dublin, Ireland
- 1995: Santa Cruz, California, U.S.A.
- 1998: Amsterdam, The Netherlands
- 2001: Grahamstown, South Africa
- 2004: Newcastle upon Tyne, U.K.
- 2007: Panama City, Republic of Panama
- 2010: Prague, Czech Republic
- 2013: Detroit, U.S.A.
- 2017: Naples/Florence, Italy

ISIRD Committee, 2018-2025

- President: Prof. Valerio Zupo (Stazione Zoologica Anton Dohrn, Napoli, Italy)
- Past-president: Prof. Jeffrey L. Ram (Wayne State University, Detroit, U.S.A.)
- Vice-president: Dr. Joris M. Koene (Vrije Universiteit, Amsterdam, The Netherlands)
- Vice-president: Dr. Janet Leonard (UC Santa Cruz, U.S.A.)
- Vice-president: Dr. Olga Yurchenko (Russian Academy of Sciences Vladivostok, Russia)
- Secretary: Dr. Florian Raible (Max F. Perutz Laboratories, Austria)
- Treasurer: Prof. Ulrich Hoeger (Johannes Gutenberg-Universität Mainz, Germany)

ISIRD: Regional Representatives

A number of regional representatives assist the ISIRD committee in promoting its activities. Current regional representatives are:

- Prof. Maria Byrne (Australia)
- Dr. Carol Simon (South Africa)
- Dr. Christopher Tudge (U.S.A.)
- Dr. Melike Erkan (Turkey)
- Prof. Valerio Zupo (Mediterranean)

ISIRD: Honorary members

The International Society for Invertebrate Reproduction & Development has awarded the following people with an honorary membership:

- 2013 – Alan Hodgson (South Africa)
- 2010 – Hans Laufer (USA)
- 2007 – Motonori Hoshi (Japan)
- 2007 – John Pearse (USA)

ICIRD-2025: 15th International Congress Invertebrate Reproduction and Development



Convenor

Christopher Tudge, American University, Washington DC, USA

Organizing Committee

- Eric Haag (University of Maryland)
- Jeff Ram (Wayne State University – Detroit)
- John Bracht (American University)
- Carlos Machado (University of Maryland)
- Alexa Bely (University of Maryland)

Scientific Committee

- Valerio Zupo (Zoological Station, Naples, Italy)
- Ulrich Hoeger (University of Mainz, Germany)
- Florian Raible (University of Vienna, Austria)
- Janet Leonard (University of California, Santa Cruz)
- Joris Koene (Vrije University, Netherlands)

Congress assistance

We especially appreciate the administrative and other assistance of Lori, Jill, Khuong, Cooky and Elizabeth (Burk Inc., VA), Michelle and Kim (AU, Conference & Guest Services), Lorraine, Bernadette, Andrea and Wyatt (AU, Hall of Sciences), AU ADVANCE and TRAC programs, and the Office of the Dean of the College of Arts and Sciences.

Congress volunteers

The following student and other volunteers are thanked for their time and assistance: Stefania Mehedincu, Siv Bjorge, Sofia Palau, Jayden Mooso, Jonathan Barley, Sonora Robles, Ariane McDermott and Karen Mudar.

Congress Symposia Organizers

The participation, dedication and hard work of the organizers of the various congress symposia is hugely appreciated, and without them there would basically not be a congress!

- SY1: Development of Exoskeleton Biomineralization (Organizers – Shai Shaked & Amir Sagi)
- SY2: Sexual Plasticity (Organizers – Eliahu Aflalo & Amir Sagi)
- SY3: Nematode Reproduction (Organizer – Eric Haag)
- SY4: Diversity & Evolution of Invertebrate Reproductive Pheromones, Chemical Cues & their Application (Organizer – Jeff Ram)
- SY5: Crustacean Reproduction (Organizer – Christopher Tudge)
- SY8: Care-full Insects—development and evolution of reproductive and caregiving behaviors in insects (Organizers – Mallory Hagadorn & Clare Rittschof)

Adiyodi Memorial Lecturer

At each ICIRD congress one oral presentation, usually a plenary or keynote, is selected to be the Adiyodi Memorial Lecture in honor of the ISIRD and ICIRD founder, Kenoth Adiyodi.



Dr Kenoth G. Adiyodi

Creator: Nadirsha.mob:9847220865

Copyright: nadairsha3737@gmail.com

Information about Dr K. G. Adiyodi can be found at:

en.wikipedia.org/wiki/Kenoth_G._Adiyodi

The 15th ICIRD 2025 Adiyodi Memorial Lecturer is:

Dr W. Malcolm Byrnes from Howard University College of Medicine, Washington DC, for his keynote oral presentation in the Diversity & Evolution of Invertebrate Reproductive Pheromones, Chemical Cues & their Application symposium. His presentation is entitled “*E. E. Just, champion of the cell surface*” will be at 11:00 AM on Tuesday morning.

General Information

Registration

ICIRD registration is in Hall of Science (HoS) 113, the Collaboration room. Registration will open at 5:00pm on Sunday, June 1, 2025. Each morning (Monday – Friday) the registration desk will open at 8:30 am, half an hour before the oral presentations start.

Badge Identification

Please wear your ICIRD 2025 congress badge at all times while on campus, as an official attendee of the congress. If you lose your badge, please see the registration desk.

Lecture Facilities

All oral presentations will be in the same lecture hall, T07, on the terrace level of HoS.

Bathroom Facilities

There are male, female and all gender bathrooms on each level of the HoS building, to the left of rooms 113 and T07, as you leave each room.

Coffee Breaks and Exhibitor Tables

The morning and afternoon coffee/refreshment breaks will be in the same room as registration, HoS 113. Each break is 30 minutes. Any exhibitor tables are also in this room.

Breakfast and Lunches

If you purchased the meal plans for breakfast and/or lunch, your meals are pre-loaded onto your swipe card provided for dorm access, or just for meals if you are staying off campus. Breakfast and lunch is served in the Terrace Dining Room (TDR) in the basement of the Mary Graydon Center.

Please wear your ICIRD badge and have your swipe card to be able to access the dining room and get your meals.

If you did not purchase any meal programs, then your nearest place to get breakfast and lunch is a 5-minute walk down to 3201 New Mexico Avenue to the shopping complex on the left of the street. You will find a Starbucks, Shemali's café (great Middle Eastern food), Wagshal's Café and bar, Al Dente Italian restaurant, Cravings (an ice cream and smoothie café) and Chef Geoff's bar and restaurant. Some will be open for breakfast, but all should be open by lunchtime. You only have 1 hour for lunch and so be mindful of time if you decide to have a sit-down lunch. Many of these establishments though are your nearest places for dinner and drinks at night.

If you are a light breakfast eater (coffee and croissant), try the student-run Davenport Coffee Lounge which is on AU's campus on the ground floor corner of the School of International Service Building. It opens at 8:30 am, just in time to make it to the 9:00 AM oral presentations.

All dinners, with the exception of the opening social BBQ (Sunday evening) and congress dinner (Thursday evening), are "on your own".

ISIRD Membership

Not yet an ISIRD Member?
Need to renew?
Scan the QR code to join!



Opening Social & Congress Dinner

Your registration paid for your attendance at the opening social event on Sunday evening (June 1), which is an outdoor (weather-permitting) BBQ with drinks. This is on the patio outside of HoS room 113 (the registration room) from 6-8 pm.

The "official" ICIRD congress dinner is on Thursday evening (June 5) from 7:00-9:00 PM at Guapo's Restaurant, Tenleytown. You must purchase a ticket to attend.

Poster Session

Posters will be displayed in HoS 113 (the Collaboration room, again!) on the walls of the room. The poster session with refreshments is Tuesday (June 3) from 6:30-7:30 pm. Please have your posters up on Monday. All posters can stay on display until Friday, when they should be removed at the end of the congress by 6:00 pm.

Oral Presentation Submission and Preview

Final oral presentations can be submitted at the computer assistance desk in the registration area in HoS 113 when you check in at the registration desk. You will be able to quickly preview your presentation on this computer. We will transfer your presentation to the lecture hall on the day of your presentation. Please bring your presentation on a USB drive in powerpoint.

Internet Access

ICIRD congress attendees will be able to log in as guests to access the internet while on campus.

AU Public Safety

You can use the following ways to contact the AU Police and Emergency Management Department.

- Non-emergency phone: 202-885-2527
- Emergency phone: 202-885-3636
- Website: www.american.edu/finance/publicsafety

The department of Public Safety is physically housed in the basement of the Don Myers Technology and Innovation Building (DMTI) at the NE corner closest to the parking lot. Ring the bell for assistance.

General Information

Congress Bulletin Board

There will be a large white board outside of HoS 113 for general announcements, corrections and cancellations and public messaging. A daily schedule of presentations will be posted here as well.

Dormitory Lodging

For those of you staying in AU's beautiful dorms you will be in Constitution Hall on the East Campus, near the corner of Nebraska Ave and New Mexico Ave. You can check in at the front desk upon arrival. If you drive to campus, you can park in the open lot near your building. All parking at AU has a fee from Monday to Friday.

Parking at AU

There is no free parking on the AU campus during the week (Monday – Friday). There are a couple of lots available to pay by phone: these are under the School of International Service Building on Nebraska Ave and in the open lot near the dorms above (see map below). Guests can purchase a parking permit through guest user registration for the days they need parking. Instructions at: american.t2hosted.com/cmn/newuser.aspx

Congress Tours/Excursions

Wednesday (June 4) is a free day for attendees to enjoy AU's campus, the surrounding neighborhoods and Washington DC, with its many attractions.

There are three special tours that have been organized by ICIRD on this Wednesday. Many of you have signed up and paid for these already. There may still be room on some of these tours and so ask at the registration desk if you can join. The tours are:

Great Falls Hike

9:00 AM departure from AU for Great Falls National Park (Maryland side) and Chesapeake and Ohio Canal National Historic Park. www.nps.gov/choh/planyourvisit/greatfallstavernvisitorcenter.htm

The vehicle(s) leave from the congress venue outside the HoS building for the 30-minute ride to the National Park and National Historic Park. Participants will get several hours to hike the Chesapeake and Ohio Canal (C & O Canal), view the Great Falls from Olmstead Island on the Maryland side, visit the old tavern and visitor's center and observe the functional lock system. Opportunities to observe wildlife will be plentiful. You will walk approximately 1-1.5 miles on gravel paths and boardwalks. Do not be late, as you will be left behind! The entrance fee to the park is part of your tour fee and lunch is provided.

Smithsonian Institution, Department of Invertebrate Zoology, Collections Tour

1:45 PM departure from the congress venue outside the HoS building for a behind-the-scenes tour of the Smithsonian Institution, National Museum of Natural History, Department of Invertebrate Zoology Collections. naturalhistory.si.edu/research/invertebrate-zoology

ICIRD volunteers will guide you from campus to the museum. Alternatively, participants can meet at 2:45 PM at the Constitution Avenue entrance to the National Museum of Natural History, inside the foyer once you have passed through the security checkpoint. Your volunteers and museum host will meet you there. Do not be late, as you will be left behind! Entrance to the Natural History Museum is free, but security passes will be issued for the behind-the-scenes tour. The tour duration is 1-1.5 hours.

Smithsonian Institution, National Zoo and Conservation Biology Institute, Migratory Bird House Tour:

9:00 AM departure from the congress venue outside the HoS building for a guided tour of the National Zoo's new bird house. nationalzoo.si.edu/animals/exhibits/bird-house

ICIRD volunteers will guide you from campus to the zoo. Alternatively, participants can meet at 10 AM outside the entrance to the National Zoo and Conservation Biology Institute's Migratory Bird House. Your zoo hosts will meet you there. Do not be late, as you will be left behind! Entrance to the National Zoo is free, but timed entry passes are required. Tour duration is 1-1.5 hours in duration. nationalzoo.si.edu/passess

Pre- and Post-Congress Birding Trips

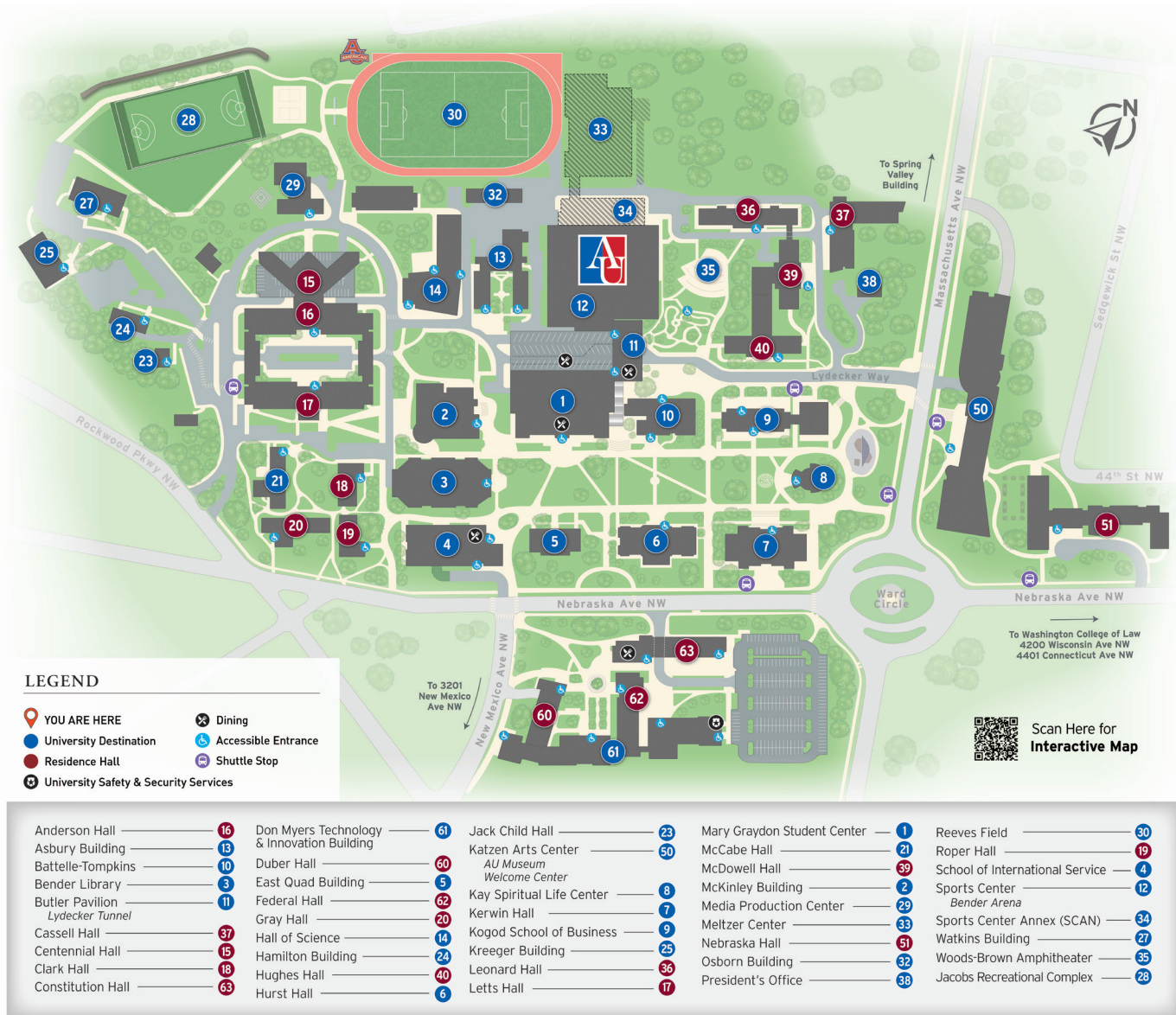
- **Pre-Congress local birding tour (7:15 AM Sunday June 1)**
Led by your local birding guide (Chris Tudge) we will visit a local birding hotspot, Huntley Meadows, in Alexandria, Virginia. www.fairfaxcounty.gov/parks/huntley-meadows-park

We leave from the congress venue outside of HoS. Wear comfortable outdoor shoes and clothing. A hat is recommended. Binoculars and bird guides provided to all participants. You will walk approximately 1 mile on gravel paths and boardwalks. Total tour duration is 3-4 hours.

