Simultaneously Hermaphroditic Organisms Workshop

17-19 February 2016

Zentrum für interdisziplinäre Forschung, Universität Bielefeld

Universität Bielefeld

CONTENTS

WORKSHOP ORGANIZERS

Athina Giannakara
Yumi Nakadera
Bahar Patlar
Steve Ramm
Michael Weber
& Lennart Winkler

ANNOTATED MAP https://goo.gl/hzdMDk

THIS PROGRAMME https://goo.gl/rjejXr

WORKSHOP NOTES https://workflowy.com/s/rWWXdq5Vwy

VENUE INFO

All workshop sessions will take place at the Centre for Interdisciplinary Research (ZiF) of Bielefeld University, located on the hillside above the main university campus. Social events will take place in the city centre *(see next page)*.

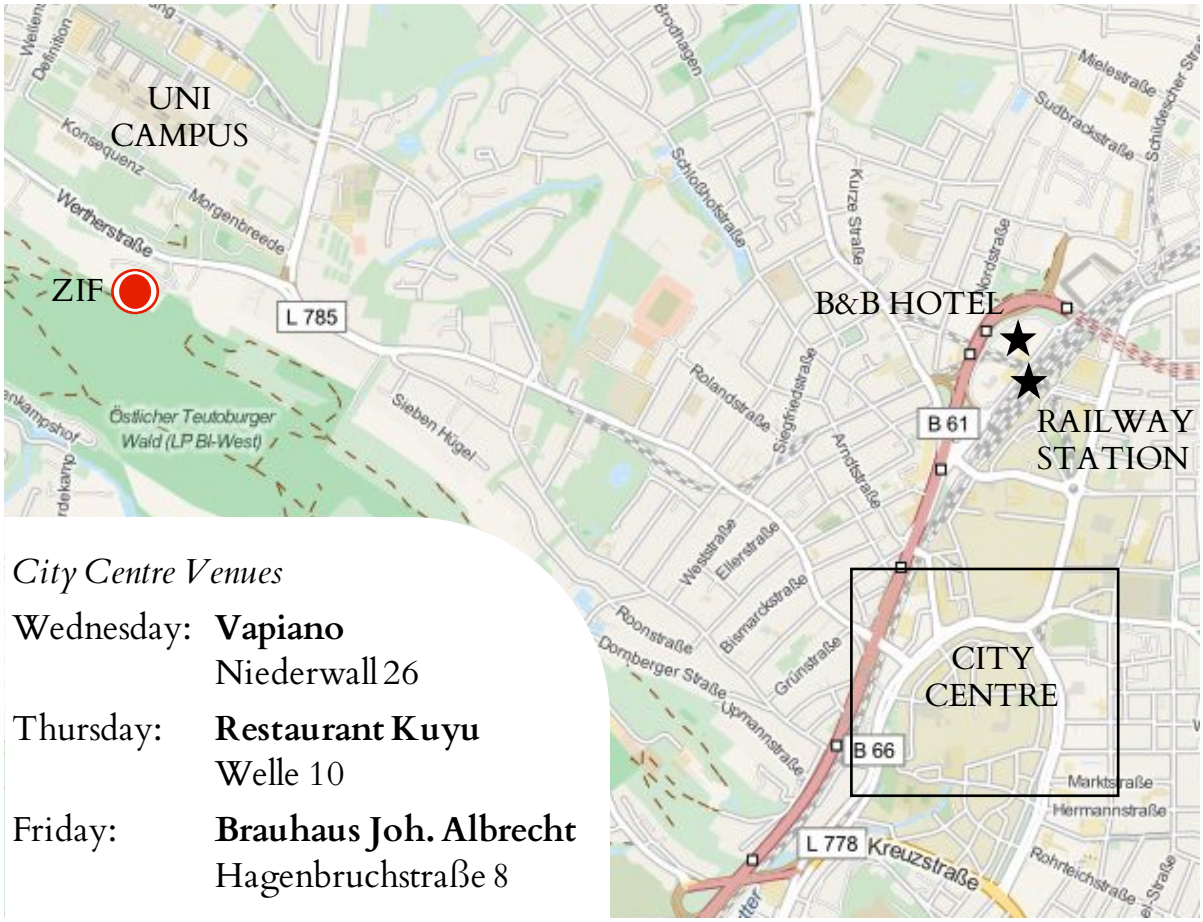
TRAVEL INFO

To reach the ZiF from the main railway station (*Hauptbahnhof*) or from the city centre (*Jahnplatz*), please take either the Tram (Line 4, red) in the direction *Universität-Lohmannshof* or a Bus (Lines 21, 61, 62) in the direction of Werther, Halle or Borgholzhausen. Both routes take ca. 20-25mins in total (but it is a longer walk from the tram stop).

If travelling by tram: Owing to construction work, the usual route via the university is blocked. Instead, get off at the stop *Bültmannshof*, continue walking west along Kurt-Schumacher Str. and then turn south up the hill onto Volltmannstraße. Using the pedestrian crossing at the top of the hill, turn right on to Wertherstraße, and the ZiF is ca. 200m further along, up the hill on the left hand side.

If travelling by bus: Get off at the stop *Universität/Studenten-Wohnheim* and continue walking along the road westwards for around 200m, until you see the ZiF up the hill on the left hand side.

Tickets: For the bus or tram, you need an adult zone 1 ticket (valid for 90 mins anywhere in the city). If travelling by bus, you can buy these from the driver as you get on (€2.60 single, or €8.40 for a "4er" ticket valid for 4 separate journeys). Or they're a bit cheaper if you buy them in advance from the machines at all tram stops and some bus stops (e.g. Jahnplatz; €2.40 single, or €8.20 for a 4er ticket). Remember to validate ("entwerten") your ticket by stamping it in one of the small, usually orange, machines on board (with the 4er ticket you just stamp it in a different orientation for each journey, eventually filling both ends on both sides).

