

2nd International *Macrostomum* Meeting

Program, Participants, Abstracts and General Information

31. October - 02. November 2008, Basel, Switzerland



an unknown species of *Macrostomum* from Tvärminne, Finland

Organizers:

Lukas Schärer, Tim Janicke, Peter Sandner,
Kiyono Sekii and Dita Vizoso

PROGRAM

Friday, October 31, 2008

Taxonomy workshop (with Tom Artois and Lukas Schärer)

- 9:00-12:30 Collection, extraction, and observation of free-living flatworms from samples contributed by the participants
- 12:30-14:00 Individual Lunch at the Basler Herbstmesse (Basel Autumn Fair)
- 14:00-15:00 Digital documentation, fixation and serial sectioning of free-living flatworms
- 15:00-16:00 The Macrostomorpha.info website: features and functions
- 16:00-16:30 Coffee Break
- 16:30-17:30 Steps required to taxonomically describe a free-living flatworm
- 18:00-19:30 Welcome Drinks and Registration at the Zoological Institute
- 20:00- Individual Dinner in the City

Saturday, November 1, 2008

- 09:00-10:00 **Invited Talk:** *Drosophila* Sex-Peptide: A 'Swiss Army Knife - Peptide' for Reproduction
KUBLI, E.
- 10:00-10:30 *Macrostomum* in Innsbruck: current advancements and future goals
LADURNER, P.
- 10:30-11:00 Coffee Break
- 11:00-11:30 RNA interference analysis of stem cell and germ line genes in *Macrostomum*
PFISTER, D., DE MULDER, K., KUALES, G., SEKII, K., SENN, C. AND LADURNER, P.
- 11:30-12:00 The exceptional capacity of *Macrostomum lignano* to recover from radiation
DE MULDER, K., PFISTER, D., KUALES, G., EICHBERGER, P., SEKII, K., BORGONIE, G. AND LADURNER, P.
- 12:00-12:30 *Macrostomum* EST database
OSTERMANN, T. AND LADURNER, P.
- 12:30-14:00 Lunch

PROGRAM

Saturday, November 1, 2008, cont.

- 14:00-14:30 Social group size affects sex allocation and mating behaviour in *Macrostomum lignano*
JANICKE, T. AND SCHÄRER, L.
- 14:30-15:00 Experimental tests of sex allocation theory using the RNAi-knockdown approach
SEKII, K., DE MULDER, K., SALVENMOSER, W., LADURNER, P. AND SCHÄRER, L.
- 15:00-15:30 Genome duplication in *Macrostomum*. A genus-wide phenomenon?
VIZOSO, D. B., SANDNER, P. AND SCHÄRER, L.
- 15:30-17:00 Posters and Coffee
- 17:00-18:00 Lab Demonstrations and Beer
- 19:00 Joint Dinner

Sunday, November 2, 2008

- 9:00-9:30 *Macrostomum lignano*: capable of virgin reproduction?
HOUBEN, A. M., PLUSQUIN, M., SMEETS, K. AND ARTOIS, T.
- 9:30-10:00 Lifespan, survival curve and age-related mortality rate of the flatworm *M. lignano*
MOUTON, S., WILLEMS M., BACK, P., BRAECKMAN, B. AND BORGONIE, G.
- 10:00-10:30 Effect of cadmium on neoblasts and oxidative stress at the level of gene-expression
PLUSQUIN, M., GEERDENS, E., HOUBEN, A., CUYPERS, A., ARTOIS, T. AND SMEETS, K.
- 10:30-11:00 Coffee Break
- 11:00-11:30 Flatworm as ideal model organisms: Real-time RT-PCR gene expression after exposure to different stress factors
GEERDENS, E., PLUSQUIN, M., DEGHESELLE, O., CUYPERS, A., ARTOIS, T. AND SMEETS, K.
- 11:30-12:00 Are there stem cell niches in *Macrostomum lignano*?
SALVENMOSER, W., ADAMSKI, Z., PFISTER, D., EGGER B. AND LADURNER, P.
- 12:00-12:30 Postembryonic development of the gonads in *Macrostomum lignano*
HEISS, H., SALVENMOSER, W., PFISTER, D., DE MULDER, K., KUALES, G. AND LADURNER, P.
- 12:30-14:00 Lunch
- 14:00-15:30 General Discussion
- 16:00 Departure or some sightseeing

PROGRAM

Posters and Coffee (Saturday, 15:30-17:00)

Determinants of mating group size and sperm transfer success in a hermaphroditic flatworm
JANICKE, T. and SCHÄRER, L.

Sperm precedence in *Macrostomum lignano*
SANDNER, P. and SCHÄRER, L.

Does non-random segregation of DNA-strands occur during stem cell division in *Macrostomum lignano*?
VERDOODT, F., WILLEMS, M., MOUTON, S., DE MULDER, K., BORGONIE, G. and LADURNER, P.

