Simultaneously Hermaphroditic Organisms Workshop

23-24 April 2024
OSUR, Campus Beaulieu, Université de Rennes

Programme

Organized by

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UMR 6553 ECOBIO – Ecosystèmes, Biodiversité, Evolution
Université de Rennes
Beaulieu Campus

Sponsors

We gratefully acknowledge financial support for the workshop from UMR 6553 ECOBIO – Ecosystèmes, Biodiversité, Evolution and from the Observatoire des sciences de l'univers de Rennes (OSUR).

Programme Overview

Tue 23rd April	09:00	Welcome
	09:15	**Marie-Agnès Coutellec:** FMRFamide-like receptor expansion in the simultaneous hermaphroditic gastropod *Lymnaea stagnalis*
	10:00	**Mario Juan Gordillo-Pérez:** Genitalia functional anatomy, dart use, and conservation biology of the Cuban tree snail *Coryda alauda*
	10:30	Coffee break
	11:00	**Cristina Lorenzi:** Strategic body growth in the protandrous sequential hermaphrodite *Ophryotrocha puerilis*
	11:30	**John Hutchinson:** Sperm precedence, selfing, fertility, fecundity, and longevity in the slug *Arion hortensis*
	12:00	**Jessica Abbott:** Sex-limited experimental evolution, mating and sexual conflict in a hermaphrodite
	12:30	Lunch
	13:30	**Irene De Sosa:** Unriddling the origin of parthenogenesis in oligochaetes
	14:00	**Elpida Skarlou:** Sex, Males and Hermaphrodites in the scale insect *Icerya purchasi*
	14:30	Coffee break
	15:00	**Discussion:** Hermaphroditism in the Anthropocene *or* Approaches to mating system inference
	16:30	Poster session and apéro
	19:00	Joint dinner at Crêperie du Pont-Levis

Wed 24th April	09:00	**Sylvain Glémin:** Mating system and the dynamics of speciation
	09:45	**Fanny Laugier:** Experimental evolution reveals counter-selection on Cytoplasmic Male Sterility in resistant nuclear backgrounds as predicted by theory
	10:15	**Don Stewart:** Hermaphroditism and doubly uniparental inheritance of mitochondrial DNA in freshwater mussels
	10:45	Coffee break
	11:15	**Jeanne Tonnabel:** Sexual selection in plants: what happens after pollen land on the pistil?
	11:45	**Kora Klein:** Modelling hermaphroditic flowers and the cost of floral displays
	12:15	Lunch
	13:30	**Chenxi Wang:** Refining the demographic inference of self-fertilising species
	14:00	**Joris Koene:** Insemination by inbreds: the possible effects of selfing on seminal fluid protein responses
	14:30	**Discussion:** Conclusion of the scientific discussions from Tuesday; followed by general discussion, including of joint funding applications
	ca. 16:30	Close of meeting

Practical Information

Welcome meet-up

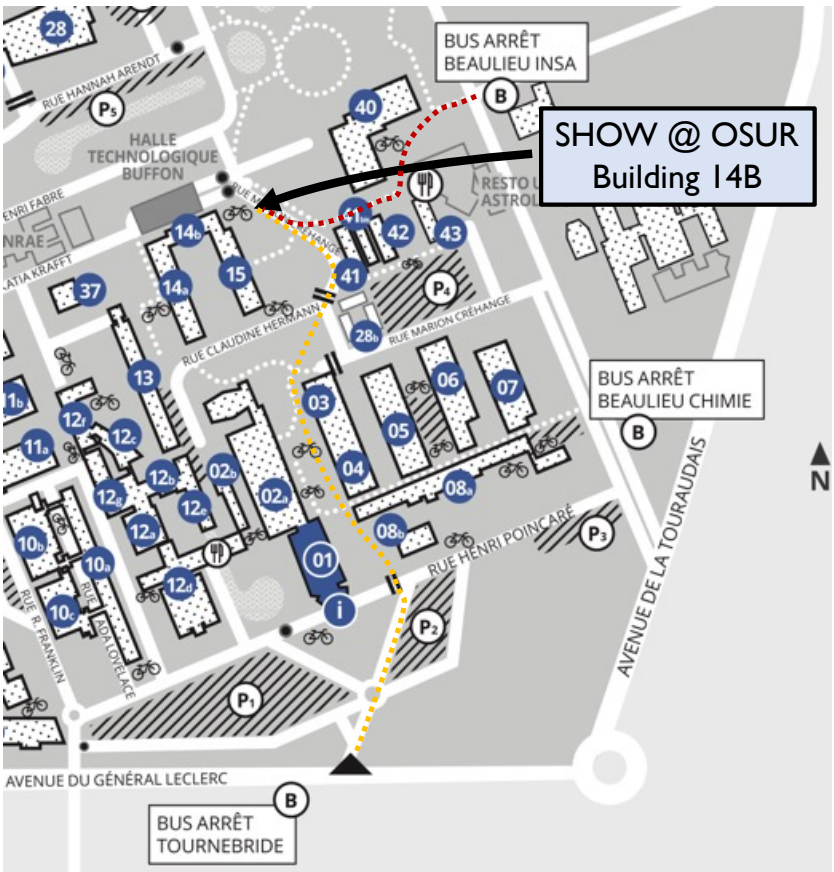
If you'd like to meet up for a drink and/or meal upon arrival, we've reserved some tables at the city centre bar *Le Hangar* (1 Rue de Dinan, https://maps.app.goo.gl/8bzXGhq4S3hJxxvD8) – we'll be there from 7pm and you're welcome to join us then, or just come whenever suits your travel plans.

