Simultaneous and Sequential Hermaphroditic Organisms Workshop

The University of Salford, MediaCityUK Campus

24th-26th April 2019

Organizers Dr Chiara Benvenuto
Dr Ilaria Coscia

With the precious help of Kelly Millward, Stacey Winnard-Corlett and Emma Looskin

Funded by the **School of Environmental and Life Sciences** and the **Ecosystems and Environment Research Centre** (EERC) https://www.salford.ac.uk/research/eerc

Contents

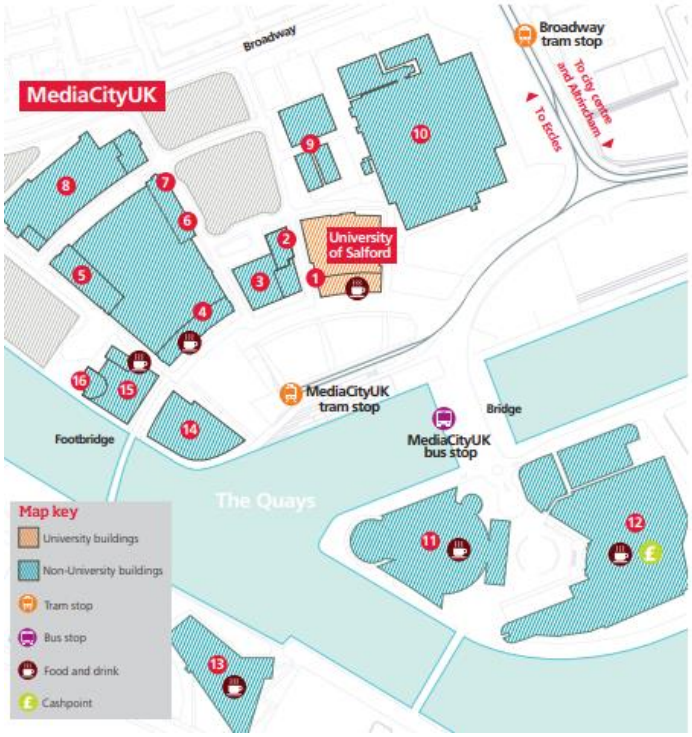
Practical Info

Venue

The workshop will take place at the University of Salford MediaCityUK campus in Salford https://www.salford.ac.uk/mediacityuk, easily reached by taxi, Metrolink (tram) and train from the airport (or the station).

Metrolink (tram) runs every six minutes in both directions between Manchester city centre and MediaCityUK

Bus The CityConnect number **50**, runs every 10 minutes during the day Monday – Friday and every 15 minutes in the evening and weekends



Guide to buildings

1 University of Salford
Orange Tower
ITV - Offices
Wagamama
Prezzo

2 Number One - Residential

3 BBC - Offices

4 The Studio
WH Smith
Booths

5 Blue Tower - Offices

6 Holiday Inn

7 White Tower - Offices

8 The Garage - Car Park

9 The Greenhouse

10 The Pie Factory

11 The Lowry
Theatre
Art Galleries
The Lowry Restaurant
The Terrace Bar
Tower Coffee Bar

12 The Lowry Outlet Mall
Costa Coffee
Bella Italia
Lime
Pizza Express
Café Rouge
Pond Quay Oriental Restaurant
Burger King
Esporta Fitness
VUE Cinema

13 The Imperial War Museum North
WaterShard Café

14 BBC - Offices

15 BBC - Offices

16 The Heart - Residential

We will be hosted on the 3rd floor of the University of Salford building (B4, Orange Tower, Salford Quays, Salford M50 2HE).

We will have an informal welcome drink on the 24th in the evening (from 7:00 pm) in the same location as the workshop.

The social dinner will be hosted at VERO MODERNO, a fine Italian restaurant at Unit 4 Vimto Gardens, in Chapel Street Salford, MCR, M3 5JF (http://www.veromoderno.co.uk/#sectionHome).

The restaurant is easily reached by bus (n. 50) from the workshop location.

Schedule at a glance

SHOW SALFORD 24th April 2019

19:00	Welcome drinks

SHOW SALFORD 25th April 2019

8:30-9:00	Breakfast
9:00-9:30	Welcome and introduction to the workshop
9:30-10:10	**Sex determination in nematode species with males, females and hermaphrodites** - Andre Pires da Silva
10:10-10:50	**How transitions from dioecy to hermaphroditism are mediated by sex chromosome evolution** - Stephen C. Weeks
10:50-11:20	Coffee break
11:20-12:00	**Reduced plasticity in sex allocation as a result of sex-specific selection** - Jessica Abbott
12:00-12:40	**Gene expression changes in a dioecious plant suggest trade-offs between reproductive functions in its cosexual ancestor** - Deborah Charlesworth
12:40-13:40	**Lunch**
13:40- 14:20	**Why animals abandon sex? On the interconnection between asexuality, hybridization and speciation** - Karel Janko
14:20-15:00	**The Model of the Conserved Epigenetic Regulation of Sex** - Francesc Piferrer, Dafni Anastasiadi, Alejandro Valdivieso, Núria Sánchez-Baizán, Javier Moraleda-Prados & Laia Ribas
15:00-15:30	Coffee break
15:30-16:10	**Plasticity of gonad development in fish – studies on medaka and hermaphrodite killifish** Manfred Schartl
16:10-16:50	**The constraints of sexual plasticity** - Chiara Benvenuto
16:50-17:30	Brain storming
17:30-18:00	**Posters**
19:30	**Dinner at VERO MODERNO**