Laboratory Demonstrations and Beer (Saturday, 17:00-18:00)

PARTICIPANTS

Johannes G. ACHATZ

Institute of Zoology
University of Innsbruck
Technikerstrasse 25
6020 Innsbruck
Austria
Tel: ++43 676 3661579
Fax: ++43 512 507 2930
Email: csac2718@uibk.ac.at

Tom J. ARTOIS

Centre for Environmental Sciences
Hasselt University
Universitaire Campus Gebouw D
3590 Diepenbeek
Belgium
Tel: +32-11-268309
Fax: +32-11-268301
Email: tom.artois@uhasselt.be

Mieke L. F. BOONE

University of Ghent
Biology Department
Nematology Section
K. L. Ledeganckstraat 35
9000 Ghent
Belgium
Tel: +32 9264 8740
Fax: +32 9264 5344
Email: mlboone.Boone@UGent.be

Maria del Mar DE MIGUEL-BONET

The Hartenstein Lab, Department of Molecular,
Cell and Developmental Biology
University of California, Los Angeles (UCLA)
621 Charles E. Young Drive South. LSB 4214
Los Angeles, CA 90095
USA
Tel: +1 310 206 7376
Email: demiguelbm@ucla.edu

Katrien K. DE MULDER

1) Institute of Zoology
University of Innsbruck
Technikerstrasse 25
6020 Innsbruck
Austria
Tel: ++43 512 507 6170
Fax: ++43 512 507 2930

2) Department of Biology

Nematology Section
University of Ghent
Ledeganckstraat 35
9000 Ghent
Belgium
Tel: ++3292648740
Fax: ++3292645344
Email: katrien.demulder@Ugent.be

Micha EICHMANN

Evolutionary Biology
Zoological Institute
University of Basel
Vesalgasse 1
4051 Basel
Switzerland
Tel: ++41 786 282 467
Fax: ++41 61 267 03 62
Email: micha.eichmann@stud.unibas.ch

Ellen GEERDENS

Research Group Biodiversity
Phylogeny and Population Studies
Department SBG, Hasselt University
Campus Diepenbeek
Agoralaan - Building D
3590 Diepenbeek
Belgium
Tel: +32-11268383
Fax: +32-11268301
Email: ellen.geerdens@student.uhasselt.be

Helene HEISS

University of Innsbruck
Institute of Zoology
Technikerstrasse 25
6020 Innsbruck
Austria
Tel: ++43-512-507-6161
Fax: ++43-512-507-2930
Email: helene.heiss@student.uibk.ac.at

Abrecht M. HOUBEN

Research Group Biodiversity Phylogeny and
Population Studies
Centre for Environmental Sciences
Department SBG, Hasselt University
Campus Diepenbeek, Agoralaan – Building D
3590 Diepenbeek
Belgium
Tel: +32-11-268383
Fax: +32-11-268301
Email: albrechthouben@hotmail.com

Tim JANICKE

Evolutionary Biology
Zoological Institute
University of Basel
Vesalgasse 1
4051 Basel
Switzerland
Tel: ++41 61 267 03 75
Fax: ++41 61 267 03 62
Email: tim.janicke@unibas.ch

PARTICIPANTS

Georg KUALES

Institute of Zoology
University of Innsbruck
Technikerstrasse 25
6020 Innsbruck
Austria
Tel: ++43 512 507 6163
Fax: ++43 512 507 2930
Email: georg.kuales@uibk.ac.at

Eric KUBLI

Institute of Zoology
University of Zürich
Winterthurerstrasse 190
8057 Zürich
Switzerland
Tel: ++41 44 635 48 92
Fax: ++41 44 635 59 09
Email: ekubli@zool.uzh.ch

Peter LADURNER

Institute of Zoology
University of Innsbruck
Technikerstrasse 25
6020 Innsbruck
Austria
Tel: ++43 512 507 6170
Fax: ++43 512 507 2930
Email: peter.ladurner@uibk.ac.at

Stijn M. MOUTON

University of Ghent
Biology Department
Nematology Section
K. L. Ledeganckstraat 35
9000 Ghent
Belgium
Tel: +32(0)92648740
Fax: +32(0)92645344
Email: Stijn.Mouton@ugent.be

Thomas OSTERMANN

Institute of Zoology
University of Innsbruck
Technikerstrasse 25
6020 Innsbruck
Austria
Tel: +43 512 507 6178
Fax: +43 512 507 94906178
Email: thomas.ostermann@uibk.ac.at

Daniela PFISTER

Institute of Zoology
University of Innsbruck
Technikerstrasse 25
6020 Innsbruck
Austria
Tel: +43 512 507 6170
Fax: +43 512 507 2930
Email: Daniela.Pfister@uibk.ac.at