For those of you arriving earlier in the day with a bit of time to spare, we'd recommend a stroll around the city centre (roughly the area between *Sainte-Anne* to the north, *République* to the south, the *Cathédrale Saint-Pierre* to the west and the *Parc du Thabor* to the east – the park itself is also well worth a visit).

Public transport

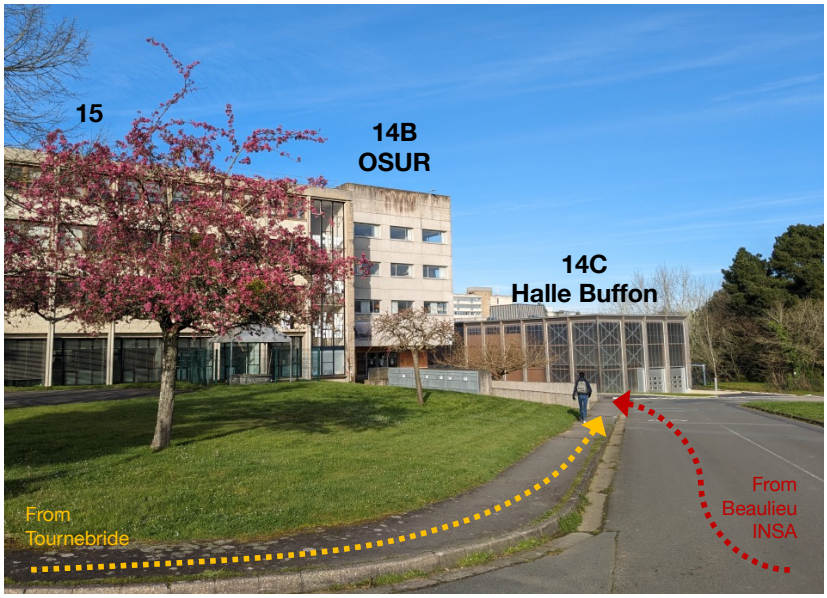
The workshop will take place on the Beaulieu Campus of the Université de Rennes, located to the east of the city centre. Unfortunately, ongoing repair works on the metro line B mean that the campus cannot currently be reached by metro, so you should take a bus instead. From the city centre, lines C4 and C6 will be the most convenient. If travelling on the C6, get off at *Tournebride* and enter the university campus by crossing the road and walking uphill. For the C4, you should get off instead at *Beaulieu INSA*, walk over the zebra crossing and go down the path onto the campus.

Full details of public transport in Rennes at: https://www.star.fr



A map of the campus can be found at:
https://www.univ-rennes.fr/plan-du-campus-de-beaulieu

Venue All talks and the poster session will take place in the OSUR conference room (*salle de conférence*) on the ground floor of building 14B. The refreshments and lunch will be served in the foyer directly outside the conference room. For the discussion session on Tuesday, one group will stay in the conference room and the other will move to the seminar room *salle Bernard Auvray* on the ground floor of the adjacent building 15.



Conference dinner As communicated when we announced the workshop, there will be no charge for attending SHOW and all refreshments and lunches are included in the registration. However, everyone will need to pay for their own dinner on Tuesday evening (we'll organise with the restaurant how best to do that).

