

Simultaneous Hermaphrodite Organisms Workshop

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Thanks to the following for sponsorship



Practical information

Conference diner All refreshments and lunches are included in the registration. On Tuesday there will be pub outing to the Brass Monkey Grange, and Wednesday's conference dinner will be held at Tuk Tuk Indian Street Food (see Detailed programme for the locations).

Location Nucleus building: Elm LT for the talks and Rowan Studio Classroom for the discussion. Visit the website for directions:
<https://show2025.github.io/directions/>

Online For those of you attending online, we'll use the following zoom links for the main conference sessions:

Day 1:

Main:

<https://ed-ac-uk.zoom.us/j/81283328480>

Meeting ID: 812 8332 8480

Passcode: qV090sf52x

Discussion: <https://ed-ac-uk.zoom.us/j/85953899845>

Meeting ID: 859 5389 9845

Passcode: 46sd8pw2q2

Day 2:

<https://ed-ac-uk.zoom.us/j/86137727108>

Meeting ID: 861 3772 7108

Passcode: 4N3Kh1bMfa

Drop box for the posters:

<https://www.dropbox.com/scl/fo/dmwru7akuukrrex9rkmx2/ANnIV3XsGii dAiLcOgwaNQ8?rlkey=nby4pbok155qivlpzcgIk4pbb&st=9pyg8s3z&dl=0>

Programme overview

Wed 7th May	8:45	Breakfast
	9:00	Welcome
	9:15	Deborah Charlesworth: Inferring trade-offs between male and female function in plants
	10:00	Crispin Jordan: What is sexual dimorphism?
	10:30	Coffee Break
	11:00	George Jarvis: Hermaphrodites have lower metabolic rates than gonochores (online)
	11:30	Cristina Lorenzi: Full compensation of parental care following partner loss in a simultaneous hermaphrodite
	12:00	Takumi Saito: How is reproductive isolation established in the hermaphroditic freshwater snail <i>Lymnaea stagnalis</i> ?
	12:30	Lunch
	13:30	Elise Jeanne: Fertility under stress: sex-specific responses to environmental change
	14:00	Sophie Bererd: Temperature-dependent benefits and costs of cytoplasmic male sterility in snail <i>Physa acuta</i> (online)
	14:30	Yinghui Wang: Spawning is regulated by light level regardless of body size and morphology in the ctenophore <i>Mnemiopsis leidyi</i> (online)
	15:00	Coffee Break
	15:30	Steven Ramm: ESEB STN Talk
	15:45	Discussions: <ul style="list-style-type: none"> ○ Plasticity and resource allocation in hermaphrodites ○ Causes and consequences of reproductive mode transition
	17:00	Poster session and apero
	18:30	Close of day
	19:30	Joint Diner at TukTuk Indian Street Food (Leven Street)

Thu 8th May	9:15	Laura Ross: Insects can self-fertilize too; the evolution and evolutionary consequences of androdioecy in scale insects
	10:00	Donald Stewart: Mitochondrial genomes of hermaphroditic freshwater mussels exhibit evidence of stronger selective constraints than their dioecious relatives.
	10:30	Coffee Break
	11:00	Colin Olito: Effects of inbreeding on the probability of balancing selection: insights from Fisher's Geometric Model
	11:30	Patrice David: Challenging classical views on inbreeding depression
	12:00	Roman Stetsenko: Effect of selfing on the deleterious load around regions under balancing selection
	12:30	Lunch
	13:30	Kat Lund-Hansen: Have experimental evolution changed the plasticity in sex allocation in the flatworm <i>Macrostomum lignano</i> ?
	14:00	Irene de Sosa: Molecular toolkits for gametogenesis in sexual and parthenogenetic earthworms: comparative gene expression in gonadal tissues
	14:30	Jose María Lorente-Sorolla Pons: Unraveling the molecular basis of sex determination in <i>Ophryotrocha</i> : differential gene expression across three sexual systems
	15:00	Coffee Break
	15:30	Discussion
	16:00	Close of meeting
	16:30	Post-conference drinks for those not leaving Edinburgh immediately

Detailed programme

Tue 6th May

From 18:00 Welcome drinks at Brass Monkey Grange,
1 Grange Rd, Edinburgh EH9 1UH,
<https://g.co/kgs/GUdehTn>

Wed 7th May

From 8:45 Registration + Breakfast

9:00 Welcome

9:15 **Deborah Charlesworth:** Inferring trade-offs between male and female function in plants

Abstract: The idea of trade-offs between male and female function is central to models for the evolution of separate sexes (dioecy) and to the hypothesis that sexually antagonistic polymorphisms in such species may have selected for suppressed recombination between the sex chromosomes. However, it is difficult to test these ideas in dioecious species. I will describe how studies in closely related derived dioecious and functionally hermaphroditic cosexual species (reflecting the likely ancestral state) can provide evidence that such a male-female trade-off was present in the ancestral cosexual.