Michelle PLUSQUIN

Research Group Biodiversity Phylogeny and
Population Studies
Centre for Environmental Sciences
Department SBG, Hasselt University
Campus Diepenbeek, Agoralaan – Building D
3590 Diepenbeek
Belgium
Tel: +32-11268383
Fax: +32-11268301
Email: michelle.plusquin@uhasselt.be

Willi SALVENMOSER

Institute of Zoology
University of Innsbruck
Technikerstrasse 25
6020 Innsbruck, Austria
Tel: ++43 512 507 6162
Fax: ++43 512 507 2930
Email: willi.salvenmoser@uibk.ac.at

Peter SANDNER

Evolutionary Biology
Zoological Institute
University of Basel
Vesalgasse 1
4051 Basel
Switzerland
Tel: ++41 61 267 03 75
Fax: ++41 61 267 03 62
Email: Peter.Sandner@unibas.ch

Lukas SCHÄRER

Evolutionary Biology
Zoological Institute
University of Basel
Vesalgasse 1
4051 Basel
Switzerland
Tel: ++41 61 267 03 66
Fax: ++41 61 267 03 62
Email: lukas.scharer@unibas.ch

Kiyono SEKII

Evolutionary Biology
Zoological Institute
University of Basel
Vesalgasse 1
4051 Basel
Switzerland
Tel: ++41 61 267 03 61
Fax: ++41 61 267 03 62
Email: kiyono.sekii@unibas.ch

Claudia SENN

Institute of Zoology
University of Innsbruck
Technikerstrasse 25
6020 Innsbruck, Austria
Tel: ++43 512 507 6173
Fax: ++43 512 507 2930
Email: Claudia.senn@student.uibk.ac.at

PARTICIPANTS

Karen SMEETS

Research Group Biodiversity Phylogeny and
Population Studies
Centre for Environmental Sciences
Department SBG
Hasselt University
Campus Diepenbeek, Agoralaan – Building D
3590 Diepenbeek
Belgium
Email: Karen.smeets@uhasselt.be

Frank VANBELLEGHEM

Research Group Biodiversity Phylogeny and
Population Studies
Centre for Environmental Sciences
Department SBG
Hasselt University
Campus Diepenbeek, Agoralaan – Building D
3590 Diepenbeek
Belgium
Email: frank.vanbelleghem@uhasselt.be

Freija VERDOODT

University of Ghent
Biology Department
Nematology Unit
K.L. Ledeganckstraat 35
9000 Ghent, Belgium
Tel: ++32 9264 8740
Fax: ++32 9264 5344
Email: freija.verdoodt@ugent.be

Dita B. VIZOSO

Evolutionary Biology
Zoological Institute
University of Basel
Vesalgasse 1
4051 Basel
Switzerland
Tel: ++41 61 267 03 76
Fax: ++41 61 267 03 62
Email: dita.vizoso@unibas.ch

ABSTRACTS

Talk: The exceptional capacity of *Macrostomum lignano* to recover from radiation

DE MULDER, K.^{1,2}, PFISTER, D.¹, KUALES, G.¹, EICHBERGER, P.³, SEKII, K.⁴, BORGONIE, G.², AND LADURNER, P.¹

¹Nematology Section, Department of Biology, Ghent University, Belgium

²Institute of Zoology, University of Innsbruck, Austria

³Department of Radiotherapy and Radiooncology, University Hospital Innsbruck, Austria

⁴Zoological Institute, University of Basel, Switzerland

Since neoblasts are the only dividing cells in flatworms, irradiation is a commonly used method to obtain stem-cell-free organisms. Trying to establish a lethal radiation protocol for *M. lignano*, an unexpected high resistance/ recover capacity was discovered. During the first week post irradiation, active stem cells were almost undetectable, comparable with the well-studied planarians (Triclad). Interestingly, during the second week postirradiation, the stem cell system gradually recovered, as shown by re-appearance of stem cell specific gene expression as well as proliferation activity. At the moment, a closer look is taken at the stem cell dynamics during the first month postirradiation, trying to understand how this recovery is taking place. A newly developed “fractionated” radiation protocol, in which worms were repeatedly radiated for 10 times in a total timeframe of 1 week, resulted in a lethal phenotype. Radiated worms were unable to reproduce and regenerate, showed an irreversible down-regulation of stem cell specific gene expression and gradually disintegrated during the third week postirradiation. Both gDNA isolation and housekeeping gene expression confirmed the stem cell specific effect of irradiation at this high dose. In the future a closer look at the differentiation potential of neoblasts could be taken, by analyzing the fate of neoblasts, injected into stem cell free hosts. This will contribute to a better understanding of the organization of the stem cell system in *M. lignano* and provides a basis for comparison with planarian and higher organisms.