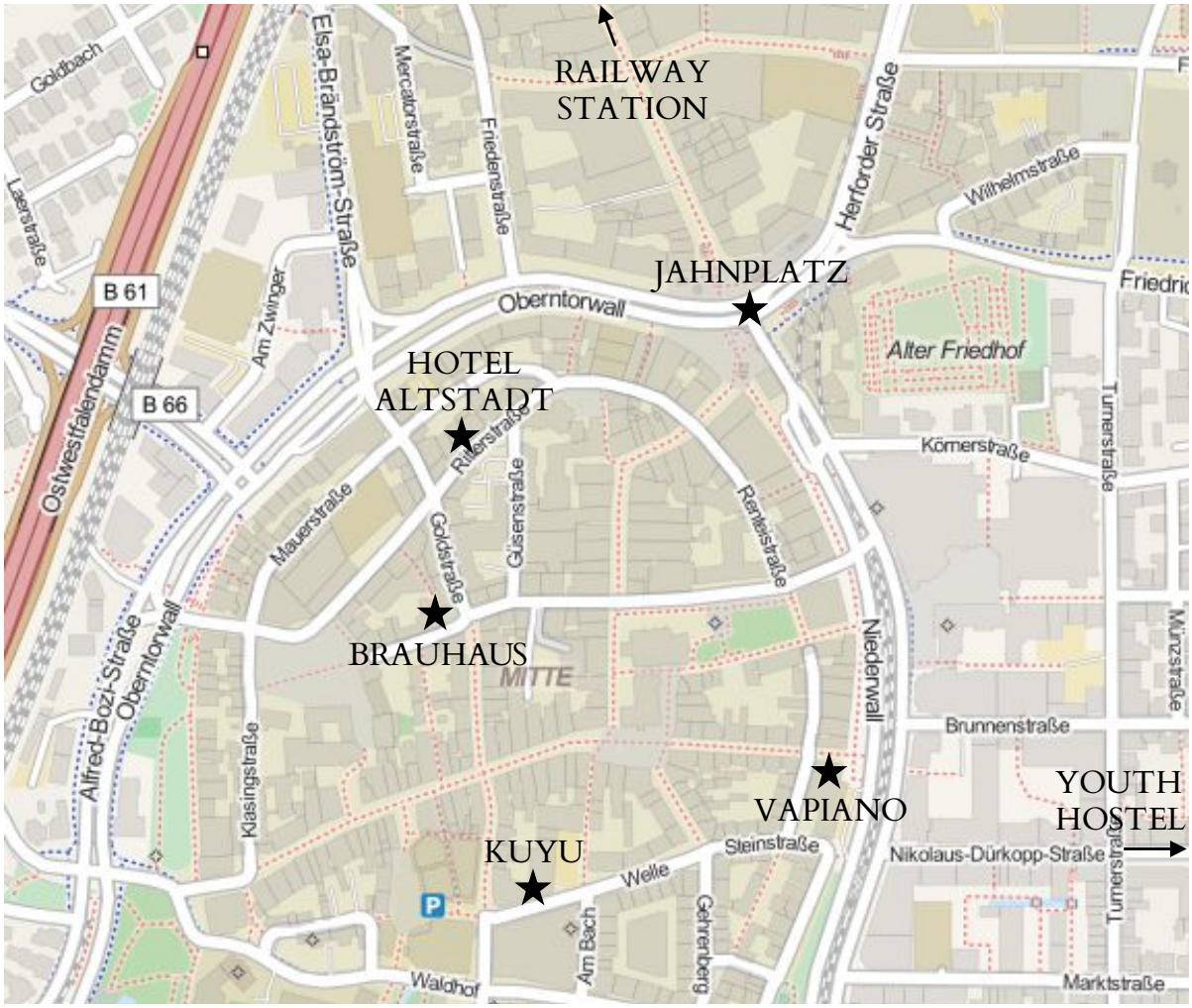


City Centre Venues

Wednesday: **Vapiano**
Niederwall 26

Thursday: **Restaurant Kuyu**
Welle 10

Friday: **Brauhaus Joh. Albrecht**
Hagenbruchstraße 8



THURSDAY	**DAY 1: Pre- and post-mating conflicts and sexual selection in hermaphrodites** *Abstracts on page 11 – 16*
09:00-09:15	*Welcome:* Steve Ramm
09:15-10:00	**Hidden female resistance in a simultaneous hermaphrodite** Monica Lodi *(VU Amsterdam)*
10:00-10:45	**Seasonal increase in rates of multiple paternity in a wild population of the simultaneously hermaphroditic freshwater snail *Radix balthica*** Anja Bürkli *(EAWAG, Zürich)*
10:45-11:15	Coffee break
11:15-12:00	**Sex allocation and sperm production plasticity: remarkable differences in the reproductive biology of two *Macrostomum* species** Athina Giannakara *(Bielefeld)*
12:00-12:45	**Measuring heritability of, and correlations between, realized female and male fitness in the hermaphrodite plant, *Solanum carolinense*** Crispin Jordan *(Edinburgh)*
12:45-14:15	Lunch
14:15-15:00	**Mutual mate choice and speciation** Oscar Puebla *(GEOMAR, Kiel)*
15:00-15:45	**DISCUSION 1:** **Sexual selection in hermaphrodites and gonochorists: Is there any difference?** *(see next page)*
15:45-16:15	Coffee break
16:15-17:00	**DISCUSION 1** *continued*
17:15-19:00	**POSTER SESSION** *(see page 8)*
19:30	**WORKSHOP DINNER** Venue: Restaurant Kuyu, Welle 10

THURSDAY
15:00

DISCUSSION SESSION 1
Sexual selection in hermaphrodites and gonochorists: Is there any difference?

