EuroEEFG closing conference:

Frontiers in Ecological and Evolutionary Genomics

Venue and dates:

Conference Hotel "De Leeuwenhorst", 26-31 May 2013, Noordwijkerhout, the Netherlands.

Proposer and Local organiser: Paul L.E. Bodelier (PL, MECOMECON).

Organising committee:

Paul Bodelier
Oliver Bossdorf
George Coupland
Gerhard J. Herndl
Jan Kammenga
Luc De Meester
Luisa Orsini
Jakob Pernthaler
Thorsten Stoeck
Rita Volkers
Jörg Wunder
Michail Yakimov

Local organisers:

Sabine van Wegen (ISME Events Assistant)
Paul Bodelier (NIOO-KNAW, Netherlands Institute of Ecology)

ESF
Maria Manuela Nogueira (Science Officer)

Summary

The EuroEEFG closing conference was organized to report on the research carried out within EuroEEFG to the members of the programme and to the outside scientific world. The programme consisted of keynote, contributed talks by EuroEEFG members as well as 2 poster sessions around three major themes being; ***Evolution and Adaptation***, ***Response to the Environment***, and ***Communities and Ecosystem functioning.*** The keynote talks as well as the research presented by the various CRP's in the programme were all centered around the "the genetic basis of adaptation, speciation and evolution". The strength of the conference, as also stated by the world leading scientist in the field of ecological genomics, was the unprecedented amount of genomic work in natural non-model organisms and ecosystems. The quantitative genetic approach taken on complex natural communities is unique and of great value for understanding and managing ecosystems under climate threat. The mix of very diverse scientific disciplines (plant, animal and microbial ecologists) worked out very well and was also noted as being worthwhile continuing on the European level. The large number of PhD students and post-doc present at the conference and their possibilities to present and interact, contributed largely to the education and network building of the young scientist within EuroEEFG. With respect to the latter a very successful course was held during the last 2 days of the conference in which 10 EuroEEFG members were educated in the annotation of microbial genomes using the Microscope platform, as organized by teachers from Genoscope, Evry, France.

Part I Conference: Final programme of the event:

Sunday 26 May

15.00 Registration Open
Coffee and Tea

17.00 Welcome
By Paul Bodelier

Keynote Lecture
Chaired by: Luc De Meester
17.15 **Thomas Mitchell-Olds**, USA
Pathway Function Influences Complex Traits and Fitness in Plant Populations

18.15 Dinner Buffet

Monday 27 May

Keynote Lecture
Chaired by: Oliver Bossdorf
09.00 **Angela Hancock**, Austria
Adaptation to the Environment in Arabidopsis thaliana

Adaptation and Evolution 1
Chaired by : Philippine Vergeer

09.40 **George Coupland**, Germany
Effect of variation at the PEP1 locus on seasonal flowering of perennial Arabis alpine

10.00 **Rita Volkers**, the Netherlands
Gene-environment interactions drive genomic and transcriptomic diversity in wild Caenorhabditis elegans populations

10.20 **Christoph Haag**, Switzerland
Evolution of partial genetic sex determination in Daphnia magna

10.40 **Jörg Wunder**, Germany
Natural variation in flowering time in Arabis alpina and its adaptive value

11.00 Coffee and tea break

Keynote Lecture
Chaired by: Luisa Orsini
11.20 **Louis Bernathez**, Canada
Investigating adaptive evolutionary response to environment at different temporal scales using NGS genomic approaches

Response to Environment 1
Chaired by: Ester Eckert

12.00 **Christian Laforsch**, Germany
Predation and proteomics: progress towards the understanding of the molecular basis of inducible defenses in Daphnia

12.20 **Oliver Bossdorf**, Switzerland
How general and how predictable are parental environmental effects? A comprehensive test with Arabidopis thaliana

12.40 **C A N C E L L E D**
Hans-Peter Grossart, Germany
Occurance and potential ecological role of actinorhodopsins in freshwater ecosystems

13.00 Lunch

Keynote Lecture
Chaired by: Thorsten Stoeck
14.30 **Martin Polz**, USA
Ecology and Evolution of Bacterial Populations in the Wild

Communities and Ecosystem Functioning 1
Chaired by: Sascha Krause

15.10 **Peter Frenzel**, Germany
Classify! A strategy and workflow to interpret NGS data

15.30 **Gerhard Herndl**, Austria
Chemolithoautotrophic communities and activity in the deep water- water masses of the North Atlantic

15.50 **Ruth Henneberger**, Switzerland
Who is doing the job? - Field-scale labelling and activity quantification of methanotrophs in a landfill-cover soil

16.10 **Jerone Pinhassi**, Sweden
Response of prokaryotic function and diversity to organic and inorganic nutrients inputs in the upper mesopelagic Atlantic

16.30 Poster Presentations
Coffee, Tea and Drinks Break

19.00 Dinner

Tuesday 28 May

Keynote Lecture
Chaired by: Gerhard Herndl
09.00 **Mary Ann Moran**, USA
Quantitative Meta-omics

Communities and Ecosystem Functioning 2
Chaired by: Pierre Offre

09.40 **Klaus Jürgens and Carlo Berg,** Germany
Chemolitoautotrophic microbes contral the biogeochemistry of marine oxygen depletion zones

10.00 **Jose M. Gonzalez**, Spain
Lifestyle of Flavobacteria in the marine environment

10.20 **Mette Svenning**, Norway
Adaptation and function of methane oxidising communities in Arctic terrestrial ecosystems

10.40 **Michail Yakimov**, Italy
Partaking of chemolithotrophic and anapleurotic pathways to dark primary production in aphotic zones of the Mediterranean Sea

11.00 Coffee and tea break

Keynote Lecture
Chaired by: Paul Bodelier

11.20 **Andreas Brune**, Germany
The symbiotic gut microbiota of termites: Evolutionary origin and functional adaptations

Response to Environment 2
Chaired by: Ruth Henneberger

12.00 **Claudia Lüke**, Germany
Macroecology of wetland rice methanotrophs: the β-diversityof pmoA genotypes in tropical and subtropical soils

12.20 **Philipp Dirksen**, Germany
The influence of naturally associated microbes on Caenorhabditis elegans life history

12.40 **Pierre Offre and Christa Schleper**, Austria
Metagenomic insights into an uncultivated group of ubiquitous oceanic Thaumarchaeota

13.00 Lunch

Keynote Lecture
Chaired by: Jan Kammenga

14.30 **Ary Hoffmann**, Australia
From ecological genetics to ecological genomics of climate change adaptation: Drosophila and beyond

Adaptation and Evolution 2
Chaired by: Jörg Wunder

15.10 **Rania Nakad,** Germany
The genetic architecture of natural variation in pathogen avoidance behaviour of the nematode Caenorhabditis elegans

15.30 **Ellen Decaestecker**, Belgium
Host allelic diversity drives long-term host-parasite coevolutionary dynamics

15.50 **Thomas Odong**, the Netherlands
Genomic variations in European Arabis Alpina natural populations: A Resequencing Study

16.10 **Per Toräng**, Sweden
Strong adaptive differentiation between populations of the alpine perennial herb Arabis alpina in northern and southern Europe

16.30 Poster Presentations
Coffee, Tea and Drinks Break

19.00 Dinner

Wednesday 29 May

Keynote Lecture

Chaired by: Jakob Pernthaler

09.00 **Siv Andersson**, Sweden
Evolution and adaptation of bacteria with small genomes