- **Post-Congress local birding tour (7:00 AM Saturday June 7)**
Led by your local birding guide (Chris Tudge) we will visit a local birding hotspot, McKee-Beshers Wildlife Management Area, Poolesville, Maryland. dnr.maryland.gov/wildlife/pages/publiclands/central/mckeebeshers.aspx

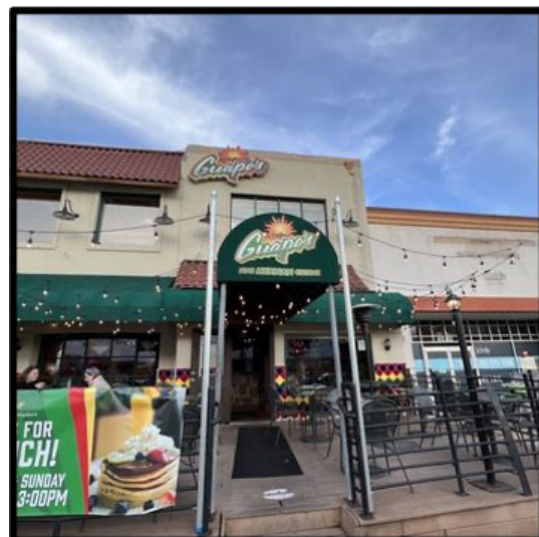
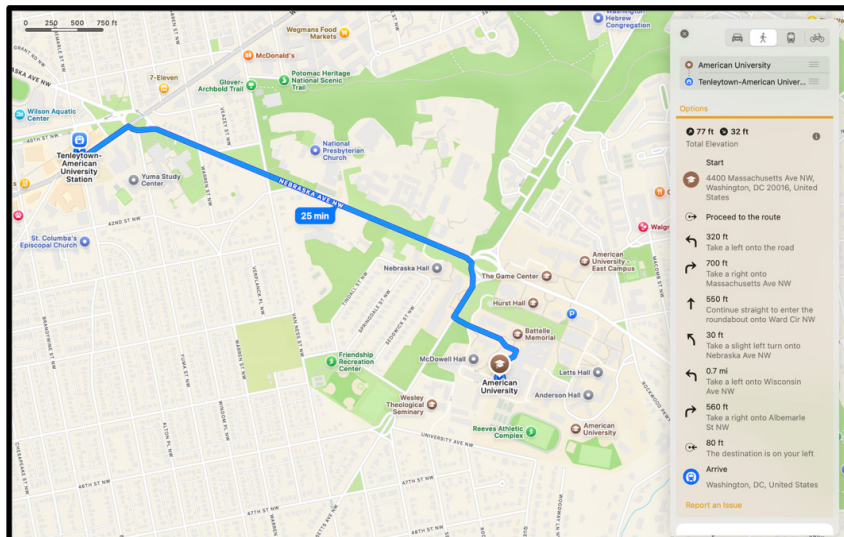
We leave from the congress venue outside of HoS. Wear comfortable outdoor shoes and clothing. A hat is recommended. Binoculars and bird guides provided to all participants. You will walk approximately 1 mile on grass and gravel paths. Total tour duration is 3-4 hours.

Map of American University Campus



Restaurants

Directions from American University to Tenleytown-AU Metro



Tenleytown Restaurants and Getting There

- A 25-minute walk from American University to Tenleytown-AU Metro is possible:
 - Refer to the screenshot above!
 - You get to see the Embassy of Japan.
 - Not a strenuous or uphill walk, wide paved sidewalks are available the entire way.
- The AU shuttle system also provides transport to Tenleytown:
 - Exit through the Hall of Science main entrance.
 - Anderson hall is to your right, walk through the courtyard and behind the building to the left is the bus stop for the AU shuttle.
 - Board the bus and stay on until the end, the final stop brings you right to the Tenleytown AU metro station (look for the Wawa convenience store).

Refreshments and Fare

- Guapo's Restaurant:
 - Mexican Cuisine
 - Click [here](#) for the menu and directions

Other options in the same area can be found [here](#).

If coffee is what you are looking for, head to the [Davenport Coffee Lounge](#) on campus in the lobby of the SIS building.

Spring Valley Restaurants and Getting There

Spring Valley is accessible by AU shuttle bus (Red Line), or 15-minute walk down Massachusetts Ave

- **Millie's** – lunch and dinner, new American cuisine, gluten-free options available. milliesdc.com
- **Playa Bowls** – coffee, smoothies, bowls, breakfast, vegan and gluten-free options available. playabowls.com/location/spring-valley
- **Bluestone Lane** – breakfast, coffee and lunch, vegan, gluten-free and kosher options available. bluestonelane.com/cafes/spring-valley

New Mexico Ave Restaurants and Getting There

A 5-minute walk from campus, down the hill past the Don Meyers Innovation and Technology building.

- **Starbucks** – Coffee and breakfast, vegan, gluten-free and kosher options available. www.starbucks.com
- **Shemali's** – Mediterranean food, lunch and dinner, vegan options available. shemalis.com
- **Wagshal's** – Sandwiches, soups, coffee and groceries, bar with happy hour. www.wagshals.com
- **Al Dente** – Italian food, lunch and dinner, gluten-free, vegetarian and kosher options available. www.aldentedc.com/
- **Chef Geoff's** – American food, lunch and dinner, vegan and gluten-free options available. www.chefgeoff.com/cgmenu

Schedule-at-a-Glance

June 1 Sunday

7:00 AM – 10:00 AM	Bird Tour	Offsite
5:00 PM – 6:00 PM	Registration	Hall of Science 113
6:00 PM – 7:30 PM	BBQ	Hall of Science 113 Patio

June 2 Monday

8:30 AM – 9:00 AM	Registration	Hall of Science 113
9:00 AM – 9:30 AM	Opening Ceremony	Hall of Science T07
9:30 AM – 1:00 PM	SY1: Development of Exoskeleton Biomineralization	Hall of Science T07
1:00 PM – 2:00 PM	LUNCH	Terrace Dining Room
2:00 PM – 3:30 PM	SY1: Development of Exoskeleton Biomineralization	Hall of Science T07
3:30 PM – 4:00 PM	BREAK	Hall of Science 113
4:00 PM – 6:30 PM	SY8: Care-full Insects—development and evolution of reproductive and caregiving behaviors in insects	Hall of Science T07

June 3 Tuesday

8:30 AM – 9:00 AM	Registration	Hall of Science 113
9:00 AM – 1:00 PM	SY4: Diversity & Evolution of Invertebrate Reproductive Pheromones, Chemical Cues & their Application	Hall of Science T07
1:00 PM – 2:00 PM	LUNCH	Terrace Dining Room
2:00 PM – 5:30 PM	SY4: Diversity & Evolution of Invertebrate Reproductive Pheromones, Chemical Cues & their Application	Hall of Science T07
5:30 PM – 6:30 PM	SY6: Contributed Papers	Hall of Science T07
6:30 PM – 7:30 PM	Poster Session	Hall of Science 113

June 4 Wednesday

8:30 AM – 9:00 AM	Registration	Hall of Science 113
9:00 AM – 9:30 AM	Great Falls Tour	Offsite
10:00 AM – 10:30 AM	Zoo birdhouse Tour	Offsite
3:00 PM – 3:30 PM	SI IZ tour	Offsite

June 5 Thursday

8:30 AM – 9:00 AM	Registration	Hall of Science 113
9:00 AM – 1:00 PM	SY2: Sexual Plasticity	Hall of Science T07
1:00 PM – 2:00 PM	LUNCH	Terrace Dining Room
2:00 PM – 2:40 PM	SY2: Sexual Plasticity	Hall of Science T07
3:00 PM – 3:30 PM	BREAK	Hall of Science 113
3:30 PM – 6:30 PM	SY3: Nematode Reproduction	Hall of Science T07
7:00 PM – 9:30 PM	Congress Dinner	Guapo's, Tenleytown

June 6 Friday

8:30 AM – 9:00 AM	Registration	Hall of Science 113
9:00 AM – 1:00 PM	SY6: Contributed Papers	Hall of Science T07
1:00 PM – 2:00 PM	LUNCH	Terrace Dining Room
2:00 PM – 5:00 PM	SY5: Crustacean Reproduction	Hall of Science T07
5:00 PM – 5:30 PM	Closing Ceremony	Hall of Science T07

June 7 Saturday

7:00 AM – 10:00 AM	Bird Tour	Offsite
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In Memoriam Prof. Hans Laufer

October 18, 1929 – August 17, 2024

Prof. Hans Laufer Professor in the Department of Molecular & Cell Biology at the University of Connecticut

Hans completed his PhD at Cornell University, his Postdoctoral studies at Johns Hopkins University, and then became a member of the Department of Molecular and Cell Biology at the UConn Storrs campus. Every year during the summer months Hans conducted research at the Marine Biological Laboratory in Woods Hole.

Hans's main interests include gene regulation during development and reproduction; hormonal and molecular interactions; and comparative endocrinology of invertebrates, particularly hormones affecting reproduction, morphogenesis and metamorphosis. He was particularly interested in elucidating the signal transduction pathways involved in the regulation of reproductive and developmental events of crustacea and how environmental endocrine disrupting compounds interact with the normal pathways and their hormonal receptors.

He was a member of the Connecticut Academy of Science and Engineering, a Fellow of the American Association for the Advancement of Science and Elected Fellow of the Royal Entomology Society of London. In 2010 was awarded an Honorary Membership of the International Society for Invertebrate Reproduction and Development (ISIRD), where he served as president and was a member since 1978, and editor of the society journal of Invertebrate Reproduction and Development since 1998.

Hans was born in Silesia, Germany. In 1939, after Kristallnacht, he and his family immigrated to the United States. He was married to Evelyn Green and they had three children Jessica, Marc, and Leonard, and six grandchildren.

More about Hans and his family can be found on these websites:

- tbewellesley.org/hans-laufer
- www.isird.org/members/Laufer_honorary_ISIRD_member.pdf


ICIRD Congress Dinner

You're Invited!

Join us for an evening of great food, conversation, and connection as we celebrate the ICIRD Conference!

 **Date:** Thursday, June 5

 **Time:** 7:00 PM

 **Dinner Buffet:** \$40 (includes your first drink)

 **Venue:** Guapo's Restaurant, Tenleytown

 [Guapo's Website](#)



The dinner will be held in the upstairs function room at Guapo's. Please pre-pay by scanning the QR code or using [this link](#) so we can plan accordingly and get an accurate headcount. It will be a wonderful chance

to unwind and enjoy the evening with fellow conference participants. We look forward to seeing you there!

Getting There from American University

Walking

- A 25-minute walk from American University to the Tenleytown-AU Metro Station is possible. (Refer to the screenshot above if available.)

By AU Shuttle

- Exit the Hall of Science main entrance. Walk to Anderson Hall, which will be on your right. Pass through the courtyard; the shuttle stop is located behind the building to the left.
- Board the shuttle and ride to the final stop at the Tenleytown-AU Metro Station. From there, turn right onto Wisconsin Ave—Guapo's will be immediately on your right.

Scientific Program

June 2 Monday

9:00 AM – 9:30 AM

Opening Ceremony

Welcome to delegates

Christopher Tudge
Professor of Biology, American University & Chair of ICIRD
2025 Organizing Committee

Welcome to AU

Vicky Wilkens
Provost American University

Welcome to the College of Arts and Sciences and the new science building

Katie DeCicco-Skinner
Associate Dean of Faculty Affairs

Welcome from ISIRD

Valerio Zupo
Scientist, Stazione Zoologica Anton Dohrn, Napoli & President ISIRD.

Welcome to campus & emphasis on public safety while at ICIRD

Lt. Pamela Salazar
American University Public Safety

SY1

9:30 AM – 3:30 PM

Development of Exoskeleton Biomineralization

Organizers: Shai Shaked & Amir Sagi

9:30 AM

SY1-1

The structure and formation of the mineralized exoskeleton of crustaceans during development and growth

Robert Roer
University of North Carolina Wilmington

10:00 AM

SY1-2

Impact of heavy metals on exoskeletal mineralization in the post-ecdysial blue crab, *Callinectes sapidus*: insight into epidermal metal transporters

Enmin Zou
Nicholls State University

10:30 AM

BREAK

11:00 AM

SY1-3

Possible interactions between limb regeneration and exoskeleton synthesis determining molt stage transitions

Donald L. Mykles
Colorado State University

11:40 AM

SY1-4

Mechanical properties of biomineralized crustacean exoskeletons

Jennifer R. A. Taylor
University of California San Diego

12:00 PM

SY1-5

An evolutionarily conserved mineralization pathway in balanomorph barnacles

Gary H. Dickinson, Jazmine Shaw, Phylcia Menendez, Corin Hoppe, Nick Aldred, Beatriz Orihuela, Daniel Rittschof, Rebecca A Metzler
The College of New Jersey

12:20 PM

SY1-6

The combined effects of global warming and pollution on development and biomineralization of sea urchins

Chiara Martino, Roberto Chiarelli, Dario Savoca, Manuela Mauro, Maria Byrne, Thorsten Hüffer, Rosario Badalamenti, Antonella Maccotta, Vincenzo Arizza, Mirella Vazzana
University of Palermo, Italy

1:00 PM

LUNCH

2:00 PM

SY1-7

Unraveling the genomic toolkit of crustacean exoskeleton biomineralization: Ion transporters in *Cherax quadricarinatus*

Amir Sagi, Shai A. Shaked, Rivka Manor, Simy Weil and Eliahu D. Aflalo
Ben-Gurion University of the Negev

2:30 PM

SY1-8

A tale of two functional proteins involved in crayfish exoskeleton scaffold formation and mineralization

Shai A. Shaked, Rivka Manor, Simy Weil, Eliahu D. Aflalo and Amir Sagi
Ben-Gurion University of the Negev

3:00 PM

WRAP UP & DISCUSSION

SY8**4:00 PM – 6:30 PM**

**Care-full Insects—
development and evolution of reproductive
and caregiving behaviors in insects**

Organizers: Mallory Hagadorn & Clare Rittschof)

4:00 PM**SY8-1**

Phenotypic plasticity and behavior in horned beetles

Sofia Casasa

Boston University

4:30 PM**SY8-2**

Maternal Care Enhances Starvation Resistance in Bumble Bees:
The Role of Early Rearing History in Metabolic Resilience

Claudineia P Costa, S Hollis Woodard

University of California, Riverside

5:00 PM**SY8-3**

A welfare-focused lens on insect reproduction

Meghan Barrett

Indiana University Indianapolis

5:30 PM**SY8-4**

Using social bees to explore the impacts of pre-adult experience
on developing neural systems

Mallory A. Hagadorn and Clare C. Rittschof

University of Kentucky

6:00 PM**SY8-5**

Offspring overcome poor parenting by being better parents in a
subsocial beetle

Ahva L. Potticary, C.B. Cunningham, A.J. Moore

Northern Michigan University

June 3 Tuesday

SY4**9:00 AM – 5:30 PM**

**Diversity & Evolution of Invertebrate
Reproductive Pheromones,
Chemical Cues & their Application**

Organizer: Jeff Ram

9:00 AM**SY4-1**

Sagi Laufer Tribute Lecture

9:30 AM**SY4-2**

Peptide Hormone Shapes Lipid-Steroid Metabolic States to
Trigger Sexual Maturation

Jie Sun, Wen-Kan Liu, Calder Ellsworth, Adam Aldahir, Hong-Cun Bao,
Yi-Chun Huang, Wu-Min Deng

Tulane University

10:00 AM**SY4-3**

Day/night cycles regulate pheromone acuity to gate rhythmic
courtship behavior

Renny Ng, Margarita Bellah, and Chih-Ying Su

University of California San Diego

10:30 AM**BREAK****11:00 AM****SY4-4**

E. E. Just, champion of the cell surface

W. Malcolm Byrnes

Howard University

11:45 AM**SY4-6**

What is a worm pheromone doing in humans? Spawning of *Alitta succinea* elicited by modulators of renal calcium sensing

Jeffrey L Ram and Michal L. Ram

Wayne State University

12:30 PM**SY4-7**

The physiological and molecular mechanisms of Juvenile
hormone regulation of reproduction in bumble bees

Hagai Y. Shpigler, Eran Levin, Gene E. Robinson, Guy Bloch

Agricultural Research Organization – The Volcani Institute

1:00 PM**LUNCH**

2:00 PM **SY4-8**
Substances in the mandibular glands mediate queen effects on larval development and colony organization in an annual bumble bee
Bloch Guy, Franco Maayan, Fassler Rosi, Goldberg Tzvi, Reichmann Dana
Hebrew University of Jerusalem