Online For those of you attending online, we'll use the following zoom link for the main conference sessions:
https://univ-rennes1-fr.zoom.us/j/64849593993
Meeting ID: 648 4959 3993
Passcode: 178610

For the discussion session held in salle Auvray, please use:
https://univ-rennes1-fr.zoom.us/j/67006348172
Meeting ID: 670 0634 8172
Passcode: 611333

Detailed Programme

Monday 22nd April

from 19:00 Pre-conference drinks and dinner at *Le Hangar*
1 Rue de Dinan
https://maps.app.goo.gl/8bzXGhq4S3hJxxvD8

Tuesday 23rd April

from 08 :45 Registration

09:00 Welcome address

09:15 **Marie-Agnès Coutellec**
UMR 0985 DECOD, Rennes, France

FMRFamide-like receptor expansion in the simultaneous hermaphroditic gastropod *Lymnaea stagnalis*
Co-authors : J.M. Koene, D. J. Jackson, Y. Nakadera, N. Cerveau, M.-A. Madoui, B. Noel, V. Jamilloux, J. Poulain, K. Labadie, C. Da Silva, A. Davison, Z.-P. Feng, C.M. Adema, C. Klopp, J.-M. Aury, P. Wincker

The great pond snail *Lymnaea stagnalis* has served as a model organism for over a century in diverse disciplines such as neurophysiology, evolution, ecotoxicology and developmental biology. To support both established uses and newly emerging research interests we have performed whole genome sequencing (~ 176 x depth), assembly and annotation of a single individual derived from an inbred line. These efforts resulted in a nal assembly of 943 Mb (L50 = 257; N50 = 957,215) with a total of 22,499 predicted gene models. As a rst step towards understanding the hermaphroditic reproductive biology of *L. stagnalis*, we identified molecular receptors, specifically nuclear receptors (including newly discovered 2xDNA binding domain-NRs), G protein-coupled receptors, and receptor tyrosine kinases, that may be involved in the cellular specification and maintenance of simultaneously active male and female reproductive systems. A phylogenetic analysis of one particular family of GPCRs (Rhodopsin neuropeptide FMRFamide-receptor-like genes) shows a remarkable expansion that coincides with the occurrence of simultaneous hermaphroditism in the Euthyneura gastropods. As some GPCRs and NRs also showed qualitative differences in expression in female (albumen gland) and male (prostate gland) organs, it is possible that separate regulation of male and female reproductive processes may in part have been enabled by an increased abundance of receptors in the transition from a separate-sexed state to a hermaphroditic condition. These ndings will support efforts to pair receptors with their activating ligands, and more generally stimulate deeper insight into the mechanisms that underlie the modes of action of compounds involved in neuroendocrine regulation of reproduction, induced toxicity, and development in *L. stagnalis*, and molluscs in general.

10:00 **Mario Juan Gordillo-Pérez**
CMK Centre for environmental Sciences, Hasselt University, Belgium

Genitalia functional anatomy, dart use, and conservation biology of the Cuban tree snail *Coryda alauda*
Co-authors: Natalie Beenaerts, Bernardo Reyes-Tur

Coryda alauda is a simultaneous hermaphroditic, dart-possessing, Cuban tree snail. Here, we study the genitalia functional anatomy and describe the mating behavior of four *C. alauda* subspecies in order to explore potential evolutionary significant units (ESU). Animals were collected between August 2014 – April 2019 in nine localities of Eastern Cuba. The length of the flagellum, bursa copulatrix, oviduct and spermoviduct were the traits with more variability, which suggest the potential action of sexual selection mechanisms. A Discriminat Analysis allowed separating the subspecies *C. alauda* weeksiana from the rest, and based on that is proposed as ESU. In addition, we identify not only different types of dart gland cells and secretions but also the presence of a nervous plexus covers the dart apparatus. These histological elements plus the diversity

of the epithelial tissues suggest a high and unexplored reproductive functional complexity of Cuban dart-possessing species. The mating behaviour has three stages: courtship, copulation and post-copulation. The dart apparatus is used throughout mating and has been described to perform 'wiping', 'rubbing' and 'stabbing'. Rubbing is the most common contact type and it takes place majorly in the cephalic region of the mate, probably to guarantee substances transference close to target organs.