This work was supported by a predoctoral FWO grant to Katrien De Mulder (Belgium), an LFU-Nachwuchs Forschungsförderung-Stipendium to Daniela Pfister and FWF grant 18099 to Peter Ladurner (Austria).

Talk: Flatworm as ideal model organisms: Real-time RT-PCR gene expression after exposure to different stress factors

GEERDENS, E., PLUSQUIN, M., DEGHESELLE, O., CUYPERS, A., ARTOIS, T. AND SMEETS, K.
Research Group Biodiversity, Phylogeny and Population Studies, Hasselt University

As flatworms become more and more important as genetic and experimental model organisms, both *Schmidtea mediterranea* and *Macrostomum lignano*, were used to evaluate the effects of different stress factors using real time quantitative PCR. Because of their unique properties to regenerate, genetically identical organisms can be produced and stress responses can be studied over generations using the same animal. However, accurate quantification by real-time qPCR relies on the normalisation of the measured gene expression data. Therefore, the expression stability of a number of candidate reference genes was studied using the computer algorithms geNorm and NormFinder. We identified the best combination of candidate reference genes in different environmental stress situations: cadmium, hexavalent chromium, salinity and temperature. The preference genes most suited for gene expression analyses varied between the used model organisms, although within one organism no specific treatment patterns were observed.

ABSTRACTS

Talk: Postembryonic development of the gonads in *Macrostomum lignano*

HEISS, H., SALVENMOSER, W., PFISTER, D., DE MULDER, K., KUALES, G. AND LADURNER, P.
Institute of Zoology, University of Innsbruck, Austria

Gonads of hermaphrodites can be formed either during embryogenesis or during postembryonic development. In Platyhelminthes, namely in triclads, an exclusively epigenetic (post embryonic) germ cell development was proposed. In contrast, there is good evidence of an embryonic development of a so called gonad anlage in *Macrostomum* and *Schmidtea* (Pfister et al. 2008, Handberg-Thorsager and Salo 2007). In *Macrostomum* a 4-6 macvsa positive cell cluster was found in freshly hatched embryos and in *Schmidtea* Smednos was found in stage 8 embryos. Which cells are present in this gonad anlage? Are they a mixture of male and female precursor cells, or are there male/female precursors and the other germ cell line will grow later on epigenetically? To elucidate this question we have investigated the postembryonic development of the gonad anlage by semithin sections and TEM. The gonad anlage in *Macrostomum* in a 10 minutes old hatchling consists of 4 to 6 cells covered by an extra-cellular matrix and tunica cells. The germ line cells are morphologically different and we propose an anterior male and a posterior female part. During day 2 and day 3 the anlage elongates and mitotic activity can be observed in the male part and at day 3 the first spermatocytes can be recognised. At day 4 or day 5, tunica cells migrate into the anlage and start to separate the male from the female part. At day 5 pachytene spermatocytes can be observed and also the first oocytes with their typical nuclear structure. At day 7 the first spermatids can be seen and at day 8 spermiogenesis has fully started.

Talk: *Macrostomum lignano*: capable of virgin reproduction?

HOUBEN, A, M., PLUSQUIN, M., SMEETS, K. AND ARTOIS, T.
Research Group Biodiversity, Phylogeny and Population Studies, Hasselt University

During experiments on general sensitivity to heavy metal exposure, a number of virgin specimens of *M. lignano* were observed to lay eggs. As this phenomenon was not yet known from literature, we designed an experiment to see whether *M. lignano* is capable of laying eggs as a virgin, to what extent they do this, and whether the eggs can hatch. Ninety-two juvenile worms of 4 days old were put in individual wells and were followed every other day for 31 days after isolation. We found that 41 animals laid at least one egg, and they laid a total of 94 eggs during this experiment. The first worm that laid an egg was 21 days old. In total 48 juveniles hatched from these eggs. We are now repeating this experiment with animals from UHasselt and from Basel to see whether our results are based on a mutation in our culture.

ABSTRACTS

Talk: Social group size affects sex allocation and mating behaviour in *Macrostomum lignano*

JANICKE, T. AND SCHÄRER, L.