10:00 **Crispin Jordan:** What is sexual dimorphism?

Abstract: Sexual dimorphism is ubiquitous among species with separate sexes, where functional differences between females and males and genetic constraints between them both present functional (ecological) diversity and restrictions to their evolution. This talk generalizes the concept of sexual dimorphism beyond species with separate sexes. In the first part, I synthesize theoretical and empirical literature to demonstrate that the genetic mechanisms known to produce sexual dimorphism in separate sexed species may also occur in other breeding systems, including simultaneous hermaphrodites. Second, I test these predictions using quantitative genetic data from a hermaphrodite plant, and demonstrate "sexual dimorphism" in this hermaphrodite population. The synthesis and empirical results reveal new perspectives to study evolution of hermaphrodite populations and sexual dimorphism, generally, and suggest the presence of 'cryptic' sexual functional diversity in hermaphrodite populations.

10:30 Coffee Break

11:00

George Jarvis (Dustin Marshall): Hermaphrodites have lower metabolic rates than gonochores (online)

Abstract: Simultaneous hermaphroditism reduces the costs of mating by guaranteeing that every adult can mate with another – a particular advantage for conspecifics with low rates of encountering each other. Meanwhile hermaphroditism is relatively rare in animals. This paradox has long been explained by an energetics argument: hermaphrodites require more energy to fuel two reproductive roles, which favours the evolution of separate sexes. However, this argument has never been tested. We compared resting metabolic rates between hermaphrodites and gonochores across 536 species of marine invertebrates, spanning 11 phyla. Our analyses, which control for body size, environmental temperature, and phylogeny, directly contradict predictions from classic theory: instead of requiring more energy than gonochores, hermaphrodites require only half as much. These findings overturn a 150-year-old argument that hermaphroditism is rarer in animals because it is more costly, and highlight the need to reconsider the role of energetics in the evolution of sexual systems.

11:30

Cristina Lorenzi: Full compensation of parental care following partner loss in a simultaneous hermaphrodite

Abstract: Sexual conflict extends beyond fertilization when parents are in conflict over who provides parental care. In species with biparental care, if one parent deserts or reduces its investment, the partner is forced to compensate for the loss of partner-care effort. We tested this in a simultaneously hermaphroditic annelid worm, where both parents care for egg clutches. Experimentally widowed parents significantly increased the time they spent attending their brood compared to control (paired) parents and provided levels of clutch attendance statistically equivalent to that provided by paired parents. These results conform to full compensation, a response which is expected to undermine the evolutionary stability of biparental care.

12:00

Takumi Saito: How is reproductive isolation established in the hermaphroditic freshwater snail *Lymnaea stagnalis*?

Abstract: Reproductive isolation is a key mechanism of speciation and a foundational concept in evolutionary biology. However, it comprises multiple distinct phases, and its formation process is inherently complex. Consequently, few studies have comprehensively elucidated the detailed mechanisms underlying its development. In this study, we aim to investigate the formation of reproductive isolation using

seven standardized populations of the hermaphroditic freshwater snail *Lymnaea stagnalis*, a model organism in behavioural ecology and reproductive biology. We assessed mating behaviour through systematic observation, quantifying male and female reproductive behaviours and measuring mating success. Post-mating reproductive outcomes, including egg production and hatching rates, were also quantified. Additionally, we conducted artificial injection experiments to evaluate the role of seminal fluid proteins in reproductive isolation. By integrating these results, we seek to clarify the formation process of reproductive isolation in hermaphroditic organisms, with emphasis on the role of sexual conflict.