Response to Environment 3

Chaired by: Abishek Srivastava

09.40 **Anne Roulin**, Switzerland
Local adaptation of sex-indiction in a facultative sexual crusacean: insights from QTL mapping and natural population of D. magna

10.00 **Martin Hahn**, Austria
Photosynthesis genes in pelagic freshwater bacteria affiliated with genus Polynucleobacter (Burkholderiaceae, Betaproteobacteria)

10.20 **Andreas Reim**, Germany
Effect of energy flow on the susceptibility of aerobic methanotrophic communities to disturbance

10.40 **Stefan Bertilsson**, Sweden
Functional comparison of freshwater SAR11 (LD12) and Actinobacteria ac1 using single cell genomics

11.00 Coffee and tea break

Keynote Lecture

Chaired by: Michail Yakimov

11.20 **Steven Hallam**, Canada
Oceans of Information

Communities and Ecosystem Functioning 3

Chaired by: Sabine Filker

12.00 **Thorsten Stoeck**, Germany
Assesing marine Protistan Grazing on Marine Prokaryotes in the Bathypelagic Realm of the eastern Mediterranean Sea

12.20 **Sascha Krause**, the Netherlands
Trait-based approaches in microbial ecology: a case study on methane oxidizing bacteria testing the phylogenetic signal on functional traits

12.40 **Ester Eckert,** Switzerland
Feeding on their competitor's remains: grazing resistant freshwater bacteria profit from organic carbon possibly released through protistan foraging

13.00 Lunch

Evolution and Adaptation 3

Chaired by: Rita Volkers

15.00 **Vit Latzel**, Switzerland
Rapid epigenetically-based evolution in experimental plant populations

15.20 **Philippine Vergeer**, the Netherlands

New challenges in ecological epigenetics: epigenetics linked to inbreeding depression

15.40 **Luisa Orsini**, Belgium
Environmental and ecological genomics of Daphnia magna: identifying processes and mechanisms of adaptation to stress

Keynote Lecture
Chaired by: George Coupland
16.00 **Felicity Jones**, Germany
Genetics and Genomics of Stickleback Adaptations

16.30 Coffee and Tea Break
17.00 Award Ceremony - Best posters
17.20 Wrap up and Closing
19.00 Dinner

Scientific content

The current unprecedented scope of genomic information allows ecologists and evolutionary biologists to connect ecological-evolutionary questions and approaches with genomic tools and to address fundamental and exciting questions about the **genomic basis of phenotypic variation, ecological interactions, adaptation to changing environments, and speciation**. Moreover, metagenomic techniques are currently revolutionizing the study of microorganisms and our ability to quantify biodiversity, multitrophic organization and evolutionary change of taxonomic groups and entire ecosystems that used to be inaccessible and little understood. The four-day EuroEEFG conference gave an overview of the most significant scientific achievements within EuroEEFG, complemented with plenary presentations of world leading scientist in the field of ecological genomics. In total 111 delegates participated in the conference of which 21 were open registrants not affiliated to any EuroEEFG CRP. The programme was centered on 3 themes ***Evolution and Adaptation***, ***Response to the Environment***, and ***Communities and Ecosystem functioning***. A mix of project leaders, principal investigators, post-docs and PhD students from within the 8 CRP's presented the research they performed within EuroEEFG within 3 sessions per conference theme, all preceded by a keynote speaker. The keynote speakers were carefully selected by the organizing committee to have representative world leading scientist covering all fields studied within the 8 CRP's. It is not the intent of this report to summarise all talks, for this we refer to annex 1 of this report where all abstracts of the presentation and posters are listed. The general main ecological question addressed by all keynotes, microbial as well as macro-ecologists was **"what is the genetic basis of adaptation and speciation" mainly in natural communities".** This topic was broadly covered by means of examples on model organisms (e.g. Drosophila, Sticklebacks, Daphnids, Arabidopsis) as well as many presentations on ecological genomics work on natural communities of plants, animals and microbes. Many important novel and important findings were presented on topics like models of evolutionary change, genetic basis of flowering in perennial plants, genetics of inducible defenses in Daphnids, role of epigenetics in parental effects in plants, methods of classification of NGS data, possible mechanisms of microbial primary production in oceans in the absence of light, single cell genomics of uncultured freshwater bacteria, host-parasite evolution, resilience of methane consuming microbial communities. The general consensus of all the scientific talks as well as the results presented in the poster sessions was that in all fields of ecology we are moving to quantitative genetics in natural, often unexplored communities of plants, animals

and microbes enabled through the application of genomic techniques. This is also the main achievement of the EuroEEFG research, delivering a wealth of information on non-model systems which is highly needed in able to understand ecosystem functioning in order to predict and possibly mitigate the consequences of climate change. At the same time it was concluded that the more we measure and the more data are obtained, it gets more apparent how variable and complex natural systems are and how challenging it will be link genetics to adaptations and behavior. Probably, the most important statement in the meeting was made at the end; "to get a good understanding ……we do not need to know everything and we should also not strive to measure everything".

Results and impact on the EUROCORES programme

The conference brought together scientist from 8 CRP's and the outside scientific world with completely different scientific backgrounds. However, there was great interest for each others' research and the conference attendance was the same, no matter whether there was a plant, animal or microbial oriented talk. This is actually a great achievement form this conference. There was a lot of "looking over the fence", facilitated by the common ecological approaches through the use of genomic techniques. This was also the common feeling of the participants, who realized that we are all interested in the same ecological questions, no matter what the systems of study is. It was suggested to try to create a European Eco-genomics network on the basis of the EuroEEFG conference. Hence, the networking goal of the progarmme was achieved successfully. Next to this it should be noted that, as also discussed with the review panel members, the variety of CRP's involved in EuroEEFG did not facilitate cross-CRP networking. However, the networks within the CRP's have strengthened and expanded enabling them to carry out unique science of high quality which was not possible without the EuroEEFG programme. The collaborative effort within the individual CRP's is very strong with large scientific output.

Besides the CRP scientific networking, this conference contributed significantly to the education of the young scientist in the programme, who could present their research, who could chair sessions and who could talk to world leading scientist during the conference in a informal meeting between key-notes and post-docs and PhD students only. The announcement of the conference, the distribution within the various scientific networks and the presence of open-registrants contributed successfully in the dissemination of the programme.

Part II Workshop:

ESF Eurocores Ecological and Evolutionary Functional Genomics (EuroEEFG) conference workshop:

Annotation and analysis of microbial genomes using the MicroScope platform

Venue and dates: Conference center 'NH Hotel Leeuwenhorst", 30-31 May 2013, Noordwijkerhout, the Netherlands.

Proposer and organizer: Paul L.E. Bodelier (PL, MECOMECON) and Sascha Krause (Postdoc, MECOMECON)

Course leader: Dr. Alexandra Calteau (CEA, Institut de Génomique, Genoscope) and Audrey Capitaine (Université d'Evry-Val-d'Essonne)

Scientific content of the workshop

MicroScope is a web-based platform for microbial comparative genome analysis and manual functional annotation. The platform was developed by the Laboratory of Bioinformatics Analyses for Genomics and Metabolism (LABGeM) at the French National Sequencing Center (Genoscope). In the framework of the final ESF EuroEEFG customized 2-day training was offered to give an overview about the Microscope pipeline on several microbial model organisms. The course introduced the MicroScope web interface Magnifying Genomes (MaGe). Practical exercises allowed participants to get familiar with the use the MicroScope platform and the MaGe graphical interfaces in the presence of 2 experts from the LABGeM bioinformatics team which led the course.
This workshop aimed to introduce the EuroEEFG microbial oriented CRP's (FREDI, MOCA, STRESSFLEA, and MECOMECON) to the MicroScope platform.