SHOW SALFORD 26th April 2019

8:30-9:00	Breakfast
9:00-9:40	**Simultaneous hermaphroditism in fish and maximum body size -** Susanna Pla & Francesc Piferrer
9:40-10:20	**Contrasting rates of molecular evolution in reproduction-related genes in *Macrostomum* flatworms with different mating strategies -** R. Axel W. Wiberg, Jeremias Brand, Peter Ladurner, Steven A. Ramm, Christian Beisel & Lukas Schärer
10:20-11:00	Coffee break
11:00-11:40	**Evolution of sex allocation and its correlates in the flatworm genus *Macrostomum* -**Jeremias N. Brand, Gudrun Viktorin, Christian Beisel, Luke J. Harmon & Lukas Schärer
11:40-12:20	**The importance of assortative mating in reciprocating hermaphrodites -** Maria Cristina Lorenzi, Dasa Schleicherovà & Heiko Rödel
12:20-13:00	**What is a Species? Biological and Phylogenetic Data in the Genus *Helisoma* (Planorbella) -** Cynthia Norton, Robert T. Dillon, Jr., Kay Tweeten & Ngozika Ezenagu
13:00-14:00	**Lunch**
14:00-14:40	**The reproductive strategies of the ctenophore *Mnemiopsis leidyi*, a holopelagic simultaneous hermaphrodite capable of self-fertilization -** Daniel A. Sasson & Joseph F. Ryan
14:40-15:20	**Evolution of waiting time to selfing - Can bottlenecks and density fluctuations explain trait variation?** - Chantal Stock
15:20-16:30	Final discussion, including coffee break

Abstracts

TALKS

Thursday 9:30

Sex determination in nematode species with males, females and hermaphrodites

Andre Pires da Silva

School of Life Sciences, University of Warwick, UK
https://rivak.lnx.warwick.ac.uk/Pires/index.html ✉ Andre.Pires@warwick.ac.uk

Androdioecy, gynodioecy and trioecy are usually thought to be intermediate evolutionarily unstable strategies, but offer important systems to test models of the causes and consequences of mating system on the evolution of populations. Free-living trioecious nematodes, such as *Auanema rhodensis* and *A. freiburgensis* are experimentally and genetically tractable and may permit delineation of the mechanisms of sex determination and of the processes that underlie the origin and maintenance of alternative mating systems. In *Auanema*, females and hermaphrodites are karyotypically identical, with two X chromosomes (XX), whereas males have only one X chromosome (XO). In the nematode *Auanema freiburgensis*, stress-resistance and sex of the offspring depend on environmental cues experienced by the mother. Maternal sensing of high population densities results in the production of stress-resistant larvae (dauers) that develop into hermaphrodites. We will provide recent data on how the neurons sense environmental information to reprogram epigenetically the germline to generated different types of offspring.

Thursday 10:10

How transitions from dioecy to hermaphroditism are mediated by sex chromosome evolution

Stephen C. Weeks

Department of Biology, The University of Akron Akron, OH, USA
https://blogs.uakron.edu/weeks/✉ scw@uakron.edu

Evolutionary transitions among breeding systems are common in plants and animals. In animals, one of the most common breeding system transitions is between gonochorism (or dioecy) and hermaphroditism. Authors considering transitions between the “endpoints” of simultaneous hermaphroditism and dioecy have posited various intermediate reproductive forms, such as environmental sex determination, sequential hermaphroditism, gynodioecy and androdioecy. The underlying genetics of sex determination must be considered when assuming the likely evolutionary transitions between dioecy and hermaphroditism. Understanding the mechanism of sex determination (e.g., a master sex-determining gene or many linked sex-limited genes) is critical for determining which transitions are probable and

noting potential constraints on such transitions. This transition will be explored by considering various sex determining options.

Thursday 11:20

Reduced plasticity in sex allocation as a result of sex-specific selection

Jessica Abbott

Department of Biology, Section for Evolutionary Ecology, Lund University, Lund, Sweden
http://jessicakabbott.com ✉ jessica.abbott@biol.lu.se

The early stages of sex chromosome formation are predicted to involve linkage of sex-specific and sexually antagonistic fitness loci to the sex determining region. In order to mimic the early stages of sex chromosome evolution, we have carried out experimental evolution in *Macrostomum lignano*, using a GFP marker as a proxy for a sex determining locus. Male-selected (M-lines) and female selected (F-lines) populations should therefore evolve to move close to their respective sex-specific optima. We hypothesized that this would entail a change in baseline sex allocation and/or loss of plasticity in sex allocation (SA). To test this hypothesis, we allowed juvenile worms to develop in isolation in a common garden setting, recording their initial SA; we subsequently obtained individuals' new SA in response to a factorial combination of food availability (ad libitum/restricted) and mating group size (pairs/octets). We found that 1/ There was no initial difference in SA among evolved lines. 2/ In the restricted food treatment, M-line worms showed significantly more male-biased SA after experiencing mating opportunities compared to the F-lines and controls. 3/ When food was restricted, F-line worms did not increase their allocation to testes after being offering mating opportunities. This suggests that when food is restricted, M-line and F-line worms adjust their sex allocation as predicted.