12:30 Lunch

13:30 **Elise Jeanne:** Fertility under stress: sex-specific responses to environmental change

Abstract: To evaluate the potential for adaptation to global change, fertility has emerged as a better predictor of species distribution than survival, as it reflects a key aspect of population persistence: reproduction. While several studies have explored the effects of temperature, pH or salinity, on fertility, few have examined the impact of multiple stressors and their potential interactions. Here, we investigated how salinity and temperature influence fertility in the simultaneous hermaphrodite *Macrostomum lignano*. First, we exposed an inbred line to a range of salinities and temperatures to identify the most impactful sublethal combination. Then, we exposed multiple inbred lines and outcross population to this combination to assess standing genetic variation and sex-specific effects. While fertility declined at high temperatures, salinity appeared to mitigate temperature stress. The female fertility presented a similar trend, whereas male fertility was reduced by both stressors, with stronger effects when combined.

14:00 **Sophie Bererd:** Temperature-dependent benefits and costs of cytoplasmic male sterility in snail *Physa acuta* (online)

Abstract: Cytoplasmic male sterility (CMS) originates from a mito-nuclear conflict where mitochondrial genes induce male sterility and nuclear genes restore male fertility in hermaphrodites. The first observation of CMS in animals was reported recently in the freshwater snail *Physa acuta* where it is associated with two extremes divergent mitotypes D and K. The D individuals are male-steriles while male fertility is restored by nuclear genes in K and are found mixed with the

most common male-fertile N mitotype in natural populations (i.e. gynodioecy). We compared male and female fitness, growth rate and metabolism between the three mitotypes at two temperatures as this factor influences CMS in gynodioecious plants via alteration of mitochondrial functioning. Temperature did not affect male sterility which depended only on the mitotype and the presence of restorers. Our results provided evidence that CMS is beneficial to female fitness in the absence of restorers while it is costly in their presence, and furthermore driven by body mass, fulfilling a key theoretical condition for the long-term maintenance of gynodioecy. Fitness benefits and costs mediated by differences in body mass are enhanced at cold temperature, suggesting that the system dynamics may vary according to thermal conditions in nature.

14:30 **Yinghui Wang:** Spawning is regulated by light level regardless of body size and morphology in the ctenophore *Mnemiopsis leidyi* (online)

Abstract: The ctenophore *Mnemiopsis leidyi* is an emerging important simultaneous hermaphrodite model. It is native to the western Atlantic but well known for successfully invading seas in western Asia and Europe. *M. leidyi* can spawn starting from a relatively small size (~1 mm). They also undergo an obvious morphological change as they develop. Reproduction at different sizes was thought to be discontinuous, but recent work shows continuity of reproduction across stages. However, many outstanding questions remain about whether there are physiological differences defining these morphological stages. While it is known that light to darkness transition regulates spawning of larger *M. leidyi*, we investigated whether light cues similarly regulate reproduction at earlier stages. We found that constant exposure to light inhibits *M. leidyi*'s reproduction and that the light to darkness transition initiates spawning, regardless of size or morphology. These results indicate continuity in the mechanism that regulates reproduction across *M. leidyi*'s lifetime.

15:00 Coffee Break

15:30 **Steven Ramm:** ESEB STN Talk

15:45 **Discussions:**

- Plasticity and resource allocation in hermaphrodites
- Causes and consequences of reproductive mode transition

17:00

Poster session and apero

Chenxi Wang: Inferring the demographic history of *Caenorhabditis elegans* while accounting for its self-fertilising reproductive mode

Self-fertilisation extensively influences the evolutionary history of hermaphroditic species, including reducing population diversity and the effective recombination rate (and hence also the effective population size and the ability to generate new genotypes), which can bias demographic inference methods that assume outcrossing. Here, we tried a new method for jointly inferring the demographic history and the self-fertilising rate of the population on the whole-genome data of *Caenorhabditis elegans*, a predominantly selfing nematode. The population structure analysis suggests a much higher level of genetic divergence in strains from different Hawaiian islands than strains outside Hawaii. A preliminary test on the Big Island population from Hawaii has yielded a selfing rate of 99% and a population decline followed by a massive increase. Further investigations using different methods and data from different genetic lineages will provide important information on how to best infer species demographic history with reproductive mode considered.

Freya Way: How does inbreeding affect local adaptation in the mountain plant *Arabis alpina*?