Final programme of the event

Thursday May 30, 8.30-18.00.

- **Welcome**

- **Course 1** : Introduction: The MicroScope platform for genome annotation and comparative genomics
  - Microbial genome annotation and worldwide platforms
  - MicroScope platform overview: annotation pipeline, PkGDB multi-genome database, MaGe graphical interface

- **Course 2** : Introduction to the MicroScope query system : keyword search using single and multiple modes.

- **Course 3** : Functional annotation of microbial genomes
  - Similarity search in generalist public databanks – Result interpretation – Drawbacks of automatic functional annotation
  - Orthologous genes and gene/protein families – COG classes – HAMAP families – Protein domain detection
  - Exercises and corrections for everyone.

- **Course 4** : Relational annotation of microbial genomes
  - Synteny algorithm and its usage in the framework of genome annotation – Fusion/fission events detection
  - Exercises and corrections for everyone.

- **Course 5**: Gene content comparison of microbial genomes
  - Phylogenetic profiles: searching for common genes among several species or strain specific genes.
  - Exercises and corrections for everyone.

- **Course 6**: Core-Pan genome
  - Compute pan-genome and core-genome sizes and their evolutions for a genome set
  - Exercises and corrections for everyone.

Friday May 31, 8.15-17.00.

- **Course 7** : Expert gene annotation
  - The different levels of manual annotation
  - Annotation rules used in MicroScope
  - Expert annotation of gene fusions, pseudogenes and duplications.

  Live expert annotation examples using MIcroScope
  - Exercises and corrections for everyone.

- **Course 8** : Relational annotation of microbial genomes
  - Metabolic pathway reconstruction with BioCyc and KEGG – Combination of synteny and metabolism for functional annotation – Completion of metabolic pathways and/or search for alternative pathways – Metabolic pathway comparison
  - Exercises and corrections for everyone.

- **Tour of MicroScope system functionalities**
  - RNAseq data exploration
  - Exporting data from the PkGDB database

- **Open questions**

Annex 1: Abstracts of keynote, oral contributions and posters.

Keynotes

Pathway Function Influences Complex Traits and Fitness in Plant Populations

Thomas Mitchell-Olds, C. Olson-Manning, K. Prasad, B-H Song, J. Anderson, C-R Lee, M.E. Schranz and A. Windsor
Institute for Genome Sciences and Policy, Department of Biology, Duke University, Durham, USA

To understand the functional and evolutionary basis of complex trait variation in nature, we studied regulation of defensive metabolites that influence resistance to herbivore damage in plant populations. We analyzed genetic and environmental control of pathway flux in *Arabidopsis*, then quantified fitness variation in natural populations of a close wild relative, *Boechera stricta*. Theoretical analyses suggest that adaptive substitutions should be concentrated in the enzymes that exert the greatest control over flux. Although several studies have found a correlation between position in a pathway and evolutionary rate, these investigations have not examined the relationship between evolutionary rate and flux control. Here, we perturbed the enzymes in the glucosinolate pathway, and showed that flux control is focused in the first step in the pathway. Next, we showed that flux control of these defensive phenotypes is robust across five environmental treatments. Furthermore, signatures of selection showed that this enzyme is the only one in the pathway that shows convincing evidence of selection. In *Boechera stricta* we cloned a QTL that controls plant defensive chemistry, damage by insect herbivores, survival, and reproduction in the natural environments where this polymorphism evolved. This QTL encodes the first enzyme in the pathway, orthologous to the flux-controlling enzyme in *Arabidopsis*. These ecological effects are driven by gene duplications controlling this variation and by two selectively favored amino acid changes in the proteins that they encode. These changes cause a gain of novel enzyme function, modulated by allelic differences in catalytic rate and gene copy number, which control survival and reproduction in nature. Our results support the hypothesis that natural selection preferentially acts on enzymes with high control over flux and phenotype, as suggested by non-neutral evolution at orthologous genes in *Boechera* and *Arabidopsis*.

Adaptation to the Environment in Arabidopsis thaliana

Angela Hancock
University of Vienna, Austria

A major goal of evolutionary biology is to understand the phenotypic and genetic bases of adaptation to the environment. Since plants have limited mobility, aspects of climate play an especially important role in their survival and reproductive success. Some of the earliest evidence for this comes from phenotypic clines with the environment. To better understand the genetic basis for climate adaptation in plants, we conducted genome---wide scans to identify climate--adaptive genetic loci and pathways in the plant *Arabidopsis thaliana*. Variants that result in amino acid changes are significantly enriched among the loci most strongly correlated with climate, suggesting that this scan effectively detects adaptive alleles. Moreover, from our results, we successfully predict relative fitness among a set of geographically diverse *A. thaliana* accessions when grown together in a common environment. Our results provide a set of candidates for dissecting the molecular bases of climate adaptations, as well as insights about the prevalence of selective sweeps, which has implications for predicting the rate of adaptation to environmental change.

Investigating adaptive evolutionary response to environment at different temporal scales using NGS genomic approaches

Louis Bernatchez
Institut de Biologie Intégrative et des Systèmes (IBIS), Université Laval, Québec, Canada

The main focus of this talk will be on providing an empirical overview of recent NGS-based applications towards highlighting adaptive evolutionary response to variable environments at different temporal scales. At the deeper scale (thousands of generations), results will be presented to illustrate how extent of genomic

differentiation between diverging populations has been shaped according to predicted differences based on levels of environmental heterogeneity. At an intermediate scale (tens of generations), I will present results providing evidence that human driven environmental change has led to rapid evolution detectable as selective footprints at the genome level. Finally, I will provide results of an ongoing research program providing evidence for the role of spatially variable selection in shaping patterns of genetic differences during the time course of a single generation and discuss the relevance of such findings for management and conservation. I will also briefly comment on challenges being faced in interpreting signals of selection with current analytical methods at hands and in establishing causal links between genotype and phenotypic response, especially in non-model species.

Ecology and Evolution of Bacterial Populations in the Wild

Martin Polz
Parsons Laboratory for Environmental Science and Engineering, Massachusetts Institute of Technology, USA

Microbes dominate biogeochemical processes and are far more diverse than anticipated. Yet the absence of a workable species concept, largely due to the potential for rampant horizontal gene transfer among distantly related genomes, precludes facile ordering of this diversity into ecologically cohesive populations. Using coastal vibrios as a model, we show that in spite of horizontal gene flow, coexisting microbes are organized into genotypic clusters, which display many fundamental properties typically ascribed to animal and plant populations. They act as ecologically cohesive units with high predictability of environmental associations and metabolic functions. Moreover, using antibiotic production as an example, we demonstrate that social interactions structure these populations. Finally, this population model has also afforded us the opportunity to study the microevolutionary processes leading to ecological specialization with the surprising result that the process of sympatric speciation is highly similar among bacteria and sexual eukaryotes.