2:30 PM **SY4-9**
Exposure to miticides and agrochemicals during development affects the reproductive health of honey bee (*Apis mellifera*) queens and drones
Juliana R., Elizabeth M. Walsh, Tonya S. Shepherd
Texas A&M University

3:00 PM **SY4-10**
Impact of stress on division of labour in honeybee colonies
Zeynep N. Ulgezen, Coby van Dooremalen, Frank van Langevelde
Wageningen University & Research

4:00 PM **SY4-11**
Testing the reproduction-immunity trade-off in honey bee queens using lipidomics and proteomics
Leonard J. Foster
University of British Columbia

4:30 PM **SY4-12**
Peptide Pheromones, Endoproteinases, and Egg Hatching: Barnacles and Pea Crabs
Daniel Rittschof, Joshua Osterberg, Beatriz Orihuela, Desa Bolger
Duke University

5:00 PM **WRAP UP & DISCUSSION**

SY6

5:30 PM – 6:30 PM

Contributed Papers

5:30 PM **SY6-1**
Toxicological Response of the Sea Urchin *Paracentrotus Lividus* to Virgin and Commercially Derived Polystyrene Microplastics
Maria Costantini, Amalia Amato, Simone Gioia, Roberta Esposito, Immacolata Liotta, Mariacristina Cocca, Davide Caramiello, Loredana Manfra, Giovanni Libralato, Valerio Zupo
Stazione Zoologica Anton Dohrn

6:00 PM **SY6-2**
Effects of *Chlorella vulgaris* bioremediation on alterations induced by microplastics in the gills of *Mytilus galloprovincialis*
Rachele Macirella, Mariarosaria F. Muoio, Federica Talarico, Elvira Brunelli
University of Calabria

6:30 PM – 7:30 PM

Poster Session

P1
Organization of the ovaries in earthworms belonging to the family Acanthodrilidae – a preliminary study
Dominika Raś, Shweta Yadaw, Pooja Tiwari, Anupam Verma, Piotr Świątek
University of Silesia in Katowice

P2
Comparative Morphology of Land Hermit Crab (Genus *Coenobita*) Larval Development
Anna Clark, Christopher Tudge
American University

P3
The toxic effect of an oxylipins-containing macroalgae extract on sea urchin reproduction and development
Chiara Martino, Rosario Badalamenti, Roberto Chiarelli, Antonio Palumbo Piccionello, Manuela Mauro, Vincenzo Arizza, Mirella Vazzana
University of Palermo, Italy

P4

Why do microplastics affect the symbiosis between corals and Symbiodiniaceae?

Nami Okubo
Rikkyo University

P5

Insights into the origin of nuclear-encoded mitochondrial genes from genomic analyses of two closely related species of *Drosophila*

Javier Carpinteyro-Ponce, Fatemeh Yosefy, Carlos A. Machado
university of Maryland college park

P6

Sex ecology in the European green crab *Carcinus maenas* in its native range.

R Graves, O Medlar, SC Culloty, SA Lynch*
University College Cork

P7

Use of gonad and brood pouch placement to identify platyctene genera

Nicholas Bezio, Gustav Paulay, Allen Collins
University of Maryland

P8

Reproductive biology of the rock oyster *Striostrea prismatica* in two populations of the Mexican Central Pacific

Karla Genoveva Ríos-González, Jorge Chávez-Villalba, María Cristina Chávez-Sánchez, Selene María Abad-Rosales, Saúl Guerrero-Galvánb, Elaine Espino-Barre and Ernesto López-Uriarte
Universidad de Guadalajara

June 5 Thursday

SY2**9:00 AM – 3:00 PM****Sexual Plasticity**

Organizers: Eliahu Aflalo & Amir Sagi

9:00 AM**SY2-1**

The constraints of sexual plasticity: a crustacean perspective

Chiara Benvenuto, Stephen C. Weeks, Cristiana Ramalho Maciel
University of Salford

9:30 AM**SY2-2**

Extreme sexual plasticity and flexibility within an annelid genus

Maria Cristina Lorenzi
University of Sorbonne Paris Nord

9:50 AM**SY2-3**

Sexuality and Sexual Plasticity in peppermint shrimps *Lysmata* spp.: the role of sexual selection in driving sex allocation

Baeza, J. A.
Clemson University

10:30 AM**BREAK****11:00 AM****SY2-5**

Keynote Lecture

11:30 AM**SY2-6**

Sex allocation in a hermaphroditic host barnacle shows flexible changes in response to parasitic infestation

Masami M. Tamechika, Hiroyuki Yamada, Shigeho Ijiri, Yoichi Yusa
Hokkaido University

11:50 AM**SY2-7**

Ferroptosis facilitates sexual plasticity in *Hippolyte inermis* (Leach, 1815): patterns of proteins and genes

Valerio Zupo, Marialuisa Lusito, Stefania Crispi., Roberta Esposito, Amir Sagi, Eliahu D. Aflalo, Anna Italiano, Bruno Pinto, Maria Costantini
Stazione Zoologica Anton Dohrn

12:10 PM **SY2-8**
Sexual plasticity of the decapod crustacean *Hippolyte inermis*: patterns of expression of genes involved in vitellogenin and insulin-like hormone metabolism

Bruno Pinto, Marialuisa Lusito, Amalia Amato, Roberta Esposito, Amir Sagi, Eliahu D. Aflalo, Ulrich Hoeger, Takashi Gojobori, Robert Hoehndorf, Maria Costantini, Valerio Zupo
Stazione Zoologica Anton Dohrn (Naples, Italy)

12:30 PM **SY2-9**
Isolation, characterization and aging of germline stem cells in a hermaphrodite protochordate

Tom Levy, Chiara Anselmi, Katherine J. Ishizuka, Tal Gordon, Karla J. Palmeri, Yotam Voskoboynik, Erin McGeever, Angela M. Detweiler, Liron Levin, Daniel D. Liu, Rahul Sinha, Benjamin F. Ohene-Gambill, Tal Raveh, Maurizio Morri, Virginia Vanni, Lucia Manni, Norma F. Neff, Benyamin Rosental, Irving L. Weissman, Ayelet Voskoboynik
Stanford University

1:00 PM **LUNCH**

2:00 PM **SY2-10**
Sexual differentiation in decapod crustaceans exhibiting ZW/ZZ sex inheritance: the case of the IAG-sexual switch in the prawn *Macrobrachium rosenbergii*

Sagi A
Ben Gurion University of the Negev

2:20 PM **SY2-11**
Sexual Differentiation in *Macrobrachium rosenbergii*: Unravelling the Link Between the IAG-Switch and W/Z-Linked Regulatory Elements

Eiahu D Aflalo, Melody Wahl, Tom levy Yaniv Borojovich, Rivka Manor, Barak Rotblat, Vered Chalifa-Caspi, Noa Asculai, Amir Sagi
Ben Gurion University of the Negev

SY3

3:30 PM – 6:30 PM
Nematode Reproduction
Organizer: Eric Haag

3:30 PM **SY3-1**
Strongyloides spp. – a life between sex and parthenogenesis

Adrian Streit
Max Planck Institute for Biology Tübingen

4:00 PM **SY3-2**
Potential amplification of collagen gene copy number in the elongated nematode, *Caenorhabditis inopinata*

Gavin C. Woodruff
University of Oklahoma

4:30 PM **SY3-3**
Natural variation reveals hidden divergence in the evolution of a polyphenism

Stephen M. Dreyer, Erik J. Ragsdale
Indiana University

5:00 PM **SY3-4**
Exploring the mechanisms through which MSS glycoproteins modulate sperm competitiveness

Asan Turdiev, Justin Van Goor, Savannah J. Speir, Jillian Manning, Eric S. Haag
University of Maryland, College Park

5:30 PM **SY3-5**
The Devil (Worm) in the (Genetic) Details: Breaking Muller's Ratchet

Ali Amini and John R. Bracht
American University

June 6 Friday

SY6

9:30 AM – 12:30 PM
Contributed Papers

9:00 AM **SY6-1**
Characterizing the phenotypic effects of disrupting conserved long noncoding RNAs in *Drosophila melanogaster* and *D. pseudoobscura*

Yuxi Wang, Carlos Machado
University of Maryland, College Park

9:30 AM **SY6-2**
Developmental repurposing of larval neurons for adult sexual behaviors in *Drosophila*

Kara E. Miller, Troy R. Shirangi
Villanova University

10:00 AM **SY6-3**
High-resolution single-cell transcriptomic comparison of double-sex-expressing neurons across *Drosophila* species

Justin Walsh, Ian P. Junker, Yu-Chieh David Chen, Yen-Chung Chen, Helena Gifford, Yun Ding
University of Pennsylvania

10:30 AM **BREAK**

11:00 AM **SY6-4**
Can exposure to seaweed biocompounds and selenium influence reproductive development in the European oyster *Ostrea edulis*?
A Mitchell, C Collins, SC Culloty, SA Lynch*
University College Cork

11:30 AM **SY6-5**
Sexual reproduction in the earliest stages of a cricket radiation
E. Dale Broder, Gabrielle T. Welsh, Harper Johnston, Aaron W. Wickle, James H. Gallagher, Tessa Appel, Rose Keaton, Victoria Rockwell, and Robin M. Tinghitella
American University

12:00 PM **SY6-6**
Millipede on your back: Testing the energetic costs of Male Mate-Guarding in *Nyssodesmus python* (Peter, 1864) (Myriapoda, Diplopoda, Polydesmida)
Pooja A. Anilkumar, Jessica G. Murray, J. Edgardo Arévalo, Anita Aisenberg
The George Washington University

12:30 PM **SY6-7**
Organization of an unusual female reproductive system in earthworms – the ovo-spermathecal apparatus in *Eudrilus eugeniae*
Dominika Raś, Anna J. Phillips, Piotr Świątek
University of Silesia in Katowice

SY5

9:30 AM – 12:30 PM Crustacean Reproduction Organizer: Christopher Tudge

2:00 PM **SY5-1**
Pumping, pipes, precision – New insights into the function and morphology of male crab copulatory organs
Svetlana Gruetzke, Peter Michalik, Dirk Brandis
Kiel University

2:30 PM **SY5-2**
Population dynamics of *Hippolyte inermis* (Leach, 1815) in two areas of the Mediterranean basin: a long-term study
Bruno Pinto, Marialuisa Lusito, Giuseppe Trotta, Elvira Brunelli, Ilaria Cuomo, Takashi Gojobori, Robert Hoehndorf, Maria Costantini, Valerio Zupo
Stazione Zoologica Anton Dohrn (Naples, Italy)

3:00 PM **SY5-3**
Pycnogonida Developmental Biology
John A. Fornshell
National Museum of Natural History

3:30 PM **BREAK**

4:00 PM **SY5-4**
Wolbachia sp. and microsporidia are not the cause of parthenogenesis in the terrestrial crustacean *Talitroides topitotum* (Burt, 1934) (Amphipoda, Peracarida)
Gláucia Brisotto, Marlise L. Bartholomei-Santos, Sandro Santos
Federal University of Santa Maria

4:30 PM **SY5-5**
Development of the marsupium of the parthenogenetic amphipod *Talitroides topitotum* (Amphipoda: Peracarida)
Gláucia Brisotto, Marlise Ladvoat Bartholomei-Santos, Sandro Santos
Universidade Federal de Santa Maria

5:00 PM **SY5-6**
Temporal gene expression in Atlantic fiddler crab larvae
Caitlin BrabbleRose, Quinton Krueger, Adam Reitzel, and Paola López-Duarte
University of North Carolina at Charlotte

Abstracts

Millipede on your back: Testing the energetic costs of Male Mate-Guarding in *Nyssodesmus python* (Peter, 1864) (Myriapoda, Diplopoda, Polydesmida)

Pooja A. Anilkumar, Jessica G. Murray, J. Edgardo Arévalo, Anita Aisenberg

The George Washington University

Mate-guarding is a post-copulatory behavior in which one sex remains attached to its mate for a period, preventing or reducing further mating attempts of the other sex. *Nyssodesmus python* is a blind millipede with chemical defenses, found abundantly in the forests of La Selva Biological Station, Costa Rica. After copulation, males of this species attach dorsally to females, who may carry them for up to five days. The objective of this study was to examine the energetic cost of mate-guarding for *N. python* females by assessing the impact of male body mass on female locomotion. We tested the hypothesis that male mate-guarding increases the energetic cost of locomotion in females, and that they therefore compensate energetic expenditure by slowing down the walking speed. We predicted that greater male body mass would be related with slower female locomotory speed. To test this, we conducted experiments on 20 *N. python* females in a controlled lab setting. Females were introduced to a 1 m runway in four different conditions: carrying plasticine male models on their dorsum (2g, 4g, 6g) and without male models (control). Walking speed was recorded, with each female undergoing randomized trials, generating 80 data points. A linear regression model in R analyzed the correlation between male body mass and female speed. Our findings corroborated our predictions: increased male body mass significantly reduced female walking speed ($p = 0.0161$). Further field observations indicated that mating pairs could climb trees and often remained immobile on algae-covered surfaces or decomposing logs, suggesting that females might be compensating for the energetic costs of carrying males by staying in resource-rich areas. This study is the first to assess the energetic cost of mate-guarding in millipedes, and provides insights into sexual selection mechanisms, an understudied aspects of millipede behavior.

Sexual Differentiation in *Macrobrachium rosenbergii*: Unravelling the Link Between the IAG-Switch and W/Z-Linked Regulatory Elements

Eiahu D Aflalo, Melody Wahl, Tom Levy Yaniv Borojovich, Rivka Manor, Barak Rotblat, Vered Chalifa-Caspi, Noa Asculai, Amir Sagi Ben Gurion University of the Negev

Sex determination initiates transcriptional cascades that dictate male or female phenotypes. Our previous work identified the insulin-like androgenic gland (IAG) hormone as a master regulator of sex differentiation in the freshwater prawn *Macrobrachium rosenbergii*, termed the "IAG-switch". Manipulating this sexual switch enabled the generation of functional monosexual lines of WW females ZZ males that assisted in the present study. This study aimed to elucidate the connection between sex determination and sexual differentiation by identifying key W/Z-linked elements controlling sexual differentiation in crustaceans with ZW/ZZ sex inheritance. We constructed a transcriptomic library of early developmental stages (embryo, larva, and post-larva) using males and females lacking either the W or Z chromosome and performed clustering analysis of differentially expressed genes. Mapping these genes to the *M. rosenbergii* sex chromosomes revealed W/Z-linked candidates with a male-biased expression pattern peaking at day 10 post-larvae (PL10), the phenotypic sexual differentiation stage. Two leading candidates were identified: a W/Z-associated coding gene (cytochrome P450 4c3-like, MrCYP4) and a Z-specific long noncoding RNA (MrZlnc). Silencing of these candidates resulted in a reduction of MrIAG expression, and vice versa, demonstrating a clear crosstalk with the IAG-switch. This study represents the initial step in unravelling critical elements that govern sexual differentiation in crustaceans exhibiting ZW/ZZ sex inheritance.

Sexuality and Sexual Plasticity in peppermint shrimps *Lysmata* spp.: the role of sexual selection in driving sex allocation

Baeza, J. A.

Clemson University

This presentation discusses more than 10 years of research focusing on the sexual system exhibited by peppermint shrimps in the genus *Lysmata*, plasticity in sex allocation, and the role of sexual selection and other environmental condition in driving sex allocation patterns.

A welfare-focused lens on insect reproduction

Meghan Barrett

Indiana University Indianapolis

How might we use welfare frameworks to think about insect welfare while simultaneously addressing our other aims in research or in farming? This talk will briefly introduce the concept of welfare for insects before diving into the Five Domains framework as a way to think about animal welfare in the context of research or farming. Some brief examples of how this framework could guide us in considering an animal's plausible affective state, and how that state could bear on their reproduction, will be provided. Finally, data on the effects of providing accessible and preferred nutrition for black soldier flies (BSF) farmed as food and feed will be provided. BSF are currently starved to death over eight to twelve days in industry conditions; lack of access to nutrition has been identified as a welfare concern for this species on farms. We assessed the preferred diet of adult BSF and the effects of providing this preferred diet on longevity, observed mating pairs, and egg output (compared to the industry standard of only providing water). We found that fed flies lived longer, mated more frequently, and laid 3.8-fold more eggs on days 2 – 5 post-eclosion compared to starved flies provided only water. These data suggest we could improve BSF welfare and increase production on farms by providing access to nutrition for adult BSF breeders.

