



**Simultaneously Hermaphroditic Organisms Workshop**  
10 - 12. February 2012, University of Torino, Italy  
Programme, Participants, Abstracts



**Organizers:**

**Gabriella Sella, Maria Cristina Lorenzi, Dáša Schleicherová**

**Meeting location: Museo Regionale di Scienze Naturali, Via Giolitti, 36, Torino**

# PROGRAMME

## Friday, 10. February 2012

**18:00 – 19:30** Welcome reception and registration

**20:00-** Individual Dinner in the City

## Saturday, 11. February 2012

**9:25-9:30** Presentation of the meeting

**9:30-10:10** Janet L. Leonard: The Evolution of Sexual Selection in Simultaneous Hermaphrodites

**10:10-10:50** Sara Carvalho and Henrique Teotónio: Life-history Evolution of *C. elegans* populations with varying outcrossing rates

**10:50-11:10** Coffee break

**11:10-11:50** Tim Janicke, Nikolas Vellnov, Patrice David: Inbreeding depression on male and female reproduction: insights from a hermaphrodite

**11:50-12:30** Yumi Nakadera, Christiaan Blom, Joris M. Koene: Quantifying male reproductive success of a simultaneously hermaphroditic snail species *Lymnea stagnalis*

**12:30-13:30** Lunch break

**13:30-14:10** Lucas Marie-Orleach, Tim Janicke, Micha Eichmann, Dita B. Vizoso, Katrien de Mulder, Eugene Berezikov, Peter Ladurner, Lukas Schärer: Fluorescent sperm in a transparent worm – Post copulatory sexual selection in a simultaneous hermaphrodite

**14:10-14:50** Alexandra Staikou and Marina-Elena Garefalaki: Determinants of paternity success in land snails

**14:50-15:10** Coffee break

**15:10-15:50** Rolanda Lange, Johanna Werminghausen, Ines Häderer, Nils Anthes: Traumatic mating strategies in gastropodid sea slugs

**15:50-16:10** Coffee break

**16:10-18:10 Discussion Session 1**

**Evening Conference Dinner**

**Sunday, 12. February 2012**

**9:30-10:10 Nausicaa Poulet, Nicolas Callemeyn-Torre, Clotilde Gimond, Anne Vielle, Christian Braendle:** Evolution and developmental plasticity of germline and reproduction in *Caenorhabditis* nematodes

**10:10-10:40 Maria Cristina Lorenzi, Dáša Schleicherová, Gabriella Sella:** Plasticity in sex allocation: comparisons among three species of polychaete worms

**10:40-11:00** Coffee break

**11:00-13:00 Discussion Session 2**

**13:00-** Lunch, departure

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# ABSTRACTS

## **The Evolution of Sexual Selection in Simultaneous Hermaphrodites**

**Janet L. Leonard**

*Long Marine Laboratory, University of California at Santa Cruz*

Over the last 30 years sexual selection in simultaneously hermaphroditic organisms has become a flourishing field. Initially, research into sexual selection in simultaneous hermaphrodites was premised on transferring then-current views of sexual selection in dioecious species to hermaphrodites. However, more careful analysis, and data, suggests that this is too simple. Here I suggest that the study of sexual selection in simultaneous hermaphrodites offers a useful way of testing hypotheses derived from dioecious species and can extend our understanding of sexual selection in general. In particular, early hypotheses such as egg-trading and sperm-trading were based on the general assumption of “all else being equal”. After 30 years of work, on an increasing range of taxa, we can begin to identify which factors are important in determining the strength and direction of sexual selection in simultaneous hermaphroditism and which factors play a role in determining which sexual role is preferable under which conditions. One factor which needs further consideration is the effect of the capacity for self-fertilization on the potential for, and strength of, sexual selection in simultaneous hermaphrodites.