Chair: Tim Janicke *(CEFE, Montpellier)*

This discussion session aims to sharpen our understanding of the differences in the form, strength and direction of sexual selection between gonochorists and simultaneously hermaphroditic animals. We will tackle this by trying to answer three main questions:

(1) **Where do our ideas come from?** Compiling a (ideally complete) list of verbal hypotheses and theoretical work suggesting that sexual selection differs between simultaneous hermaphrodites and gonochorists. This includes the discussion of the proposed ideas/models. Special focus will also be given to the relative importance of pre- versus postcopulatory sexual selection and to consider which of our predictions depend on mating type (i.e., reciprocal versus unilateral mating species).
(2) **Is there empirical evidence in support of these hypotheses?**
(3) **What approaches are (now) required to test the discussed hypotheses?** The final aim will be to design experiments/ comparative studies/meta-analyses that allow us to test for differences in sexual selection between gonochorists and simultaneous hermaphrodites (e.g., comparisons of standardized metrics of sexual selection, proxies of sperm competition intensities, mating differentials, and selection differentials of post-copulatory traits).

Suggested Reading

Greeff, J. M. and N. K. Michiels. 1999. Low potential for sexual selection in simultaneously hermaphroditic animals. *Proc. R. Soc. B* 266:1671–1676.

Morgan, M. T. 1994. Models of sexual selection in hermaphrodites, especially plants. *Am. Nat.* 144:S100–S125.

Schärer, L. and I. Pen. 2013. Sex allocation and investment into pre- and postcopulatory traits in simultaneous hermaphrodites: the role of polyandry and local sperm competition. *Philos. Trans. R. Soc. B* 368: 20120052.

THURSDAY
17:15–19:00

POSTER SESSION

Abstracts on page 21 – 30

Learning in sex role decisions
Beatriz Alvarez *(Oviedo)*

Experimental evolution of resource allocation in a simultaneously hermaphroditic freshwater snail: preliminary results
Nicolás Bonel *(Montpellier)*

Sperm competition and cryptic female choice in *Cornu aspersum:* Inter-population variation in reproductive traits
Marina-Elena Garefalaki *(Thessaloniki)*

A rigorous comparison of sexual selection indexes via simulations of diverse mating systems
Jonathan Henshaw *(ANU, Canberra)*

Size-assortative mating in simultaneous hermaphrodites: an experimental test and a meta-analysis
Tim Janicke *(Montpellier)*

Selective sperm storage influences paternity in a simultaneous hermaphrodite
Ruben Janssen *(Basel)*

Sex allocation, plasticity and the preference for the male role in simultaneous hermaphrodites
Cristina Lorenzi *(Paris)*

Estimating the response to sex-limited experimental evolution in a hermaphrodite
Anna Nordén *(Lund)*

Differential gene expression analysis of seminal fluid proteins in a simultaneously hermaphroditic marine flatworm *Macrostomum lignano*
Bahar Patlar *(Bielefeld)*

A targeted in situ hybridization screen identifies putative seminal fluid proteins in a simultaneously hermaphroditic flatworm
Michael Weber *(Bielefeld)*

FRIDAY

DAY 2: Genetic and evolutionary constraints on hermaphroditism

Abstracts on page 17 – 20

09:00–09:45 **Models of egg trading: sex allocation, reciprocity, and the stability of hermaphroditism**
Jonathan Henshaw *(ANU, Canberra)*

09:45–10:30 **Models of sexual antagonism in hermaphrodites**
Jessica Abbott *(Lund)*

10:30–11:00 Coffee break

11:00–11:45 **Experimental evidence for the negative effects of self-fertilization on the adaptive potential of populations**
Elsa Noël *(Montpellier)*

11:45–12:30 **DISCUSSION:**
Future developments of the SHOW

12:30–13:30 Lunch

13:30–15:00 **DISCUSSION 2:**
Why be a hermaphrodite? Revisited *(see next page)*

Close of Workshop

15:00 Coffee and further discussion for those who want to stay, or free-time to explore….

19:00 *Optional joint dinner in the city centre for those not travelling home until Saturday*

Venue: Brauhaus Joh. Albrecht, Hagenbruchstraße 8

FRIDAY
13:30–15:00

DISCUSSION SESSION 2
Why be an hermaphrodite? Revisited

Chair: Lukas Schärer *(Basel)*

This discussion session aims to consider how we can explain the distribution of gender expression (gonochorists, sequential- and simultaneous hermaphrodites) in nature. We will follow the same general format as the first discussion session:

(1) **Where do our ideas come from?** Compile the main theories to account for hermaphroditism, and the main ecological/life history traits we expect to be associated with it.
(2) **Is there empirical evidence in support of these hypotheses?** How well do these ideas seem to explain the distribution of gender expression patterns in animals and plants? How are transitions in gender expression achieved? How important are phylogenetic constraints?
(3) **What approaches are (now) required to test the discussed hypotheses?** Can we design experiments, comparative studies or meta analyses to test the current hypotheses? What can we do in taxa having gonochoristic and hermaphroditic species? What can we do in exclusively/predominantly hermaphroditic taxa?

Suggested Reading

Charnov, E. L., Bull, J. J., and Maynard Smith, J. 1976. Why be an hermaphrodite? *Nature* 263:125–126.