The symbiotic gut microbiota of termites: Evolutionary origin and functional adaptations

Andreas Brune
Department of Biogeochemistry, Max Planck Institute for Terrestrial Microbiology, Marburg, Germany

The recent finding of termite-specific bacterial lineages in the gut of cockroaches prompts the hypothesis that the symbiotic gut microbiota of termites is derived from a common ancestor. However, the microbial diversity in many termite families has not been fully explored, and even less is known about that in cockroaches. We characterized the bacterial communities in the hindguts of 34 host species by pyrotag sequencing of the 16S rRNA gene (V3–V4 region) using a modified primer set. A comprehensive analysis based both on phylogeny and hierarchical classification revealed that the gut microbiota of termites is less diverse and more specialized than that of cockroaches. The gut communities of different host lineages formed distinct clusters. Although community structure differed strongly already at the phylum level, we identified numerous genus-level taxa that were present in almost every host. Remarkable changes in their relative abundance correlate with major events in the evolutionary history of termites, such as acquisition and loss of cellulolytic flagellates or dietary diversification. The emerging patterns document a long
history of co-evolution of termites and their gut microbiota, which provided a reservoir of bacterial diversity that was exploited whenever new functions were required.

Quantitative Meta-omics

Mary Ann Moran
University of Georgia, Athens, USA

The short half lives of microbial mRNAs, on the order of just a few minutes, means that transcriptional changes relative to gene copies provide a highly sensitive bioassay for environmental signals that are relevant to microbes. We are assembling a quantitative inventory of genes and transcripts in the Amazon River Plume to measure gene expression patterns in this globally-important ecosystem. The 1×10^{13} microbial genes in a liter of nearshore plume water were 84% bacterial in origin, 15% eukaryotic, and 1% archaeal. While the eukaryotic cells contributed fewer genes than bacteria, they had greater per gene expression, with 55% of the 1×10^{12} transcripts per liter assigned to eukaryotes, 45% to bacteria, and 0.3% to archaea. The particle-associated microbes in the plume community were considerably more active transcriptionally than free-living cells, with an average gene

expression ratio (transcripts L^{-1}/genes L^{-1}) that was five-fold higher. Functional difference in their transcriptomes suggest different contributions to biogeochemical processes within these microhabitats of the Amazon plume.

From ecological genetics to ecological genomics of climate change adaptation: Drosophila and beyond

Ary Hoffmann
University of Melbourne, Australia

Drosophila studies have featured prominently over the last few decades in attempts to link genetic variation to traits and processes involved in climate change adaptation through functional analyses. In this talk I provide an overview of what the studies have achieved and some current limitations. I cover early and more recent studies on specific genetic polymorphisms that have provided links between variation and traits important in adaptation and provided information on the nature of selection acting on these polymorphisms. I then consider early efforts to look across the genome including QTL mapping and analyses of selection lines. I address major gaps in our current knowledge and how attempts are being made to fill these through more recent –omics and phylogenetic approaches. The new studies promise to provide a much deeper understanding of adaptive changes and allow an entire new set of questions to be considered, but they also raise challenges when studies are designed to generate masses of data using relatively simple experimental designs but without considering a relevant ecological context.

Oceans of Information

Steven Hallam
Department of Microbiology & Immunology, Life Sciences Institute, University of British Columbia, Vancouver, Canada

Although the vast majority of microbes in nature resist laboratory cultivation, they represent an almost limitless reservoir of genetic diversity and metabolic innovation. Next generation sequencing technologies are rapidly expanding our capacity to access genotypic and phenotypic information directly from environmental samples. However, to effectively interpret and apply this increasing volume of information, new analytical tools and services must be developed with the end user in mind. Here, I explore problems and solutions in environmental sequence analysis spanning different levels of biological organization from genomes to biomes. I highlight emerging open source tools for integrating and visualizing multimolecular (DNA, RNA and protein) and environmental parameter data and consider the future of data intensive computation as it relates to pathway reconstruction, ecosystem modeling and synthetic ecology.

Oral contributed papers from EuroEEFG partners

Effect of variation at the PEP1 locus on seasonal flowering of perennial *Arabis alpina*

George Coupland [1], Stefan Wötzel [1], Maria Albani [1], Per Toräng [2], Jon Ågren [2], Michel Herzog [3], José Ramón Obeso [4], Marco Bink [5], Thomas Odong [5], Roeland Van Ham [6], Yiannis Kourmpetis [6] and Jörg Wunder [1]

[1] *Department of Plant Developmental Biology, Max-Planck-Institute for Plant Breeding Research, Cologne, Germany*

[2] *Evolutionary Biology Centre, Department of Ecology and Evolution, Uppsala University, Uppsala, Sweden*

[3] *Laboratoire d'Ecologie Alpine (LECA), Université Joseph Fourier, Grenoble, France*

[4] *Unidad Mixta de Investigación en Biodiversidad, Universidad de Oviedo - Campus de Mieres, Mieres, Spain*

[5] *Biometris, Wageningen*

[6] *Laboratory of Bioinformatics, Wageningen University and Research Centre, Wageningen, the Netherlands*

Higher plants exhibit a variety of different life histories. Annual plants live for less than a year and after flowering produce seeds and senesce. By contrast perennials live for many years, dividing their life cycle into episodes of vegetative growth and flowering. Environmental cues control key check points in both life histories. Genes controlling responses to these cues exhibit natural genetic variation that has been studied most in short-lived annuals. For example in *Arabidopsis thaliana* variation at a small number of genes, including *FLOWERING LOCUS C* (*FLC*), *FRIGIDA* and *FLOWERING LOCUS T*, has been shown to confer dramatic differences in flowering time or responses to low winter temperatures (vernalization response). We have been characterizing natural genetic variation conferring differences in the perennial life cycle of *Arabis alpina*, a member of the same family as *A. thaliana*. We are also contrasting flowering control in *A. alpina* with that of its sister annual species *A. montbretiana*, and have been able to form hybrids between the two species allowing the direct identification of genes that contribute to the divergence of annual and perennial life history.

We showed that the *A. alpina* Pajares accession flowers after prolonged vernalization treatment and only for a limited period before returning to vegetative growth. This behavioural pattern requires the *PERPETUAL FLOWERING 1* (*PEP1*) gene, which encodes a MADS box transcription factor orthologous to *FLC* of *A. thaliana*. PEP1 prevents flowering before vernalization, falls in expression during vernalization and then rises again afterwards promoting the return to vegetative development. This pattern of expression differs from FLC in A. thaliana which is stably repressed by vernalization allowing the plant to flower continuously until senescence.

We have performed molecular-genetic analysis on five accessions of *A. alpina* collected across its European range that do not require vernalization to flower and flower continuously (perpetual flowering) rather than returning to vegetative growth (seasonal flowering). Genetic complementation showed that these accessions carry mutant alleles at *PEP1*. Each accession carries a different mutation at *PEP1*, suggesting that such variation has arisen independently many times. More intensive collecting in selected populations showed that both vernalization-requiring and perpetual flowering types exist in the same populations, and we are intensively analyzing two populations in the alps to determine the distribution of alleles of PEP1 and related genes.

Characterization of these alleles demonstrated that in most accessions, including Pajares, the *PEP1* locus contains a tandem arrangement of a full length and a partial *PEP1* copy, which give rise to two full-length transcripts that are differentially expressed. This complexity contrasts with the single gene present in the annual species *A. thaliana* and *A. montbretiana* and might contribute to the more complex expression pattern of *PEP1* that is associated with the perennial life cycle. Our work demonstrates that natural accessions of *A. alpina* exhibit distinct life histories conferred by differences in *PEP1* activity, and that continuous flowering forms have arisen multiple times by inactivation of the floral repressor PEP1.