Thursday 12:00

Gene expression changes in a dioecious plant suggest trade-offs between reproductive functions in its cosexual ancestor

Deborah Charlesworth

Institute of Evolutionary Biology, University of Edinburgh, UK
https://www.ed.ac.uk/profile/deborah-charlesworth ✉ deborah.charlesworth@ed.ac.uk

The evolution of separate sexes may involve changed expression of many genes, as each sex adapts to its new state. Evidence is accumulating for sex differences in expression even in organisms that have recently evolved separate sexes from hermaphrodite or monoecious (cosexual) ancestors, such as some dioecious flowering plants. I will describe evidence that a dioecious plant species with recently evolved dioecy, *Silene latifolia*, has undergone adaptive

changes that improve functioning in females, in addition to changes that are probably pleiotropic effects of male-sterility. The results suggest pervasive adaptations in the genome as soon as males and females evolve. This supports the hypothesis that trade-offs occurred in the cosexual ancestor, and that release from these constraining pleiotropic effects occurs when dioecy evolves. Some increases in expression in the dioecious species may reflect pleiotropic effects in which presence of a gynoecium in the ancestral cosexual suppresses expression of genes in developing stamens, or vice versa.

Thursday 13:40

Why animals abandon sex? On the interconnection between asexuality, hybridization and speciation

Karel Janko[1,2]

[1]Laboratory of Fish Genetics, Institute of Animal Physiology and Genetics of the CAS, Rumburská 89, 277 21 Liběchov, Czech Republic; [2]Department of Biology and Ecology, Faculty of Science, University of Ostrava, Chittussiho 10, 710 00 Ostrava, Czech Republic

✉ janko@iapg.cas.cz

Although genetic machinery controlling production of recombined gametes is conserved, it has been repeatedly disrupted in all animal and plant phyla leading to emergences of organisms, which possess a wide array of gametogenetic aberrations more or less alleviating the classical sexual reproduction. Despite intensive investigation, little is known about the proximate mechanisms which made these so-called asexual lineages switching from sex to asexuality. Our research revealed molecular and cytogenetic mechanisms explaining why the distortion of reproductive modes towards asexuality is often bound to interspecific hybridization. As a model organism we use loach fishes (Cobitidae) with several species pairs forming independent hybrid zones and producing wide array of hybrid forms ranging from sexual, through (hemi)clonal to sterile ones. Comparative analysis of hybrids and parental species employing phylogenomics, gene expression profiling and experimental crossings along with functional analyses of the gametogenetic pathways, provided some general clues explaining the link between hybridization and asexuality. First, clonal gametogenesis is not restricted to only some 'pre-adapted' parental genomes, but evolves gradually as a side-effect of accumulation of reproductive incompatibilities between species. Hybrid's asexuality may thus be viewed as a special type of Bateson-Dobzhansky-Muller incompatibility. Second, successful establishment of hybrid clones requires certain level of cis-regulatory divergence between parental genomes, which ensures partially independent execution of co-inherited developmental programs in hybrids. We further revealed that the efficiency of trans regulatory pathways in a hybrid were systematically affected by polyploidization, which explain why polyploidy is so common among asexuals. Finally, asexuality typically emerges in one hybrid sex but evolves hand-in-hand with sterility of the other hybrid's sex. Although

various types of epistatic incompatibilities are likely involved in independent cases of sterility and clonality, they are developmentally canalized into only several types of gametogenetic aberrations. Our study points at tight association between asexuality and other postzygotic reproductive barriers.

Thursday 14:20

The model of the conserved epigenetic regulation of sex

Francesc Piferrer, Dafni Anastasiadi, Alejandro Valdivieso, Núria Sánchez-Baizán, Javier Moraleda-Prados, Laia Ribas

Institute of Marine Sciences (ICM), Spanish National Research Council (CSIC), Barcelona, Spain
http://www.icm.csic.es/en ✉ piferrer@icm.csic.es

Genomic and environmental information is integrated through epigenetic regulatory mechanisms to produce a given phenotype. Here, we present the model of Conserved Epigenetic Regulation of Sex (CERS), which concerns genes involved in sexual development and on epigenetic gene expression activation and silencing. This model was recently postulated to be applied to sexual development of both gonochoristic and hermaphroditic fish species and it states that epigenetic and gene expression patterns are more associated with the development of a particular gonadal phenotype, e.g., during testis differentiation, rather than with the intrinsic or extrinsic causes that lead to the development of this phenotype. This involved genes with different possible epigenetic modifications, for example, changes in DNA methylation levels, associated with the development of a particular sex. Focusing on DNA methylation, the identification of sex-linked methylation differences in specific CpGs constitutes the basis for the identification of Essential Epigenetic Markers (EEM), defined as the number and identity of informative epigenetic marks that are strictly necessary to bring about a specific, measurable, phenotype of interest. We provide a summary of the genes where DNA methylation has been investigated so far, focusing on fish. We found that cyp19a1a and dmrt1, two key genes for ovarian and testis development, respectively, consistently show an inverse relationship between their DNA methylation and gene expression levels, thus following CERS predictions. However, in foxl2a, a pro-female gene and amh, a pro-male gene, such relationship is not clear. The available data pertaining to other genes related to sexual development such as sox9, gsdf and amhr2 is also discussed. Next, we discuss the use of CERS to make testable predictions of how sex is epigenetically regulated and to better understand sexual development, as well as the use of EEMs as tools for the diagnosis and prognosis of sex. We argue that CERS can aid in focusing research on the epigenetic regulation of sexual development, not only in fish but in vertebrates in general, particularly in reptiles with temperature sex-determination. This knowledge can be used for