10:30 Coffee break

11:00 **Maria Cristina Lorenzi**
LEEC - Sorbonne Paris Nord University, France

Strategic body growth in the protandrous sequential hermaphrodite *Ophryotrocha puerilis*
Co-authors: Pooja Radhakrishnan, Noé Saboul, Raquel Monclùs, Soumeya Ahmed, Sergi Taboada

Sequential hermaphrodites opportunistically adjust their sex allocation by adjusting the time they spend as females and males to current condition, including social environment. In protandrous sequential hermaphrodites, individuals mature as males and change sex to females when they reach a certain body size; typically, the largest individual is a female and the next a male, where individuals organize in size-hierarchy. We tested this hypothesis in the protandrous, sequentially hermaphroditic polychaete worm Ophryotrocha puerilis, by following the development of size-matched juvenile worms kept either in pair or triplet (and in isolation or with adults, as controls). Worms in pairs and triplets did adjust their growth rate so that they rapidly developed size differences and kept such a size hierarchy until the larger worm changed sex. Preliminary analyses suggest that behavioral interactions among paired individuals may play a role in the negotiations on who occupies which position in the size hierarchy. Strategic body growth has been described in both protogynous and protandrous sequentially hermaphroditic fish as well as in group-living separate-sex animals with high reproductive skew, suggesting it is an evolutionary convergence to solve reproductive conflicts.

11:30 **John Hutchinson**
Senckenberg Museum of Natural History Görlitz, Germany

Sperm precedence, selfing, fertility, fecundity, and longevity in the slug *Arion hortensis*

A Mendelian-inherited colour morph of the terrestrial slug *Arion hortensis* was found at several sites in the British Isles: the pink colour of hatchlings is recessive to wild type. This enabled estimation of sperm precedence when isolated individuals mated with two different partners successively. When offered a partner, slugs only sometimes mated, and attempts often failed to lead to copulation. Copulation itself typically lasts around 90 min and involves continuous stroking of the partner, so also cyptic female choice seems plausible. Intervals between successful matings were up to 100 days apart, but some slugs continued laying fertile eggs for over a year. A full range of mixed paternities was observed but often only one partner (either the first or second) fathered offspring after the second mating, even if its competitor was seen to have transferred a spermatophore. Selfing was rare, with unmated slugs usually producing no eggs or a small number of infertile ones. However, some mated individuals switched to selfing late in life. Aged slugs scarcely lost weight, but the proportion of fertile eggs in a clutch, clutch size, and laying frequency declined; typically in their last few weeks slugs laid no eggs.

12:00 **Jessica Abbott**
Lund University, Sweden

Sex-limited experimental evolution, mating and sexual conflict in a hermaphrodite
Co-author: Aivars Cirulis

Sexual selection in hermaphrodites can act through both male competition and female choice, and seems to drive the evolution of male-biased traits in particular. Nevertheless, sexual selection in simultaneous hermaphrodites is still poorly studied compared to gonochorists. We therefore investigated changes in mating behaviour and male copulatory organ called stylet in lines of *Macrostomum lignano*, a simultaneous hermaphrodite, exposed to sex-limited experimental evolution. We recorded mating videos of worms after more than 40 generations and took photographs of the stylet after

more than 50 generations of selection for male- or female-specific fitness. The selection lines changed in mating frequency and duration, where the female-selected lines decreased their mating effort, although the difference was not statistically significant. However, we did find a significant difference in the post-copulatory sucking behaviour in the wild-type mating partner, in which the worm covers its female antrum with mouth and seemingly tries to remove the received ejaculate. After mating with the male-selected worms the mating partners sucked the most, while after mating with the female-selected worms – the least. Additionally, we found that the probability of sucking decreased with increasing mating duration, but increased with increasing number of matings. These results are consistent with previous speculation that the post-copulatory sucking behaviour is likely a result of sexual conflict over fertilization, which is expected to be the most prominent conflict in hermaphrodites. We also found differences in the shape of the stylet, thus confirming that sexual selection can drive rapid evolution of the male copulatory organ.

12:30 Lunch at the *Club des Professeurs*
Building 1, Beaulieu Campus

13:30 **Irene De Sosa** *(via Zoom)*
MNCN-CSIC/Universidad Autonoma de Madrid, Spain

Unriddling the origin of parthenogenesis in oligochaetes
Co-authors: Patricia Álvarez-Campos, Marta Turon, Sergi Taboada, Marta Novo, Natasha Tilikj, Rosa Fernández, Jose Lorente-Sorolla, María Conejero, Cristina Díaz-Vives, Ana Riesgo

Oligochaete earthworms are considered hermaphrodites with cross-fertilisation, as this is the most common mode of reproduction. However, hermaphroditism is not the only reproductive mechanism in oligochaetes. Nowadays, numerous parthenogenetic species have been discovered, most of which are polyploid. There are even species that maintain both modes of reproduction. This makes them excellent candidates for studying the molecular and evolutionary mechanisms associated with parthenogenesis. We studied three populations of Aporrectodea trapezoides with sexual and parthenogenetic specimens of Argelia, Plasencia and Mallorca. We found 5297 ZOTUs grouped by populations. The phyla most present were Actinobacteriota, Firmicutes and Proteobacteria. No significant results were obtained for earthworms from Plasencia and Mallorca. However, Algerian earthworms presented more than 500 ZOTUs with differential abundance (314 sexual and 440 parthenogenetic). Genome-wide differences have been found in the Algerian population between the different types of reproduction. The sexual specimens from Algeria presented a different composition to the rest of the individuals studied. High levels of heterozygosity were obtained for parthenogenetic populations, indicating a hybrid or contagious origin of parthenogenesis in this earthworm species.