Ghiselin, M. T. 1969. The evolution of hermaphroditism among animals. *Quart. Rev. Biol.* 44:189–208

Henshaw, J. M., Marshall, D. J., and Jennions, M. D. 2014. Local gamete competition explains sex allocation and fertilization strategies in the sea. *Am. Nat.* 184: E32–E49.

Talk cancelled

Improving the interpretability of Bateman and other sexual selection metrics

Nils Anthes[1], Ines Häderer[1], Nico Michiels[1], Tim Janicke[2]

[1]Institute of Evolution and Ecology, University of Tübingen, Auf der Morgenstelle 28, D-72076 Tübingen, Germany
[2]Centre d'Ecologie Fonctionnelle et Evolutive, CNRS-UMR 5175, 1919 Route de Mende, 34293 Montpellier, France

Population wide measurements of mating success (MS) and reproductive success (RS) are central to almost all available metrics serving as proxies for the opportunity for, or intensity of, sexual selection. The resultant parameter estimates, however, are rather sensitive to how exactly, and under which conditions, MS and RS are measured and analysed. Slight modifications in methods can substantially affect our qualitative interpretation of (sex differences in) sexual selection. Taking advantage of a recent meta-analysis assembling published estimates of the classic Bateman metrics, we evaluate the potential impact of different study approaches, and discuss guidelines for "best practice" that can help improving the interpretability and comparability of sexual selection metrics.

Thursday
09:15-10:00

Hidden female resistance in a simultaneous hermaphrodite

Monica Lodi, Joris Koene

Department of Ecological Science, VU University, Amsterdam, The Netherlands

Conflict of interests between the sexes over reproduction is widespread. This sexual conflict leads to co-evolution between the manipulative traits of males and the mate-selection traits of female. Simultaneous hermaphroditic land snails with love-darts are a case in point where conflict between partners occurs over sperm storage. The dart introduces mucus when stabbed into the partner's body wall during courtship. This mucus induces physiological changes that ultimately increase the amount of sperm that avoid digestion by the female. To regain control over fertilization processes or to screen good males, the female system evolved a spermatophore-receiving organ, called diverticulum, that increases the distance to the sperm-storage organ. The snail *Eobania vermiculata* possesses a diverticulum that is three times longer than the spermatophore it receives. As we show here, the love-dart mucus of this species evolved a substance that shortens the diverticulum. However, this effect is only detectable when the mucus is applied to another helicid species, *Cornu aspersum*. This latter finding suggests that *E. vermiculata* evolved a resistance to the male manipulation via the love dart by becoming insensitive to the substance. This gives useful insight into sexual co-evolution in simultaneous hermaphrodites, indicating that it can follow the same patterns as those predicted for separated-sexes species.

Thursday
10:00-10:45

Seasonal increase in rates of multiple paternity in a wild population of the simultaneously hermaphroditic freshwater snail *Radix balthica*

Anja Bürkli

Department of Aquatic Ecology, EAWAG & Institute of Integrative Biology, ETH Zürich, Switzerland

Mating systems are treasure troves of evolutionary insight, but analysing them can be technically challenging. We characterised the mating system in a natural population of the annual, simultaneously hermaphroditic freshwater snail *Radix balthica* in detail. Specifically, we assessed variation in the rate of multiple paternity and selfing while accounting for seasonality. We also estimated potential fitness effects of these mating system parameters. We achieved this by measuring and genotyping large numbers of field-collected egg clutches and adult snails using ten highly polymorphic microsatellite markers. Overall, 50% of the clutches had multiple fathers, but both the frequency of multiple paternity (20 – 93% of clutches) and its magnitude (mean 1.3 – 3.8 fathers per clutch) strongly increased during the breeding season. Simultaneously, mean clutch size decreased by half, causing expected paternity shares to plummet. True to the predicted decline in the number of male-acting snails, we saw a decrease in mating frequency over time, measured as the frequency of reciprocally reproducing snails. Meanwhile, reproduction was almost exclusively biparental, as evidenced both by progeny arrays and null-allele corrected population-level selfing rates. These data reveal a surprisingly complex mating system, in which individual sex-role decisions may be fine-tuned to specific time windows.

Thursday
11:15-12:00

Sex allocation and sperm production plasticity: remarkable differences in the reproductive biology of two *Macrostomum* species

Athina Giannakara[1], Lukas Schärer[2], Steven A. Ramm[1]

[1]Department of Evolutionary Biology, Bielefeld University, Germany
[2]Zoological Institute, University of Basel, Switzerland

Simultaneous hermaphrodites are expected to respond to sperm competition by investing more of their reproductive resources into the male sex function. Such increase in male investment is often manifested as testis size adjustment and most research has focused on this parameter, ignoring another important factor as a potential target of sperm competition, i.e. spermatogenesis speed. To investigate the plasticity potential of spermatogenesis kinetics we used *M. lignano*, already known for adjusting testis size when exposed to different sperm competition environments. A proliferation marker allowed us to estimate the speed of spermatogenesis across social groups differing in sperm competition level. We find that in this species spermatogenesis speed upregulation and testis size adjustment are indeed two complementary mechanisms employed together to maximize sperm production in competitive conditions, explaining ca. 45% and ca. 55% of the variation in sperm production between worms kept in different sperm competition conditions, respectively. Intrigued by these results, we went on to investigate whether such sperm competition-driven sex allocation and spermatogenesis plasticity is exhibited by other *Macrostomum* species as well. Similar experimental approaches in the self-fertilizing *M. pusillum* have yielded opposite results: this species exhibits no plasticity in either parameter. Our results highlight the remarkable sexual diversity characterizing the *Macrostomum* clade and suggest differences in fertilizing mode and breeding system as its driving forces.

