Simultaneous and Sequential Hermaphroditic Organisms Workshop

Center for Evolutionary and Functional Ecology (Cefe)
CNRS Campus, Montpellier, France

March 2-4 2020

Organizers Philippe Jarne and Patrice David

With the precious help of the administrative staff of Cefe and Labex Cemeb

Funded by:

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Practical information

The workshop will take place on the CNRS campus in Montpellier (1919 route de Mende), on the second floor of the Cefe (Center for Evolutionary and Functional Ecology; https://www.cefe.cnrs.fr/) building.

The CNRS campus can easily be reached by tram from down town – take tram line 1 (direction "Mosson"). Then two options:

- Exit at "Université des sciences et lettres". Take bus 22 (direction "Clapiers / Jacou"; every 30 min) or "La Navette" (direction "Agropolis via Vert-Bois"; every 10 min) – the bus station is adjacent to the tram station. Exit at "Vert-Bois". Then walk up the street "route de Mende" (lots of roadwork) for 400 m till CNRS main entrance (on your left). Same ticket for tram and bus.
- If you don't mind walking for 15-20 min. Exit at Saint Eloi, and then walk along the "avenue du Dr Pezet" (perpendicular to tram line; just before the tram station), pass a kind of roundabout (lots of work) and then follow the "route de Mende" in between the beautiful university buildings on both road sides (several buildings under construction). Pass a second roundabout (bus stop Vert-Bois) and continue straight ahead. Pass a third roundabout, and go on your left, still on "route de Mende" until reaching the CNRS main entrance (400 m; on your left).

Map at http://www.cnrs.fr/languedoc-roussillon/09com-presen-deleg/09-6-plan/Plan_acces_CNRS.pdf (in French … but it's a map)

Cefe is the first building (1960's architectural style, greenish looking; tripod on the picture below) on your right, 50 m down once you have passed the entrance barrier (bottom left on the picture).

We will have an informal welcome drink (and some food) on March 2nd from 7:30 pm at the Broc Café (2 boulevard Henri IV, https://www.facebook.com/broccafemontpellier) down town Montpellier, just in front of the "Jardin des plantes" (botanical garden), and not far away from the Peyrou place and its Arc de Triomphe (see below). It can easily be reached by tram or by foot, if your hostel is down town.

The social dinner on March 3rd will be hosted at Al Manara (4 rue du Clos René; http://www.almanara.fr/), a Libanese restaurant down town Montpellier. Al Manara is in the only small street on your left when going down the Place de la Comédie (below) to the railway station. It can easily be reached by tram or by foot, if your hostel is down town.

Schedule at a glance

SHOW MONTPELLIER MARCH 2nd	
19:30	**Welcome drink – Broc Café**
SHOW MONTPELLIER MARCH 3rd	
9:00-9:30	Welcome and introduction to the workshop
9:30-10:10	**The evolution of hermaphroditism in tardigrades -** Matteo Vecchi, Sara Calhim
10:10-10:50	**Lessons from 100+ worms: reproductive trait evolution in simultaneous hermaphrodites -** Jeremias N. Brand, Pragya Singh, R. Axel W. Wiberg, Gudrun Viktorin, Lukas Schärer
10:50-11:10	Coffee break
11:10-11:40	**Sex-limited experimental evolution on a simultaneous hermaphroditic flatworm leads to differential sex allocation responses -** Qinyang Li, Jessica Abbott
11:40-12:20	**Evolution with different sexes and sex ratios -** Sophie Labaude, François Mallard, Luke Noble, Christian Braendle, Henrique Teotónio
12:20-13:00	**Mito-nuclear interactions and gynodioecy in the freswhater snail –** Patrice David, Mathilde Düfay, Philippe Jarne, Tristan Lefébure, Emilien Luquet, Sandrine Plenet, Jonathan Romiguier
13:00-14:00	Lunch
14:00- 14:40	**Effects of sex-limited experimental evolution on a hermaphrodite transcriptome -** Aivars Cīrulis, Anna K. Nordén, Lukas Schärer, Steve Ramm, Jessica K. Abbott
14:40-15:20	**How fast does the "speciation clock" tick in selfing versus outcrossing lineages? -** Lucas Marie-Orleach, Christian Brochmann, Sylvain Glémin
15:20-16:00	**Self-fertilization and morphological stasis in an ancient group of worldwide freshwater snails -** Pilar Alda, Antonio A. Vázquez, Robert T. Dillon Jr., Patrice David, Philippe Jarne, Jean-Pierre Pointier, Sylvie Hurtrez-Boussès
16:00-16:30	Coffee break
16:30-17:10	**Hermaphrodites in undergraduate biology education: broadening horizons in first- and second-year courses** - Cynthia G. Norton
17:10-17:30	**Sex shells: Gender fluidity in the modern age (poster on screen) -** Jonathan Ho, Joris M. Koene
17:30-18:00	Brain storming
19:30	**Dinner at Al Manara**