Zoological Institute, University of Basel, Switzerland

Sex allocation theory predicts that simultaneous hermaphrodites should allocate their reproductive resources to male versus female function depending on the social conditions individuals experience. Higher levels of sperm competition should lead to a higher investment into the male function on the cost of the female function. Among many studies aiming to show this trade-off in *Macrostomum lignano*, only in one of them this pattern could be observed and exclusively under certain conditions. Furthermore, nothing is known on whether changes in sex allocation covary with differences in mating behaviour in hermaphrodites. According to Bateman's principle one would expect that more male-biased individuals are more eager to mate whereas female-biased individuals are more choosy. In this study we addressed two questions. First, we tested whether there is a trade-off in sex allocation in *M. lignano* using an experimental design that ensures a higher statistical power compared to previous experiments. Second, we asked whether sex allocation affects copulation rate, copulation duration and post-copulatory mating behaviour in *M. lignano*. The morphological comparison of worms that were raised in pairs versus worms raised in octets confirmed that social group size affects sex allocation in our model species. Worms from octets (i.e. higher level of sperm competition) had larger testes but smaller ovaries compared to worms from pairs. Moreover, differences in sex allocation were accompanied by differences in mating behaviour. As expected from theory, pairs formed by more male-biased individuals copulated more often than pairs formed by more female-biased individuals. In addition, pairs of more female-biased individuals showed a post-copulatory behaviour more frequently than more male-biased pairs. Our results suggest that there is a trade-off in resource allocation between the male and the female function in *M. lignano* and that sex allocation affects mating behaviour in accordance to theory originally formulated for gonochorists.

Poster: Determinants of mating group size and sperm transfer success in a hermaphroditic flatworm

JANICKE, T. AND SCHÄRER, L.

Zoological Institute, University of Basel, Switzerland

A crucial question in sex allocation theory for simultaneous hermaphrodites is how social group size translates into mating group size. We studied how mating group size and sperm transfer success are affected by social group size, density and morphological traits such as body size, testes size and genital morphology in the outcrossing hermaphroditic flatworm *Macrostomum lignano*. Furthermore, we tested whether the mating role predicts sperm transfer success. To do this we used a sperm-tracking method based on labelling of S-phase cells in the testes. We found that mating group size increases linearly with social group size. Moreover, we demonstrate that sperm transfer success depends on testes size and the shape of the male copulatory organ. Finally, in mating trials with two competitors, the individual that is in the second role on average managed to successfully transfer twice as many sperm to a mate than the individual that mated in first role. This suggests that second male sperm precedence operates in *M. lignano*.

ABSTRACTS

Invited Talk: Drosophila sex-peptide: a 'Swiss Army Knife-peptide' for reproduction

KUBLI, E.

Institute of Zoology, University of Zürich, Switzerland

Mating elicits a variety of post-mating responses in the *Drosophila* female. The seminal substance Sex-Peptide (SP) is one of the major players with many effects in the female: 1) the N-terminal end of SP stimulates juvenile hormone synthesis in the corpora allata. It is also responsible for binding to sperm and, thus, for the molecular basis of the sperm-effect; 2) an intact C-terminal end of SP is necessary to stimulate oocyte production and to elicit egg laying and reduction of receptivity. It is this part of SP that binds to specific sites of the central and peripheral nervous system; 3) The C-terminal part of SP binds also to the genital tract of the female. But here the binding constant K_d is different, and the binding less stringent in terms of amino acid sequence; 4) The innate immune response is stimulated by SP in the first few hours after mating. The N-terminal part of SP is not needed for this effect. The accumulation of several functions in a relatively small peptide, comparable to a Swiss-Army-Knife, may be the reason for its conserved amino acid sequence in comparison with other proteins and peptides involved in reproduction. In my talk I will concentrate on the molecular basis of the sperm-effect and the innate immune response and also discuss our results in relation to the recent isolation of a SP receptor by the group of Barry Dickson (Yapici et al., Nature, 451, 33-37, 2008).

Talk: Macrostomum in Innsbruck: current advancements and future goals

LADURNER, P.

Institute of Zoology, University of Innsbruck, Austria

For established model organisms a number of methodological tools are available. Among those large-scale gene expression, large-scale RNA interference (RNAi), transgenics, and mutagenesis are important. Knowledge on the spatial and temporal expression patterns is necessary to understand the molecular mechanisms that control developmental processes. For *Macrostomum* we have performed a pilot study demonstrating its suitability for high-throughput whole-mount in situ hybridization (HTISH). Our goal is to study the expression (HTISH) and function (RNAi) of many genes in order to identify cell- tissue and organs specific markers. Furthermore, the promoters of the respective genes will be isolated using targeted microarray-based enrichment of genomic 5'-regions. Knowledge on gene expression, function and the availability of the promoter region of the respective gene will help to select genes for the generation of transgenic *Macrostomum*. Current attempts to introduce of foreign DNA into the genome of *Macrostomum* will be discussed. A powerful approach for determining the biological functions of genes is to produce mutants. For *Macrostomum* a pilot study using EMS mutagenesis is currently performed. Success in the establishment of the suggested methods will be of great benefit for *Macrostomum* research and call the attention of additional research groups.