Gene-environment interactions drive genomic and transcriptomic diversity in wild *Caenorhabditis elegans* populations

Rita J.M. Volkers [1,], L. Basten Snoek [1], Caspara J. van Hellenberg Hubar [1], Renata Coopman [2], Wei Chen [3], Mark G. Sterken [1], Hinrich Schulenburg [3], Bart P. Braeckman [2] and Jan E. Kammenga [1]

[1] *Laboratory of Nematology, Wageningen University, Wageningen, The Netherlands*

[2] *Department of Biology, Ghent University, Belgium*

[3] *Department of Evolutionary Ecology and Genetics, Zoological Institute, Christian Albrechts-Universitaet zu Kiel, Kiel, Germany*

Analyzing and understanding the relationship between genotypes and phenotypes is at the heart of genetics. Research in the nematode *Caenorhabditis elegans* has been instrumental for unravelling many genotype-phenotype relations. But almost all studies, including forward and reverse genetic screens, are dominated by investigations in one canonical single strain. In order to explore the full potential of the natural genetic variation and evolutionary context of the genotype-phenotype map, it is important to study these relations in wild populations. We used multiple wild strains freshly isolated from local habitats to investigate the gene sequence polymorphisms and a multitude of phenotypes including the transcriptome, fitness and behavioural traits. The genotype, transcriptome and a number of fitness traits showed a direct link with the original habitat of the strains. The separation between the isolation sites was prevalent on all chromosomes however chromosome *V* contributed the most to this variation. These results were supported by a differential food preference of the wild isolates for naturally co-existing bacterial species. Moreover, we show that genomic and transcriptomic diversity was driven by genes involved in gene-environment interactions, like c-type lectins and fbox genes. Importantly, where wild *C. elegans* strains show a broad range of genotype-phenotype relations the widely studied canonical genotype N2 covers only a diminutive part of the myriad of genotype-phenotype relations which are present in the wild.

RJMV was funded by the NWO-ALW (project 855.01.151) NEMADAPT, RC and BPB were funded by ESF-EEFG (09-EuroEEFG-FP-002 / G.0998.10N) NEMADAPT. JEK and LBS were funded by the ERASysbio-plus ZonMW project GRAPPLE (project nr. 90201066). MGS was supported by Graduate School Production Ecology & Resource Conservation. WC and HS were funded by NEMADAPT (DFG grant SCHU 1415/11-1).

Evolution of partial genetic sex determination in *Daphnia magna*

Christoph Haag
University of Fribourg and CNRS Monpellier, Switzerland

In *Daphnia*, parthenogenetic reproduction alternates with sexual reproduction, and the sex is usually determined by the environment. However, some lines never produce males, and these non-male producing ("NMP") genotypes can only persist through phases of sexual reproduction if they co-occur with normal ("MP") genotypes that produce both males and females. The distinction between MP and NMP is genetically determined by a single chromosomal region carrying a dominant NMP allele and showing characteristics of a young sex chromosome. In particular, there is evidence for strong suppression of recombination in this region, which may favor the accumulation of further sex-specific loci. However, surprisingly, recombination in this region is also suppressed between the normal MP-chromosomes (analogous of Z-chromosomes in birds). Thus, contrary to the common assumption, recombination suppression may not necessarily evolve after the appearance of sex-determining mutations, but, rather, sex determining mutations may have an advantage if they happen to occur in a region in which recombination is already suppressed.

Natural variation in flowering time in *Arabis alpina* and its adaptive value

Jörg Wunder [1], Stefan Wötzel [1], Maria Albani [1], Per Toräng [2], Jon Ågren [2], Michel Herzog [3], José Ramón Obeso [4], Marco Bink [5], Thomas Odong [5], Roeland van Ham [6], Yiannis Kourmpetis [6] and George Coupland [1]
[1] *Department of Plant Developmental Biology, Max-Planck-Institute for Plant Breeding Research, Cologne, Germany;*
[2] *Evolutionary Biology Centre, Department of Ecology and Evolution, Uppsala University, Uppsala, Sweden;*
[3‡] *Laboratoire d'Ecologie Alpine (LECA), Université Joseph Fourier, Grenoble, France,*
[4] *Unidad Mixta de Investigación en Biodiversidad, Universidad de Oviedo - Campus de Mieres, Mieres, Spain;*
[5] *Biometris, Wageningen,*
[6] *Laboratory of Bioinformatics, Wageningen University and Research Centre, 6708 PB Wageningen, the Netherlands*

In order to learn about the natural variation in the perennial life-history strategy of plants, we analysed populations of *Arabis alpina* L. (Brassicaceae) along an environmental gradient. We selected natural populations in its natural distribution range from Spain to northern Scandinavia to analyse traits related to perennial growth,

with a particular focus on flowering traits. Therefore, we planted several thousand individuals of *A. alpina*, originated from various natural populations, reciprocally into four different experimental gardens. These experimental sites are located in alpine environments with the plants exposed to almost natural, species specific habitat conditions. In addition to these plantations, we analysed the plants growing *in-situ* in their natural habitat and grew plants in controlled greenhouse conditions. Among various other traits which differ among populations, we observed also differences in the timing of flowering between individuals from the far north or high Alps compared to individuals from Spain. The latter started flowering later and proceeded for a longer period than the ones from Scandinavia or the Alps. This correlates with the length of the growing season in these regions and therefore starting to flower earlier and finishing the process of seed production faster is likely an adaptation to the very short summers in these extreme habitats and contributes to the pronounced fitness differences we observed, indicating local adaptation (see abstract of Per Toräng). Moreover, we identified natural mutants which lack the necessity to be exposed to a period of cold to flower in the following spring (vernalisation requirement). Allelism tests suggest that the gene PEP1 is involved, the orthologue of FLC in *Arabidopsis thaliana* (see abstract of George Coupland). The analysis of the PEP1 locus in different natural accessions revealed a particular gene structure with the first exon duplicated in some populations but not in all. Furthermore, the cDNA of PEP1 is not always impaired when vernalisation requirement is absent. This suggests that in at least some natural mutants which lack vernalisation requirement an additional regulator maybe involved. The geographical distribution of individuals lacking vernalisation requirement is not random but shows both regional (all Spanish population show vernalisation requirement) and habitat specific patterns. We found such individuals preferentially in habitats which exhibit an extremely prolonged period of snow coverage. Therefore, we propose that PEP1 provides an advantage to *A. alpina* plants growing in habitats where they experience a long growing season.

Predation and proteomics: progress towards the understanding of the molecular basis of inducible defenses in *Daphnia*

Christian Laforsch [1], Kathrin A. Otte [1,2], Thomas Fröhlich [2] and Georg J. Arnold [2]
[1] *Department of Animal Ecology, University of Bayreuth, Germany*
[2] *Laboratory for Functional Genome Analysis (LAFUGA), Gene Center, Ludwig-Maximilians-University Munich, Germany*

Predation is a key factor in the evolution of prey species and the dynamics of prey communities. In both animals and plants, different defensive mechanisms have evolved in response to this selection pressure. Phenotypic plasticity in defensive traits appears to be an appropriate mechanism to cope with the variable hazard of a frequently changing predator system. A textbook example for these so called inducible defences is the waterflea *Daphnia*. However, our understanding of the mechanisms underlying plastic responses in *Daphnia* is still in its infancy. Given that *Daphnia* is emerging as the key model invertebrate system in ecological genomics/proteomics, our study aims to discover candidate proteins linked to predator induced morphological defences. We apply a proteomic "2D Fluorescence Difference Gel Electrophoresis" (DIGE) approach followed by mass spectrometric (MS) analysis to unravel patterns of differentially expressed proteins in predator (*Triops cancriformis*) exposed *Daphnia magna.* Combining proteomics and genomics provides an exceptional opportunity to reveal the nature and complexity of plastic defensive traits in *Daphnia* on a molecular basis.