Thursday
12:00-12:45

Measuring heritability of, and correlations between, realized female and male fitness in the hermaphrodite plant, *Solanum carolinense*

Crispin Y. Jordan[1], Elizabeth Elle[2], Tom R. Meagher[3]

[1]Institute of Evolutionary Biology, University of Edinburgh, UK
[2]Simon Fraser University, Canada
[3]University of St. Andrews, UK

In sexual species, the magnitude of genetic variation for realized fitness and genetic correlations between female and male fitness represent strong constraints for adaptive evolution. Several studies have estimated genetic variance for realized female and male fitness in animal species, but no estimates exist for plant species. Using a previously published dataset, we estimated genetic variation for realized female (seed production) and male (siring success) fitness in a hermaphrodite plant. We detected little evidence for genetic or environmental correlations between female and male fitness, but had little power to do so. As expected, genetic variance for both female and male fitness was small, as environmental effects dominated variance in fitness. Moreover, the environmental variance of 'per flower' male fitness exceeded that of 'per flower' female fitness, whereas the environmental variances of female and male fitness were equal on the scale of entire floral displays. We discuss the implications of these findings for the evolution of floral displays.

Thursday
14:15-15:00

Mutual mate choice and speciation

Oscar Puebla

GEOMAR Helmholtz Centre for Ocean Research, Kiel, Germany

The award of the 2012 Nobel Prize in Economic Sciences to the two main founders of matching theory, Alvin E. Roth and Lloyd S. Shapley, establishes the fundamental importance and specificity of this theoretical framework. In evolutionary biology, matching theory provides a very distinctive opportunity to address sexual selection and speciation in a context of mutual mate choice. I will illustrate this potential using the hamlets (*Hypoplectrus* spp, Serranidae), brightly colored reef fishes from the tropical western Atlantic, as a model system. A combination of short- and long-term field observations reveal complex pairing and mating dynamics in the hamlets. Mating decisions are highly context-dependent, individuals involved in stable pairs mate more than individuals who tend to switch partners, and assortative mating is not necessarily costly for rare phenotypes. Matching theory is particularly well suited to explicitly address context-dependent mating and pair stability. Individual-based simulations adopting this approach suggest that the pairing dynamics observed in the field provide a robust mechanism for both the evolution and maintenance of assortative mating in the presence of gene flow. In the hamlets, ongoing gene flow is suggested by the occurrence of hybridization. Genomic analysis reveals that sympatric hamlets can be extraordinarily similar, suggesting extensive introgression across most – but not all – of the genome. I propose that the mechanism outlined here for the evolution and maintenance of assortative mating in the presence of gene flow may apply to variety of species with different mating systems.

Friday
09:00–09:45

Models of egg trading: sex allocation, reciprocity, and the stability of hermaphroditism

Jonathan Henshaw, Michael Jennions, Hanna Kokko

Research School of Biology, Australian National University,
Australian Capital Territory 0200, Australia

Egg trading (the alternating exchange of egg parcels during mating by simultaneous hermaphrodites) is one of the best-documented examples of reciprocity between nonrelatives. By offering eggs only to partners who reciprocate, traders increase their reproductive success in the male role, but at a potential cost of delaying or reducing fertilisation of their own eggs. To investigate when egg trading might evolve, we built a model that takes into account mating frequencies and the potential presence of 'streakers' (unpaired individuals that join mating pairs but contribute only sperm). Frequent mate encounters have a direct effect of making it easier for egg trading to invade, because the costs of delaying egg release are reduced at high mate encounter rates. Simultaneously, however, we expect an indirect and opposite effect caused by increased streaking rates. Our model suggests that the direct effect typically outweighs the indirect effect, such that egg trading as a whole is more likely when mate-encounter rates are high. However, this result leads to a second problem: egg trading obviously requires the organism to be hermaphroditic, and hermaphroditism itself is less likely to be stable when mate-encounter rates are high. We propose a potential solution to this conundrum by showing that egg trading 'protects' hermaphroditism by changing the sex allocation to be female-biased. This makes it harder for gonochorism to invade.

Friday
09:45-10:30

Models of sexual antagonism in hermaphrodites

Jessica Abbott[1], Sam Tazzyman[2]

[1]Department of Biology, Section for Evolutionary Ecology, Lund University
Sölvegatan 37, 223 62 Lund, Sweden
[2]Institute of Integrative Biology, Department of Environmental Sciences, ETH Zürich, Switzerland

Sexually antagonistic alleles result in increased fitness in one sex at the cost of decreased fitness in the other, and sexual antagonism is by now a well-established phenomenon in separate-sexed organisms. Because hermaphrodites gain fitness via both male and female sex functions, it has long been thought that sexually antagonistic alleles would not show any interesting dynamics in hermaphroditic populations. However in recent years there has been an increased interest in the potential for sexual conflict and sexual antagonism in hermaphrodites. We have therefore developed a population-genetic model of the fate of sexually antagonistic alleles in hermaphroditic populations. Pleiotropic genes that both bias sex allocation and affect fitness in a given sex role can be at a stable polymorphism, depending on parameter values. This is to our knowledge a new finding, but it is consistent with a verbal argument developed by Bedhomme et al. It has previously been shown that sexually antagonistic alleles can coexist at stable polymorphisms in separate-sexed species, but we can show that where one of the alleles also biases sex role investment, there is a wider range of parameters that will lead to polymorphism. This increase in the region of parameter space leading to stable polymorphism is especially notable for weak selection coefficients. This means that there could potentially be large amounts of standing sexually antagonistic genetic variation in hermaphrodites, primarily via alleles with small effects.