ABSTRACTS

Talk: Lifespan, survival curve and age-related mortality rate of the flatworm *M. lignano*

MOUTON, S., WILLEMS M., BACK, P., BRAECKMAN, B. AND BORGONIE, G.
Nematology Section, Department of Biology, Ghent University, Belgium

Ageing is often defined as the increased probability of dying with advancing age and this can be quantified by determining the age-related accelerations of the mortality rate. Most species show an exponential increase of mortality rate as a function of age after maturation. This is expressed by the Gompertz model, which is the major mortality rate model in gerontology. The slope of the Gompertz curve (G) can be used to calculate the mortality rate doubling time (MRDT). Both, G and MRDT, express the rate of senescence, but the MRDT is preferred because this parameter is expressed in years and allows for comparing the rate of senescence of different species. In this talk, we will present the mean and maximum lifespan, the survival curve and the age-related mortality rate of *M. lignano*. Furthermore, we have plotted the Gompertz model on the survival data and calculated the MRDT. *M. lignano* is the first flatworm in which the rate of senescence is characterised and we will compare this to other ageing model organisms. Moreover, the usefulness of the survival curve for future ageing and rejuvenation research and the possibilities and restrictions of *M. lignano* for ageing research will be discussed.

Talk: *Macrostomum* EST database

OSTERMANN, T. AND LADURNER, P.
Institute of Zoology, University of Innsbruck, Austria

We have generated a public *Macrostomum* sequence database available at <http://flatworm.uibk.ac.at/macest/>. More than 23.000 Expressed Sequence Tags (EST) of *Macrostomum lignano* are available from different sequencing projects. These data were processed using different bioinformatic methods. The idea was to analyze all available EST's with the same pipe of bioinformatic tools and provide the analyzed data to the community. We used "EST2uni" (<http://bioinf.comav.upv.es/est2uni/>) to run programs needed for analysis (EST preprocessing, clustering, annotation). EST2uni is an open source tool for automated EST analysis and database creation, with a data mining web interface. All results provided by different programs are parsed and feeded into a relational MySQL database. EST2uni provides a web page connected to the database. This end-user interface it allows to run queries for sequence names, blast results, Gene Ontology categories in all available *Macrostomum* libraries. Furthermore EST2uni provides statistics on gene sequence numbers, quality, length, singletons and contigs. In addition BLAST searches against the MAC-EST database can be performed. Finally all search results, sequence data and chromatograms can be downloaded by any user. A demonstration on how to use the library will be shown at the *Macrostomum* meeting.

ABSTRACTS

Talk: RNA interference analysis of stem cell and germ line genes in *Macrostomum*

PFISTER, D.¹, DE MULDER, K.^{1,2}, KUALES, G.¹, SEKIL, K.³, SENN, C.¹ AND LADURNER, P.¹

¹ Institute of Zoology, University of Innsbruck, Austria

² Nematology Section, Department of Biology, Ghent University, Belgium

³ Zoological Institute, University of Basel, Switzerland

The stem cell system of *Macrostomum* is remarkable. Its multipotent/totipotent features have been studied in great detail by ultrastructural and histological methods, by immuno- and BrdU-stainings. These findings have led to a substantial knowledge about cell cycle, stem cell distribution, migration, morphology and the stem cell role during development and regeneration. Recently, molecular markers have broadened the understanding of the spatial and temporal expression of stem cell and germ line genes in *Macrostomum*. However, little is known about their function or molecular regulation. We have used an optimized irradiation scheme for the generation of animals lacking stem cells and germ cells. With these animals a subtractive cDNA library enriched for stem cell and germ line genes was produced. So far 3072 clones of differentially expressed genes have been sequenced. Similarity search using BLAST was performed and ESTs were annotated according to GO categories. Candidate genes will be used for gene knock-down by RNA interference. The goal is to analyze the function and molecular regulation of pluripotent/totipotent stem cells. Several stem cell and germ line genes show interesting phenotypes and were analyzed by morphology, BrdU stainings, in situ hybridization and immunocytochemistry. Our analysis will help to better understand stem cells in flatworms and also higher organisms, including human.

The work was supported by LFU-Nachwuchsforschungsförderung to D.P., FWO to K.D.M. and FWF project 18099 to P.L.

Talk: Effect of cadmium on neoblasts and oxidative stress at the level of gene-expression

PLUSQUIN, M., GEERDENS, E., HOUBEN, A., CUYPERS, A., ARTOIS, T. AND SMEETS, K.

Research Group Biodiversity, Phylogeny and Population Studies, Hasselt University

Cadmium is a toxic metal and an environmental pollutant. At the cellular level it can indirectly result in the production of reactive oxygen species (ROS). As a consequence, enhanced level of lipid peroxidation, DNA damage and protein oxidation can occur. The mechanisms and genetic pathways of flatworm stem cells (neoblasts) are being intensively studied. These results need to be confirmed in studies with different conditions and stress factors. Therefore, a study was started to evaluate the cellular effects of cadmium in the free-living flatworm *M. lignano*. To unravel the effect of cadmium on neoblasts and the importance of oxidative stress in these pathways, real time RT-PCR will be used.