possible practical applications including sex control in aquaculture and also in conservation biology. *Research supported by MINECO grant AGL2016-787107-R to FP*.

Thursday 15:30

Plasticity of gonad development in fish – studies on medaka and hermaphrodite killifish

Mateus Adolfi, Meng Qu, Peter Fischer, Manfred Schartl

University of Wuerzburg, Physiological Chemistry, Biocenter, Am Hubland, D-97074 Wuerzburg, Germany
https://www.biozentrum.uni-wuerzburg.de/pch1/mitarbeiter/manfred-schartl/
✉ phch1@biozentrum.uni-wuerzburg.de

Fish show a remarkable plasticity of reproductive organ development, which allows functional sex change and even simultaneous hermaphroditism. To contribute to a better understanding of the underlying molecular processes, we have studied the development of the gonad of the so far only known vertebrate, which exists as a simultaneous hermaphrodite, the mangrove killifish *Kryptolebias marmoratus*. Analysing the temporal and spatial expression patterns of known critical male and female sex determining genes we find that these genes follow the known signature of gonochoristic gonads. In the rare primary males, testis development also shows no deviation from males of gonochoristic species. A special focus is put on the Anti-Müllerian hormone (AMH) and its receptor because we hypothesize that absence of the Müllerian duct in teleosts is a precondition for hermaphroditic development and that release of this molecular pathway from the function in duct regression has allowed its recruitment for other important processes in gonad development. Reasoning that environmentally induced sex reversal shares common features with processes of sex change we analysed the mechanism of temperature dependent sex reversal in medaka, *Oryzias latipes*, which has a stable XX/XY genetic sex determination system, but still responds to environmental cues. High temperatures lead to female-to-male sex reversal. However, the mechanisms behind are still unknown. We show that high temperature during the critical stage of sex determination increases PGC numbers before they reach the genital ridge, which, in turn, regulates germ cell proliferation. Complete ablation of PGCs led to XX males with germ cell less testis, while experimentally increased PGC numbers did not reverse XY genotypes to female. For the underlying molecular mechanism we provide support for the explanation that activation of the dmrt1a gene by cortisol during early development of XX embryos enables this autosomal gene to take over the role of the male determining Y-chromosomal dmrt1bY.

Thursday 15:30 16:10

The constraints of sexual plasticity

Chiara Benvenuto

School of Environment & Life Sciences, University of Salford, Salford, M5 4WT, UK

https://sites.google.com/view/benvenutoresearchgroup/home ✉
c.benvenuto@salford.ac.uk

One of the main aims of life history theories is to investigate how, given specific trade-offs and constraints, life history traits and strategies can maximise fitness. Let's consider, for example the extraordinary diversity of animal sexual systems. Among vertebrates, fish show the most variable repertoire of reproductive modes, from unisexual reproduction (gynogenesis and hybridogenesis) to gonochorism, simultaneous hermaphroditism, sequential hermaphroditism (sex-change) and mixed mating system (androdioecy, when males coexist with hermaphrodites). An even larger range is found in crustaceans (with the inclusion of obligate and cyclical parthenogenesis and protandric simultaneous hermaphroditism, when males become hermaphrodites). In the attempt to explain so much variability, we have started studying sex in an evolutionary perspective, as a continuum of plastic traits affecting sex determination and sex differentiation. Here I propose a further switch in perspective. If sex can be so plastic, what are its constraints? Why certain reproductive modes, common in certain taxa are not found in others? Using fish and crustaceans as reference, I propose a multi-taxa approach to explore what limits sexual plasticity. In particular, I will focus on why so few crustacean species are simultaneous hermaphrodites, why no decapods are protogynous and why in fish protandry is not as common as protogyny, based on physiological, developmental and evolutionary constraints.

DISCUSSION AND BRAINSTORMING

Friday 9:00

Simultaneous hermaphroditism in fish and maximum body size

Susanna Pla, Francesc Piferrer

Institute of Marine Sciences (ICM), Spanish National Research Council (CSIC), Barcelona, Spain
http://www.icm.csic.es/en ✉ spla@icm.csic.es