SHOW MONTPELLIER MARCH 4th	
9:00-9:40	**How sexual selection shapes sexually-dimorphic and non-dimorphic plant traits? -** Jeanne Tonnabel, Patrice David, Etienne Klein, Mathilde Dufaÿ, John Pannell
9:40-10:00	**Comparison of the effect of seminal fluid proteins on the suck behaviour in two flatworm species of the genus *Macrostomum* (poster on screen)** - Jeanne Brülhart, Lukas Schärer
10:00-10:40	**Changing sex in different directions –** Chiara Benvenuto
10:40-11:00	Coffee break
11:00-11:40	**Mate choice and sex role preference in hermaphroditic worms -** Laura Picchi, Guénaël Cabanes, Loïc Planche, Adélia Delouche and Maria Cristina Lorenzi
11:40-12:20	**Mining transcriptome assembly data for insights on genome evolution in the genus *Macrostomum* -** R. Axel W. Wiberg, Jeremias N. Brand, Lukas Schärer
12:20-13:00	**Divergence of seminal fluid expression and function among natural snail populations -** Yumi Nakadera, Alice Smith, Lea Daupagne, Marie-Agnes Coutellec, Joris M. Koene, Steven A. Ramm
13:00-14:00	Lunch
14:00-14:40	**Seminal fluid-mediated fitness effects in *Macrostomum* flatworms** - Steve Ramm, Bahar Patlar, Michael Weber, Athina Giannakara, Tim Temizyürek
14:40-15:20	**Cuban cupids: accessory gland product transfer in hermaphroditic snails and their potential behavioural and physiological avoidance -** Joris M. Koene, Bernardo Reyes-Tur
15:20-16:30	Final discussion, including coffee break

Abstracts

Tuesday 9:30

The evolution of hermaphroditism in tardigrades

Matteo Vecchi & Sara Calhim

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Until the late 1970s, it was thought that the taxa in the phylum Tardigrada were either bisexual or parthenogenetic. Since then, hermaphroditic species have been described, but these are rare and mostly limited to the class Eutardigrada. Although the patterns of gamete maturation in hermaphroditic eutardigrades have been described, the evolutionary patterns of this sexual condition has never been analysed. Taking advantage of the many currently available DNA sequences, we reconstructed the phylogeny of 140 eutardigrade species and mapped the presence of hermaphroditism in order to infer the number of gains and losses of this trait. Since there were no conclusive data about the sexual condition for many of the species (~40%), we used a Bayesian stochastic mapping approach to simultaneously reconstruct the ancestral and the unobserved tips states. Our analyses show that hermaphroditism evolved independently in the four Eutardigrada orders. Furthermore, its overall rarity (<3% of all species) and absence at ancestral nodes suggest a long-term disadvantage of this self-fertilizing reproductive mode.