How general and how predictable are parental environmental effects? A comprehensive test with *Arabidopsis thaliana*

Oliver Bossdorf [1,2], Vít Latzel [1], Markus Fischer [1], Maartje Groot [3], Ruben Gutzat [4], Christian Lampei [5], Ortun Mittelsten-Scheid [4], Joop Ouborg [3], Karl Schmid [5], Philippine Vergeer [3] and Yuanye Zhang [1]
[1] *Institute of Plant Sciences, University of Bern, Bern, Switzerland*
[2] *Plant Evolutionary Ecology, University of Tübingen, Tübingen, Germany*
[3] *Molecular Ecology, Radboud University Nijmegen, Nijmegen, The Netherlands*
[4] *Gregor Mendel Institute of Molecular Plant Biology (GMI), Austrian Academy of Sciences, Vienna, Austria*
[5] *Institute of Plant Breeding, Seed Science and Population Genetics, University of Hohenheim, Stuttgart, Germany*

Phenotypes of plants, and thus their ecology and evolution, can be influenced by the environmental conditions

experienced by their parents, a phenomenon called parental environmental effects. In some cases, parental effects have been found to be adaptive, i.e. to increase offspring fitness, which has led to much speculation about their evolutionary role, and about the underlying, possibly epigenetic, mechanisms. However, there is still much uncertainty about how common parental environmental effects actually are. Previous studies found mixed results, and it is not clear whether this is because of differences in the design and statistical power of these studies, or whether it is a real phenomenon. Also, for investigating underlying mechanisms, it would be desirable to focus on environmental stresses that reliably trigger the strongest parental effects. Both questions can only be addressed if many different environmental factors are tested for their transgenerational effects in a rigorous, comparative framework. Here, we carried out a comprehensive test for parental effects of different environmental stresses in the model plant *Arabidopsis thaliana*. We subjected plants of three different *Arabidopsis* ecotypes to 24 different biotic or abiotic stresses, or their combinations, and then grew the offspring of all plants in a common environment to test for divergence of phenotypes. We found that the majority of environmental stresses caused either positive or negative parental effects (ranging from -50% to +50% changes in fitness), but that oftentimes these effects were only observed in some ecotypes but absent, or even in the opposite direction, in others. Parental effects of multiple environmental stresses often differed from the combination of their single effects, which indicates that multiple stresses act in non-additive ways, and their effects can thus not be predicted from what is known about individual stresses. Most intriguingly, the direction and magnitude of parental effects was unrelated to how stresses affected the parents. Some stresses hardly affected the parents but caused substantial effects on offspring. For others the situation was reversed. In summary, parental effects can be substantial and appear to be fairly common in *A. thaliana*, but they are strongly genotype-dependent, and appear to be rather difficult to predict.

Occurrence and potential ecological role of actinorhodopsins in freshwater ecosystem

Hans-Peter Grossart
Leibniz Institute of Freshwater Ecology and Inland Fisheries, Stechlin, Germany

Freshwater Actinobacteria are an important and dominant group of bacterioplankton in most temperate freshwater systems. Recently, metagenomic studies discovered rhodopsin-like protein-coding sequences present in Actinobacteria which could be a decisive hint for their success in freshwater ecosystems. We analysed the diversity of actinorhodopsin (ActR) in Lake Stechlin (northern Germany) and assessed the *actR* expression profile during a diurnal cycle. We obtained 85 positive *actR* clones and the phylogenetic analysis points to a close relationship of all obtained sequences to the acI lineage of Actinobacteria. For the first time, we followed *in situ* transcription of *actR* in Lake Stechlin revealing a rather constitutive circadian gene expression. Furthermore, we were successful in heterologous overexpression of the *actR* in *E. coli* for further analysis of their potential ecological function. The knowledge on distribution and functionality of actinorhodopsin is a prerequisite to better understand the ecological success of this abundant and ubiquitous group of freshwater ultramicrobacteria.

Classify! A strategy and workflow to interpret NGS data

Peter Frenzel and Claudia Lüke
Max-Planck-Institute for Terrestrial Microbiology, Marburg, Germany

Deep-sequencing techniques allow an unprecedented coverage of molecular diversity. They promise a breakthrough in molecular ecology, but application is still hampered by technical shortcomings and the sheer number of sequences that have to be quality checked and classified before interpretation. Manual classification is tedious and comes to its limits soon. Here, we use a subunit of the key gene of methane oxidizing bacteria, the *pmoA*, as molecular marker and example for a robust work flow to classify amplicon sequences. Beside the *pmoA*, we considered the *amoA* encoding for a subunit of the closely related ammonium monooxygenase.
We started with a manually curated set of 5000 *pmoA*- and *amoA*-sequences retrieved from public databases. These sequences were quality-checked and aligned using ARB. Based on this data set, we identified the major *pmoA*-lineages, an amendment to previous work. The data set is referred to as 'ARB' below. A second data set comprises 6900 pyrosequencing-reads that were manually quality-checked and assigned to lineages. This data

set is referred to as 'PYRO'. The classifier should be robust enough to tolerate frame shifts and some variation in sequence length, too. In a first step, we calculated for all sequences the frequencies of possible nucleotide n-mers (n=3). This is equivalent to move a sliding window of length n along a sequence and estimating frequencies of the respective n-mers. This technique has originally been developed for text mining, but only occasionally used for nucleotide sequences. For n=3, this procedure results in a total of 64 variables. These variables were used to build two random forest classifiers. Both the classifiers for low (9 putative *pmoA* lineages and *amoA*) and high phylogenetic resolution (67 putative *pmoA* lineages, *amoA*, and 3 putative alkane monooxygenase lineages) showed an excellent agreement with the *a-priori* classification. Applying the classifiers to the PYRO data set (that had been classified manually, but not used for building the classifier) gave comparably good results. The classifiers' performance degraded only marginally upon introducing errors by randomly deleting 1% of nucleotides. Removing up to 10% of nucleotides from one end of the sequences had only minor effects.
In summary, the classifiers showed an excellent performance. We expect more uncertainties studying environments that's diversity is as yet underrepresented in the public databases. Hence, we have developed a criterion to export those sequences for further analyses. Current work is focusing on a spin-off project: We have experienced a diversification in type II methanotrophs that is not yet resolved in our classification. Hence, we have started exploring techniques to generate from yet available and future sequence data useful operational taxa within this lineage.