Adaptation to the environment is impacted by many factors, one of which is the species' mating system. Self-fertilisation is a mating system that occurs in 42% of angiosperms, including rare species and crops. On one hand selfing causes inbreeding and reduces the efficacy of selection, however theory also indicates that it may increase the fixation of recessive beneficial mutations and promote local adaptation by limiting pollinator-mediated gene flow. To better understand how inbreeding affects adaptation in nature, this project uses the wild mountain plant *Arabis alpina* to assess if local adaptation to elevation has been affected by regional differences in selfing rate. We sampled leaf tissue, phenotype and site data at high and low elevations, from self-compatible populations in France and self-incompatible populations in Italy. We are obtaining genome sequences, combining our new dataset with existing data, and then will use genome-environment associations and scans for selective sweeps to test how intraspecific variation in self-compatibility affects genetic signals of local adaption to elevation.

Fanny Laugier: The role of linkage in shaping selection in *Caenorhabditis* nematodes

Self-fertilisation is a common reproductive strategy with profound effects on genetic diversity and evolutionary processes. In highly selfing species, strong genetic linkage results in genetic associations and atypical adaptation patterns. Little is still known about how these linkage effects influence the response to polygenic adaptation. This study investigates the extent to which genome-wide adaptation occurs under self-fertilisation by developing theory that incorporate linkage effects into polygenic selection frameworks.

Donald Stewart: Searching for molecular signatures of hermaphroditism in the freshwater mussel *Nephronaias tempisquensis* from Costa Rica

Emma-Jean G. Freeman, William G. Bauer, Russell H. Easy, Rebeca Quesada Céspedes, Sidey Arias Valverde, Sophie Breton and Donald T. Stewart (presenter)

Many bivalves exhibit a unique mode of mitochondrial DNA transmission called doubly uniparental inheritance (DUI), in which females retain a female-transmitted (F-type) mtDNA genome and males retain both the F-type genome and a male-transmitted (M-type) genome. These sex-associated mtDNA genomes contain traits unique to DUI, including two sex-specific protein-coding open reading frames (*orfs*) found in the F- and M-type genomes, respectively. During the transition to hermaphroditism, the M-type genome is lost, and the F-type genome, now called the hermaphrodite (H-type) mtDNA genome, exhibits a highly divergent *orf* (the *H-orf*) compared to the *F-orf*. Recent research on freshwater mussels (Unionidae) led to a proposed method for using hydrophobicity profiles of ORFs to identify possible transitions from dioecy to hermaphroditism. The present study used this method to look for molecular signatures of hermaphroditism in *Nephronaias tempisquensis* from Costa Rica. Minimal differences in ORF and *cox1* divergence were observed among *N. tempisquensis* haplotypes. The divergence patterns were consistent with those of dioecious unionid species leading us to conclude that this particular population of *N. tempisquensis* is not transitioning to hermaphroditism although hermaphrodites have been observed in this species in other regions of Costa Rica.

Joris Koene: Investigating the effects of accessory gland proteins on ejaculate processing in *Lymnaea stagnalis*

Accessory gland proteins (ACPs) are socially transferred materials that act during and/or after mating to increase a sperm donor's chance at fertilisation. In simultaneous hermaphrodites ACPs can affect a recipient's female as well as male functions, as has been shown in the great pond snail *Lymnaea stagnalis*. A potential ACP function that has not been tested in this species is the ability to directly affect the reproductive physiology related to sperm processing after receipt. Therefore, in this study we used a quantifiable sperm substitute, inert non-toxic carbon particles, to visualise and quantify active uptake into the recipient's reproductive tract after artificial insemination and in semi-intact preparations. The uptake into the tract was found to be similar in presence and absence of ACPs and did not correlate with the overall volume of the bursa copulatrix, which was previously assumed. The latter warrants further investigation to understand the sperm-digestion process.

Sarthak Grover: Does conspecific encounter rate influence mating system expression and reproductive timing?

Abstract: For self-compatible hermaphrodites, an individual's age at reproductive onset is influenced by the availability of outcrossing opportunities and the magnitude of inbreeding depression in selfed offspring. Generally, when outcrossing opportunities are low, individuals can switch to selfing after a delay, with the waiting period commensurate with the magnitude of inbreeding depression.

Studies typically contrast treatments with either no or constant mate availability, as these conditions are expected to produce the greatest differences in age at reproductive onset. Using a highly inbred population of *Macrostomum hystrix* flatworms, we additionally tested a treatment in which individuals were grouped intermittently. As expected for an inbred population, age at reproductive onset did not differ significantly between isolated and always-grouped individuals. However, individuals in the intermittently grouped treatment began reproducing much earlier than those in the other two treatments, likely in response to cues of an unstable social and physical environment.