Tuesday 10:10

Lessons from 100+ worms: reproductive trait evolution in simultaneous hermaphrodites

Jeremias N. Brand, Pragya Singh, R. Axel W. Wiberg, Gudrun Viktorin, and Lukas Schärer

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Recent efforts in our lab have focussed on assembling a large comparative data set in the free-living flatworm genus *Macrostomum*, with the aim of studying reproductive trait evolution and coevolution in this group of copulating simultaneous hermaphrodites, with respect to both morphology and behavior. Briefly, we have collected tissue samples and phenotype data on ~150 *Macrostomum* species from all over the world, and generated a highly resolved phylogenomic phylogeny using transcriptome data from ~100 species, complemented with Sanger sequences from an additional ~50 species. This data set reveals many independent shifts from reciprocal copulation to hypodermic insemination—representing two alternate states of sexual conflict resolution—and shows that these shifts are associated with shifts, not only in sperm and genital morphology, but also in sex allocation. Moreover, we find evidence for coevolution between a range of different aspects of mating behavior. Finally, and taking advantage of the many independent shifts we observe from the reciprocal to the hypodermic

mating syndrome, we are now trying to use the extensive transcriptome data to identify candidate transcripts that may underlie the two syndromes.

Tuesday 11:10

Sex-limited experimental evolution on a simultaneous hermaphroditic flatworm leads to differential sex allocation responses

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Sex allocation (SA) and sexual conflict are theorized to be linked in simultaneous hermaphrodites. Given the fundamental assumption of trade-off between female and male functions, intensified mate competition should male-bias sex allocation. Using sex-limited experimental evolution, we generated lines where worms were free to evolve toward the optimum of their female or male function, hereafter f-line and m-line worms respectively. We hypothesize that f-line worms would show a less male-biased SA than m-line worms. To test this hypothesis, I firstly subjected juvenile worms to develop as virgins in common garden, recording their initial SA; I subsequently obtained individuals' new SA in response against ecological factor combinations of food availability (present/absent) and mating group size (pair/octets). Results show that 1/ No initial difference in SA among evolved lines. 2/ When food was absent, f-line worms showed significantly less male-biased SA, regardless of mating group size. 3/ In paired mating groups, f-line worms showed a trend of relative testes reduction, regardless of the presence of food. In general, our results indicate f-line worms were less capable of male-biasing their sex allocation.

Tuesday 11:40

Evolution with different sexes and sex ratios

Sophie Labaude, François Mallard, Luke Noble, Christian Braendle, Henrique Teotónio

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Evolution of sexual functions in hermaphrodites can be explained in part by antagonistic selection between individuals assuming different genders or between individuals of different sexes. In part, it can also be due to selection on conflicting sexual allocation within each individual. We conducted 100 generations of experimental evolution in the nematode *C. elegans* under dioecy (males and females), androdioecy (males and hermaphrodites ; hermaphrodites that can only outcross with males), and monoecy (hermaphrodites only capable of self-fertilization). Through back genetic-transformation we were able to transform females to the ancestral hermaphroditic condition, after evolution, as well as obtain males from the monoecious populations. We will present (very) preliminary results on the evolution of

hermaphrodite « selfing » function and « outcrossing » function as well as male function, as assayed under competition and gene expression of germline development.

Tuesday 12:20

Mito-nuclear interactions and gynodioecy in a freswhater snail

Patrice David, Mathilde Düfay, Philippe Jarne, Tristan Lefébure,
Emilien Luquet, Sandrine Plenet, Jonathan Romiguier

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Genetic conflicts occur within organisms because different genes maximize their transmission in different ways, especially when they are not equally transmitted by the two sexes. A spectacular example of genetic conflict is gynodioecy in hermaphroditic plants, with interactions between cytoplasmic genes causing male sterility (CMS) and nuclear genes restoring it. Gynodioecy has extensively been studied in plants, and sophisticated models have been developed to predict the evolutionary dynamics of gynodioecy. We recently discovered the first animal example of gynodioecy due to CMS in a population of the hermaphroditic freshwater snail Physa acuta. CMS is associated with a single mitotype, widely divergent from other mitotypes, but restorer genes have not been characterized. We will present the first results on gynodioecy in Physa acuta, as well as the main lines of our ongoing research program on this topic.