Chemolithoautotrophic communities and activity in the deep water-water masses of the North Atlantic

Gerhard J. Herndl, Roberta Hansman, Eva Sintes and Thomas Reinthaler
Department of Marine Biology, University of Vienna, Austria

Dark ocean microbial organic carbon demand has been shown to be much higher than supplied by sinking organic matter flux in the Atlantic and the Pacific. One potential source of organic matter in the deep ocean, currently not taken into account, might be the large amount of buoyant or only slowly sinking organic matter in the meso- and bathypelagic ocean and the role of chemoautotrophy of deep-water prokaryotic plankton. Chemlithoautotrophy in the meso- and bathypelagic ocean is comparable to the magnitude of heterotrophic prokaryotic metabolism in the deep-waters of the Atlantic. While some studies have implicated the oxidation of ammonia by members of Thaumarchaeota as fueling dissolved inorganic carbon (DIC) fixation, the estimated nitrogen flux into the deep ocean is about one order of magnitude too low to fuel the measured dark DIC fixation. Thus, the nature and extent of in situ energy sources for chemoautotrophy in the dark ocean are only partially identified and quantified. With a combined –omics and biogeochemical approach, it is possible to identify the energy sources for deep-water chemoautotrophic microbes and ultimately, arrive at a mechanistic understanding of the functioning of the microbial food web in the deep ocean.

Who is doing the job? - Field-scale labelling and activity quantification of methanotrophs in a landfill-cover soil

Ruth Henneberger and Martin H. Schroth
Institute of Biogeochemistry and Pollutant Dynamics (IBP), Swiss Federal Institute of Technology (ETH), Switzerland

Methane (CH_4) is a major contributor to global climate change, and its atmospheric concentration is increasing. Yet, a large portion of produced CH_4 is consumed by methane-oxidizing bacteria (MOB) before it can reach the atmosphere. MOB are ubiquitous in oxic soils and sediments, and they utilize CH_4 as their sole energy and carbon source. In general, MOB may be differentiated based on characteristic phospholipid fatty acids (PLFA). Stable-isotope probing (SIP) on PLFA has been widely applied in laboratory incubations to identify active MOB populations, but results can be difficult to extrapolate to the field. Therefore it is crucial to complement laboratory studies with field-scale approaches, *e.g.* the gas push-pull test (GPPT) method, which allows determining *in-situ* CH_4 oxidation rates. Here, we combined GPPTs with SIP on PLFA by using $^{13}CH_4$, in order to label active MOB at the field-scale while simultaneously quantifying turnover rates. The SIP-GPPT was applied to a landfill-cover soil at several locations along a gradient of MOB activity during summer and winter. Soil samples were collected before and after GPPT, total PLFA were extracted, and incorporation of ^{13}C in the polar lipid fraction was analysed. Methane oxidation rates derived from the $^{13}CH_4$-GPPTs were similar to those derived from CH_4-GPPTs that were performed at the same location prior to the SIP experiments, showing that the ^{13}C-label did not adversely affect CH_4 oxidation. Oxidation rates at the different locations ranged from 0.2

to 55.7 mmol CH_4 (L soil air)$^{-1}$ day^{-1} during summer and from 0.06 to 11.3 mmol CH_4 (L soil air)$^{-1}$ day^{-1} during winter. Clear ^{13}C incorporation was detected into different 14C and 16C fatty acids (FA), typical for Type I MOB, and 18C FAs, typical for Type II MOB. The amount of ^{13}C incorporated into biomass thereby clearly increased with increasing oxidation rates, and $\delta^{13}C$ values of >1500 ‰ were observed for selected FAs. In addition, the range of labelled FAs changed with activity, and no Type II MOB specific FAs were labelled at the low-activity location. The novel SIP-GPPT approach was shown to be a suitable field-scale method to label active MOB over a wide range of activities. Short incubation times required for measurable ^{13}C incorporation into FAs make PLFA-SIP preferable compared to DNA-, RNA- or protein-SIP for field applications. Our findings indicate a positive correlation between CH_4 oxidation rate and diversity of the active MOB community at this site. Type I MOB appear to be dominant in the active MOB community at all locations and seasons, which was also confirmed by microarray analysis of *pmoA* transcripts.

Response of prokaryotic function and diversity to organic and inorganic nutrients inputs in the upper mesopelagic Atlantic

Jarone Pinhassi [1], Federico Baltar [1], Gerhard J. Herndl [2,3], Itziar Lekunberri [2] and Thomas Reinthaler[2]
[1] *Centre for Ecology and Evolution in Microbial model Systems – EEMiS, Linnaeus University, Kalmar, Sweden*
[2] *Dept. of Marine Biology, University of Vienna, Vienna, Austria*
[3] *Dept. of Biological Oceanography, Royal Netherlands Institute for Sea Research (NIOZ), Den Burg, The Netherlands*

To decipher the response of mesopelagic prokaryotic communities to pulses of nutrients, we tracked changes in prokaryotic abundance, extracellular enzymatic activities, heterotrophic production, dark dissolved inorganic carbon fixation and prokaryotic community composition (454 tag pyrosequencing of 16S rRNA genes) in three microcosm experiments with water from the oxygen minimum zone of the subtropical North Atlantic. Four different treatments were established, each amended either with thiosulfate, ammonium or organic matter (i.e. pyruvate plus acetate), and one treatment served as an unamended control. No stimulation was found for any of the prokaryotic parameters examined in the thiosulfate treatment. However, the addition of ammonium resulted in a >two-fold increase in dissolved inorganic carbon fixation, but not in other activity measurements, as compared to the controls in all experiments. The strongest stimulation of bacterial activity was found in the organic matter enrichments, where all measured rates increased >10-fold. Alkaline phosphatase was the measured activity that was most stimulated by organic matter, indicating a coupling between this hydrolytic ectoenzyme and organic matter availability in the dark ocean. Strikingly, in the organic matter treatment, the dark dissolved inorganic carbon fixation rates —assumed to be related to autotrophic metabolisms— were equally stimulated as all the other heterotrophic-related parameters. Our results suggest an important role of dark dissolved inorganic carbon fixation in the carbon flux of the subtropical North Atlantic oxygen minimum zone related to ammonium availability and/or organic matter pulses. Only the addition of organic carbon compounds changed the prokaryotic community structure in the oxygen minimum zone, with some members that belonged to the 'rare biosphere' in the original communities increasing in relative abundance (i.e., Alteromonodales, Rhodobacteraceae, Oceanospirillales and Vibrionaceae). Hence, specific members of the 'rare biosphere' potentially contribute to efficient utilization of pulsed organic matter supply in the mesopelagic realm of the ocean via a wide range of metabolic activities.

Chemolithoautotrophic microbes control the biogeochemistry of marine oxygen depletion zones

Klaus Jürgens, Ruth Anderson, **Carlo Berg**, Christian Bruckner, Sabine Glaubitz, Matthias Labrenz
Leibniz Institute for Baltic Sea Research Warnemünde (IOW), Rostock-Warnemünde, Germany

Marine oxygen-deficient water columns occur near coastal upwelling areas, in enclosed basins, marginal seas and fjords with restricted water circulation. Oxygen-deficient waters are currently expanding on a global scale due to climate change and eutrophication. Microbial community metabolism in oxygen deficient systems impacts marine nutrient and energy flow patterns, resulting in biological nitrogen loss, climate active trace gas production, H_2S detoxification and significant chemoautotrophic production. Recent research combining "metaomics" data with process rate measurements has provided new insights into coupled biogeochemical cycling in different types of oxygen-deficient marine waters and revealed general patterns in global distributions of microbial biodiversity. Our studies on the anoxic basins of the central Baltic Sea revealed that the microbial community of the pelagic oxic-anoxic interface is dominated by chemolithoautotrophic microorganisms. Several groups can be considered as key players for distinct biogeochemical processes. These include ammonia-

oxidizing Thaumarchaeota, chemoautotrophic denitrifying Epsilonproteobacteria (*Sulfurimonas* sp.) and potentially sulfur-oxidizing Gammaproteobacteria (SUP05 cluster). Accounting for up to one fourth of total cell counts in their respective redox zones, these organisms link the carbon, nitrogen and sulfur cycles, and their chemoautotrophic production is the basis of a microbial food web. Close relatives of these groups have been found in marine oxygen minimum zones worldwide. As such, the Baltic Sea represents an ideal model system for an in-depth understanding of the structure and regulating mechanisms of the biogeochemistry and microbiology of marine pelagic anoxia. By a combination of cultivation-dependent and independent approaches we obtained insights into the general structure of the communities, the functional performance of the identified key players and the regulation of their abundance and vertical distribution by bottom-up and top-down factors.