The constraints of sexual plasticity: a crustacean perspective

Chiara Benvenuto, Stephen C. Weeks, Cristiana Ramalho Maciel University of Salford

The diversity of sexual systems and reproductive strategies in animals and plants is extraordinary. Invertebrates in general, and crustaceans in particular, are excellent model systems to explore such variety in reproduction: crustaceans show one of the broadest repertoires of reproductive modes, from asexuality (obligate and cyclical parthenogenesis), gonochorism (fixed separate sexes), simultaneous hermaphroditism, sequential hermaphroditism (sex change from male to female or vice-versa), protandric simultaneous hermaphroditism (when males become hermaphrodites) to mixed mating systems (i.e., androdioecy, when males coexist with hermaphrodites). In an attempt to explain such variability, in recent years sex has been analysed, using an evolutionary approach, as a continuum of plastic traits affecting sex determination and sex differentiation. The broad array of reproductive types begs more questions, which can bring a further switch in perspective: if sex can be so plastic, what are its constraints? Why are certain reproductive modes common in certain taxa but are not found in others? A multi-taxa approach is presented here to explore what limits sexual plasticity, considering possible physiological, developmental, and/or evolutionary constraints. Examples from androdioecious, protandric simultaneous, and sequential hermaphroditic species will be presented, together with experimental work in the lab as well as a discussion of parasitic feminization.

Use of gonad and brood pouch placement to identify platytene genera

Nicholas Bezio, Gustav Paulay, Allen Collins
University of Maryland

Morphologically distinct from the rest of the phylum Ctenophore (comb jellies), Platyteneida, is a benthic order that exhibits notable morphological diversity, particularly in the arrangement of their reproductive structures and early development stages. Differentiation among Platytene families (i.e., Benthoplanidae, Coeloplanidae, Ctenoplanidae, Lyroctenidae, and Tjalfiellidae) can be reliably achieved by examining the location, shape, and arrangement of gonads and brood pouches across the body. Of the dominant families, the oligospecific Benthoplanidae, female gonads and reproductive tissue is located close to the center of the body with testes developing along the peripheral oral sole. Ctenoplanidae exhibit gonads both along the meridional canals and pharyngeal regions, with internal brood pouches housed near the base of the comb rows. Coeloplanidae possess both male and female gonads on the meridional canals, but are distinguished by the presence of lateral brood chambers located externally along the oral sole. Lyroctenidae, a more derived lineage, have a gonadal distribution along the meridional canals diverticula network and possess internalized brood chambers that align parallel with the female gonads. Tjalfiellidae house their gonads subepithelially along the gastro-vascular canals, often with encapsulated brooding directly within the mesoglea. These distinctions in reproductive morphology offer crucial diagnostic characters for taxonomic identification and provide insights into the evolutionary adaptations of benthic ctenophores to their substrate-bound lifestyles.

Substances in the mandibular glands mediate queen effects on larval development and colony organization in an annual bumble bee

Bloch Guy, Franco Maayan, Fassler Rosi, Goldberg Tzvi, Reichmann Dana
Hebrew University of Jerusalem

Social organization is commonly dynamic, with extreme examples in annual social insects, but little is known about the underlying signals and mechanisms. Bumble bee larvae with close contact to a queen do not differentiate into gynes, pupate at an earlier age, and are commonly smaller than siblings that do not contact a queen. We combined detailed observations, proteomics, microRNA transcriptomics, and gland removal surgery to study the regulation of brood development and division of labor in the annual social bumble bee *Bombus terrestris*. We found that regurgitates fed to larvae by queens and workers differ in their protein and microRNA composition. The proteome of the regurgitate overlaps significantly with that of the mandibular (MG) and hypopharyngeal glands (HPG), suggesting that these exocrine glands are sources of regurgitate proteins. The proteome of the MG and HPG, but not the salivary glands, differs between queens and workers, with caste-specificity preserved for the MG and regurgitate proteomes. Queens subjected to surgical removal of the MG showed normal behavior, brood care, and weight gain, but failed to shorten larval development. These findings suggest that substances in the queen MG are fed to larvae and influence their developmental program. We suggest that when workers emerge and contribute to larval feeding, they dilute the effects of the queen substances, until she can no longer manipulate the development of all larvae. Longer developmental duration may allow female larvae to differentiate into gynes rather than to workers, mediating the colony transition from the ergonomic to the reproductive phase.

Peptide Pheromones, Endoproteinases, and Egg Hatching: Barnacles and Pea Crabs

Daniel Rittschof, Joshua Osterberg, Beatriz Orihuela, Desa Bolger
Duke University

Crustacean pheromones can be very different from those of insects. Crustacean larval release pheromones are peptides generated by exogenous trypsin acting on structural proteins that are associated with brooded eggs. The proteases generate a suite of arginine carboxyl peptides which are the pheromones. The closest well-studied analogs or potentially homologs to these pheromones are leucocyte chemo-attractants in vertebrates. Enzymes and their products coordinate behavioral, physical and biochemical processes involved in egg hatching and larval release. We have probed the enzymes involved in egg hatching and larval release using a proteomics approach. Non-ovigerous, ovigerous, and egg hatching pea crabs and barnacles were incubated with micromolar amounts of pure proteins for 20 to 30 minutes. Eggs near the normal time of hatching hatched within minutes of incubation. The peptides generated from the pure proteins were identified by high resolution LC, MS, MS. Endoproteinases generating peptides were identified by the carboxyl terminal amino acids of the peptides. Here we report the suites of exogenous proteases associated with barnacles and pea crabs and changes in enzymes observed at the time of egg glue degradation and larval release.

Temporal gene expression in Atlantic fiddler crab larvae

Caitlin BrabbleRose, Quinton Krueger, Adam Reitzel, and Paola López-Duarte
University of North Carolina at Charlotte

Larval behaviors of many estuarine species are timed with tidal and solar cycles to export out of and transport into estuaries. While the behavioral components of the circadian and circatidal clocks have been extensively studied, limited work has been conducted to understand the genetic mechanisms that underlie these behaviors in early life history stages of intertidal crustaceans. Previously, we examined gene expression in Atlantic fiddler crab (*Leptuca pugilator*) larvae maintained under light:dark (LD) conditions to identify genes with a circadian (24, +/- 2hr) or circatidal (12, +/- 2hr) periodicity of expression. Larval tissues were collected upon hatching every 2-hr across 4 tidal cycles (48-hr). A comparative transcriptomics analysis was used to identify changes in gene expression, and cyclically expressed genes were identified by periodogram analysis. More genes were identified as having a circadian periodicity than a circatidal periodicity. This experiment was replicated under total darkness conditions (DD) to investigate changes in gene expression in the absence of an exogenous light cue. We identified a substantial decrease in genes with a circadian periodicity and increase in those with a circatidal periodicity. Genes associated with transcription and glycogen synthesis were upregulated in both LD and DD experiments. Since genes expressed under both conditions are candidates for core components of the circadian and/or circatidal clocks in *L. pugilator*, we are cross-referencing them with other studies to identify similarities in other species. This work is key to understanding how these clocks are expressed and function during the crucial larval dispersal phase of intertidal crustaceans.

The Devil (Worm) in the (Genetic) Details: Breaking Muller's Ratchet

Ali Amini and John R. Bracht
American University

Muller's Ratchet is a generally thought to limit the long-term survival of asexual species, as they cannot remove deleterious mutations and are destined to undergo genetic meltdown and extinction. However, the subterranean nematode, *Halickephalobus mephisto*, reproduces parthenogenetically and has existed as a species for an estimated minimum of 22 million years. With a genome of 115% SNP heterozygosity, this organism provides a unique opportunity to query genomic dynamism in an asexual organism. To test for loss of heterozygosity, we PCRtyped 56

individual animals at two different loci; no homozygotes were observed in the population. Furthermore, whole-genome analysis of parent and progeny demonstrated no transition from heterozygote to homozygote across over 560,000 SNPs in the entire genome. We discovered that *H. mephisto* still performs chromosomal synapsis during meiosis, raising the possibility that recombination may still occur. However, extensive analysis of Nanopore long-read data showed no evidence of any recombination within the genome. This parthenogenetic organism appears to clone itself each generation, which would be expected to lead to the rapid extinction of the species by mutational meltdown. The mechanism by which *Halicephalobus mephisto* avoids this fate remains unknown.

Wolbachia sp. and microsporidia are not the cause of parthenogenesis in the terrestrial crustacean *Talitroides topitotum* (Burt, 1934) (Amphipoda, Peracarida)

Gláucia Brisotto, Marlise L. Bartholomei-Santos, Sandro Santos
Federal University of Santa Maria

Recent studies of the terrestrial amphipod *Talitroides topitotum* have shown that this crustacean reproduces by parthenogenesis. One way for parthenogenesis to arise in a sexual lineage is through an infectious origin, as there are endosymbionts that can modify reproductive aspects of the host, including the induction of asexual reproduction. Among the endosymbionts that induce parthenogenesis are the bacteria *Wolbachia* sp., already recorded in terrestrial isopods, and a microsporidian fungus, found in aquatic amphipods. To investigate the possible cause of parthenogenesis in *T. topitotum*, we tested the presence of both endosymbionts in the DNA of a parthenogenetic population of this species. To do this, we extracted the DNA of 35 individuals and tested the quality of the samples amplifying the mitochondrial COI gene by PCR (Polymerase Chain Reaction) with universal primers. We tested the presence of *Wolbachia* sp. by amplifying the bacterial genes 16S rDNA, *CoxA*, and *ftsZ*. We used a sample of *Drosophila melanogaster* infected with *Wolbachia* as a positive control. We also tested for the presence of microsporidia by PCR-amplifying the gene SSU rDNA and used a sample of the infected amphipod *Gammarus duebeni* as a positive control. None of the 35 samples showed amplification of the endosymbionts' genes. Assuming a low rate of individuals infected in the population (5%), the probability of finding at least one infected individual among the 35 tested is 82%, indicating that parthenogenesis in this group may have another origin. Additional studies are needed to understand how and when a sexual ancestor gave rise to an asexual lineage and what consequences this had on the genetic diversity of the population and, consequently, in the evolutionary success of the group.

Development of the marsupium of the parthenogenetic amphipod *Talitroides topitotum* (Amphipoda: Peracarida)

Gláucia Brisotto, Marlise Ladvoat Bartholomei-Santos, Sandro Santos
Universidade Federal de Santa Maria

Talitroides topitotum is a terrestrial crustacean from the order Amphipoda. Recently, a population of this species in southern Brazil was characterized as parthenogenetic. Observations carried out in the laboratory followed at least six generations of a single female for 18 months. During this period, we observed the development process of the female marsupium, which is described next in four stages: Stage I: Diapause. The female is not reproducing, and the marsupium is transparent and translucent. The oostegites are reduced. In some cases, it is possible to notice that the oostegites have remaining setae. Stage II: Initial Development. The female prepares to produce eggs. The marsupium is dark purple, possibly due to the accumulation of vitellogenin in the female's sternum. The oostegites are more robust and the setae are easily visible. Stage III: Eggs production. The eggs are formed and remain in the cavity of the marsupium. The color of the eggs varies from dark purple to black. The oostegites form the floor of the marsupium and hold the eggs. Stage IV: Egg maturation. It is possible to observe the development of the eggs, and its final phase culminates in the visualization of the juveniles, who have already formed inside the eggs.

The eggs' color becomes lighter, allowing easy identification of some structures of the juveniles, such as the eyes. These preliminary observations may help us better understand how the reproductive process occurs in this parthenogenetic species and, in the future, compare these mechanisms with their sexual counterparts.

Sexual reproduction in the earliest stages of a cricket radiation

E. Dale Broder, Gabrielle T. Welsh, Harper Johnston, Aaron W. Wikle, James H. Gallagher, Tessa Appel, Rose Keaton, Victoria Rockwell, and Robin M. Tinghitella
American University

The evolution of novel traits is key to understanding biodiversity, yet many questions persist regarding the origins of novelty since we so rarely observe such events. It is particularly challenging to imagine how novel sexual signals might arise since receivers should select against rare signals that deviate from the norm. How does reproduction/courtship facilitate the success of novel morphs? We have the unique opportunity to test this question using the Pacific field cricket, native to Australia, which is in the earliest stages of a radiation event in Hawaii. In response to an introduced acoustically hunting lethal parasitoid, at least six novel male morphs (song and wing) have evolved in HI the past 20 years. Using one-on-one courtship experiments in the field and in the lab, we compared behavior of the ancestral morph to that of two novel morphs. We found that novel morphs use alternative combinations of behavior to achieve mating success. For example, purring males (quietest novel morph) are much more likely than ancestral males to use substrate drumming. However, plasticity plays a role; when we remove males' ability to produce airborne song, ancestral males increase drumming behavior. In common garden lab experiments, female crickets from HI are much more accepting of novel males than Australian females (parasitoid not present), suggesting that evolved relaxed female preferences may play a critical role in facilitating novel sexual signals. This study system provides a rare window into the nuanced ways in which both evolved and plastic reproductive behaviors facilitate novelty.

E. E. Just, champion of the cell surface

W. Malcolm Byrnes
Howard University

Known for his work in cell and developmental biology and, in particular, for his discovery of the fast block to polyspermy in the eggs of marine invertebrates, E. E. Just (1883-1941) was a giant in the history of modern biology. An ardent believer in the holistic integrity of the cell and embryo, he was not afraid to take on giants in the field, including T. H. Morgan and Jacques Loeb, whose views he felt were too reductionistic. This stance, together with his gravitation toward European biology and the fact that he was African American—which challenged American scientists' notions of race and achievement—led to his being sidelined and treated as an outsider by his peers. His scientific ideas were outwardly rejected and his papers, though published in the best journals, were generally not cited. Despite this, I hope to show in this talk, Just's ideas, especially his concept of the "independent irritability" of the cell, i.e., its ability to respond in a physiologically relevant way to nonspecific triggers acting at its surface, have broadly infiltrated modern cell and developmental biology. After giving a biographical sketch, presenting an overview of Just's contributions, and discussing some of his lesser-known experiments, the talk will argue that Just has made an indelible mark on biology, and that he now serves as a path-breaking role model for any scientist who passionately strives for excellence amidst difficult circumstances.

Phenotypic plasticity and behavior in horned beetles

Sofia Casasa
Boston University

Phenotypic plasticity — the ability of organisms to adjust their phenotype in response to variable environmental conditions — is widespread across taxa. Morphological plasticity is often accompanied by distinct behaviors. While considerable attention has been given to the molecular

mechanisms underlying morphological plasticity, many questions remain about the associated behaviors, the neural and molecular pathways that regulate them, and how morphology and behavior are integrated at the molecular level. In this talk, I use horned beetles as a model to investigate the mechanisms and evolution of phenotypic plasticity. Beetles in the tribe Onthophagini exhibit a high degree of nutritional plasticity across several morphological traits, including body size and horns. I will first discuss known molecular mechanisms that regulate body and horn size plasticity. I will then examine the alternative reproductive tactics associated with horn morphology, and highlight our ongoing research on the neural and molecular mechanisms that regulate these alternative behaviors. Ultimately, this work aims to uncover how organisms coordinate the development of morphological and behavioral plasticity at the molecular level.

Comparative Morphology of Land Hermit Crab (Genus *Coenobita*) Larval Development

Anna Clark, Christopher Tudge
American University

Hermit crabs (Crustacea: Decapoda: Anomura) can be aquatic or terrestrial, with different morphology in order to survive in these very different environments. The main terrestrial hermit crab family, Coenobitidae, is composed of two genera: Coenobita and Birgus (the latter with only one species, Birgus latro). My research project has been a comparative anatomical study of the aquatic larval and juvenile stages of three species in Coenobita: C. clypeatus, C. perlatus, and C. lila using scanning electron microscopy (SEM). These specimens were all captive-bred, raised in the US, and fixed for SEM at different stages of their development. The comparative ultrastructure of the first two zoea stages and the megalopa stage of each species is illustrated via scanning electron micrographs with any significant differences between the three species described. A comparison to wild caught larval stages in other species, previously described in the literature, is made.