Tuesday 14:00

Effects of sex-limited experimental evolution on a hermaphrodite transcriptome

Aivars Cīrulis, Anna K. Nordén, Lukas Schärer, Steve Ramm, Jessica K. Abbott

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Simultaneous hermaphrodites, which reproduce reciprocally, are good model organisms to study evolution of sex determination. First of all, acquisition of a mutation causing sterility in one sex role (i.e. a sex determining gene) can lead to selection favouring linked sex-specific fitness alleles. This is expected to be followed by recombination suppression between these loci, leading to the evolution of sex chromosomes. Later acquirement of an opposite sex role sterility mutation in the hermaphroditic partner leads to the final evolution of two separate sexes. The non-recombining sex-determining region later expands by acquiring more sex-specific genes, thus causing sex chromosomes to become heteromorphic. However, as both sexes still share most of the genome, shared genes with opposite fitness effects between the sexes must acquire sex-biased or sex-specific gene expression. Thus, to experimentally observe this process, we subjected a simultaneous reciprocally mating hermaphrodite *Macrostomum lignano* to sex-limited selection using a GFP marker as a sex-determining locus. In this setup

male-limited selection resembles XY chromosome evolution, while female-limited selection – ZW. After 21-22 generations of sex-limited experimental evolution, we performed RNA-seq to see if we can indeed observe divergence in expression profiles of male- and female-selected lines. Our results show that there is an evolving divergence between male- and female-selected lines compared to controls.

Tuesday 14:40

How fast does the "speciation clock" tick in selfing versus outcrossing lineages?

Lucas Marie-Orleach, Christian Brochmann, Sylvain Glémin

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Self-fertilisation is relatively common in simultaneously hermaphroditic plants and animals. This sexual system has important ecological and evolutionary consequences. On the one hand, selfing allows lone individuals to reproduce, and allows individuals to rechannel resources invested into finding and attracting sexual partners or pollinators into other fitness functions. On the other hand, selfing increases inbreeding and genetic drift, and decreases effective recombination and migration rates, which are expected to elevate the rate of species extinction in the long run. However, it is less clear how selfing affects speciation rates. Conflicting arguments predict that natural selection will either speed up or slow down speciation rates in selfing lineages. Here, we aim to use theoretical models to test how fast the "speciation clock" ticks in selfing versus outcrossing lineages. Specifically, we use the Bateson-Dobzhanzky-Muller model, and test how fast genetic incompatibilities arise between populations of different selfing rates. We will show preliminary results, and discuss future development.

Tuesday 15:20

Self-fertilization and morphological stasis in an ancient group of worldwide freshwater snails

Pilar Alda, Antonio A. Vázquez, Robert T. Dillon Jr., Patrice David, Philippe Jarne, Jean-Pierre Pointier, Sylvie Hurtrez-Boussès

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Self-fertilization is generally thought to be a derived character tending to drive species towards extinction by loss of evolutionary potential over time scales on the order of 1–2 Myr. In a group of freshwater pulmonate snails—the lymnaeid genus *Galba*—, however, selfing seems to have remained stable for a much longer period of time. All *Galba* species worldwide (except one) have also retained a cryptic phenotype, *i.e.* a very similar shell morphology and reproductive

anatomy. Contrary to the well-documented long-term negative effects of self-fertilization, populations of *Galba* have not merely thrived but invaded suitable habitat on six continents. In this study, we investigate the origin of such exceptional mating system stasis, together with morphological crypsis. We develop an integrative approach combining morphological observations with molecular markers (microsatellites and DNA sequences for four genes) to analyze *Galba* samples using Bayesian phylogenetic reconstruction. Our analysis suggested that the most recent common ancestor of *Galba* already has the cryptic phenotype and evolved ca. 22 Myr ago. We conclude that crypsis in *Galba* may best be explained by shared morphological stasis. *Galba* populations live in temporary habitats which may mitigate both predation and interspecific competition. Adaptation to such habitats may impose strong stabilizing selection for a shell morphology able to resist desiccation and concomitant morphological stasis. Static reproductive anatomy may have initially reflected a shared adaptation to self-fertilization in unpredictable habitat patches. Once established, however, self-fertilization would promote rapid erosion of genetic variation to reinforce morphological stasis that may have originated for other reasons.