14:00 **Elpida Skarlou**
University of Edinburgh, UK

Sex, Males and Hermaphrodites in the scale insect *Icerya purchasi*
Co-authors: Andrew Mongue, Laura Ross

Icerya purchasi is the only insect unequivocally described as androdioecious (i.e., its populations consist of males and hermaphrodites) and haplodiploid (i.e., males originate from unfertilized eggs). With males being rare and hermaphrodites capable of producing both sperm and eggs, self-fertilization emerges as the predominant mode of reproduction in this species. The mechanism by which a female can produce sperm has been an enigma for over a century! The prevailing hypothesis suggests that the sperm present in hermaphrodites originate from the individual's father, who "infects" his offspring with sperm-producing cells during fertilization. Nevertheless, alternative mechanisms have been proposed, necessitating further investigation to shed light on this intriguing phenomenon. In this initial phase of our study, we employed both experimental and genomic approaches to distinguish between the proposed mechanisms of sperm production in *I. purchasi*. These findings will initially elucidate the mating system of the only known androdioecious insect, paving the path for future population genetic inquiries into wild populations. Therefore, allowing us to better understand the evolution of androdioecy in animals, particularly in the context of haplodiploidy.

14:30 Coffee break

15:00 Discussion topics

Topic 1: Hermaphroditism in the Anthropocene

What unique insights can be gained from hermaphrodites for understanding the impacts of Anthropogenic activities? Are hermaphrodites differentially impacted compared to separate-sexed organisms? What could be the effects of these Anthropogenic activities on evolutionary transition between hermaphroditism and dioecy?

Topic 2: Mating system inference in the wild

What exactly is measured as proxies of self-fertilisation rates? How can we determine if these proxies are accurate estimators? What are the latest methods for estimating selfing rates, and what advantages do they offer over classical methods? What are the limitations of each method, and when should one be preferred over the other? Depending on the model system (animals, plants, or clonal species), are there any special considerations to be aware of?

16:30 Poster session

Significant mito-nuclear discordance within hermaphrodite freshwater snails in Asia

Takumi Saito *(VU Amsterdam)*
Co-authors: Bin Ye, Larisa Prozorova, Tu Van Do, Mohammad Shariar Shovon, Purevdorj Surenkhorloo, Kazuki Kimura, Yuta Morii, Osamu Miura, Takahiro Hirano, Satoshi Chiba

Mitochondrial DNA (mtDNA) has recently been suggested to be associated with various evolutionary processes, such as positive selection, although it has traditionally been considered neutral. Due to such evolutionary processes, mtDNA may contribute to genetic diversification, including speciation, and the resulting evolutionary histories may be detected phylogenetically as mito-nuclear discordance, a phenomenon commonly observed in recent decades. Recently, cytoplasmic male sterility (CMS), a genomic conflict resulting from mito-nuclear interactions at the basis of sexual conflict, was first discovered in a hermaphrodite animal, the hygrophilid freshwater snail Physella acuta. While CMS is common in higher plants and has the potential to dramatically accelerate molecular evolution, only the first example is known in animals, and the generality of CMS in animals remains uncertain. Here, we report widespread mito-nuclear discordance and remarkable divergence of mtDNA within two hermaphrodite freshwater snails, *Gyraulus chinensis* (Planorbidae) and *Radix plicatula* (Lymnaeidae), in Asia. We sampled two hygrophilid snails from their entire distribution range and estimated CO1 phylogenies and genomic phylogenies using ddRAD-seq. Consequently, outstanding CO1 diversities (approximately 20% or more) with no or weak geographical relevance were detected in these two species, and the phylogenetic structures were inconsistent with the genomic phylogenies. Although shell and genital morphology, as well as reproductive features, have not been examined, and this phenomenon cannot be immediately concluded to be CMS; the widespread mito-nuclear discordance in these two species suggests that there are mechanisms promoting discordance in some hermaphrodites. Additionally, these two snails, closely related to *Physella acuta*, might provide good model systems for understanding the impacts of CMS on evolutionary histories.