The incidence of simultaneous hermaphroditism (SH) in fish is not fully resolved at the taxonomic level. It is not clear either whether fish exhibiting SH tend to have a smaller maximum body size (MBS) than closely related gonochoristic species, as it occurs in some invertebrates. Here, we conducted and extensive survey of the primary literature and could confirm the presence of SH in 4 out of the 59 orders of the Class Actinopterygii with 64

confirmed species and three species that would require further verification. The orders/families where SH is present in fish are the F. Serranidae, F. Gobiidae, O. Aulopiformes, F. Muraenidae and F. Rivulidae. Using data on the sexual pattern and MBS of species belonging to these families, we utilized phylogenetic analysis procedures to reconstruct the ancestral sexual pattern and MBS of each family to determine, first, whether species with SH have a smaller MBS than gonochoristic conspecifics and, if so, whether this can be attributed to a small-sized ancestor or to a further reduction in size during the evolution to SH. Results showed that Serranidae species with SH had a smaller MBS than gonochoristic species, that the ancestral state was protogyny, estimated in a mid-sized fish, and that there was further reduction of MBS during the evolution to SH. A similar situation was observed in the F. Gobiidae, the major difference being that in this family the ancestor was a both-way sequential hermaphrodite and that analysis included only two SH species for which this condition would require further confirmation. In the other cases examined, no differences in MBS were found. In Aulopiformes (gonochoristic ancestor) comparisons had to involve SH and gonochoristic species of different families, but no further reduction in MBS was evidenced during the evolution of species with SH. In Muraenidae and Rivulidae (protogynous and gonochoristic ancestors, respectively) no changes in MBS were evidenced either. Our results provide an updated account of the incidence of SH in Actinopterygian fishes and, aside the situation in Serranidae, where a reduction in MBS in SH species could be documented, such a reduction is either only marginally supported (F. Gobiidae) or not apparent at all in the rest of taxa examined. Thus, in fish as a whole, with the currently available data, a reduction in MBS in SH species cannot be supported. *Research supported by MINECO grant AGL2016-787107-R to FP.*

Friday 9:40

Contrasting rates of molecular evolution in reproduction-related genes in *Macrostomum* flatworms with different mating strategies

R. Axel W. Wiberg, Jeremias Brand, Peter Ladurner, Steven A. Ramm, Christian Beisel, Lukas Schärer

Zoological Institute, Department of Environmental Sciences, University of Basel, Switzerland
http://evolution.unibas.ch/scharer/index.htm ✉ raw.wiberg@unibas.ch

Sexual conflict and sexually antagonistic co-evolution are potent diversifying forces, and their study has begun to employ comparative genomics to identify signatures of adaptation. Free-living flatworms of the genus *Macrostomum* are simultaneous hermaphrodites and display an extraordinary diversity of mating behaviours and reproductive morphologies. These are likely the result of diversifying sexual conflict over sex roles during mating, leading to two main mating strategies. Specifically, “reciprocal copulation” involves both partners donating and

receiving sperm at the same time and “hypodermic insemination” involves unilateral sperm transfer via a hypodermic male reproductive organ, with the latter strategy having at least five independent origins. In the genome-sequenced model species *M. lignano* several RNA-Seq and in situ hybridisation studies have identified transcripts expressed in reproductive tissues (e.g. gonads, and prostate glands). These are good candidates for loci involved in sexual conflict, which may therefore be evolving at different rates throughout the genus *Macrostomum*. Here we assemble transcriptomes from 97 *Macrostomum* species and several out-groups. We then identified ortholog groups that contain reproduction-related genes, by transferring annotations from *M. lignano*. Next, we estimated non-synonymous (dN) and synonymous (dS) substitution rates within these genes, to evaluate the evidence for positive selection, and contrasted dN/dS ratios in lineages that differ in their mating strategies. These analyses test the importance of sexual conflict in different lineages of *Macrostomum*, allow identification of candidate genes for traits involved in mating strategy evolution, and contribute more generally to our understanding of the manifestations of sexual

---

Friday 11:00

Evolution of sex allocation and its correlates in the flatworm genus *Macrostomum*

Jeremias N. Brand, Gudrun Viktorin, Christian Beisel, Luke J. Harmon, Lukas Schärer

Zoological Institute, Department of Environmental Sciences, University of Basel, Switzerland
http://evolution.unibas.ch/scharer/index.htm ✉ jeremias.brand@unibas.ch

Many simultaneous hermaphrodites have the ability to adjust their sex allocation in response to changes in the environment. Indeed, there is a growing body of evidence for phenotypically plastic sex allocation in response to changes in the social environment. Given this intra-species plasticity we could expect that sex allocation also evolves on an evolutionary time-scale in response to the characteristic social environment of a particular lineage. However, investigating the rate of evolution of sex allocation is challenging, since in many organisms measuring both male and female gonads simultaneously is difficult and laborious. We here present comparative work using the free-living flatworm genus *Macrostomum*, which are small and transparent with distinct ovaries and testes, thus allowing estimation of gonad volume in vivo using a light microscope. In a global sampling effort, we have collected 96 Macrostomum species, estimated their sex allocation, and also collected data on sperm and copulatory organ morphology. We combined this data with a molecular phylogeny derived from de novo assembled transcriptomes, thus allowing for phylogenetic correction in our analysis. We show that sex allocation evolves at a homogeneous rate across the genus, with a bimodal distribution representing species with a more or less strongly female-biased allocation respectively. This is indicative of disruptive selection towards two optima. Furthermore, we show that both sperm length and male copulatory organ length are associated with an increased investment into the male function, as estimated by testis size.