TOXICOLOGICAL RESPONSE OF THE SEA URCHIN *PARACENTROTUS LIVIDUS* TO VIRGIN AND COMMERCIALY DERIVED POLYSTYRENE MICROPLASTICS

Maria Costantini, Amalia Amato, Simone Gioia, Roberta Esposito, Immacolata Liotta, Mariacristina Cocca, Davide Caramiello, Loredana Manfra, Giovanni Libralato, Valerio Zupo
Stazione Zoologica Anton Dohrn

The ecological risks of plastic contamination come not only from degraded microplastics or nanoplastics, but also from additives used to modify their attributes and stability. Polystyrene (PS) is a plastic widely used for foodservice, medicine and toy industries; it poses serious threats both to the environment and the human health. Here, we applied a polyphasic approach to evaluate the impacts of PS microplastics on early embryonic stages of the sea urchin *Paracentrotus lividus*, an important species in shallow benthic environments. Sea urchin eggs were exposed ten minutes to microplastics obtained from virgin PS and from disposable PS plates, at increasing concentrations, and further fertilized. The embryonic development was followed until the pluteus stage (48 hours post fertilization). Our results showed that microplastics from disposable PS plates induced a stronger effect with respect to virgin PS, affecting the first mitotic division yet at the lowest concentrations tested. A delayed embryonic development was also recorded. Post-recovery experiments showed that embryos can recover after exposure to PS, indicating that harmful effects depend both on concentrations and the exposure time of the embryos. The molecular targets and gene pathways affected by these plastics were detected by following the expression levels of eighty-seven genes involved in stress response, development and differentiation, skeletogenesis and detoxification. Overall, the outcomes of this work provide the basis for future investigations on PS and its additives ecotoxicity, showing *P. lividus* as a promising model to evaluate risks for human health caused by plastics and, more in general, by different contaminants in the marine environment.

Research conducted in the framework of the Project – Biomonitoraggio di micro e nanoplastiche biodegradabili: dall'ambiente all'uomo in una prospettiva one health (BioPlast4Safe) – with the technical and economic support of the Italian Ministry of Health – PNC.

Peptide Hormone Shapes Lipid-Steroid Metabolic States to Trigger Sexual Maturation

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Tulane University

Steroid hormones are ancient signaling molecules that regulate sexual development. Since steroidogenic tissues have limited storage of steroid hormones, endocrine cells must rapidly mobilize lipid droplets (LD) to release free cholesterol (FC) for steroid synthesis. Here, we show that the epithelial-derived peptide hormone Unpaired (Upd), the fly leptin analog, plays a key role in balancing LD and sterol pools in the steroidogenic prothoracic gland (PG). Upd activates JAK/STAT signaling in the PG to control developmental transitions, with this regulation amplified under tissue damage or tumorigenesis.

JAK/STAT signaling reprograms PG metabolism, regulating the LD-cholesteryl ester (CE)-FC-steroid axis to fine-tune steroid pulse frequency, amplitude, and duration. The sex determination factor Fruitless (Fru), previously shown by us to regulate pheromone production and perception in *Drosophila*, functions as a downstream effector of JAK/STAT signaling, dynamically controlling sex hormone synthesis. We identified the conserved hormone-sensitive lipase (Hsl) as the key rate-limiting enzyme mediating the conversion of CEs to FC. Deleting JAK/STAT or Fru in PG cells disrupts LD and sterol pool homeostasis, leading to premature sexual maturation, which can be partially rescued by exogenous Hsl.

High JAK/STAT activity delays developmental transitions by inducing Fru and Hsl expression—a mechanism conserved across processes involving injury, inflammation, and cancer. Using steroidogenic tissue models from German cockroaches and mice, we found that the JAK/STAT-Hsl axis is conserved in balancing LD-CE-FC-steroids, guiding steroidogenesis and sexual maturation in both insects and vertebrates. These findings reveal a conserved regulatory network that links metabolism, sexual development, and environmental stress responses.

An evolutionarily conserved mineralization pathway in balanomorph barnacles

Gary H. Dickinson, Jazmine Shaw, Phylcia Menendez, Corin Hoppe, Nick Aldred, Beatriz Orihuela, Daniel Rittschof, Rebecca A Metzler
The College of New Jersey

Balanomorph (acorn) barnacles play important ecological roles in coastal benthic communities. Their success depends on defense provided by a mineralized outer shell, which must form rapidly after metamorphosis. We report on parallel assessments of shell structure, mechanics, and composition in adult and juvenile barnacles. Seven species of adult balanomorph barnacles, including representatives from each of the major monophyletic groups of Balanomorph, were examined. At the macro- and meso-scales, shell properties varied dramatically among species, with differences in the number of shell plates, canals within plates, and base plate mineralization. At the micron-scale, however, structure was remarkably similar. Plates of all species were constructed of irregular micron-scale calcite crystallites, with a broad range of crystallite dimensions within the same shell. Early shell development was examined in *Amphibalanus amphitrite*. Juveniles were grown for 1 hour, 1 day, or 6 days. Plates were thin and largely unmineralized at 1 hour post-metamorphosis. Calcite crystallites emerged at 1-day post-metamorphosis, forming first at the exterior edge of the plate. Plate thickness increased roughly three-fold over the first 6 days of development, and calcite crystals grew to fill the entirety of the plate. Correspondingly, calcium content and shell hardness increased as crystals emerged. Micron-scale similarities across phylogenetic groups and over developmental time suggest an evolutionarily conserved pathway for mineralization in balanomorph barnacles.

Natural variation reveals hidden divergence in the evolution of a polyphenism

Stephen M. Dreyer, Erik J. Ragsdale
Indiana University

Background – Polyphenism, the production of discrete alternative phenotypes in response to environmental signals, exemplifies the non-linear relationship among genes, the traits they influence, and the environment. While several genes that regulate polyphenism have been identified in some systems, especially the nematode *Pristionchus pacificus*, which has a resource polyphenism that results in alternative mouth morphologies, it is mostly unknown which loci harbor intraspecific variation for polyphenism in nature.

Methods – We generated recombinant inbred lines from parental *P. pacificus* isolates with similar polyphenism (morph-frequency) phenotypes under common rearing conditions, upon which quantitative trait loci (QTL) analysis was done to reveal loci contributing to differences in morph frequencies. We then used CRISPR/Cas9-directed recombination in a near-isogenic line (NIL) to make an additional panel of recombinants to fine-map the causal locus of largest effect.

Results – Recombinants included morph-bias phenotypes outside the parental range, indicating cryptic divergence between strains. QTL analysis revealed three loci of large effect that influence morph production: two on Chr I, and a larger-effect one on Chr X that is epistatic over the other two. Although the X-linked QTL contains a region previously found to harbor variation for polyphenism, functional tests by CRISPR/Cas9-driven allelic replacement instead indicate a novel, causal locus. Successive NILs have narrowed this locus to a 100-kb interval.

Conclusion – In summary, we describe a diverging polyphenism's genetic architecture, consisting of multiple QTL that together hide genetic variation by epistasis, and we show that a narrow, X-linked locus has been a convergent target of polyphenism evolution.

Pycnogonida Developmental Biology

John A. Fornshell
National Museum of Natural History

The term anamorphic development is used to describe arthropods which add segments throughout their lives. Epimorphic development is used to describe arthropods which hatch with a set number of segments which does not increase with subsequent molts. Some arthropods add segments initially until a set number have been produced after which no further segments are produced with subsequent molts. The latter are said to have hemianamorphic development. In the Chelicerata the completion of embryonic development and hatching of the egg are not as intricately linked as in the other classes of the Arthropoda. Thus, making the distinction of which developmental pattern is occurring less obvious. The members of the Pycnogonida typically hatch as a "Protonymphon larva" having three pairs of appendages and a proboscis with a tripartite mouth. The subsequent growth of the animals displays certain developmental process typically seen in embryos: (1) Apoptosis; (2) invagination of ectodermal tissues to initiate the formation of neurogenic niches. (3) organogenesis; and (4) limb development progressing in an anterior-posterior developmental gradient.

If the "Protonymphon larva" is interpreted as an embryonic stage, then the Pycnogonida may be considered to have epimorphic development.

Testing the reproduction-immunity trade-off in honey bee queens using lipidomics and proteomics

Leonard J. Foster
University of British Columbia

Background – Honey bees (*Apis mellifera*), as social insects, use chemical cues to communicate all manner of messages, from mating to defence to health status. Here we apply new analytical methods to understand the chemical cues that honey bee workers may use to determine the health of their queen.

Methods – Honey bee queens were infected with a combination of black queen cell virus and deformed wing virus B, then cared for by attendant workers in observation colonies that could be manipulated to allow egg laying or not. In parallel, queens with natural levels of infection were collected from field colonies. Lipids, pheromones, and proteins were extracted from queens and analyzed using a timsTOF-Pro (Bruker) and the data interpreted using MetaboScape (metabolomics) and FragPipe (proteomics).

Results – Virus infections reduced the queen retinue pheromone component methyl oleate and general triglycerides. Reducing ovary mass via laying restriction was sufficient to lower methyl oleate abundance but was insufficient to reduce abundance of most triglycerides or stimulate immune effector expression. Abundance of circulating apolipoprotein III, a lipid transporter and putative potentiator of immune effectors, was lower in queens with restricted laying. Queen pheromone blends lacking methyl oleate are less attractive to workers so diminishing methyl oleate could result in a less desirable pheromone bouquet.

Conclusion – The reproduction-immunity trade off appears to only operate under immune stimulation and not restricted laying. Our data are consistent with positioning ApoLP-III as a candidate molecular switch that could be governing reproduction vs. immunity investment.

Sex ecology in the European green crab *Carcinus maenas* in its native range.

R Graves, O Medlar, SC Culloty, SA Lynch*
University College Cork

The green crab *Carcinus maenas* is an invasive species that has spread from Europe to coastal regions globally, including North America. *C. maenas* has a significant impact on introduced marine ecosystems and commercial bivalve fisheries. In this study, monthly samples (May to July) of *C. maenas* (n=427) were carried out during peak breeding season. An overall total of 331 (77.5%) living and 96 (22.5%) dead crabs were sampled. Intertidal crabs (n=214) were collected from a shipping port, marsh nature reserve and an oyster fishery, while subtidal crabs (n=213) were captured using baited pots at the oyster fishery. Crab biometrics and sex were recorded, as well as bivalve pathogen groups (*Bonamia ostreae*, *Ostreid herpesvirus-1 microVar* (OsHV-1 μ Var) and *Vibrio* spp.) by PCR. A male bias was recorded at the intertidal sites except for the marsh nature reserve, and females dominated the subtidal site. Female crabs were larger in size compared to males at all sites, however maximum size was not achieved. Co-existence of adults and juveniles of both sexes at all sites and shore heights indicated a lack of competition for resources and cannibalism. The greater abundance of males on the intertidal indicates physiological tolerance while females guard their subtidal burrows and eggs. Pathogen detection was similar in both sexes, however dead crabs at the marsh nature reserve had the highest detection of OsHV-1 μ Var, which might indicate a susceptibility to this virus and possible role of this species as a host and not just a carrier of this global virus.

Pumping, pipes, precision – New insights into the function and morphology of male crab copulatory organs

Svetlana Gruetzk, Peter Michalik, Dirk Brandis
Kiel University

Background – The eubrachyuran crabs are an extremely diverse group showing outstanding feature within the decapods: internal fertilization. For the sperm transfer male crabs developed specialized and paired copulatory organs. Each side consists of two modified pleopods, the so-called gonopods, and a penis – together forming a functional unit. The gonopods are species specific in their interaction with the female gonopore. Therefore, they have often been studied for taxonomy – mainly with focus on their outer morphology. The function of the gonopods were mainly distinguished between species that have longer second than first gonopods and those in which the second gonopods are shorter than the first. Nevertheless, regarding the diversity of eubrachyuran crabs, it can be assumed that there are more various transport types.

Methods – We examined gonopods of different crab species with μ CT, SEM, TEM and histological cross sections to find out more about the morphology and the function. The minimally invasive μ CT made it possible to study valuable and rare material.

Results – The first investigations already indicate interesting new details about the functional morphology of the gonopods including the interaction of the gonopods with each other and the process of the sperm transfer. We found differences in the structure and function of the gonopods between the species studied and got indications that the diversity of sperm transfer mechanisms is greater than previously assumed.

Conclusion – By combining different methods and analysing several crab species, we might provide new insights into the sperm transfer of eubranchyurans.

Using social bees to explore the impacts of pre-adult experience on developing neural systems

Mallory A. Hagadorn and Clare C. Rittschof
University of Kentucky

Background – Insect brains are dynamic, refining in both structure and function throughout a lifetime. This neuroplasticity includes experience-based neural refinement in adults, which promotes learning and memory formation. Amazingly, pre-adult experiences can also alter organisms developmental trajectories and behavior, leading to long-term phenotypic and fitness consequences. These impacts may be asymmetrically strong during critical periods of juvenile development. Social insects offer a unique opportunity to explore these relationships across multiple hierarchical levels—within individuals, among distinct social groups, and throughout and across colonies. Yet, how social insect early-life experiences shape juvenile brain development and baseline adult neuro-architecture is still relatively unexplored, hindering our understanding of the synergy between juvenile and adult neural states and their resiliency to changing environments.

Methods – Using honey bees, we explored the impacts of early-life experiences on developing social insect sensory systems. Larvae rely entirely on contact with adult nestmates for food acquisition and survival. Thus, to manipulate pre-adult experience, we exposed larvae to an acute bout of social deprivation by inhibiting contact with their caregivers. We used confocal microscopy, 3D brain reconstruction, and volumetric analyses to assess short and long-term effects of social deprivation on brain development.

Results & Conclusions – We collected neural tissue from samples spanning multiple developmental timepoints, including critical growth periods of larval and pupal neurodevelopment. 3D brain renderings and volumetric comparisons will map the developing honey bee brain, yielding insight into the plasticity and resiliency of social insect brains across life stages.

Isolation, characterization and aging of germline stem cells in a hermaphrodite protochordate

Tom Levy, Chiara Anselmi, Katherine J. Ishizuka, Tal Gordon, Karla J. Palmeri, Yotam Voskoboynik, Erin McGeever, Angela M. Detweiler, Liron Levin, Daniel D. Liu, Rahul Sinha, Benjamin F. Ohene-Gambill, Tal Raveh, Maurizio Morri, Virginia Vanni, Lucia Manni, Norma F. Neff, Benyamin Rosental, Irving L. Weissman, Ayelet Voskoboynik
Stanford University

Botryllus schlosseri is a hermaphrodite tunicate which undergoes a weekly stem-cell-mediated whole body regeneration process. While various stem cells regenerate different tissues, only germline stem cells (GSCs) establish gonads and pass their genes to the next generation via sexual reproduction. To isolate GSCs and explore their fitness during aging, testes were dissociated and cells were analyzed using flow cytometry. Based on morphological parameters, different cell types along the spermatogenesis process were separated. However, while sperm and spermatids were morphologically recognizable, GSCs were impossible to classify. Therefore, cells were index-sorted followed by single-cell RNA sequencing which revealed expression of germline markers, that

were proved, by in-situ hybridization, to be specific to different cell types, including GSCs, in *Botryllus* testes. Next, GSCs were sorted and transplanted into recipient animals. A week after, some of the engrafted cells underwent differentiation into sperm, indicating that the transplanted cell population was enriched with GSCs. Using our strategy to isolate GSCs, we investigated their role in gonadal aging at the single-cell level. Remarkably, the RNA footprint of young and old spermatids and sperm was pretty similar while between young and old GSCs it was more distinct. Furthermore, the higher expression levels of GSC markers like *DDX4* and *PIWIL1* in young compared to old *Botryllus* showed a similar pattern in mice and humans. This suggests the existence of a reproductive aging mechanism in the gonad, mediated by GSCs, that seems to be conserved across protochordates and mammals and has been maintained throughout evolution from invertebrates to vertebrates.