Friday
11:00-11:45

Experimental evidence for the negative effects of self-fertilization on the adaptive potential of populations

Elsa Noël, Philippe Jarne, Sylvain Glémin, Patrice David

Centre d'Ecologie Fonctionnelle et Evolutive, CNRS-UMR 5175, 1919 Route de Mende, 34293 Montpellier, France

Self-fertilization is widely believed to be an evolutionary dead-end. However we lack direct measurements of how selfing affects the response to selection within species over generations. Selfing may initially increase the available genetic variance, but can on a longer term decrease it, as it enhances genetic drift and makes recombination inefficient. We study the response to selection on a morphological trait in laboratory populations of a self-compatible snail under either selfing or outcrossing. Both treatments are applied to two types of populations: some having undergone frequent selfing and purged a substantial fraction of their mutation load in their recent history, others continuously maintained under outcrossing. As expected, populations with a history of outcrossing respond faster to selection than those which have experienced selfing before selection started. In addition, when self-fertilization occurs during selection, the response is initially faster, but then rapidly slows down while outcrossing populations maintain their response throughout the experiment. This occurs irrespective of past selfing history, suggesting that having previously purged inbreeding depression does not significantly alter the effect of current self-fertilization on the response to selection. These findings confirm the short-term positive and long-term negative effects of selfing on the adaptive potential.

Talk cancelled

Gender plasticity and the evolution of sexual systems in animals

Janet L. Leonard

University of California-Santa Cruz, USA

In the Metazoa there are two common sexual systems; dioecy and simultaneous hermaphroditism with outcrossing. These sexual systems are often characteristic of whole phyla and classes and have been very stable over evolutionarily time and a great deal of environmental change. Other sexual systems such as sequential hermaphroditism, gynodioecy, androdioecy, etc, occur more rarely and are not evolutionarily stable in the sense that they are characteristic of major clades such as phyla and classes. . In angiosperms the evolutionary paths from dioecy to simultaneous hermaphroditism are well understood and typically involve either gynodiocey or androdioecy. This is not the case in animals. Gynodioecy is extremely rare in animals. Although androdioecy has evolved repeatedly in animals, the hermaphrodites do not outcross as males, but either self-fertilize or act as females to males, except in barnacles. In barnacles androdioecy can be a transitional state between dioecy and outcrossing simultaneous hermaphroditism. Here I suggest that phenotypic plasticity in gender, based on social cues, including sequential hermaphroditism and environmental sex determination (ESD) may be a common evolutionary path between outcrossing simultaneous hermaphroditism and dioecy in animals. Recent work has demonstrated that simultaneous hermaphroditism often involves changes in sex allocation as function of age, size and social environment. These changes in sex allocation can blur the distinction between simultaneous and sequential hermaphroditism. Similarly, with ESD, a single genotype may develop as either a male, female or hermaphrodite depending on social and/or other environmental cues. The distinction between sequential hermaphroditism and ESD has again become very blurry. A plausible evolutionary path between simultaneous hermaphroditism and dioecy might start with simultaneous hermaphrodites which alter sex allocation according to social conditions evolving to sequential hermaphrodites and then these sequential hermaphrodites evolving an earlier and earlier sex change in response to social cues until there is effectively ESD of separate sexes. This could then evolve into genetically determined dioecy which is unresponsive to early social cues. Comparative studies and meta-analyses can help test this hypothesis. In animals, the evolutionarly advantage of dioecy is a largely unexplored question.

POSTER
ABSTRACT

Learning in sex role decisions

Alvarez, B[1], Koene, J. M.[2], Hollis, K.[3], & Loy, I.[1]

[1]Universidad de Oviedo
[2]Vrije Universiteit, Amsterdam
[3]Mount Holyoke College, Massachusetts

Learning via classical conditioning has been shown to increase mating chances and paternity success in non hermaphroditic animals. However, whether it can provide any benefits for hermaphroditic species has not been explored. In simultaneous hermaphrodites that mate unilateral, i.e., perform one role within one mating, conflict can arise over the division of the sex roles. The aim of this work was to assess whether Pavlovian learning can help to overcome such a conflict in the pond snail Lymnaea stagnalis. To do so, the presence of a conspecific was signalled by an odour cue. Subjects belonging to the experimental group showed an increased male mating performance compared to the control group, for which the cue and the presence of a conspecific were not contingent. The results obtained show that when subjects were able to predict a mating encounter the preferred reproductive strategy was that of the male role. This is in line with the general assumption that hermaphrodites can flexibly allocate their resources to either sexual function, maximizing their reproductive fitness.

POSTER
ABSTRACT

Experimental evolution of resource allocation in a simultaneously hermaphroditic freshwater snail: preliminary results

Nicolás Bonel[1,2], Kevin Sartori[2], Elodie Chapuis[3], Tim Janicke[2], Violette Sarda[2], Philippe Jarne[2], Patrice David[2]

[1]Laboratorio de Zoología de Invertebrados I, Universidad Nacional del Sur, Bahía Blanca, Argentina
[2]Centre d'Ecologie Fonctionnelle et Evolutive, Montpellier, France
[3]IRD, UMR186 "Résistance des plantes aux bioagresseurs", 911, Avenue Agropolis, BP 64501, 34394 Montpellier Cedex 05, France

The sex allocation theory in hermaphrodites deals with fitness optimization in the presence of tradeoffs between male and female functions under different environmental and social conditions. We sought to determine the evolutionary changes of reproductive investment in the male and female sex functions, and in their fitness, in the hermaphrodite snail *Physa acuta* (Basommatophora). We used experimental evolution lines (38th generation) in which selection was relaxed on one gender by manipulating female or male fitness and mating group size—female-biased (F lines) and male-biased (M lines) were compared to control lines. Each line included two populations (sub-lines). The experiment comprised 450 pigmented individuals (focals) equally distributed among lines and populations; 450 albino snails served as mating partners to estimate male investment and fitness of focals. We assessed female fecundity rate (female investment), survival rate of offspring (female fitness), time spent in male position when copulating (male investment), and paternity rate (male fitness). Preliminary results indicated that relaxing selection in female function (M lines) led to high male investment and fitness as expected from sex allocation theory. Relaxing selection in the male function (F lines) led to high female investment but, counter to theory, female fitness was low. These results suggest that there was a positive evolutionary change in reproductive investment and fitness towards male function in M lines. The result for female fitness could be due to suppressed sexual selection in F lines in which individuals might have accumulated deleterious mutations, decreasing fitness in the function selected. This supports the idea that sexual selection on the male function contribute to purge the genome from deleterious mutations.