Tuesday 16:30

Hermaphrodites in undergraduate biology education: broadening horizons in first- and second-year courses

Cynthia G. Norton

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With so much public discussion of gender issues in the US, it is important to introduce students to the breadth of reproductive expressions in the biological world early in their university education. I do this in two ways: first by centering a first-year research project on the reproduction of hermaphroditic snails, and second by focusing a second-year writing seminar on "Varieties of Animal Reproduction." The laboratory component of our Foundations of Biology I course is a semester-long investigation where students are guided through the process of scientific research. With support from instructors, each team frames a question, writes a proposal, carries out experiments, analyzes data and presents a poster. This year, all students in one lab section worked with my research organism, the freshwater snail Planorbella trivolvis. This focus not only challenges them to think about hermaphroditism as a natural phenomenon, but also provides valuable pilot data for ongoing work in my lab. In the seminar course, I provide background information about the diversity of animal mating strategies and sexual expressions (simultaneous and sequential hermaphroditism, same sex courtship, behavioral gender reversals, polygamy, etc) and students each choose a subtopic about which to write a review paper. While the course is focused on developing critical reading, writing, and communication skills, we also emphasize the connections between biology and society – so challenge them to broaden their views of what is "natural" and consider how an understanding of biological diversity might (or might not) be relevant to appreciation of human diversity and dignity.

Tuesday 17:10 (poster on screen)

Sex shells: Gender fluidity in the modern age

Jonathan Ho, Joris M. Koene

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As humans we tend to think in binary male and female stereotypes, but how natural is this? This question gets to the heart of our winning Bio Art & Design award project in which we use the hermaphroditic sexuality of snails as a metaphor for gender fluidity. Our aim is to take the visitor beyond the versatility of flexible sex roles in hermaphroditic snails into the ever expanding opportunities we humans have to shape our own identities. Think of how hormone therapy, artificial insemination, assisted reproduction, sperm banks and genital modification all contribute to changing the role that gender and sexual fluidity play in society. Recent medical advances, combined with the increased use of gender-fluid identities in popular culture, enables the modern human to envision a reality that extends beyond the traditional confines of one's biological sex. This project combines the philosophical inquiry of science with art, creating an immersive installation chronicling the complex and transgressive capabilities of hermaphroditic fluidity.

Wednesday 9:00

How sexual selection shapes sexually-dimorphic and non-dimorphic plant traits?

Jeanne Tonnabel, Patrice David, Etienne Klein, Mathilde Dufaÿ, John Pannell
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Following intense debates on whether and how sexual selection might operate in plants, it is now widely accepted that selection to access sexual partners is an important force in the plant kingdom. Sexual selection may drive variance in reproductive success between pollen donors both because of differences in their ability to disperse pollen to mates and to produce competitive pollen tubes that will win the race towards fertilization within maternal tissues. This seminar will present experiments aimed at testing whether sexual selection can shape sexual dimorphism in dioecious plant species and drive sexual conflict in hermaphroditic plant clades. Thanks to paternity analyses, we showed that key predictions of the theory of sexual selection, the Bateman principles, hold in the dioecious wind-pollinated plant species *Mercurialis annua*. Selection gradient analyses further revealed that morphologies allowing to disperse pollen further allowed plant to reach more sexual partners and to yield higher reproductive output. Paternity analyses also showed that plants experienced larger competition for accessing mates with higher plant density. After only three generations of evolution at low *versus* high plant density, male plants that evolved at high-density showed larger plant sizes than the original based population, possibly as a consequence of sexual selection. To study the

importance of sexual selection in driving sexual conflicts in hermaphrodites, we are currently starting an experimental evolution protocol implementing monogamy *versus* polygamy during the phase of competition among pollen tubes in the herbaceous fast-cycling *Brassica rapa*. Such experiment will aim at testing whether polygamy can drive the evolution of more selective maternal tissues, through evolution of pistil morphologies and physiology, resembling a sexy son process.