## ABSTRACTS

### **Life-history Evolution of *C. elegans* populations with varying outcrossing rates**

**Sara Carvalho and Henrique Teotónio**

*Instituto Gulbenkian De Ciência, Oeiras*

The opportunity for natural and sexual selection depends on the mating system. While species with exclusive self-fertilization or outcrossing must resolve resource allocation to male and female function through development, those with varying rates of self-fertilization and outcrossing must also resolve resource allocation among mating partners that can have several sexual functions. We will report the evolution of reproductive schedules in a *C. elegans* experimental system whereby adaptation to a novel environment occurred in the context of androdioecy or dioecy. Experimental evolution took place during 100 generations at large population sizes, abundant genetic variation, and under density-independent and discrete-time dynamics. We find that irrespective of mating system the fitness components contributing to outcrossing among individuals have all similarly increased. Results suggest that spermatogenesis in hermaphrodites has degenerated although a high rate of self-fertilization is stably maintained under androdioecy. We will discuss these findings in light of inbreeding depression and the evolution of antagonistic and mutualistic sexual interactions within and between individuals.

## ABSTRACTS

### **Inbreeding depression on male and female reproduction: insights from a hermaphrodite**

**Tim Janicke<sup>1</sup>, Nikolas Vellnov<sup>1</sup> and Patrice David<sup>1</sup>**

<sup>1</sup>*Centre d'Ecologie Fonctionnelle et Evolutive, CNRS-UMR 5175*

Inbreeding depression - the reduced survival and fertility of offspring produced by related individuals - has been argued to be a major selective agent for the evolution of mating systems in sexually reproducing organisms. Until now, our knowledge on inbreeding depression in animals is mainly restricted to its effects on female reproduction but more recent empirical work provides accumulating evidence that inbreeding also depresses male fitness. We studied inbreeding depression in the simultaneously hermaphroditic freshwater snail *Physa acuta*, which provides the excellent opportunity to explore the effects of inbreeding on male and female reproductive performance within the same individual. Using a family breeding design, we compared the reproductive success of both sex functions between selfed and outcrossed focal individuals in a series of mating regimes, which reflect different levels of male competition. Specifically, the experimental manipulation of the mating regime included situations of no competition (i.e., one virgin partner), indirect competition (i.e., one already mated individual) and direct competition (i.e., two virgin partners). This experimental setup allowed us to test (1) for effects of inbreeding on male and female reproductive success, (2) whether there is genetic variation in inbreeding depression on male and female reproductive success and (3) whether effects of inbreeding on the male sex function are specific to the competition level. Finally, using the same experimental approach, we also studied (4) whether inbreeding and the level of male competition affects the opportunity for sexual selection.

## ABSTRACTS

### **Quantifying male reproductive success of a simultaneously hermaphroditic snail species *Lymnaea stagnalis***

**Yumi Nakadera, Christiaan Blom, Joris M. Koene**

*Ecological Science, VU University*

In contrast to the historical expectation, recent theoretical studies have indicated that simultaneously hermaphroditic animals also experience sexual selection, just like separate sexed species. The empirical study of sexual selection processes in hermaphrodites is fascinating and required for a full understanding of the evolution of this mode of reproduction. However, due to the fact that these animals are male and female at the same time, examining the proposed predictions and assumptions is a challenge. In particular, many simultaneously hermaphroditic taxa fertilize internally, can store and digest sperm and can self-fertilize their eggs, making precise measurement of their reproductive success difficult. Here, we present research that quantitatively measures male reproductive success of the great pond snail, *Lymnaea stagnalis*. In particular, we focused on how long they use sperm from a mating partner (allosperm) using a microsatellite marker. Previous work indicated that these snails store and use allosperm for about three months, but in that study the author used albino mutants (as genetic marker) and the mating history of the animals was unclear. Our results reveal that most individuals stop to use allosperm well before three months after they received sperm only once. Interestingly, we found considerable variation in the duration that received sperm were used (paternity longevity), as well as in male reproductive success. This suggests that there is potential for sexual selection on sperm storage traits in this species. Moreover, there is a significant correlation between paternity longevity and body size of donors and recipients. The latter is indicative of a male reproductive strategy via seminal fluid. We will discuss how these findings will form a good basis for further research into sexual selection in this simultaneous hermaphrodite, including more realistic experimental settings (e.g., multiple mating, different mating history).