Friday 11:40

The importance of assortative mating in reciprocating hermaphrodites

Maria Cristina Lorenzi[1], Dasa Schleicherovà[2], Heiko Rödel[1]

[1]LEEC - Laboratoire d'Ethologie Expérimentale et Comparée, University of Paris 13, Villetaneuse, France; [2]DBIOS - Department of Life Sciences and Systems Biology, University of Turin, Turin, Italy

http://leec.univ-paris13.fr/new/pages/lorenzi/CLorenzi.html ✉ cristina.lorenzi@leec.univ-paris13.fr

The alternation of sexual roles in unilaterally mating hermaphrodites may be explained by reciprocation, where the amount of eggs donated is adjusted to that of eggs received. This suggests that reciprocation will only occur between partners matched by quality; for example, partners of different size (i.e., with different prospective egg output) will be less likely to cooperate than size-matched partners. We tested this hypothesis in the obligately outcrosser, hermaphroditic, polychaete worms *Ophryotrocha diadema*, that reciprocate egg clutches by alternating sexual roles with their partners (i.e., at each mating, each worm plays the sexual role opposite to that of the partner). We set up pairs where focal worms were ready to lay and fertilize eggs and their partners were either equally mature (symmetrical pairs), or ready to fertilize, but not yet ready to lay eggs (asymmetrical pairs). Focal worms in symmetrical pairs laid significantly more eggs than those in asymmetrical pairs, as expected under the reciprocation paradigm. Moreover, focal worms in symmetrical pairs laid their first clutch significantly earlier than those in asymmetrical pairs, suggesting that focal worms were less likely to donate eggs soon to partners unable to reciprocate.These results confirm egg trading as an example of reciprocation occurring in cognitively unsophisticated animals, and emphasize the importance of assortative mating in reciprocating hermaphrodites and in the maintenance of hermaphroditism.

Friday 12:20

What is a Species? Biological and Phylogenetic Data in the Genus *Helisoma* (Planorbella)

Cynthia Norton, Robert T. Dillon, Jr., Kay Tweeten, Ngozika Ezenagu

Biology Department, St. Catherine University St. Paul, MN USA

https://www.stkate.edu/academics/our-faculty/cynthia-norton ✉ cgnorton@stkate.edu

Biologists have argued for years about what constitutes a species. Evolutionary biologists have focused on both the biological species concept (BSC), which maintains that species are reproductively isolated groups and the phylogenetic species concept (PSC) which relies on molecular analysis to group individuals based on shared ancestry. Historically (and for

practical reasons), freshwater snails have been grouped by differences in morphology - often differences in shell shape and size and/or genitalia. We address this question of species identity broadly, providing an example of some of the dilemmas posed by these definitions using closely related members of the genus *Helisoma* (also known as Planorbella). We examined *Helisoma duryi* collected in Florida (HdF), *H. trivolvis* from South Carolina (HtC), and an albino lab population of *H. trivolvis* (HtA) to investigate the extent of genetic similarity as well as reproductive isolation. Wild-caught HdF and HtC were mated to HtA individuals from the lab, and offspring were collected and examined for eye pigmentation. Any pigmented embryos produced by albino individuals would indicate cross-fertilization, whereas albino progeny presumably resulted from matings with other albinos in the tanks (self-fertilization is extremely rare in this species). 5/8 crosses with HtC partners yielded pigmented offspring, while 4/7 crosses with HdF led to pigmented progeny, indicating that successful partner recognition, copulation, transfer of sperm, fertilization, and early development had occurred. Sequence analysis of mitochondrial genes cytochrome oxidase subunit I and 16sRNA showed close phylogenic similarity (100 and 91% respectively) between HtC and HtA and lesser similarity (95 and 91%) between these two and HdF. Additional genetic markers need to be analyzed to determine if there are sufficient genetic differences to suggest HtF be designated a separate phylogenetic species from HtC and HtA, even though the three populations appear not to be distinct biological species.

Friday 14:00

The reproductive strategies of the ctenophore *Mnemiopsis leidyi*, a holopelagic simultaneous hermaphrodite capable of self-fertilization

Daniel A. Sasson, Joseph F. Ryan

Whitney Laboratory for Marine Bioscience, University of Florida, St Augustine, FL, 32080, USA
http://ryanlab.whitney.ufl.edu/ ✉ http://ryanlab.whitney.ufl.edu/

To avoid inbreeding depression, some simultaneously hermaphroditic species with the ability to self-fertilize employ behavioral strategies like gamete trading. Little is known about reproductive behavior or conspecific communication in ctenophores. We used aquaria with sealed and permeable dividers to measure individual egg output in paired the ctenophore *Mnemiopsis leidyi*. We found that, when not allowed to interact (sealed barrier), size-matched individuals produced similar numbers of eggs on each side of the arena. However, if allowed to interact for a short period and then share water (permeable barrier), size-matched pairs produce significantly different numbers of eggs on each side of the barrier. In addition, zygote survival was higher in aquaria with permeable barriers suggesting that inbreeding depression was a factor in the isolated animals. Together, these results suggest that *Mnemiopsis leidyi* individuals use chemical communication to modulate reproduction in the presence of conspecifics as would be expected in gamete trading. Given that all but a very few of the most

mobile ctenophore species are simultaneous hermaphrodites, and that *Mnemiopsis leidyi* is of intermediate mobility, these results may offer insight into the transition between a hermaphroditic and gonochoristic sexual mode.