POSTER
ABSTRACT

Sperm competition and cryptic female choice in *Cornu aspersum*: Inter-population variation in reproductive traits

Marina-Elena Garefalaki, Sofia Kalyva, Alexandra Staikou

Department of Zoology, School of Biology, Aristotle University of Thessaloniki, 540 06 Thessaloniki, Greece

Cornu aspersum is a simultaneous hermaphroditic land snail. Reproduction of this species involves multiple mating and prolonged sperm storage. Thus, sperm competition and cryptic female choice seem important factors driving the evolution of reproductive traits. We compared behavioral (reproductive activity period, number of matings, number of partners, mating frequency and mating duration), and anatomical reproductive traits (spermatophore producing organs of the 'male' part of the reproductive system, and spermatophore receiving and sperm storage organs of the 'female' part) from populations of two geographically distant regions with distinct habitat microclimatic conditions and predictability. Our results showed that inter-population variation of *Cornu aspersum* in both behavioral and anatomical reproductive traits is evident. Post-copulatory sexual selection is reflected in the increased variance of both behavioral and anatomical reproductive traits. In *Cornu aspersum* both sexual functions respond to increased sexual selection, providing evidence of the potential antagonistic co-evolution of these reproductive traits.

POSTER
ABSTRACT

A rigorous comparison of sexual selection indexes via simulations of diverse mating systems

Jonathan Henshaw*,1, Andrew Kahn[1], Karoline Fritzsche*,2

*joint first author
[1]Research School of Biology, Australian National University, Australian Capital Territory 0200, Australia
[2]Institute of Zoology, University of Graz, Graz 8010, Austria

Sexual selection is a cornerstone of evolutionary theory, but measuring it has proved surprisingly difficult and controversial. Various proxy measures – e.g. the Bateman gradient and the opportunity for sexual selection – are widely used in empirical studies. However, we do not know how reliably these measures predict the strength of sexual selection across natural systems, and most perform poorly in theoretical worst-case scenarios. Here we provide a rigorous comparison of eight commonly used indices of sexual selection. We simulated 500 biologically plausible mating systems, based on the templates of five well-studied species that cover a diverse range of reproductive life histories. We compared putative indices to the actual strength of premating sexual selection, measured as the strength of selection on a simulated 'mating trait'. This method sidesteps a key weakness of empirical studies, which lack an appropriate yardstick against which proxy measures can be assessed. Our model predicts that, far from being useless, the best proxy measures reliably track the strength of sexual selection across biologically realistic scenarios. The maximum intensity of precopulatory sexual selection (the Jones index) outperformed all other indices and was highly correlated with the strength of sexual selection. In contrast, the Bateman gradient and the opportunity for sexual selection were poor predictors of sexual selection, despite their continuing popularity.

POSTER
ABSTRACT

Size-assortative mating in simultaneous hermaphrodites: an experimental test and a meta-analysis

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Assortative mating by size has been argued to be widespread in the animal kingdom. However, the strength of size-assortative mating is known to vary considerably between species and the underlying mechanisms promoting this inter-specific variation remain largely unexplored. Size-assortative mating has been proposed to be particularly strong in simultaneous hermaphrodites, i.e. organisms that produce male and female gametes at the same time. Here, we build on this hypothesis by arguing that size-assortative mating mediated by sexual selection is generally stronger in reciprocally mating hermaphrodites compared with unilaterally mating species and separate-sexed organisms. We show a series of empirical tests suggesting that size-assortative mating in the unilaterally copulating freshwater snail *Physa acuta* is caused by spatial clustering of similar-sized individuals and not by mate choice. In addition, we present a meta-analysis testing, the hypothesis that sexual selection-mediated size-assortative mating is stronger in reciprocally copulating simultaneous hermaphrodites. Overall, we found significant size-assortative mating across 18 tested species and substantial inter-specific variation. Importantly, part of this variation can be explained by mating type, providing support for the hypothesis that size-assortative mating is stronger in reciprocally mating hermaphrodites compared with unilaterally mating species.

POSTER
ABSTRACT

Selective sperm storage influences paternity in a simultaneous hermaphrodite

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Studying the mechanisms of storage, use and digestion of allosperm is essential to understand postcopulatory sexual selection processes. Stylommatophoran gastropods have specialized organs to store and digest allosperm. Therefore the female function potentially has the ability to manipulate sperm use. We recently adjusted an immunocytochemical staining method to detect sperm of two different donors in the reproductive tract of the simultaneously hermaphroditic land snail *A. arbustorum* (Kupfernagel et al. 2013). In this study, we experimentally investigated patterns of sperm storage in the spermatheca of double-mated individuals of *A. arbustorum* (S2, measured as the proportion of sperm stored from the second of two donors) and compared that to patterns of paternity (P2, measured as the proportion of offspring assigned to the second of two donors). In doing so we are able to investigate whether biased sperm use occurs. First results show that S2 varies substantially between individuals, and that sperm storage also varies spatially within the spermatheca. This differential sperm storage in turn influences paternity patterns.