18:30 Close of day

19:30 Conference diner at Tuk Tuk Indian Street Food,
1 Leven Street, Edinburgh EH3 9LH,
<https://g.co/kgs/pFuobDb>

Laura Ross: Insects can self-fertilize too; the evolution and evolutionary consequences of androdioecy in scale insects

Hermaphroditism in insects is virtually nonexistent—except for a remarkable exception. In stark contrast to its prevalence among many invertebrate groups, hermaphroditism is almost entirely absent in insects, one of the most diverse and widespread animal clades. Yet, an extraordinary evolutionary anomaly has emerged in a small group of hemipteran plant parasites: the fluted scale insects (Hemiptera: Monophlebidae: Iceryini). Several species in this clade possess female-like hermaphrodites capable of producing both sperm and oocytes, enabling self-fertilization. Notably, males also persist in most species and can successfully mate with hermaphrodites—indicating a rare case of androdioecy in insects. This mating system has evolved in parallel with haplodiploidy: males are completely haploid, as is the sperm-producing tissue of the hermaphrodites. I will explore the evolutionary dynamics and turnover of androdioecy within this lineage, by discussing the turnover between species and population genetic analyses of selfing rates in natural populations of a widespread and invasive agricultural pest; *Icerya purchasi*. Furthermore, I will present recent genomic evidence demonstrating that androdioecy in this clade is tightly linked to extensive genome expansion and structural rearrangement. These findings not only illuminate the evolution of a rare reproductive strategy in insects but also reveal its genomic consequences.

10:00

Donald Stewart (Emily Chase, Brent Robicheau, Sophie Breton and Don Stewart): Mitochondrial genomes of hermaphroditic freshwater mussels exhibit evidence of stronger selective constraints than their dioecious relatives.

Abstract: Many bivalves possess an unusual system of mitochondrial DNA transmission called doubly uniparental inheritance (DUI). Within the freshwater mussels, hermaphroditism has evolved several times. Dioecious FWM with DUI and closely-related hermaphroditic species with maternal inheritance present the opportunity to examine patterns of molecular evolution related to reproductive mode. Previous studies have shown that the paternal mtDNA transmitted via sperm (Male- or M-type) evolves faster than maternal mtDNA transmitted via eggs (Female or F-type). Here we compare the DNA substitution patterns between F-type and closely-related mtDNAs of hermaphrodites (H-type) and test the “cellular arenas hypothesis.” According to this hypothesis, the mtDNA of a hermaphrodite must operate in more cellular arenas (i.e., eggs, somatic tissues and sperm)

and will therefore experience stronger selective constraints than F-type mtDNA of diecious species. Four of five test groups showed stronger selective constraints on H-type versus M-type mtDNA, consistent with the cellular arenas hypothesis.

10:30 Coffee Break

11:00 **Colin Olito:** Effects of inbreeding on the probability of balancing selection: insights from Fisher's Geometric Model

Abstract: Balancing selection is widely invoked as an important mechanism maintaining genetic variation for fitness and as a genetic basis for inbreeding depression. We combine classical population genetics theory for inbreeding populations with Fisher's geometric model to quantify the probability of balancing selection by heterozygote advantage in inbreeding relative to outcrossing populations. We find that the prevalence of balancing selection and its contribution to fitness variation is consistently, and often substantially, lower within inbred populations than predicted by classic theory, which suggests that detection of balanced genetic polymorphisms in inbred species should be more challenging than currently thought.

11:30 **Patrice David:** Challenging classical views on inbreeding depression

Abstract: Inbreeding depression (ID), the decline in fitness upon inbreeding, is thought to result from a decrease in genetic heterozygosity enhancing phenotypic effects of recessive deleterious mutations. However, emerging evidence suggests that mutations may not explain ID completely. In this study, we test whether ID can emerge even in contexts where genetic heterozygosity does not vary. To that end, highly inbred lines ($F=0.99999997$) of the freshwater snail *Physa acuta* were used to produce individuals with varying levels of parental relatedness (self-fertilization, sibling crosses, and cousin crosses), though with identical genomic heterozygosity. Several fitness traits declined significantly with increasing parental relatedness, a pattern characteristic of ID, and quantitatively representing a non-negligible fraction of the ID usually observed in natural, genetically diverse populations of *Physa acuta*. Individual-based simulations showed that mutation rates compatible with values of ID found in natural populations are way too low to generate as much ID as observed in our experiment. These findings are consistent with the hypothesis that epigenetic changes, in addition to mutations, could contribute to a rapid regeneration

of ID and explain the persistence of detectable ID in sets of genetically identical individuals.