Friday 14:40

Evolution of waiting time to selfing: Can bottlenecks and density fluctuations explain trait variation?

Chantal Stock

Faculty of Biology, Theoretical Biology, University in Bielefeld, Germany

https://www.uni-bielefeld.de/biologie/theoreticalbiology/team.html ✉ chantal.stock@uni-bielefeld.de

When the chances of finding a mating partner are low, an alternative to outcrossing can grant reproductive assurance. Self-fertilization ("selfing") is one such alternative. Offspring produced through selfing are not asexual clones of their parents, but genes are reshuffled and thus liable to a loss of heterozygosity. The resulting expression of recessive deleterious alleles leads to reduced fitness especially for selfed offspring. This inbreeding depression frequently includes high mortality at early life stages, reduced fertility, or an overall shortened lifespan. Deleterious alleles, however, can be purged out in populations that self frequently over several generations. Selfing then no longer has any disadvantage, but rather has a transmission advantage as selfed offspring carry exclusively the selfer's genes. For a mixed mating system where both strategies, outcrossing and selfing, to persist, inbreeding depression has to remain high enough for selfing to be generally avoided, but mate finding needs to be uncertain enough for selfing capability to occasionally required. Inspired by the mixed mating strategy of the flatworm *Macrostomum hystrix*, we constructed a stochastic individual-based model for the evolution of waiting times to selfing in the absence of mating partners, a measure of selfing avoidance. This trait shows wide, heritable variation in M. hystrix, as well as in other selfing taxa. We explored different fluctuating density and bottleneck scenarios. Features of the model are a detailed genetic system and a sperm storage mechanism. We also looked at the role of sex allocation, spatial patterns, and explored parameter space. For any given density, we did not find optimal (i.e., intermediate) waiting times. Rather, selection was either towards absolute avoidance of selfing or towards immediate selfing. We show that under appropriate parameter combinations, fluctuations in density can maintain variation in waiting time to selfing and thus a mixed mating system.

Posters

Sex-limited experimental evolution of sex-biased gene expression in a simultaneous hermaphrodite

Aivars Cīrulis, Anna K. Nordén, Jessica K. Abbott

Department of Biology, Lund University, 223 62 Lund, Sweden

https://www.biology.lu.se/aivars-cirulis ✉ aivars.cirulis@biol.lu.se

Simultaneous hermaphrodites, which reproduce reciprocally, are good model organisms to study evolution of sex determination. First of all, acquisition of a mutation causing sterility in one sex role (i.e. a sex determining gene) can lead to selection favouring linked sex-specific fitness alleles. This is expected to be followed by recombination suppression between these loci, leading to the evolution of sex chromosomes. Later acquirement of an opposite sex role sterility mutation in the hermaphroditic partner leads to the final evolution of two separate sexes. The non-recombining sex-determining region later expands by acquiring more sex-specific genes, thus causing sex chromosomes to become heteromorphic. However, as both sexes still share most of the genome, shared genes with opposite fitness effects between the sexes must acquire sex-biased or sex-specific gene expression. Thus, to experimentally observe this process, we subjected a simultaneous reciprocally mating hermaphrodite *Macrostomum lignano* to sex-limited selection using a GFP marker as a sex-determining locus. In this setup male-limited selection resembles XY chromosome evolution, while female-limited selection – ZW. After 21-22 generations of sex-limited experimental evolution, we performed RNA-seq to see if we can indeed observe divergence in expression profiles of male- and female-selected lines. Our results show that there is an evolving divergence between male- and female-selected lines compared to controls, although it is difficult to know the function of these genes due to poor annotation in this species. The experimental evolution is still ongoing, so since we know that organ size affects sex-specific fitness and the lines have changed somewhat in organ size, we also plan to investigate organ-specific expression in the future.

Intraspecific variation in waiting times and evidence for purging of inbreeding depression in a self-fertilizing flatworm

Athina Giannakara, Steven A. Ramm

University of Bielefeld, Department of Evolutionary Biology, Bielefeld, Germany

https://macrostomum.wordpress.com/ ✉ athina.giannakara@uni-bielefeld.de

Self-fertilization (selfing) is thought to evolve as a way of guaranteeing reproduction when mate availability is low. Because selfing often leads to inbreeding depression in the progeny,

many self-compatible hermaphrodites chose to postpone reproduction when mates are scarce and employ so-called delayed selfing. Species exhibiting delayed selfing may consequently 1) show inter-population variation in waiting time (WT) due to varying demographic histories and mate availabilities causing differing optimal selfing propensities, and 2) under sustained selfing, be able to purge the deleterious recessive alleles causing inbreeding depression (ID). We therefore set out to test both predictions in the simultaneously hermaphroditic flatworm *Macrostomum hystrix*, based on comparing previously published data on waiting times (WT) and inbreeding depression (ID) from one population to a) a second, geographically distinct population and b) an inbred line derived from the first population through several generations of selfing. By comparing the two natural populations, we find clear evidence for variation in selfing propensity, as indicated by substantial WT in one but no WT in the other population. By comparing the first natural population to the inbred line derived from it, we demonstrate that sustained selfing has led to the apparent purging of deleterious alleles causing ID as well as to the elimination/depletion of genetic variation for WT. Our study thus supports evidence in gastropods and plants that selfing propensity can exhibit substantial intraspecific variation and moreover confirms the effectiveness of selfing for purging inbreeding depression in the long term.