Reciprocation and partner recognition: do reciprocating partners recognize each other?

Malo Loubière, Maria-Cristina Lorenzi *(LEEC - Sorbonne Paris Nord University)*

Conditional reciprocity is one of the evolutionary mechanisms explaining cooperation between individuals, even unrelated ones. It explains cooperative behavior where individuals appear to follow the rule “help who helped you before”, that is cooperative behaviors which are performed in response to cooperative behaviors offered by a partner before. Therefore, the exchanges should occur between the same partners and should require individual recognition mechanisms. Some species of outcrossing, simultaneous hermaphrodites trade eggs following reciprocity rules, i.e., one individual offers its eggs to the partner conditionally upon receiving eggs before from the partner. However, we do not know whether they discriminate their partner from other conspecifics. We tested this in the marine polychaete worm *Ophryotrocha diadema*, where monogamous partners engage in egg trading by exchanging eggs every two days. By switching

reciprocating partners between pairs, we showed that worms did not delay egg exchanges nor diminish clutch size compared to control (not switched) pairs. These results suggest that worms do not discriminate between familiar and unfamiliar partners. We discuss these results in the perspective of the mechanisms of egg trading in rare hermaphroditic species living in sparse populations.

Cimate change and fertility
Elise Jeanne, Sarthak Grover, Steve Ramm *(UMR 6552 Ecobio, Université de Rennes)*

The impact of climate change on fertility has been investigated across a wide range of taxa, revealing that fertility limits provide more accurate predictions of species distributions than survival limits. However, several questions remain controversial or overlooked, including which sex is more affected and the natural potential for recovery. Hermaphrodites represent a unique model to investigate these questions, as a single individual possess both sexual reproductive systems, circumventing potential confounding effects present in separate-sex species. However, most studies conducted on hermaphrodites predominantly focus on overall fertility impact rather than sex-specific effects. While *Macrostomum lignano*, a simultaneous hermaphroditic marine flatworm, stands as a potential candidate, we needed further characterization of abiotic stressors affecting its fertility and potential interactive effects between these stressors. In this study, we assigned worms to control conditions (20°C; salinity 32‰) or to range of high temperature (35, 37.5, 40°C) and/or salinity (48, 64 ‰) for 8 hours, with one or two shocks to test for the impact of stressor frequency. Following the shock, worms were returned to baseline conditions with a partner to measure their fertility (i.e. number of larvae) and their potential for recovery over time. Worms exposed to heat/salinity shocks are likely to have a decreased fertility compared to control worms. We also expect that the severity of this decline will increase with more intense, frequent and combined stressors. We expect the worms to be able to recover the loss in fertility when returned to baseline conditions after the shock, but their potential for recovery might be negatively correlated with the intensity, frequency and number of stressors. This study establishes the baseline for future work on the short- and long-term effects of climate change on hermaphrodite fertility.

Impacts of heat and salinity stress on inbreeding depression in a free-living hermaphrodite flatworm
Sarthak Grover, Steve Ramm *(UMR 6552 Ecobio, Université de Rennes)*

Climate change presents an urgent threat to the survival of many organisms, including through its impact on increasing the frequency and intensity of stressful events. Inbred individuals tend to be more sensitive to stress compared to their outbred counterparts. In other words, stress amplifies the magnitude of inbreeding depression. Generally, the more stressful an environment, the greater the increase in inbreeding depression. However, the exact nature of this relationship, and whether it varies depending on the stressor, remains understudied. We plan to conduct a study using *Macrostomum hystrix*, a free-living hermaphrodite flatworm, as our model organism. We will expose both outbred and inbred *M. hystrix* individuals at different life stages to varying levels of salinity and heat stress and measure their fitness. *M. hystrix* individuals can self-fertilize (or self) their gametes, and those from primarily outcrossing populations can utilize selfing as a reproductive assurance strategy when outcrossing opportunities are limited. However, selfing can impose severe fitness costs due to inbreeding depression, as it is the strongest form of inbreeding. Understanding how environmental conditions change these costs is crucial because inbreeding depression levels play a vital role in many evolutionary processes like extinction risk, purging of inbreeding depression and mating systems evolution.

ca. 18:30 Depart together for city centre

19:00 Conference dinner at *Crêperie du Pont-Levis*
6 Rue des Portes Mordelaises, 35000 Rennes
https://maps.app.goo.gl/fgKFbQVe6xXn5Bp36

Wednesday 24th April

09:00 **Sylvain Glémin**
UMR 6553 Ecobio, Université de Rennes, France

Mating system and the dynamics of speciation
Co-authors: Lucas Marie-Orleach, Lovisa Gustafsson, Abel Gizaw Seid, Siri Birkeland, Loren Rieseberg, Anne Brysting and Christian Brochmann

Selfing has many consequences on the genetic diversity and the evolutionary dynamics of populations, which may in turn affect macroevolutionary processes such as speciation. I will first present population genetics models on the effect of mating system on the accumulation of genetic incompatibilities during population divergence in allopatry, which shows under which conditions selfing is expected to facilitate speciation. Then, I will present empirical results from literature surveys and original datasets aiming at testing the effect of mating systems on reproductive isolation.