Extreme sexual plasticity and flexibility within an annelid genus

Maria Cristina Lorenzi
University of Sorbonne Paris Nord

Gonochoirists, simultaneous hermaphrodites, sequential hermaphrodites: the genus *Ophryotrocha* encompasses multiple sexual systems within relatively closely related species. By exposing individuals of a gonochorist, a sequentially hermaphroditic and a simultaneously hermaphroditic species (*O. labronica*, *O. puerilis*, *O. diadema*) to different social environments, we also documented high levels of plasticity and flexibility within each sexual system: among gonochorists, females and males express (possibly vestigial) hermaphroditic traits when kept in isolation; among sequential hermaphrodites, individuals adjust their body growth, size at and timing of sex change to their partners'; among simultaneous hermaphrodites, individuals adjust their sex allocation to a more female- or male-biased one, depending on whether they are kept in pairs or larger groups, respectively. While plasticity might mechanistically easily evolve from a relatively simple anatomical and physiological substrate (e.g., lack of sexual organs) and accurate social monitoring, the functional adaptive value likely emerged under the pressure to respond to fluctuating social condition in the hermaphroditic species. Where tested, population size varies largely in the wild, from sparse colonizer individuals to population outbursts, where the ability to quickly adjust individual sex allocation to current social condition (i.e., mating opportunity) – and re-adjust them as soon as the condition varies again – allowed individuals to maximize their reproductive success.

Effects of *Chlorella vulgaris* bioremediation on alterations induced by microplastics in the gills of *Mytilus galloprovincialis*

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University of Calabria

Plastic is an essential material of the modern era whose global production reached 400.3 million tons in 2022, posing serious environmental challenges. Once released, plastic waste enters aquatic environments through various pathways, including wastewater treatment plants and atmospheric transport. Microplastics (MPs), particles <5 mm, make up 93% of marine plastic waste, with polyethylene (PE) being the most prevalent. Mussels, due to their filter-feeding behavior, easily accumulate dissolved contaminants, making them effective bioindicators of pollution. Current research overlooks realistic environmental MP concentrations and frequently investigates their effects with other stressors. Recent studies have begun to explore the protective potential of natural products against MP toxicity, including the microalgae *Chlorella* successfully used in aquaculture to improve nutrition and alleviate damage.

On this basis, we investigated PE-induced gill damage in *Mytilus galloprovincialis* and the protective role of *Chlorella vulgaris*. Mussels were exposed to two environmentally relevant PE concentrations (20 and 100 μ g/L) over 7, 14, and 21 days, with and without co-exposure to *C. vulgaris*. We revealed that PE exposure led to severe histopathological changes, including increased thickened gill filaments and mucus-secreting cells, epithelial disarrangement, granulocyte proliferation, and epithelial

detachment. Such structural impairment adversely affects energy storage and metabolism with obvious consequences on development and reproduction. Co-exposure with *C. vulgaris* partially mitigated these damages, thus suggesting that natural agents could be a promising avenue to mitigate the impact of microplastic pollution in the marine environment.

The toxic effect of an oxylipins-containing macroalgae extract on sea urchin reproduction and development

Chiara Martino, Rosario Badalamenti, Roberto Chiarelli, Antonio Palumbo Piccionello, Manuela Mauro, Vincenzo Arizza, Mirella Vazzana

University of Palermo, Italy

Background – Algae possess complex and highly evolved chemical defenses against their grazers, including the production of bioactive molecules such as oxylipins. *Ericaria brachycarpa*, a canopy-forming brown algae, contribute to form the habitat in the Mediterranean Sea for the sea urchin species *Arbacia lixula*. The aim of this work was to evaluate the effects of an oxylipins-containing extract from *E. brachycarpa* on the reproduction and development of *A. lixula*, evaluating the fertilization success and the embryotoxic activity from fertilization (0 h) to the pluteus stage (72 h).

Methods – The extract was tested in gametes to test the fertilization success and at three developmental endpoints (zygote, gastrula and pluteus). The range of concentrations tested was chosen to cover a full 0-100 % abnormality curve, with doses ranging from 0 to 40 µg/mL.

Results – We found a strong dose-dependent effect on the fertilization success and on development. Gastrulae were the most sensitive to the extract with the lowest EC50. At low doses we found an impairment in development and biomineralization, while the highest concentration tested caused 100% mortality of the embryos at all stages. Western Blot experiments showed the modulation of different molecular markers (HSP60, LC3, p62, CHOP and cleaved caspase-7), indicating enhanced autophagy at low concentrations and apoptosis at high concentrations. The TUNEL assay confirmed high levels of fragmented DNA.

Conclusion – These data support the hypothesis that macroalgae may exert a sort of population control against their grazers, releasing in the marine environment toxic compounds, such as oxylipins, following their tissue disruption by sea urchins and other grazers.

The combined effects of global warming and pollution on development and biomineralization of sea urchins

Chiara Martino, Roberto Chiarelli, Dario Savoca, Manuela Mauro, Maria Byrne, Thorsten Hüffer, Rosario Badalamenti, Antonella Maccotta, Vincenzo Arizza, Mirella Vazzana

University of Palermo, Italy

Background – Global warming represents major threat for marine organisms already facing chemical contamination in coastal areas. Here I report the combined effects of thermal stress and exposure to three different pollutants of the marine environment (gadolinium, vanadium and phthalates) on embryos of two common Mediterranean sea urchin species with predicted opposite responses to warming, the temperate *Paracentrotus lividus* and the sub-tropical *Arbacia lixula*.

Methods – Embryos were exposed to several treatments of three temperatures (18°C, 21°C, 24°C) and different concentrations of the three pollutants (from environmentally relevant to cytotoxic). The single and combined effects to thermal stress and pollutants were tested at three functional levels: i) exposure–response relationships, ii) morphological, iii) biochemical/cellular.

Results – With respect to developmental progression, elevated temperatures at near-future projections accelerated development while extreme warming at present-day marine heatwave conditions breached the thermotolerance threshold of both species. We found a fascinating double side effect of increased temperature combined to pollution: a mild temperature increase reduced the negative effects of pollutants on development with a lower percentage of abnormality and improved skeleton growth, while

combined heatwave conditions and pollution resulted in a lower proportion of embryos reaching the advanced larval stages.

Conclusion – Our data highlight the need for a better understanding of the interactions between the multiple stressors faced by marine species in coastal environments.

Developmental repurposing of larval neurons for adult sexual behaviors in *Drosophila*

Kara E. Miller, Troy R. Shirangi

Villanova University

As juvenile animals mature into adults, they gain a variety of new innate behaviors like mating and aggression. How does the brain change during maturation? In different animal species, many new neurons are born during maturation and contribute to neural circuits for adult behaviors. However, there are also examples of juvenile neurons that are recycled and reprogrammed during maturation for use in adults. The molecular mechanisms by which neurons reprogram during maturation are unknown.

We recently identified a population of neurons in the abdominal ganglion of the *Drosophila* larval nerve cord. During metamorphosis, a subset of these neurons acquires sexual identity by expressing the sexual differentiation gene, doublesex. These neurons then undergo apoptosis in males, but in females they reprogram for functions in adult mating behaviors. The transformation of these neurons from functions in larvae to functions in adult female mating provides a system to study the mechanisms that regulate neuronal reprogramming during animal maturation.

Here, I will present my work using single-cell RNA sequencing to molecularly categorize these neurons in larvae and adults. Their molecular identities have allowed me to precisely match individual adult neurons to their counterparts in larvae. I will discuss our progress in understanding the larval functions of these neurons, and how these neurons are transcriptionally reprogrammed during *Drosophila* metamorphosis. Our findings provide insights into neuronal plasticity during development and how common neural substrates can construct a variety of circuits whose use is specific to different sexes and stages of life.

Can exposure to seaweed biocompounds and selenium influence reproductive development in the European oyster *Ostrea edulis*?

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University College Cork

Widely distributed within Europe and cultured in North America, the European oyster *Ostrea edulis* is an important commercial species that has been impacted by habitat loss, inconsistent seed supply and the parasite *Bonamia ostreae*. Current efforts to restore *O. edulis* populations throughout Europe depend on wild capture and hatchery supply of seed. *O. edulis* are notoriously difficult to maintain and to induce reproduction artificially. Hatchery production is erratic and depends on successful broodstock conditioning and nutrition. In this study, the treatment of *O. edulis* with seaweed biocompounds (fucoidan, k-carrageenan) and the mineral selenium (Se) to enhance oyster survival and gonad development in a pilot “hatchery” setting were investigated. A ten-week laboratory trial was carried out with weekly samples of control and treated oysters being screened for sex ratio, gonad development, fecundity (egg and sperm volume) and pathogens (*Vibrio* spp. and *B. ostreae*). Survival (%) was also recorded. Gametogenesis was investigated by histology and pathogens were screened by PCR and heart imprints. Control oysters had the highest mortality. Treated oyster cohorts progressed to later maturation stages (ripe and spawning) and had higher fecundity compared to the control oysters. Similar gonad development patterns were observed in the treated and the wild control oyster cohort screened at Week 5 from an oyster farm. Both pathogens were significantly reduced in the fucoidan and k-carrageenan treated oysters compared to the control oysters. Findings highlight the value of using health boosting and reproduction enhancement supplements to support hatchery production of *O. edulis* and associated restoration efforts.

Possible interactions between limb regeneration and exoskeleton synthesis determining molt stage transitions

Donald L. Mykles

Colorado State University

Molting is a unidirectional process characterized by phenotypic changes in the Y-organ (YO). These phenotypes have distinct transcriptomic and proteomic profiles: (1) basal state during intermolt; (2) activated state during early premolt; (3) committed state during mid- and late premolt; and (4) repressed state during postmolt. The YO is kept in the basal state by molt-inhibiting hormone (MIH), resulting in low 20-hydroxyecdysone (20E) titers in the hemolymph. Reduced MIH activates the YO, resulting in increased mTOR-dependent ecdysteroidogenesis. The activated YO remains sensitive to MIH and to a peptide factor produced regenerating limb buds (LBs). The transition from the activated to committed YO requires Myostatin signaling; further increases in 20E titer initiate synthesis of the exoskeleton and continued LB growth. The committed YO is less sensitive to MIH, assuring that premolt proceeds without delay. The repressed YO is physiologically and transcriptionally inactive, resulting in very low 20E titers. Transition from the committed to repressed state coincides with the peak in 20E titer in late premolt, suggesting that 20E and methyl farnesoate signaling genes are involved. Upon completion of exoskeleton synthesis and calcification, the YO transitions back to the basal state at the end of postmolt. As the YO expresses receptor tyrosine kinases, it is hypothesized that the integument produces a putative peptide growth factor that restores the YO to the basal state.

Why do microplastics affect the symbiosis between corals and Symbiodiniaceae?

Nami Okubo

Rikkyo University

The first experimental evidence is presented showing that microplastics interfere with the establishment of symbiosis between anthozoans and Symbiodiniaceae. In both *Aiptasia* and the coral *Favites chinensis*, symbiont acquisition was significantly suppressed by exposure to microspheres, either directly or via *Artemia*. Similar inhibition was observed using microplastics from commercial facewash. In bleached *Seriatopora caliendrum* polyps, microplastic incorporation was enhanced. Transmission electron microscopy revealed that microplastics and symbionts were endocytosed into the same endodermal cells via shared phagocytosis zones, suggesting that microplastics block symbiont uptake by occupying their niche.

Maternal Care Enhances Starvation Resistance in Bumble Bees: The Role of Early Rearing History in Metabolic Resilience

Claudineia P Costa, S Hollis Woodard

University of California, Riverside

Background – Eusocial insects, such as bumble bees, rely on both maternal and alloparental care. Early in colony development, queens provide direct care, but once workers emerge, sibling care takes over. Bumble bees (*Bombus impatiens*) offer a model to study how rearing conditions shape adult traits. Here, we examined how maternal versus sibling brood care affects starvation resistance in workers.

Methods – We reared bees under three caregiving conditions: queen (maternal care), a single worker, or five workers. To assess metabolic resilience, adult bees underwent either a 6- or 12-hour starvation period, during which we measured blood sugar concentrations and metabolic reserves.

Results – While body size was influenced by the number of caregivers, starvation resistance was more directly linked to maternal care. Bees reared by queens exhibited greater metabolic flexibility, maintaining stable blood sugar levels and efficiently shifting to alternative fuel sources during starvation. In contrast, those reared by sibling workers showed reduced metabolic adaptability. However, metabolic rates and blood sugar levels remained unchanged under normal conditions, suggesting that maternal investment specifically enhances resilience to nutritional stress rather than altering baseline metabolism.

Conclusion – Our study demonstrates that maternal care during development enhances starvation resistance in bumble bees. These findings highlight the lasting effects of early rearing history on stress responses, emphasizing the role of maternal investment in shaping adult metabolic resilience in eusocial insects.

Sexual plasticity of the decapod crustacean *Hippolyte inermis*: patterns of expression of genes involved in vitellogenin and insuline-like hormone metabolism

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Hippolyte inermis is a shrimp inhabiting *Posidonia oceanica* meadows. It is a protandric hermaphrodite characterized by a complex life cycle. Diatoms of the genus *Cocconeis* produce a still unidentified 20-carbon PUFA which, upon ingestion by young post-larvae, triggers the disruption of its AG germ cells, naturally inducing an early sex-reversal. We isolated sex-specific genes from the transcriptome of shrimps at various steps of their life cycle, and evaluated their expression to identify molecular markers and understand the mechanisms underlying the sexual plasticity of this peculiar crustacean. The expression levels of Insuline-like-hormone (IAG), Vitellogenin-1 (VG1), two Uncharacterized -male and -female genes (UCM and UCF), and two Uncharacterized -male and -female eyestalks-genes (UCMe and UCFe) were firstly evaluated by Real Time q-PCR on wild adults, after determination of their sex, based on external sexual characters. VG1 and UCM were the only two genes consistently related to the sexual phenotype of adults. However, their expression provided contrasting results in wild specimens and in young shrimps cultured in the laboratory. Physiological and developmental patterns of VG1 and UCM, along with the expression of several vitellogenin variants, have been investigated in shrimps at various developmental stages to disclose the mechanisms underlying the complex regulation of sex in this species. Although the results show complex patterns of responses, they permit to develop tools for a rapid determination of sex of specific age classes and represent a base to comprehend the relationships between the expression of key genes and the maturation of sex in this puzzling species.

Population dynamics of *Hippolyte inermis* (Leach, 1815) in two areas of the Mediterranean basin: a long-term study

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The caridean shrimp *Hippolyte inermis* produces stable populations in seagrass meadows off the coasts of the Mediterranean Sea, Marmara Sea and the north-eastern Atlantic. It is a protandric hermaphrodite characterized by two spawning seasons and a peculiar mechanism of sex reversal triggered by the ingestion of diatoms. Its life cycle was first investigated in Ischia (Bay of Naples) in 1994, showing the yearly succession of cohorts and the monthly growth patterns. Thirty years later (2024), we have collected the same species in southern Italy (Calabria) to partially repeat the study of the population dynamics in two additional areas, one pertaining to the same Tyrrhenian basin (Cirella), and another in the Ionian Sea, physically separated by the Messina strait. New shrimp collections revealed average size differences among the studied populations. Taking into account this long-term study, a possible effect of global warming on the size of shrimps might be considered. However, latitudinal differences produce warmer seasons in southern Italy, as compared to the bay of Naples (central Italy). Consequently, shrimp populations have been analyzed to detect the effect of global warming, as compared to physiologic variations due to latitudinal differences of temperature and even to a possible separation of the genetic pools, especially referred to the Ionian population. In this view, the reproductive and developmental biology of this peculiar invertebrate calls for reflections about the actual meaning of “species” when referred to time and space variations.

Offspring overcome poor parenting by being better parents in a subsocial beetle

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Background – The evolutionary repercussions of parental effects—the impact of the developmental environment provided by parents on offspring—are often discussed as static effects that can have negative influences on offspring fitness that may even persist across generations. However, individuals are not passive recipients and may mitigate the persistence of parental effects through their behavior. Here, we show how poor parenting influences subsequent generations in a species with complex parental care, the burying beetle, *Nicrophorus orbicollis*.

Methods – We cross-fostered young and manipulated the duration of parental care received and measured the impact on morphological and behavioral traits of both F1 and F2 offspring to experimentally extricate the effect of poor parenting from other parental effects. We conducted targeted behavioral observations of the parenting behaviors exhibited by F1 offspring when they were adults and providing care to F2 offspring.

Results – As expected, reducing parental care negatively affected traits that are ecologically important for burying beetles, including F1 offspring development time and body size. However, F1 parents that received reduced care as larvae spent more time feeding F2 offspring than parents that received full care as larvae. As a result, both the number and mass of F2 offspring were unaffected by the developmental experience of their parents.