## ABSTRACTS

### **Fluorescent sperm in a transparent worm – Post-copulatory sexual selection in a simultaneous hermaphrodite**

**Lucas Marie-Orleach<sup>1</sup>, Tim Janicke<sup>1,2</sup>, Micha Eichmann<sup>1</sup>, Dita B. Vizoso<sup>1</sup>,  
Katrien de Mulder<sup>3,4</sup>, Eugene Berezikov<sup>3</sup>, Peter Ladurner<sup>4</sup> & Lukas Schärer<sup>1</sup>**

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Post-copulatory sexual selection is an important evolutionary force, but in most species the relevant processes are internal and cryptic, and thus difficult to observe. Here we studied post-copulatory processes in the outcrossing hermaphroditic flatworm *Macrostomum lignano*, taking advantage of a transgenic line with ubiquitous green-fluorescent protein (GFP) expression. The transparency of the worms enables us to quantify *in vivo* the proportion of GFP-positive sperm relative to wild-type sperm a recipient has received. Furthermore, given that the GFP marker is genetically dominant we can use it to measure the resulting proportion of offspring sired by a GFP-positive donor. We used this tool to study two questions, namely sperm displacement and Bateman gradients. First, in a sperm displacement experiment we showed that the number of sperm received from a first donor decreased when a recipient copulated with a second donor. This suggests that by copulating, donors can displace sperm previously stored by competitors. Second, in a Bateman gradient experiment, we created groups of five individuals (one focal GFP and four wild-type individuals), and then performed sperm tracking and paternity analysis. We found a strong positive relationship between genetic mating success (i.e., the number of individuals from which the focal worm obtained paternity) and reproductive success for the male sex function, whereas the same relationship was not observed for the female sex function. Interestingly, this relationship was weaker when the mating success was assessed through the sperm mating success (i.e., the number of individuals in which the focal worm stored sperm). Finally, the male reproductive success depended to a large extent on the sperm-storage success (i.e., proportion of sperm stored in the mate by a focal sperm donor). These studies clearly document, that the addition of GFP expressing lines to the *M. lignano* model system offers tremendous opportunities to study the processes of post-copulatory sexual selection in simultaneous hermaphrodites.

# ABSTRACTS

## **Determinants of paternity success in land snails**

**Alexandra Staikou and Marina-Elena Garefalaki**

*Department of Zoology, School of Biology, Aristotle University of Thessaloniki*

Sperm competition is important in species with reproductive strategies that involve multiple mating and prolonged sperm storage such as simultaneously hermaphroditic land snails. In such species several traits of their mating behaviour and/or reproductive morphology may affect the outcome of sperm competition. Elaborate courtship behaviour such as dart shooting is known in several land snail species. In *Cornu aspersum* effective dart shooting behaviour has been shown to increase paternity success through a manipulative function of the mucus with which darts are coated, on the reproductive tract of dart recipients. Apart from dart shooting, other behavioural traits like mating order have been tested as potential determinants of paternity success in land snails. Double mating trials in *Cornu aspersum* have revealed that mating order may also affect paternity success but a clear priority pattern has not been demonstrated. We investigated the effect of behavioural and anatomical reproductive traits on paternity success of snails from triple mating trials. In 58% of the egg clutches analyzed all three sperm donors sired a variable proportion of offspring while in the remaining egg clutches sperm from only one or two sperm donors was used for the fertilization of the recipients' eggs. Zero paternity was observed in 16% of sperm donors. Mating order and the length of the epiphallus, which is the organ responsible for the formation of the spermatophore body, were identified as traits affecting paternity success