09:45 **Fanny Laugier**
UMR 5175 Centre d'Ecologie Fonctionnelle et Evolutive, Montpellier, France

Experimental evolution reveals counter-selection on cytoplasmic male sterility in resistant nuclear backgrounds as predicted by theory
Co-authors: Patrice David, François Rousset

Gynodioecy, the coexistence of hermaphrodites with females, often reflects conflicts between cytoplasmic male sterility (CMS) genes and nuclear genes restoring male fertility. CMS is frequent in plants, and has been recently discovered in one animal: the freshwater snail Physa acuta. In this system, a CMS-associated mitogenome (K) show nuclear polymorphisms contributing to the restoration of male function. Through a 11-generation experimental evolution, we monitored in real time changes in the frequency of CMS and explored empirically the type of evolutionary trajectories predicted by theoretical models. Our results highlight a decrease in CMS frequency in populations with high restoration potential. In congruence with this decline, we estimated a strong negative selection coefficient (s = -0.18) affecting the K mitotype. Our findings are consistent with theoretical expectations and demonstrate a CMS cost.

10:15 **Donald Stewart**
Acadia University, Canada

Hermaphroditism and doubly uniparental inheritance of mitochondrial DNA in freshwater mussels
Co-authors: Emma-Jean Freeman, Russell Easy, Emily Chase, Brent Robicheau, Sophie Breton

Many freshwater mussels (FWM) of the order Unionida exhibit doubly uniparental inheritance of mitochondrial DNA. Males in these species possess both a female-transmitted or F-type mt genome as well as a male-transmitted or M-type mt genome. Phylogenetic analysis shows that the M- and F-type lineages diverged over 200 MYA. These mt genomes contain atypical genes, referred to f-orf and m-orf genes (for open reading frame), present in F and M mt genomes, respectively. These genes are not involved in ATP synthesis. Instead, they may be involved in sexual development in FWM. When dioecious FWM species evolve hermaphroditism, which has happened independently several times, the M-type mt genome is lost and the f-orf gene of the remaining F-type genome evolves dramatically. We refer to these genes as hermaphroditic or h-orfs. Inferred amino acid sequences and hydrophobicity properties of h-orf proteins are frequently dramatically different. Using a method to quantify the divergence in hydrophobicity profiles of h-orfs we hope to detect cryptic hermaphrodites. FWM are among the most threatened animals worldwide due in part to invasive species such Zebra mussels. We predict that population declines will favour evolution of hermaphroditism, which may not be an evolutionary stable strategy.

10:45 Coffee break

11:15 **Jeanne Tonnabel**
Institut des Sciences de l'Évolution de Montpellier (ISEM), France

Sexual selection in plants: what happens after pollen land on the pistil?

Sexual selection, acting through competition for the access to mates and their gametes, successfully explained numerous reproductive strategies in animals. Sexual selection typically emerges when females produce fewer numbers of larger gametes than males – a situation called anisogamy – which fosters competition among males for accessing the rare ovules. Sexual selection theory should thus be universally valid for all sexually reproducing anisogamous organisms encompassing plants. While the idea that sexual selection acts on plants is largely admitted, most predictions of the sexual selection theory remain untested in the plant kingdom. I will present results from various experiments that aim at testing fundamental predictions of the sexual selection theory in the hermaphroditic plant Brassica rapa, and decipher the mechanisms by which sexual selection may apply in plants. In particular, we combine paternity analyses, quantitative genetics and experimental evolution to test whether cryptic female choice can act through variation in simple pistil traits.