ABSTRACTS

Poster: Sperm precedence in *Macrostomum lignano*

SANDNER, P. AND SCHÄRER, L.

Zoological Institute, University of Basel, Switzerland

Sperm competition is a major force of sexual selection since it has implications for mating system and life-history evolution. Mechanistically, it can operate as a *fair raffle* with equal chances of each sperm to fertilize an egg or as a *loaded raffle* process, whereby one donor's sperm has a fertilization advantage. We here provide data on relative offspring (paternity) numbers of competing donors in the repeatedly mating turbellarian flatworm *Macrostomum lignano* by the use of microsatellite markers and propose a mechanism to explain the pattern of sperm utilization.

Talk: Are there stem cell niches in *Macrostomum lignano*?

SALVENMOSER, W., ADAMSKI, Z., PFISTER, D., EGGER B. AND LADURNER, P.

Institute of Zoology, University of Innsbruck, Austria

It is generally accepted that Platyhelminthes possess a totipotent stem cell system, which gives them the ability of flexible tissue homeostasis and an enormous regeneration capacity. These stem cells are the only dividing cells in the animals and the proliferating cells in S-phase are easy to visualise with BrdU labelling. In *Macrostomum lignano* BrdU-positive cells are located in two lateral bands on each side of the animal with a typically S-phase cell free area in the rostrum. But, are all these S-phase cells totipotent stem cells? At the ultrastructural level three morphologically different stem cell stages have been described. Immunogold labelling of a 30 minute BrdU-pulsed animal showed that all three stages were in S-phase, indicating that there are different stem cell populations. Comparison of BrdU and macvasa double staining also revealed that there are subpopulations of stem cells. Moreover double staining of IdU and CldU at the transition from the stem cell area to the stem cell free rostrum in pulse-chase-pulse experiments (IdU pulse-chase and CldU pulse) clearly shows that the anterior cell is only labelled with IdU and the more posterior cell is double labelled. TEM analysis with immunogold pointed out that the anterior cell complies with a stage 3 cell neoblast and the posterior cell complies with a stage 1 or a stage 2 neoblast. These data suggest that the posterior double stained stage 1 or stage 2 neoblast corresponds to a stationary continuous asymmetrically dividing neoblast, possibly situated in a niche.

ABSTRACTS

Talk: Experimental tests of sex allocation theory using the RNAi-knockdown approach

SEKIL, K.¹, DE MULDER, K.^{2,3}, SALVENMOSER, W.², LADURNER, P.² AND SCHÄRER, L.¹

¹ Zoological Institute, University of Basel, Switzerland

² Institute of Zoology, University of Innsbruck, Austria

³ Nematology Section, Department of Biology, Ghent University, Belgium

How individuals should invest their energies to male and female reproduction is an important aspect of an organism's life history, and sex allocation theory can provide successful predictions about this intriguing question. For example, it can predict the sex ratio of sons vs. daughters in gonochorists, the timing of sex change in sequential hermaphrodites and the resource allocation to male and female function in simultaneous hermaphrodites. However, the experimental examination of sex allocation theory is so far limited, especially in simultaneously hermaphroditic animals. Therefore we propose to approach this question experimentally by using the strategy as followed: (1) Scanning the EST databases for genes that are expected to be involved in male or female gametogenesis based on literature data. (2) Investigation of their gene expression pattern by *in situ* hybridization. (3) Knock down of their gene expression by using the technique of RNAi. Currently, we successfully isolated two testis-specific genes, which seem to play a role in spermatogenesis in *M. lignano*. The RNAi phenotypes of these two genes show aberrant sperm in testis and no sperm in the seminal vesicle, suggesting that these worms can't achieve reproductive success through the male function. By using these phenotypes, we will investigate trade-off between male and female function, which is one of the fundamental assumptions in sex allocation theory.

Poster: Does non-random segregation of DNA-strands occur during stem cell division in *Macrostomum lignano*?

VERDOODT, F.¹, WILLEMS, M.¹, MOUTON, S.¹, DE MULDER, K.^{1,2}, BORGONIE, G.¹ AND LADURNER, P.²

¹ Nematology Section, Department of Biology, Ghent University, Belgium

² Institute of Zoology, University of Innsbruck, Austria

The 'immortal strand hypothesis' was proposed by Cairns (1975) as a means by which stem cells might limit acquiring mutations during DNA-replication. According to this hypothesis the chromatids are segregated non-randomly during cell division: those sister chromatids containing the older template DNA-strands, are selectively retained in the daughter cell, destined to be the renewed stem cell. Labeling these strands during development, results in 'label retaining cells' (LRC's), cells which retain label through many cell divisions. The younger strands (with any mutations acquired during replication) are passed on to the tissue committed cell. In *Macrostomum lignano*, the 'immortal strand hypothesis' can be verified *in vivo*, performing sequential pulses of DNA-analogs (Iododeoxyuridine and Chlorodeoxyuridine). Exposure to the first DNA-analog at different stages of embryonic or post-embryonic development, should label cells, containing 'immortal strands'. A second continuous exposure to a different DNA-analog, weeks or months after the first pulse, allows us to identify double labeled cells and test for the existence of LRC's. The goal of this study is to determine whether non-random segregation of DNA-strands occurs in *M. lignano*, and specify in which cells this might be the case.