Wednesday 9:40 (poster on screen)

Comparison of the effect of seminal fluid proteins on the suck behaviour in two flatworm species of the genus *Macrostomum*

Jeanne Brülhart, Lukas Schärer

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Simultaneous hermaphrodites are often more eager to donate than to receive sperm, which can lead to a conflict over which role each mating partner plays during copulation. Reciprocal mating, in which the two mating partners act both as sperm donor and recipient, is a way of resolving this conflict. However, the sperm recipient can evolve post-mating mechanisms to remove unwanted received ejaculate. Such "female resistance" traits can in turn select for "male persistence" traits in the sperm donor to overcome the resistance adaptations in the sperm recipient. Post-mating sexual conflict over the fate of received ejaculate can potentially lead to countless antagonistic adaptations and counter-adaptations, as each party tries to regain control. Seminal fluid proteins (SFPs) are transferred together with sperm during mating and are key mediators of adaptations linked to post-mating sexual conflict in many groups of organisms. In the hermaphroditic flatworm genus *Macrostomum*, multiple reciprocally mating species display the "suck behaviour", likely a female resistance trait, during which individuals seem to suck some of the received ejaculate out of their female genital opening after mating. As a male persistence trait, a previous study in *M. lignano* identified two SFPs, Mlig-pro31 and Mlig-pro32, which seem to manipulate the mating partner's behaviour by decreasing its suck propensity after mating. The species *M. janickei* is closely related to *M. lignano* and also displays the suck behaviour. In this study, we tried to identify orthologs of the two identified SFPs and investigated their function in *M. janickei* by knocking them down with RNA interference to see if this male persistence trait is shared across different *Macrostomum* species.

Wednesday 10:00

Changing sex in different directions

Chiara Benvenuto

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Sequential hermaphrodites are often combined in one large group, as sex changers. However, changing sex from male to female (protandry), from female to male (protogyny), or from one sex to the other and back (bi-directional sex change) has strong ecological and evolutionary consequences. Indeed, these different life-history strategies present dissimilar social organizations and reproductive modes, from near-random mating in protandry to aggregate- and harem-spawning in protogyny. Sex change has intrigued evolutionary biologists for decades and a great amount of information has been gathered on sex determination, sex differentiation and the plasticity of sex change in many invertebrates and in fish (the only vertebrate group displaying sequential hermaphroditism), but less attention has been devoted to specific differences based on the direction of sex change. This is an important variable to predict demographic changes and resilience in sex-changing populations and should be incorporated more clearly in evolutionary theoretical work, as well as in conservation and management practices.

Wednesday 11:00

Mate choice and sex role preference in hermaphroditic worms

Laura Picchi, Guénaël Cabanes, Loïc Planche, Adélia Delouche, Maria Cristina Lorenzi

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In unilaterally mating hermaphrodites, individuals have the option to mate as females or males, and their choice may be affected by their physiological status. Depending on their choice, hermaphrodites might choose different partners: individuals willing to mate as males are expected to prefer different partners with respect to individuals willing to mate as females. We tested this hypothesis in the hermaphroditic worms, *Ophryotrocha diadema*, using the level of egg maturation as a proxy for the motivation to mate as females (when worms have ready-to-lay eggs) or as males (when worms do not have ready-to-lay eggs). We offered the worms the choice between partners with or without ready-to-lay eggs and measured their preference in a Y-maze apparatus which prevented physical contact between partners. Our results show that hermaphrodites without eggs, and therefore ready to mate in the male role only, significantly preferred partners ready to mate as females (i.e., with ripe eggs), though this preference tends to weaken when the worms mature new eggs. The fact that the worms exhibited a preference even when prevented from physically contacting other worms documents that mate choice is mediated by chemical cues released by conspecifics in the water, which convey information on the egg maturation level of potential partners. Overall, this study suggests that mate preference in *O. diadema* hermaphroditic worms is flexible and depends on their egg maturation level and

motivation to play a given sexual role: as these vary, the traits they prefer in their partners vary as well.

Wednesday 11:40

Mining transcriptome assembly data for insights on genome evolution in the genus *Macrostomum*

R. Axel W. Wiberg, Jeremias N. Brand, Lukas Schärer

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Recently, we have produced *de novo* transcriptome assemblies for ~100 species in the genus *Macrostomum*, a group of simultaneously hermaphroditic flatworms. In a broad comparative project, we focus on contrasting patterns in the two main mating syndromes of this group, namely reciprocal copulation and hypodermic insemination, that represent different outcomes of sexual conflict over mating roles and have evolved independently multiple times. Early analyses of this dataset concentrated on patterns of molecular evolution at candidate reproduction-related genes. The results suggest a general pattern of diversifying selection at reproduction-related genes due to ongoing sexually antagonistic coevolution. They also show patterns that may suggest an influence of reduced population sizes in species that are probably able to self. However, there is much more that can be learned from these rich data. In particular we are interested in contrasting patterns of gene presence/absence across species that differ in their reproductive morphologies. There are already hints at striking phylogenetic patterns with some clades apparently completely lacking some genes. If the same holds true for species that either do or do not express certain reproductive traits, then such analyses could reveal candidate genes that underlie these traits. However, distinguishing this signal from the noise of variation in assembly quality is a challenge. Here I will present our approaches to these questions and some preliminary results.