Conclusion – Our results show that flexibility in the behaviors composing parental care can overcome poor developmental environments and limit negative parental effects to a single generation. This may suggest that parental care can confer robustness to offspring development both within and across generations.

What is a worm pheromone doing in humans? Spawning of *Alitta succinea* elicited by modulators of renal calcium sensing

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Male *Alitta succinea* spawn in response to a pheromone, cysteine glutathione disulfide (CSSG), released by female *A. succinea* (Hardege et al.). Ram et al. characterized the responses (searching, faster swimming and spawning) in response to CSSG (EC50 = ~1 μ M) and to oxidized and reduced glutathione (EC50 both ~10 μ M) and related compounds.

CSSG is also present in mammalian plasma, ranging from 1 to 25 μ M. The extracellular Ca-sensing receptor (CaSR), a drug target in kidney disease, is sensitized by glutathionergic compounds. Genome Wide Association Studies (GWAS) of human metabolomes and ions, including serum CSSG and serum calcium, indicate associations with mutations of GGT1, an enzyme that can metabolize CSSG (bioinformatics analysis in collaboration with WSU colleague Xiangmin Zhang). To study possible homologies of the CaSR-modulatory sites to the pheromone receptor in *A. succinea* we tested CaSR-sensitizers AMG-416 (AMG) and Upacicalcet (UPA) on *A. succinea* spawning.

In experiments in Woods Hole, MA in 2022 & 2023, median concentrations to elicit spawning and faster swimming by CSSG, AMG, and UPA were 1 μ M (n=14), 100 μ M (n=15), and 1 mM (n=8), respectively. Alizarin red, of comparable molecular weight, did not elicit spawning or faster swimming at concentrations up to 10 mM (n=4). In collaborative experiments, Matarage et al showed competitive and cooperative binding of CSSG and other modulators (including AMG) to CaSR, possibly modeling homologous mechanisms that might be present in *A. succinea*. Studies of the peptide modulatory sites in CaSR may suggest how the pheromone receptor works in the worm.

Exposure to miticides and agrochemicals during development affects the reproductive health of honey bee (*Apis mellifera*) queens and drones

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A honey bee queen's developmental fate is highly plastic, and her reproductive physiology is greatly affected by the queen-rearing environment. Several environmental and biotic factors affect the reproductive quality of queens. We explored whether exposure of wax to field-relevant combinations of pesticides during development affects honey bee queen physiology, gene expression and behavior. We reared queens in plastic cups coated with molten beeswax that was either pesticide-free or containing field-relevant concentrations of agro-chemicals and miticides. Mated queens were placed in observation hives to measure egg-laying rate and worker retinue size. We then used the contents of their mandibular glands to measure worker attractiveness in caged bioassays and analyzed their chemical components. We then performed transcriptomic analysis of the queens' brain. We found that exposure of wax to these pesticides during development significantly lowered the adult queens' egg-laying rate and worker retinue size, lowered the attractiveness of queen mandibular gland contents to workers, and caused down- and up-regulation of genes involved in egg laying, thus supporting the idea that queen pheromones act as honest indicators of queen reproductive fitness and that pesticide exposure of wax during bee development is an important and concerning factor impairing honey bee health. Our results provide evidence that in honey bees, queen developmental plasticity influences several measures of fitness, supporting the idea that a colony can be viewed (at least in part) as the expanded phenotype of its queen, and thus, selection acting predominantly at the colony level is congruent with that at the individual level.

Organization of the ovaries in earthworms belonging to the family Acanthodrilidae – a preliminary study

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Background – Earthworms (Megadrili) are hermaphrodites with both female and male gonads. However, little attention has been paid to ovaries and the process of oogenesis in these organisms in the literature. Therefore, this research is part of a larger project that studies the organization and functioning of ovaries in many earthworm families. So far, the families Hormogastridae, Megascolecidae, and Lumbricidae have been analyzed. Another taxon chosen for the study is the species-rich and widely distributed family Acanthodrilidae.

Methods – We used light and transmission electron microscopy to describe in detail the histological and ultrastructural organization of acanthodrilid ovaries (a.o. from the genera *Diplocardia*, *Octochaetona*, *Ramiella*).

Results – Studies at the level of gross morphology indicate that ovaries are fan to rosette-shaped with numerous rows of growing oocytes (egg strings) radiating from the ovary center towards the segmental cavity. Ultrastructural analysis revealed the occurrence of germline cysts that are also present in the ovaries of other studied earthworm families. Within the cyst, each clustering cell is connected by a stable intercellular bridge to the central cytoplasmic mass – cytophore. As oocytes grow, they detach from the cysts, but the rest of the cells are still interconnected via cytophore and do not continue oogenesis. These cells are considered nurse cells, so the acanthodrilid ovaries can be classified as meroistic.

Conclusion – The occurrence of germline cysts seems to be a conservative feature of earthworm oogenesis. Further studies will be performed to describe ovary organization and the course of oogenesis in acanthodrilids in detail. The research was supported by NCN (OPUS) grant 2020/37/B/NZ4/00560

Organization of an unusual female reproductive system in earthworms – the ovo-spermathecal apparatus in *Eudrilus eugeniae*

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Background – Earthworms (Megadrili) reproduce predominantly by cross-fertilization, where the oocytes are fertilized externally. However, the family Eudrilidae is remarkable among earthworms for the occurrence of internal fertilization. Also, the female reproductive system in this family is unique due to the evolutionary tendency to unify individual elements into a complex system – the ovo-spermathecal apparatus.

Methods – We used light, transmission, and scanning electron microscopy to describe in detail the organization of the ovo-spermathecal apparatus in *Eudrilus eugeniae*.

Results – The ovo-spermathecal apparatus comprises 3 main elements: spermatheca, ovary with ovo-spermathecal duct, and ovisac with the ovisac-duct. The ovary appears to be a rudimentary organ containing only oögonia and germ cells at the early stage of meiosis. We discovered that the clusters of germline cells detach from the ovary and move via the ovo-spermathecal duct toward the spermatheca, where they continue oögenesis to some extent within small outgrowths of the spermathecal wall termed bulges. However, the main organs where oögenesis occurs are ovisacs, which function as the "true" ovary. In addition, ultrastructural analyses show that the germline cells within the ovaries and ovisacs form syncytial cysts similarly to other earthworms and other clitellates. However, the ultrastructure of late vitellogenic oocytes differs significantly from other earthworm species because of the very well-developed vitelline envelope.

Conclusion – Oögenesis in *E. eugeniae* is diffuse and takes place partly in the ovary and vaginal bulges, whereas full oögenesis occurs only in the ovisac, which can be considered a functional ovary. The research was supported by NCN (OPUS) grant 2020/37/B/NZ4/00560

Reproductive biology of the rock oyster *Striostrea prismatica* in two populations of the Mexican Central Pacific

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Background – Little is known about the reproductive cycle of the oyster *Striostrea prismatica*, that is a fishery-relevant species in the eastern tropical Pacific, especially in the Mexican Central Pacific (MCP). Consequently, at El Tizate and La Victoria in the MCP, we evaluated gametogenesis, sex ratio, sexual maturity stages, shell height at first maturity, and condition index in 2014 and 2015.

Methods – The research involved sampling two wild populations of rock oysters *S. prismatica* from the Mexican Central Pacific. One was from La Victoria, Jalisco, and the other from El Tizate, Nayarit. Monthly samples of at least 30 oysters were collected from April 2014 to August 2015 with the assistance of fishermen. A random sub-sample was taken for gonad section analysis to examine the gametogenic cycle, reproductive activity, and sex ratio. After removing the soft tissue from both valves, a 5–6 mm cross-section of the gonad was taken and preserved in a 10% formaldehyde solution for histological analysis. Gonad samples were processed using standard histological techniques.

Results – The sex ratio exhibited a considerable bias towards males at both locations, likely attributable to ontogenetic factors or fishing pressures. The peak condition index was recorded between April and May across both years, whereas diminished values were noted from September to December. The reproductive cycle at both locations was annual, with maturation and spawning occurring from June to August, followed by a degradation phase in August. The condition index demonstrated a negative correlation with temperature and chlorophyll *a* at both sites. Consequently, the peak maturation and spawning period aligned with the fishery's closure in Mexico (June–August). Females exhibited a greater

shell height at first maturity than males at both locations, potentially linked to fishing pressure. The data derived from this study constitutes a valuable contribution; however, it is imperative to expand research to populations located further south and north.

The structure and formation of the mineralized exoskeleton of crustaceans during development and growth

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Crustaceans possess an exoskeleton, but unlike molluscs, the exoskeleton does not grow incrementally with the growth of the soft tissues within. The crustacean exoskeleton must be periodically molted or shed to accommodate growth. This presents a unique challenge to this taxon. This challenge is particularly acute for larval and post-larval crustaceans. Post-larval blue crabs, for example, molt as often as once every two days. While the thickness of the exoskeleton of juvenile crabs is thinner than adults (generally scaling with carapace width), the structure and mechanisms of formation are common throughout development and will be reviewed in this address. The four requirements for forming a mineralized tissue are: (1) the establishment of a microenvironment in which biomineralization occurs; (2) the solubility product constant (SPC) for the mineral must be exceeded; (3) the component ions of the mineral must be transported into the microenvironment such that condition 2 is met; and (4) the presence of an organic matrix that facilitates the precipitation of the mineral by creating a template, referred to as heterogeneous nucleation. The crustacean exoskeleton provides a unique and particularly well-suited model for the study of biomineralization. Crustaceans are the only taxon that periodically deposits and resorbs calcified structures. The fact that mineral components are transported from the old cuticle across the hypodermis during premolt resorption and then transported from the hemolymph into the new cuticle across the same hypodermis during postmolt mineralization presents an excellent system for investigating the mechanism of bidirectional transport processes. These processes and the control of nucleation will be discussed.

Unraveling the genomic toolkit of crustacean exoskeleton biomineralization: Ion transporters in *Cherax quadricarinatus*

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Crustaceans rely on a rigid, mineralized exoskeleton for protection and muscle support. This exoskeleton, composed of chitin, minerals and proteins, is cyclically rebuilt through molting, a complex process involving mineral deposition in storage organs such as gastroliths, and upon the exoskeletal scaffold. Using the redclaw crayfish, *Cherax quadricarinatus*, as a model, we investigated the molecular mechanisms of ion transport during exoskeleton formation. We employed a novel molt-related gene expression analysis of transcriptomic libraries to identify key proteins involved in this process, focusing on ion transporters crucial for the biomineralization of crustacean exoskeletons. Specifically, we examined bicarbonate and phosphate transporters.

Our analysis revealed Solute Carrier (SLC) family transporters, SLC4 and SLC20, exhibiting distinct expression patterns correlated with the mineralization of two key exoskeleton components: the gastroliths and the cuticle. The gastroliths serve as calcium reserves, while the cuticle forms the primary protective layer. The differential expression of SLC4 and SLC20 during specific stages of gastrolith and cuticle mineralization suggests their critical roles in ion transport during molting which was supported by functional genomics through RNAi. These findings provide valuable insights into the intricate interplay of proteins supplying the crustacean exoskeleton with the necessary ions for proper mineralization. The identification of these two transporter-encoding genes illuminates the complex genetic toolkit underlying the mineralization of these vital exoskeletal tissues.

Sexual differentiation in decapod crustaceans exhibiting ZW/ZZ sex inheritance: the case of the IAG-sexual switch in the prawn *Macrobrachium rosenbergii*

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In many decapod crustaceans, ZW/ZZ sex determination dictates that ZZ individuals develop as males, a process largely driven by the androgenic gland (AG) and its secreted insulin-like androgenic gland (IAG) hormone. Conversely, WZ individuals develop as females due to the absence of IAG. The IAG encoding gene has been identified and sequenced in a wide range of decapods, including crabs, prawns, crayfish, and shrimps, exhibiting diverse reproductive strategies (gonochorism, hermaphroditism, intersexuality, parthenogenesis). Research on the prawn *Macrobrachium rosenbergii* has revealed the IAG hormone's critical role as a sexual "IAG-switch" demonstrating extraordinary phenotypic plasticity. A wide research effort in the prawns which included various manipulations of the IAG-switch have resulted in the creation of individuals with all possible genotypes (ZZ, WZ, WW) exhibiting complete and functional male or female phenotypes. Crucially, these manipulations are non-genomic, facilitating applications in aquaculture and environmental biocontrol without regulatory hurdles. This controlled sexual plasticity provides a valuable system for studying the upstream molecular regulators and downstream effectors of IAG-mediated sexual differentiation. Despite these advancements, the fundamental connection between ZW/ZZ sex determination and the IAG-switch's control of sexual differentiation remains a key unanswered question, requiring further investigation.

A tale of two functional proteins involved in crayfish exoskeleton scaffold formation and mineralization

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Crustaceans' remarkable ability to form and mineralize their exoskeletons involves a complex interplay of proteins. This study investigated the molecular mechanisms underlying exoskeleton formation and mineralization in the Redclaw crayfish, *Cherax quadricarinatus*, using a comprehensive molt-related transcriptomic database. Through binary expression pattern analysis, we identified and characterized two key proteins: Crusticuls, a novel protein family crucial for exoskeletal scaffold formation, and Craymin, a protein associated with mineralization.

Expression analysis demonstrated distinct temporal patterns: Crusticuls expression peaks during premolt, coinciding with scaffold formation, while Craymin expression increases at postmolt, aligning with the mineralization process. Temporal knockdown of these genes via RNA interference (RNAi) revealed their specific roles. Crusticuls knockdown resulted in severe malformation of the exoskeletal scaffold, while Craymin knockdown significantly reduced calcium carbonate (CaCO₃) levels and altered its polymorph composition. These findings indicate that Crusticuls and Craymin contribute to exoskeleton formation and mineralization.

This research provides novel insights into the intricate protein machinery governing crustacean exoskeletal formation and mineralization, highlighting the potential for coordinated action among proteins with specific functions. Our results pave the way for the development of self-forming, self-mineralizing bio-inspired materials, offering potential applications in various fields.

The physiological and molecular mechanisms of Juvenile hormone regulation of reproduction in bumble bees

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Juvenile hormone (JH) functions as a gonadotropin regulating reproduction in bumble bees. To investigate the mechanisms by which JH controls reproduction, we manipulated JH levels by removing the corpora allata,

the sole endocrine glands producing JH, thereby creating bees devoid of JH. We then applied topical JH-III treatment to these bees to assess its direct effects. Additionally, we treated young queens with naturally low JH levels with JH to evaluate its physiological impact. Our results show that JH accelerates metabolic rate, increases protein production, and reduces fat reserves in both workers and queens. RNA-Seq analysis of the brain and fat body of workers revealed that JH upregulates primary metabolic pathways in the fat body while downregulating ribosomal gene expression in the brain. Furthermore, we found that high JH levels correlate with reduced immune gene expression in the fat body, leading us to hypothesize that elevated JH creates conditions favorable for viral replication. To test this hypothesis, we infected bumble bee queens with Israeli acute paralysis virus (IAPV) and tracked its replication in the brain, ovaries, and fat body. Our results demonstrated that queens with high JH exhibited significantly higher viral transcript levels than those with low JH, suggesting that elevated JH levels increase susceptibility to viral infections. Together, our research elucidates the physiological and molecular functions of JH, reinforcing its role as a gonadotropic hormone while highlighting how pathogens may exploit its regulatory functions for their own benefit.

***Strongyloides* spp. – a life between sex and parthenogenesis**

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Strongyloides spp. is a nematode genus of small intestinal parasites. Among them is *S. stercoralis*, a human parasite estimated to infect more than 600 million people. Infections can be fatal, particularly in immunocompromised patients. In addition to parthenogenetic parasitic generations, *Strongyloides* spp. can also form single gonochoristic free-living generations in between parasitic generations. This gives these worms an ecologically and evolutionarily interesting "choice" between clonal and sexual reproduction and it leads to challenging life history switches. A) The parthenogenetic female produces female and male progeny. Some species have an environmentally induced XX/XO sex determining system, while in others the X chromosome is fused with one of the autosomes and in males a hemizygous region is generated through the elimination of the evolutionarily X-derived portion of one of the two homologous fusion chromosomes (chromatin diminution). B) Female progeny of the parasitic worms can either develop into infective larvae or into free-living females that mate with the males, all of which become free-living. C) Although the free-living adults reproduce sexually, they produce only female progeny that develop to infective larvae.