POSTER
ABSTRACT

Sex allocation, plasticity and the preference for the male role in simultaneous hermaphrodites

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According to theory, sexual selection should favor hermaphrodites that shift their reproductive resources between sexual functions depending on current mating-group size. Although often tested at the population level, sex allocation theory has been rarely tested at the individual level, i.e., by exposing the same individual to different levels of mating opportunities in subsequent time periods.
We tested individual plasticity in female allocation in iteroparous, simultaneously hermaphroditic polychaete worms that are obligate outcrossers and play only one sex role at each mating event. Each week, each focal hermaphrodite faced a novel social condition, where 1) their opportunities to mate as both males and females; 2) the level of competition for the male role varied. Finally, we exposed worms to simulated weekly changes in mating opportunities by manipulating the chemical signals hermaphrodites use to assess partner and/or rival numbers.
Results showed that wherever either real or simulated opportunities to mate as males increased, or competition over the male role increased, hermaphrodites produced fewer eggs (irrespective of density), suggesting they have no preference for the female role.
These results support the theoretical predictions for sex allocation plasticity in hermaphrodites, and show that hermaphrodites may have a preferred sex role. These results have implications for understanding the evolutionary transitions hermaphroditism/separate sexes.

POSTER
ABSTRACT

Estimating the response to sex-limited experimental evolution in a hermaphrodite

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The generally accepted theory about sex chromosome evolution starts with the evolution of a sex-determining locus, which follows by linkage with sexually antagonistic genes, and finally the inhibition of recombination between proto-sex chromosomes. We wanted to investigate the very initial step of sex chromosome evolution, by examining sexual antagonism and sex chromosome evolution in hermaphrodites. More specifically, we subjected populations of the simultaneous hermaphroditic flatworm *Macrostomum lignano* to sex-limited evolution in the lab. A GFP (green fluorescent protein) locus incorporated into the worm's DNA was used as a dominant sex-determining gene by only letting it pass through either eggs (in 'female' treatment) or sperm (in 'male' treatment) for each generation. Our hypothesis was that sex-specific fitness genes would accumulate and create a linkage to the GFP locus in the female and male treatments, respectively. Therefore, we expected to see an increased female fitness in the female treatment and an increased male fitness in the male treatment compared to the control treatment. The evolutionary lines (female-, male- and control lines) have been running for 1.5 years and are currently at generation 16. Here, we have estimated the response to the sex-limited evolution at generation 14, by measuring the fitness as number of offspring though eggs (female fitness) and sperm (male fitness) in each treatment.

POSTER
ABSTRACT

Differential gene expression analysis of seminal fluid proteins in a simultaneously hermaphroditic marine flatworm *Macrostomum lignano*

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The ejaculate often contains many seminal fluid proteins that are transferred along with sperm during mating. Since it is known that these proteins function in diverse ways to maximize male reproductive success, and often exerting strong effects on female reproductive physiology and behaviour, studies on Sfps have been increasing in sexual selection research for last decades. Considering that production of Sfps is costly, we expect individuals to strategically invest in Sfp production according to their social environment. This study examines the impact of changing social group size on Sfp gene expression in the simultaneously hermaphroditic marine flatworm *Macrostomum lignano*. It is well known that individuals of *M. lignano* which are kept in larger social group size invest more in male reproductive traits than individuals in smaller social group size, presumably as a consequence of sperm competition and as predicted from sex allocation theory. Thus, we expected that the transcriptional activity of Sfp genes in worms should differ between large versus small social group sizes. We measured Sfp gene expression in changing social group sizes across multiple inbred lines to assess genotypic and environmental variation and to test for genotype-environment interaction (GxE) effects. Preliminary analysis showed that a large fraction of tested genes show significant differential expression among genotypes and social group sizes, and around 15% of them show significant genotype–environment interaction.

POSTER
ABSTRACT

A targeted in situ hybridization screen identifies putative seminal fluid proteins in a simultaneously hermaphroditic flatworm

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During mating seminal fluid proteins (Sfps) are transferred along with sperm from the sperm donor to the recipient and these Sfps are thought to play a key role mediating post-mating sexual selection, by potentially modulate the recipients' behavior and physiology in ways that influence reproduction, and in sexual conflict. Despite the importance of Sfps for the reproductive physiology and behaviour of the ejaculate recipient, and the fertilizing ability of the donor, the identification and characterization of seminal fluid proteins have focused on just a few animal groups, mainly insects and mammals. Moreover, until know the seminal fluid profiling focus mainly on species with separate sexes. Here we report a comprehensive screen for putative seminal fluid proteins in the simultaneously hermaphroditic flatworm *Macrostomum lignano* based on existing RNA-seq data. For 147 transcripts known to be a) predominantly expressed in the tail region of worms, where the seminal fluid-producing prostate gland cells are located, and b) differentially expressed in social environments differing in sperm competition level, strongly implying that they represent a plastic aspect of male allocation in this species we performed whole mount in situ hybridization experiments. We identified 73 transcripts which showed prostate-specific expression expected for seminal fluid genes. Subsequent bioinformatic analysis revealed that 42 of the 70 putative seminal fluid genes are predicted to be located extracellular and at least 38 are predicted to possess a signal peptide implying secretion. Gene ontology analysis identifies genes involved in molecular processes such as oxidation-reduction processes or proteolysis and genes involved in the immune response. The ready availability of RNAi knockdown and other molecular methods in *M. lignano* means we can now begin to probe the function of this putative seminal fluid proteome, with diverse predicted roles impacting fertility and competitive fertilization success.

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