# ABSTRACTS

## **Traumatic mating strategies in gastropterid sea slugs**

**Rolanda Lange, Johanna Werminghausen, Ines Häderer, Nils Anthes**

*Animal Evolutionary Ecology Group, Institute for Evolution and Ecology, Faculty of Sciences, University of Tübingen*

We provide an update on most recent advances in understanding the evolutionary ecology of traumatic mating in the hermaphroditic sea slug family Gastropteridae. First, we will report the findings of new experiments trying to establish costs and benefits of traumatic mating. Second, we outline current progress in understanding the mechanics of traumatic injection in this group of slugs. Third, we present the initial stage of a phylogenetic analysis as a basis to reconstruct the evolutionary history of traumatic in the near future.

# ABSTRACTS

## **Evolution and developmental plasticity of germline patterning and reproduction in *Caenorhabditis* nematodes**

**Nausicaa Poulet, Nicolas Callemeyn-Torre, Clotilde Gimond, Anne Vielle & Christian Braendle**

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To better understand the role of gene-environment interactions in development and evolution, we study quantitative natural genetic variation and plasticity of the *Caenorhabditis* germline and reproductive system in different environments. Although the basic germline organization and processes are conserved among *Caenorhabditis* species, reproductive output and schedules vary both within and between species. We are interested in understanding how differences in offspring number and quality are coupled to the underlying properties and processes of the germline (e.g. sperm number and size, germ cell number, proliferation, apoptosis) and to what extent they are plastic, i.e. vary across different (ecologically relevant) environments.

In an initial analysis, we have quantified germline and reproductive phenotypes in 15 isolates of the three hermaphroditic species (*C. elegans*, *C. briggsae* and *C. sp. 11*). Overall, sperm number, germ cell number and offspring number are positively correlated; however, sperm number does not always closely match offspring number, indicating that isolates and species may differ in sperm fertility or efficiency of sperm use. The most striking observation is that many *C. sp. 11* isolates show a highly reduced offspring, sperm, germ cell number and mitotic zone relative to *C. elegans*.

To carry out an integrative analysis of germline and reproductive plasticity, we have characterized how e.g. germ cell proliferation, entry into meiosis and apoptosis are modified in N2 animals exposed to diverse conditions (such as liquid, starvation, passage through dauer, different bacterial food sources, ethanol, acetic acid, temperature shifts, osmotic or hypoxia). Our results confirm that diverse germline processes are highly environmentally sensitive. We also show that stressful conditions may reduce offspring number through reduction of either sperm fertility or number, as well as defects in germline progression. The plastic responses in reproductive features of *C. elegans* N2 may differ greatly from the ones observed in other wild isolates of *C. elegans*, *C. briggsae* or *C. sp. 11*, revealing considerable genotype-by-environment interactions. We will discuss these and other results in the context of how such differential plasticity of the reproductive system contributes to germline integrity and reproductive success in variable environments.

## ABSTRACTS

### **Plasticity in sex allocation: comparisons among three species of polychaete worms**

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Sex allocation in hermaphrodites is plastic. Phenotypic plasticity in sex allocation has species-specific properties in many simultaneous hermaphroditic taxa. Owing to the large biological diversity among the studied taxa, it is not easy to identify which factors promote or constrain plasticity in sex allocation.

We compared the ability to adjust sex allocation to mating opportunities in three closely related species of simultaneous hermaphrodites. We found that, in the polychaete worms *Ophryotrocha diadema*, *O. adherens* and *O. gracilis*, adults plastically changed their sex allocation when they were exposed to different levels of mating opportunities. However, the degrees of plasticity differed among species as well as between the male and the female functions. We discussed how the relative costs of male and female functions and ecological factors, such as habitat conditions and the prevailing population density in the wild, may influence the evolution of plasticity in sex allocation in each species.