Switches, stability and reversals: the evolutionary history of sexual patterns in teleosts

Susanna Pla[1], Isabella Capellini[2], Chiara Benvenuto[3], Stefano Mariani[3], Francesc Pifferer[1]

[1]Institute of Marine Sciences, Spanish National Research Council, Barcelona, Spain; [2]School of Environmental Sciences & Institute of Energy and Environment, University of Hull, Hull, UK; [3]School of Environment and Life Sciences, University of Salford, Salford, UK

✉ spla@icm.csic.es ✉ c.benvenuto@salford.ac.uk ✉piferrer@icm.csic.es

Most organisms reproduce sexually and individuals maintain their gender throughout their life (gonochorism). In some species, however, individuals can produce male and female gametes at the same time (simultaneous hermaphroditism) or reproduce initially as one sex and later in life switch to the other (sequential hermaphroditism, where female is the first sex in protogyny and male the first sex in protandry). Theoretical models, initially developed for plants, suggest that gonochorism and simultaneous hermaphroditism are evolutionary stable conditions, but if this is the case in animals is unclear, especially in sexually plastic taxa as teleosts. At least 5% of fish species are hermaphroditic with an over-representation of sequential hermaphrodites in the late Cretaceous radiation of marine percomorphs. Using

phylogenetic comparative methods and a sample of over 3400 teleost species, we show that gonochorism is the most likely ancestral condition from which protogyny, protandry and to a lesser extent simultaneous hermaphroditism evolved at a low rate. Unlike simultaneous hermaphroditism and protandry, that once evolved quickly move to a different sexual pattern, gonochorism and protogyny are evolutionary stable conditions that are more quickly gained than lost. These results contradict classic plant theoretical models by showing that simultaneous hermaphroditism is not an evolutionary stable state in teleosts, presumably because of the complexity of managing the opposing behavioural and physiological consequences of sexual hormones in animals. Instead, our results identify protogyny as the most stable condition among all forms of hermaphroditism in this taxon. We suggest that the greater evolutionary stability of protogyny over protandry may be explained by the differential physiological and energetic costs of male and female gametes for an animal of increasing size, with the production of numerus but cheaper sperm in protogynous males being a particularly successful strategy with the haremic mating system that many protogynous teleosts exhibit.

List of participants

Chiara Benvenuto University of Salford, UK ✉ c.benvenuto@salford.ac.uk @cbenvenuto73

Ilaria Coscia University of Salford, UK ✉ I.Coscia@salford.ac.uk @ilariacoscia

Jessica Abbott Lund University, Sweden ✉ jessica.abbott@biol.lu.se @jessicakabbott

Jeremias Brand University of Basel, Switzerland ✉ jeremias.brand@unibas.ch @Jere_Brand

Jeanne Brülhart University of Basel, Switzerland ✉ jeanne.bruelhart@stud.unibas.ch

Deborah Charlesworth University of Edinburgh, UK ✉ Deborah.Charlesworth@ed.ac.uk

Aivars Cirulis Lund University, Sweden ✉ aivars.cirulis@biol.lu.se @Cirulis_A

Athina Giannakara University of Bielefeld, Germany ✉ athina.giannakara@uni-bielefeld.de

Karel Janko University of Ostrava, Czech Republic ✉ Janko@iapg.cas.cz

Hanna Kokko University of Zurich, Switzerland ✉ hanna.kokko@ieu.uzh.ch @kokkonutter

Janet Leonard University of California-Santa Cruz, USA ✉ jlleonar@ucsc.edu

Maria Cristina Lorenzi University of Paris 13, France ✉ cristina.lorenzi@leec.univ-paris13.fr

Cynthia Norton St. Catherine University, USA ✉ cgnorton@stkate.edu

Laura Picchi University of Paris 13, France ✉ picchi@leec.univ-paris13.fr

Francesc Piferrer Institute of Marine Sciences, Spain ✉ piferrer@icm.csic.es

Andre Pires da Silva University of Warwick, UK ✉ Andre.Pires@warwick.ac.uk

Susanna Pla Institute of Marine Sciences, Spain ✉ spla@icm.csic.es

Joseph Ryan University of Florida, USA ✉ joseph.ryan@whitney.ufl.edu

Lukas Schärer University of Basel, Switzerland ✉ lukas.scharer@unibas.ch

Manfred Schartl University of Wuerzburg, Germany ✉ phch1@biozentrum.uni-wuerzburg.de

Alexandra Staikou Aristotel University of Thessaloniki, Greece ✉ astaikou@bio.auth.gr

Chantal Stock University in Bielefeld, Germany ✉ chantal.stock@uni-bielefeld.de

Sergi Taboada Natural History Museum, London, UK ✉ sergiotab@gmail.com

Stephen C. Weeks The University of Akron, USA ✉ scw@uakron.edu

R. Axel W. Wiberg University of Basel, Switzerland ✉ raw.wiberg@unibas.ch