ABSTRACTS

Talk: Genome duplication in *Macrostomum lignano*, *Macrostomum*, or Macrostromida?

VIZOSO, D. B., SANDNER, P. AND SCHÄRER, L.

Zoological Institute, University of Basel, Switzerland

Microsatellite data in *Macrostomum lignano* has shown a within-individual polymorphism suggestive of a genome duplication. New data shows that this phenomenon is not restricted to *M. lignano*, but occurs in other *Macrostomum* species. Using a Single-Nucleotide-Polymorphisms analysis of Histone 3 sequence data we present further evidence for a genome duplication in other Macrostromida. Methodologically, a genome duplication may pose difficult problems. Evolutionarily, it may explain the diversity and widespread distribution of this taxon.

GENERAL INFORMATION

Site of the Meeting

The meeting takes place in the small lecture hall (Kleiner Hörsaal) of the Zoologisches Institut der Universität Basel, Evolutionsbiologie, Vesalgasse 1, CH-4051 Basel, Switzerland.



Just follow the dots...

Arriving by train from Switzerland or France

Trains from Switzerland and France arrive at the main train station (Basel Bahnhof SBB + Gare SNCF). To get to the Institute you need to take a bus. Leave the train station in the direction of the City and, before mounting the bus, buy a short distance ticket (K - Kurzstrecke) from one of the green ticket machines. This will cost you CHF 1.90 (have coins ready, and note that a few ticket machines also accept Euro coins). Take Bus No. 30 (direction "Badischer Bahnhof"). If you leave the train station the bus stop is on the left side of the place (in front of a very nice Bakery called Bachmann). Get off at the stop "Universität" and use the map below to find the Institute. It takes about 15 minutes from the station to the Institute.

Arriving at the bus stop "Universität": Turn right and take the first street on the left ("auf der Lyss"). At the other end of the street you reach the tracks of a tram. Follow them to the left to the next stop ("Universität"). And walk up this street ("Spalenvorstadt"). The second small street on the right (the first one is a blind alley) is the Vesalgasse. Enter it and walk down to the big building which is the Vesalianum. The main entrance is to the right. Evolutionary Biology is located on the first floor.

GENERAL INFORMATION

Arriving by train from Germany

Trains from Germany arrive at the 'German' train station (Basel Badischer Bahnhof), but most of these trains also continue to the main train station (Basel Bahnhof SBB). So depending on your preference you can stay on the train for one more stop and follow the instructions above. Alternatively you get off and take a bus from here. Leave the train station in the direction of the City and, before mounting the bus, buy a ticket for zone 1 from one of the green ticket machines. This will cost you CHF 3.00 (have coins ready, and note that a few ticket machines also accept Euro coins). Take Bus No. 30 (direction "Bahnhof SBB"). If you leave the train station the bus stop is on the opposite side of the big road. Get off at the stop "Spalentor" and use the map below to find the Institute. It takes about 20 minutes from the station to the Institute.

Arriving at the bus stop "Spalentor": Walk through the old city gate (in former times you entered the city here) and into the "Spalenvorstadt" street. Follow the street and take the first small street to the left. This is the Vesalgasse. Enter it and walk down to the big building which is the Vesalianum. The main entrance is to the right. Evolutionary Biology is located on the first floor.

Arriving from the EuroAirport Basel/Mulhouse/Freiburg

Upon leaving the airport buy a ticket from the green ticket machine next to the bus stop in front of the airport. You need to buy a ticket for zone 2 which will be CHF 3.80 (have coins ready!). Usually you can also get such a ticket when you wait for your luggage at the luggage claim (there is a booth that sells them). Take Bus 50 to the main train station (Basel Bahnhof SBB). Then proceed like if you were arriving by train from Switzerland or France. The bus takes about 25 minutes from the airport to the train station.

Arriving by taxi

The little road in which our Institute is located is often not known to taxi drivers (I think we are the only house with a number in that street). Explain the taxi driver that you have to get off at the end of the main university building (the Kollegiengebäude) at the Petersgraben 50. There is a big metal gate, which should be open during normal office hours. Enter the metal gate and down to the big building which is the Vesalianum. The main entrance is to the right. Evolutionary Biology is located on the first floor.

Arriving by car

It is rather difficult to find us by car if your are unfamiliar with Basel. Moreover, there are hardly any parking places available and the city has a complicated system of one-way roads. Therefore we recommend you to use public transport.