11:45 **Kora Klein**
Johannes Gutenberg Universität Mainz, Germany

Modelling hermaphroditic flowers and the cost of floral displays
Co-authors: Raquel Nunes Palmeira, Bing Dong

Most flowers are hermaphroditic with male and female parts on the same floral display. This stands in stark contrast to animals where simultaneous hermaphroditism is relatively rare. We propose that this difference is related to the costliness of floral displays. A floral display aids both male and female reproduction because plants require pollinators to both deliver pollen from other plants (female reproduction) and to pick up pollen (male reproduction). In order to produce one male and one female flower, a monoecious plant would therefore need to invest into two floral displays, while a hermaphroditic flower only requires investment into a single floral display. Hence, hermaphroditic flowers require fewer resources to produce, particularly in plants with large floral displays. The idea presented here is not new, but was first proposed by Charnov, Bull, and Smith in 1976. Since then, there have been two published attempts to construct mathematical models to prove the conceptual validity of the argument, but these models where unable to show a causal relationship between the cost of floral displays and hermaphroditism in flowers. We have pinpointed an unrealistic assumption in these earlier models and believe that changing this assumption is essential to showing the proposed relationship.

12:15 Lunch at the *Club des Professeurs*
Building 1, Beaulieu Campus

13:30 **Chenxi Wang**
University of Edinburgh, UK

Refining the demographic inference of self-fertilising species

Self-fertilisation can have a profound effect on the evolutionary history of the hermaphroditic species. It can not only impact the genetic variation by increasing the level of homozygosity and genetic differentiation between different subpopulations, but also lead to smaller effective population sizes and lower effective recombination rate. Although selfing is common in both plants and hermaphroditic animals, popular methods for demographic inference rarely take its effect into account when looking into the changes of the past effective population size, which may cause a misinterpretation of the result. Some novel methods such as eSMC, teSMC and tsABC, have been developed recently to jointly infer the demographic history and selfing-related parameters of the species, including selfing rate and transition time from outcrossing to selfing. Although these methods perform well for both simulated and empirical dataset of highly selfing species, whether they can be used to infer the demographic history of partial selfing species including species with spatial variation of selfing rate and to infer more ancient transition from obligate outcrossing to selfing, remains unknown. Population-level genomic data from a mixed-mating plant, *Arabis alpina*, and selfing *Caenorhabditis* species will be used to test the usability of existing methods on more complex scenario of selfing evolution, including a gradual transition of mating system, an earlier origin of selfing (c.a. > 1Mya) and genome-wide linked selection. We also aim to extend the existing methods of inferring the demographic history, selfing rate and the transition time

of breeding system to mixed-mating species, and develop more general methods for evolutionary inference accounting for self-fertilisation.

14:00 **Joris Koene**
Vrije Universiteit Amsterdam, Netherlands

Insemination by inbreds: the possible effects of selfing on seminal fluid protein responses
Co-authors: Yumi Nakadera, Hedda van Noppen, Lea Rutsch, Anne Fluitman

Unlike species with separate sexes, simultaneous hermaphrodites often have the fall-back option to self-fertilise if no suitable mating partner is encountered. Some species are more robust against effects of inbreeding than others, and the great pond snail *Lymnaea stagnalis* seems particular in this respect: we show that over 40 generations of exclusive selfing is possible. Such strong selection on self-fertilisation implies that some reproductive processes are no longer in use, one being seminal fluid proteins (SFPs). These are normally transferred in the ejaculate by the sperm donor to the recipient. To assess whether the effectiveness of SFPs changed, we compared two inbred lines with our standard outcrossing line (from which the former originate). We specifically focussed on the well-established effect of the SFP Ovipostatin (or LyAcp10), which temporarily suppress egg laying and increases investment per egg. We tested for these effects using natural and artificial insemination (resp. n.i and a.i.), with standard snails as recipients. For natural inseminations we also quantified mating behaviour. Our data show that the effects induced by the ejaculate of one inbred line was consistently different from the other and the control. Snails inseminated with that line did not show the typical delay in egg laying (n.i.) and laid relatively larger eggs (a.i.). Moreover, that same line showed a stronger behavioural preference for mating in the female role. Finally, we tested whether the coding region and expression of Ovipostatin differed, which it did not. This may be explained by our Genome project's expression data (based on whole mount in situ hybridisation, WMISH) which show that Ovipostatin also plays a role during development. Taken together, we see detectable differences in egg laying and mating behaviour in only one inbred line that may be due to selection on selfing, but further work is needed to confirm this.

14:30 **Round-up of discussion topics 1 and 2**

15:30 **General discussion**
Coordinating efforts of the SHOW network, including joint funding applications

ca. 16:30 Close of meeting

from 17:00 Post-conference drinks
For those not leaving Rennes immediately, you're welcome to join us for further discussion at *A Fuoco Nero*
7 Rue Saint-Georges
https://maps.app.goo.gl/kUdegdsB9nSfWj7m6

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