With *Parastrongyloides* spp. and *Rhabditophanes* spp., *Strongyloides* spp. has close facultative parasitic and non-parasitic relatives. *Parastrongyloides* spp. is always sexual, while *Rhabditophanes* spp. (at least the species we have in culture) reproduce by meiotic parthenogenesis. Hence, within the *Strongyloididae*, there occurred a transition to parasitism, two transitions to parthenogenesis, a switch from genetic to environmental sex determination and a chromosome fusion, making this taxon very interesting from an evolutionary point of view.

Day/night cycles regulate pheromone acuity to gate rhythmic courtship behavior

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Day/night cycles profoundly impact animals' physiology and behavior to allow adaptation to rhythmic environmental cues. Daily rhythmic behaviors are believed to be patterned by central clock neurons. However, the physiology of primary sensory neurons, such as olfactory receptor neurons (ORNs), can also exhibit oscillatory changes, but the contribution of such peripheral neuromodulation to rhythmic behaviors remains undetermined. Here we showed that pheromone-sensing ORNs exhibit higher responses in flies at subjective night (henceforth referred to as night flies) than in flies at subjective day (henceforth referred to as day flies). Importantly, this heightened pheromone sensitivity in night flies in turn elevates odor-guided social behavior. Mechanistically, the day or night

modulation is respectively signaled via two biogenic amines. Furthermore, the day/night modulation of olfactory acuity requires a cation channel subunit whose expression likely causes depolarization block to reduce ORN spike response frequency. As such, day/night cycles — through antagonistic actions of two biogenic amines — up- or down-regulate the cation channel in ORNs to dynamically modulate olfactory acuity and odor-guided behavior. Importantly, our findings highlight a critical role of peripheral sensory neuromodulation in gating rhythmic social behaviors.

Sex allocation in a hermaphroditic host barnacle shows flexible changes in response to parasitic infestation

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Background — Simultaneous hermaphrodites allocate reproductive resources to both male and female functions, a process known as sex allocation. Previous research on sex allocation has predominantly focused on the effects of individual traits and intraspecific interactions. However, the influence of interspecific interactions, such as parasitism, remains largely unexplored. When parasites restrict host resources, hermaphroditic hosts may adaptively shift their allocation to minimize the reduction in reproductive success. In this study, we examined the effects of infestation by the rhizocephalan barnacle *Boschmaella japonica* on the sex allocation of the hermaphroditic acorn barnacle *Chthamalus challenger*.

Methods — We collected specimens in May, when the host reproduces and the parasite's prevalence is high. We recorded infestation status (presence or absence) and intensity (the number of and total area of parasites), along with host traits such as operculum weight (as an index of body size), testis and seminal vesicle volumes, penis length and diameter (as indices of male function), egg number and egg capsule volume (as indices of female function).

Results & Conclusion — Our results showed that infested individuals exhibited reduced reproductive investment in both male and female functions, with more male-biased sex allocation compared to uninfested individuals. The shift towards male-biased allocation might mitigate the reduction in reproductive success. Moreover, infested hosts had shorter penises and presumably smaller mating group sizes than uninfested ones, which could be the cause of their further reduced male investment.

Mechanical properties of biomineralized crustacean exoskeletons

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Crustacean exoskeletons have remarkable mechanical performance due to their structure and composition. As a biomineralized composite, the exoskeleton has ample stiffness and strength to support a range of essential functions. Strong correlations between biomineral content and mechanical properties are observed over evolutionary time scales, such as the specialized structures of claws and mandibles, and over physiological time scales, such as the molting process. Aside from these examples, there is variability in biomineral content within individuals and sometimes in response to environmental conditions. In these situations, there is not always a linear correlation between biomineral content (calcium carbonates and phosphates) and local or bulk material properties. Here I present data on lobster, shrimp, and crab that demonstrate a mismatch between biomineral content and mechanical properties. In the California spiny lobster, calcification and local mechanical properties were incongruent across different exoskeletal defensive structures, and in shrimp, warmer water temperatures induced changes in local mechanical properties without underlying changes in calcification. Lastly, crabs undergoing molting exhibit similar local and bulk exoskeleton properties despite large changes in mineral content. As these examples show, the mechanical integrity of the crustacean exoskeleton is surprisingly robust to changes in biomineral content.

Exploring the mechanisms through which MSS glycoproteins modulate sperm competitiveness

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Sperm competition is found across multicellular organisms using external and internal fertilization. Research from the past decades on several animal models, including flies, mice, and nematodes, has led to the identification of multiple genes with apparent roles in sperm competitiveness. Their molecular mechanisms are generally unknown but appear distinct from core fertilization factors and may reveal general lessons about gamete biology. *Caenorhabditis* nematodes are ideal organisms for functional characterization of these genes. Previously, we showed that male secreted short (MSS) glycoproteins enhance the competitiveness of male sperm. In obligately outcrossing *Caenorhabditis*, MSS is dispensable for baseline fertility but required for intraspecific sperm competitiveness. MSS is lost in self-fertile lineages. However, genetic restoration via transgene in the self-fertile nematode *C. briggsae* is sufficient to make MSS+ male sperm more competitive. Recently we showed that other mss-related proteins (MSRPs) found in the *C. briggsae* genome are similar in structure, expression, and localization to MSS, but are not necessary for normal sperm competitiveness. A closer examination of MSS and MRSP from multiple species identified small but evolutionary conserved differences in the signal peptide sequence. Given that MSS but not MSRPs enhance sperm competitiveness upon expression we hypothesize that the difference in apparent functions is the consequence of different post-translational modifications. Using newly generated single-copy transgenic *C. elegans* we attempt to interrogate: 1) what is the contribution of predicted glycosylation sites on MSS-dependent sperm enhancement; and 2) how does the sequence of signal peptides influence glycosylation in the endoplasmic reticulum of sperm cells.

Impact of stress on division of labour in honeybee colonies

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Background — Division of labour (DOL) in eusocial insects plays an important role in colony fitness and resilience. Chronic stressors can impair DOL flexibility by affecting task allocation and worker efficiency, potentially diminishing a colony's ability to cope with disturbances. Therefore, we investigated how chronic stress influences DOL and whether it reduces a colony's capacity to respond to environmental disturbances, focusing on honeybee colonies.

Methods — We monitored chronically stressed (high parasitic infestation) and non-stressed honeybee colonies from spring to winter. To assess colony resilience, we applied cold shock as a disturbance. DOL shifts were measured by estimating the proportion of bees involved in tasks, and categorizing them based on location in the hive and behavior. Stress effects were further assessed by measuring the rate of task switching, worker survival, foraging activity, forager efficiency and nurse effectivity.

Results — Prior to cold shock, worker survival was lower in chronically stressed colonies, but no significant colony-level changes were observed compared to controls. Post-disturbance, stressed colonies had a lower proportion of nurses, higher worker mortality, and earlier task switching. Task performance of both foragers and nurses was also reduced.

Conclusion — Our results indicate that honeybee colonies initially adapt to chronic stress by maintaining flexibility in task allocation. However, prolonged stress exposure may leave colonies more vulnerable to disturbances, exceeding their social resilience, leading to suboptimal worker composition and impaired task efficiency.

High-resolution single-cell transcriptomic comparison of doublesex-expressing neurons across *Drosophila* species

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Understanding how the cellular and molecular makeup of neuronal circuits differs among related species with divergent behaviors is essential for deciphering how behavior evolves. *Drosophila* courtship is remarkably diverse across species and many of the neurons encoding this behavior are specified through the action of the sex determination gene *doublesex* (*dsx*). Here, we employed single-cell RNA sequencing (scRNA-seq) to systematically characterize and compare the cell types of male *dsx*⁺ neurons across *Drosophila* species with divergent courtship behaviors. With a high-resolution to discern cellular heterogeneity, comparisons among four species revealed a prevailing conservation of cell types, with little gain or loss across species. An in-depth comparison between *D. melanogaster* and *D. yakuba* found that both the overall transcriptomic similarity and the individual genes differentially expressed between species are highly cell-type specific, suggesting that cell types evolve as highly independent units. We observed extensive changes in gene expression, including known courtship-related neuropeptides, layered onto a conserved circuit layout specified by the sex determination genes *fruitless* and *dsx*. Finally, by incorporating a scRNA-seq dataset of *D. melanogaster* female *dsx*⁺ neurons, we defined the sex-specificity of *dsx*⁺ cell types and present marker gene combinations for each cell type defined across species and between sexes, providing a foundational resource that enables the design of cell-type-specific genetic reagents for functional characterization. Overall, our study provides critical insights into how evolution shapes neuronal circuits and gene expression patterns in behavioral adaptations across species.

Characterizing the phenotypic effects of disrupting conserved long noncoding RNAs in *Drosophila melanogaster* and *D. pseudoobscura*

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Long noncoding RNAs (lncRNAs) are an important group of pervasive RNAs, which play vital roles in the regulation and evolution of many biological processes. Most lncRNAs exhibit tissue-specific expression, with a bias towards male reproductive tissues, and some are known to affect spermatogenesis. The general importance of lncRNAs in reproductive processes and the underlying molecular mechanism remains unclear. Using sequence similarity, synteny, and similarity in patterns of expression during development, we have identified a group of lncRNAs that are conserved in *Drosophila melanogaster* and *D. pseudoobscura*, species that diverged >50 Mya based. In this study, we characterized reproductive-related phenotypic effects of 19 lncRNA CRISPR knockouts in *D. melanogaster*.

Male fertility assay and sperm competitive assay were used to investigate potential dysfunction of lncRNA knockouts. Testis and ovary morphology were also examined by microscopy. We identified 5 out of 19 lines with significantly reduced fertility. Among the remaining 14 lines without significant fertility defects, 3 exhibited biased progeny ratios under competitive mating. While no apparent testis morphology changes were observed, two knockout strains displayed distinct ovary morphology alterations. The preliminary data suggests that lncRNA deletions may influence spermatogenesis or reproductive gland function, ultimately affecting fertility and mating success in *D. melanogaster*.

Potential amplification of collagen gene copy number in the elongated nematode, *Caenorhabditis inopinata*

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Understanding the genomic drivers of morphological change is a central goal of evolutionary developmental biology. *Caenorhabditis inopinata* is an exceptional member of its genus, with adults exhibiting a dramatic increase in length compared to its close relatives (including its sister species, *C. elegans*). Collagen genes may be important evolutionary substrates of body shape divergence. To address this possibility, I estimated the number of collagen genes among the genomes of 54 *Caenorhabditis* species. *C. inopinata* had 184 collagen genes (>2 σ from the mean (145)). Further examination of genomic assemblies revealed potential lineage-specific duplication events. Patterns of synteny suggest the existence of a highly-conserved and ancient cassette of three collagen genes that are homologous to *col-144*, *col-145*, and *col-10* of *C. elegans*. This three-gene cassette is largely stable across phylogeny, found in eleven *Caenorhabditis* species with high quality assemblies. In *C. inopinata*, this three-gene cassette has expanded to ~13 copies. To understand the role of these genes in body size regulation, I generated a 5,941 bp deletion removing the entire *col-144/145/10* cassette in *C. elegans*. Preliminary observations suggest this mutation may promote embryonic inviability and slow growth. Ongoing reverse genetic work that directly manipulates collagen copy number in *C. elegans* and *C. inopinata* will also be discussed. This work suggests the existence of a lineage-specific amplification of a highly-conserved and ancient collagen gene cassette in a species with a novel body shape, and changes in collagen gene copy number may be connected to the evolution of large body sizes in this group.

Insights into the origin of nuclear-encoded mitochondrial genes from genomic analyses of two closely related species of *Drosophila*

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Nuclear mitochondrial DNA segments (NUMT), are insertions of mitochondrial sequences in the nuclear genome. Studying the distribution of insertions in conserved chromosomal locations in sister species is important to understand their evolutionary origins and mechanisms of insertion.

Using blast we identified NUMTs in *Drosophila pseudoobscura* and its close relative *D. persimilis*. To characterize these insertions, we annotated the corresponding mitochondrial genes with tblastn, and estimated the age of insertion using the sequence divergence to the mtDNA. Transposable Element distribution in NUMTs and their flanking regions was assessed using TE annotations. The transcriptional activity of NUMTs assessed using de novo transcriptome assembly and RNA-seq data.

In *pseudoobscura* we identified 11 NUMTs on chromosome (chr) 3 and 12 on chr X. In *D. persimilis* we identified 10 NUMTs on chr 3 and 1 on chr X. In *D. pseudoobscura*, a ~77 kb region on chr 3 and a 5.9 MB on chr X contained clustered NUMTs that include all mitochondrial genes except ATP8. In *D. persimilis*, a similar ~86 kb region on chr 3 contains the whole mitochondrial genome. The insertions are located in regions of low recombination, in telomeres or pericentromeric. The insertions have high TE content, although their enrichment was not statistically significant compared to the rest of the chromosome. Divergence analysis and the length of the blast suggest the common insertion in chromosomes 3 is older. RNA seq analysis shows transcriptional activity in some NUMTs in specific loci.

Our results suggest that these NUMTs originate from two independent transfers of the whole mtDNA in the ancestor of these species. Genomic location of insertions may have played a role in their insertion mechanism and gradual degradation.

Impact of heavy metals on exoskeletal mineralization in the postecdysial blue crab, *Callinectes sapidus*: insight into epidermal metal transporters

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Background – Crustacean exoskeleton is a repository of metals. Besides calcium and magnesium that are structurally essential for exoskeletal strength through the formation of carbonate salts, a number of structurally unrelated metals have been discovered in crustacean exoskeleton, including cadmium (Cd), lead (Pb), zinc (Zn), chromium (Cr), etc. It remains unknown as to in what phase of the molting cycle heavy metals are deposited to the exoskeleton, what effects heavy metals have on exoskeletal mineralization and what metal transporters in the epidermis mediate heavy metal incorporation into the shell. This investigation sought to address these scientific questions using the blue crab, *Callinectes sapidus*, as the model crustacean.

Methods – Postmolt blue crabs were injected twice (at one-day and three-days postecdysis) with Cd (II), Pb (II), Zn (II), or Cr (II, III or VI). Carapaces and soft tissues were harvested at four-days postecdysis for Cd and five-days postecdysis for all the other metals. Exoskeletal and tissue metal contents were quantified using ICP-OES.

Results – Cd and Zn treatments significantly increased respective metal content in the exoskeleton while Cr (III) and Cr (VI), but not Cr (II), significantly elevated Cr content in the shell. Pb, Zn, Cr (III) and Cr (VI) significantly inhibited exoskeletal calcification.

Conclusion – Heavy metals are deposited to the exoskeleton during post-ecdysial mineralization. Cd appears to adversely affect the organic matrix while Zn and Pb suppress exoskeletal calcification, presumably through competitive inhibition of epidermal calcium transporters. Cr (III)'s entry into epidermal cells is most likely assisted by transferrin while epidermal sulfate transporter must facilitate Cr (VI) uptake by the epidermis.

Ferroptosis facilitates sexual plasticity in *Hippolyte inermis* (Leach, 1815): patterns of proteins and genes

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Ferroptosis has been detected here for the first time in a crustacean. It is a form of regulated cell death involving iron-dependent lipid peroxidation. Consequently, it generates lipid hydroperoxides via intermediate formation of peroxyl radicals. The functions and regulation of lipid peroxidation through ferroptosis as well as the antioxidant network were demonstrated in a range of species, from humans and other mammals to plants and lower invertebrates down to yeast, bacteria, and archaea. The potential evolutionary roles of lipid peroxidation and ferroptosis is of paramount importance to understand how programmed cell death entered and contributed to the evolution of species. In this research, we identified the activation of genes responsible for ferroptosis in the protandric shrimp *Hippolyte inermis*, where the mechanism is activated by the ingestion of specific diatoms and it leads to an early and quick destruction of the Androgenic Gland (AG) in still undifferentiated post-larvae. The mechanisms controlling the shrimp sexual differentiation have been only partially elucidated, but the main role has been attributed to the insulin-like hormone (IAG) secreted by the AG. Here, we explore genes known to be involved in ferroptosis to obtain further understanding of this newer cell death mechanism in decapod crustaceans and to shed light on the evolution of this process. It is suggested that ferroptosis might represent a primary factor during early development of invertebrates, followed by further apoptotic events. This also demonstrates a peculiar case of direct effect of ingested food on the patterns of development and sexual differentiation in a marine invertebrate.