12:00

Roman Stetsenko: Effect of selfing on the deleterious load around regions under balancing selection

Balancing selection maintains polymorphism among genetic variants and can originate from a diversity of biological mechanisms. Previous theoretical works found that a sheltered load can be maintained under tight linkage with a site under balancing selection, mainly focusing on self-incompatibility or mating-type loci in fungi. Numerous genomic regions displaying signatures of balancing selection have been described in self-fertilising species, despite very high selfing rates. It is not yet clear, however, what could be the effect of different forms of balancing selection on linked deleterious mutations in selfing species since no general model exists in this case. We first focus on the effect of selfing on the hitchhiking of deleterious mutations along a new variant under balancing selection when it spreads to reach its steady-state frequency. By using a semi-deterministic model, we derive approximations for the hitchhiking probability and sojourn time of deleterious variants near a locus under negative frequency-dependent selection.

12:30

Lunch

13:30

Kat Lund-Hansen: Have experimental evolution changed the plasticity in sex allocation in the flatworm *Macrostomum lignano*?

Abstract: During times with limited food many animals will choose survival over reproduction. An extreme example of this is the simultaneously hermaphroditic flatworm, *Macrostomum lignano*, which can decrease its body size and even shrink its gonads, during starvation, but rebuild these organs again when there is plenty of food. We have previously carried out sex-limited experimental evolution in this species, to select for fitness via the male or female sex function. We found changes in fitness, gene expression, and mating behaviour which were generally in line with our predictions. However, it is unclear if or how plasticity in sex allocation has changed in our experimental evolution lines. After 55 generations of selection, we investigated changes in morphology and gene expression due to starvation. Overall, there was evidence of decreased morphological plasticity in the male- and female-selected lines compared to the controls. We also have promising data for identifying plasticity genes.

14:00

Irene de Sosa: Molecular toolkits for gametogenesis in sexual and parthenogenetic earthworms: comparative gene expression in gonadal tissues

Abstract: Earthworms are hermaphrodites, but around 40% of species can reproduce by parthenogenesis, where only the oocytes are needed. The dynamics of gametogenesis are well known in earthworms, but the molecular underpinnings of gamete production are completely unknown. Here, our aim was to investigate the transcriptional profiles of male and female gonads in sexual and parthenogenetic earthworms. We selected two phylogenetically closely related species, each with a different strategy. We performed a differential gene expression analysis using RNAseq data. Our results indicate a remarkably different expression profile in both female and male gonads of sexual and parthenogenetic individuals, as it was expected. However, surprisingly, the transcriptional profile of both gonads was highly similar in sexual individuals, except for a few spermatogenesis-specific genes in male gonads. This indicates a largely conserved gametogenesis mechanism, with only minor genetic differences, particularly in the production of male flagellated cells, as previously observed in other invertebrates.

14:30

Jose María Lorente-Sorolla Pons: Unraveling the molecular basis of sex determination in *Ophryotrocha*: differential gene expression across three sexual systems

Species of the genus *Ophryotrocha* are marine annelids widely used as experimental model species due to their tolerance to stress, high fecundity, and the diverse sexual systems they present, which include simultaneous and sequential hermaphroditism as well as gonochorism. Despite the attention that these organisms have garnered, they have been barely studied under a genomic point of view. In this study, we analyzed differential gene expression in the transcriptomes of three *Ophryotrocha* species: *Ophryotrocha diadema* (simultaneous hermaphrodite), *Ophryotrocha puerilis* (sequential hermaphrodite), and *Ophryotrocha labronica* (gonochoristic), with the aim to identify genes and molecular pathways involved in sex determination. By comparing gene expression between sexes and between reproductive and non-reproductive individuals, we detected significant differences in the expression of genes related to sex hormones, methylation/demethylation processes, germline maintenance, sex determination, and gametogenesis. Notably, we found differential expression in well-known genes such as DMRT2 involved in sex determination in *Drosophila* and YX sex reversal in humans and FGFR2 which regulates proliferation

and Sertoli differentiation during male sex determination in mice, suggesting their potential role in the regulation of sex determination in Ophryotrocha.

15:00 Coffee Break

15:30 Discussion

16:00 Close of meeting

From 16:30 Post-conference drinks for those not leaving Edinburgh immediately