Wednesday 12:20

Divergence of seminal fluid expression and function among natural snail populations

Yumi Nakadera, Alice Smith, Lea Daupagne, Marie-Agnès Coutellec,
Joris M. Koene, Steven A. Ramm

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Seminal fluid proteins (SFPs) are proteins in ejaculated, and they trigger drastic changes in mating partner. Another peculiarity of SFPs is their rapid evolution revealed from cross-species comparisons. One of the key remaining questions is what the driving force of rapid SFP

evolution is. Based on their physiological functions, it is widely expected that SFP evolution is driven by sexual selection and sexual conflict. If so, a signature of rapid evolution driven by selection should also be detectable within species. Here we attempted to examine this hypothesis by combining the power of a common garden experiment and functional assays employing artificial injection of seminal fluid. First, we measured SFP gene expression of multiple families taken from four natural populations. Second, we artificially injected seminal fluid extracted from these populations, and assessed their impact on key fitness components of standardised mating partners. As the result, we detected divergence of SFP gene expression and seminal fluid functions in field populations, implying the association between SFP composition and functional consequences. This study contributes to filling the gap between highly controlled laboratory examinations and cross-species comparisons in SFP research, it and supports that intraspecific variation of SFPs is promising and accessible variation to test the hypotheses for revealing the processes of rapid SFP evolution.

Wednesday 14:00

Seminal fluid-mediated fitness effects in *Macrostomum* flatworms

Steve Ramm, Bahar Patlar, Michael Weber, Athina Giannakara, Tim Temizyürek

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Seminal fluid proteins (SFPs) are crucial mediators of sexual selection and sexual conflict, since they are uniquely positioned to influence female reproductive physiology and behaviour after mating. However, the complexity, functional redundancy and rapid evolution of seminal fluid means that identifying which seminal fluid proteins induce specific physiological or behavioural responses in mating partners is not straightforward, and predicting their evolutionary trajectories is further complicated by the multivariate nature of this secretion. I will here report on our recent efforts to understand the function and evolution of seminal fluid in the free-living, simultaneously hermaphroditic flatworm *Macrostomum lignano*. I will focus on 1) our use of quantitative genetics combined with molecular tools to identify novel seminal fluid-mediated effects and 2) experiments designed to partition genetic and environmental sources of multivariate variation in seminal fluid expression, which have revealed evidence of genotype-environment-interactions linked to sperm competitive ability and thus fitness.

Wednesday 14:40

Cuban cupids: accessory gland product transfer in hermaphroditic snails and their potential behavioural and physiological avoidance

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Animals display a remarkable range of complex reproductive morphology and behaviours, many of which are related to the transfer of accessory-gland products (ACPs). Such products generally affect the sperm recipient in such a way that they enhance the fertilization success of the sperm donor. When this is in disagreement with the recipient's reproductive optimum, a conflict of interest ensues. The love dart of snails is arguably one of the best examples of how such conflict can escalate evolutionarily. This sharp, calcareous device is stabbed through the partner's body wall to inject ACPs that affect the recipient's physiology. Here, we describe how such darts are used in hermaphroditic Cuban tree snails of the genus *Polymita*. The repeated stabbing of darts by these Cuban snails differs drastically from better-studied land snails, so they can offer additional insight into the evolution of this enigmatic reproductive trait. Besides reporting on the peculiarities of their dart shooting behaviour, our findings illustrate that the mating interactions and morphology of these snails offer a unique potential for avoiding the dart's effects behaviourally and physiologically. Since these species are endemic and threatened, understanding their reproductive biology is expected to contribute to conserving their role in agroecosystems.

List of participants

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