Lifestyle of Flavobacteria in the marine environment

José M. González [1], Silvia G. Acinas [2], Ivaylo Kostadinov [2], Carlos Pedrós-Alió [2] and Ramunas Stepanauskas [3]
[1] *University of La Laguna, La Laguna, Spain*
[2] *Institute of Marine Sciences, CSIC, Barcelona, Spain*
[3] *Bigelow Laboratory for Ocean Sciences, USA*

More than a decade has elapsed since the discovery of proteorhodopsin (PR) in marine bacterioplankton. PR phototrophy should have an important impact on the understanding of the roles of marine bacteria in the biogeochemistry of the ocean. Since its finding, a limited number of studies have dealt with the function of this abundant membrane protein. PR can be found in a wide diversity of marine taxa, although few are amenable to be cultured. Among these there is the Flavobacteria, which are abundant and express the PR gene. Flavobacteria are also the only taxon known to respond to light by using it to grow better than in darkness. In this study we analyzed the gene synteny of the neighborhood of the PR gene, as well as peptides that function as light sensors. Based on metagenome sequences from the global expedition Tara Oceans and genome sequence of isolates, gene context and promoter sequence analyses suggest that the physiology of Flavobacteria revolves around phototrophy. In addition, different gene patterns exist, as well as a wide diversity of PR, frequently the result of lateral gene exchange between Flavobacteria taxa.

Adaptation and function of methane oxidising communities in Arctic terrestrial ecosystems

Mette M. Svenning [1], Alexander T. Tveit [1] and Susanne Liebner [2]
[1] *University of Tromso, Department of Arctic and Marine Biology, Tromso, Norway*
[2] *Helmholtz Centre Potsdam - German Research Centre for Geosciences (GFZ), Potsdam, Germany*

Terrestrial permafrost soil ecosystems in the Arctic constitute a major carbon storage and natural GHG source globally. Arctic peat lands have acted as carbon sinks since the beginning of the Holocene and recent estimates point out that the northern circumpolar permafrost zone store vast amounts of carbon in peatlands. In combination with the current increase of surface temperatures primarily in the northern latitudes, Arctic peat lands are considered threatened environments and hot spots of Earth's climate change. In particular, they are substantial sources of the potent greenhouse gas methane (CH_4). Methane oxidizing bacteria (MOB) mitigate CH_4 emissions and hold an important function in the soil-atmosphere interaction. The performance and the robustness of MOB exposed to environmental changes will be decisive for the amount of methane emitted from Arctic terrestrial ecosystems. Our studies address the presence, activity and adaptation of MOB in the high-Arctic permanent continuous permafrost and in the low-Arctic discontinuous permafrost regions. In both regions, MOB are living under extreme conditions including strong seasonal variations in temperature and precipitation.
In the high-Arctic, the genus *Methylobacter* plays an important role and the cold adapted species *Methylobacter tundripaludum* is documented as the most active *in situ* in Svalbard peat lands. Sequences related to *Methylobacter tundripaludum* have a circumpolar distribution. In low-Arctic, acidic peat lands, different MOB species are active and they have a key role both for CH_4 oxidation and N_2 fixation. Among those species are methanotrophs of the family *Beijerinckiacae* known to lack the particulate methane monooxygenase and to utilize the soluble methane monooxgenase instead.

Partaking of chemolithotrophic and anapleurotic pathways to dark primary production in aphotic zones of the Mediterranean Sea

Michail M. Yakimov, Violetta La Cono and DEEP_C Consortium.
Institute for Coastal Marine Environment, IAMC-CNR, Messina, Italy

Since the dawn of marine biology the photosynthetic fixation of CO_2 occurred at superficial oceanic layer has received considerable attention, whereas the significance of light-independent CO_2 assimilation for sustaining of pelagic and seabed marine ecosystem is poorly understood and an object of active studies. Initially this process, termed as dark primary production (to distinguish from light-driven one), has been studied at oxic/anoxic marine interfaces, the favorable environments for chemoautotrophic activity. The aerobic oxidations of reduced metals, HS^- and intermediate sulfur species, diffusing from anaerobic interior to the oxygenated layers of the interface, are highly exergonic processes yielding of substantial energy capable to support the elevated biomass. The primary drivers, the chemolithoautotrophic communities fix CO_2 mainly via the Calvin-Benson-Bassham and the reductive TCA cycles depending on availability of the oxygen and other electron acceptors. Ammonium is less reduced compound than HS^- and intermediate sulfur species, which means that its oxidation gains much less energy. Nevertheless, recently the global importance of this process in the ocean, especially beneath the photic zone, was demonstrated. An active CO_2 fixation in the deep ocean was postulated be attributed to the ammonium-oxidizing chemoautotrophic archaeal populations, possessing 3-hydroxypropionate/4-hydroxybutyrate pathway of autotrophy. Members of this group are likely the most abundant microorganisms inhabiting meso- and bathypelagic zone of the ocean. Howbeit, it seems that chemolithotrophs are not only the key players capable of CO_2 fixing at the dark. As a part of their metabolism, virtually all microorganisms can incorporate CO_2 in variety of metabolic pathways, not necessary related to the autotrophy. Many heterotrophic bacteria rely on organic compounds for carbon supply, but also are capable to incorporate CO_2 via a variety of carboxylation reactions as part of their central or peripheral metabolic pathways involved in the synthesis of fatty acids, nucleotides and amino acids and in anaplerotic reactions, which incorporate CO_2 to replenish distinct biomass components. It is generally assumed, that the importance of heterotrophic CO_2 assimilation varies between different bacterial species, with the type of organic substrate used for growth and with the metabolic state of the organisms. Hence, dark primary production in the aphotic realm of the oceans may be relevant for a wide range of organisms not necessarily belonging exclusively to chemoautotrophs. Taking into account that anaplerotic pathways seemed to have a significant role in the life strategy of many marine heterotrophs, it is thus very challenging to estimate and distinguish the contribution of all players to the dark primary production, typically measured as rates of bicarbonate fixation and total carbon assimilated.

Macroecology of wetland rice methanotrophs: The β-diversity of *pmoA* genotypes in tropical and subtropical soils

Claudia Lüke [1,2], Adrian Ho [1,3], Bellinda Schneider [1] and Peter Frenzel [1]
[1)] *Max Planck Institute for Terrestrial Microbiology, Marburg, Germany*
[2)] *Radboud University Nijmegen, Nijmegen, Netherlands*
[3)] *Laboratory for Microbial Ecology and Technology (LabMET), Faculty of Bioscience Engineering, Ghent, Belgium*

Even though studies addressing microbial biogeography have increased during the past decade, research on microbial dispersal is still in its infancies and many aspects are only poorly understood. Here, we compared the methanotroph community in paddy soils sampled in Indonesia, Vietnam, China and Italy, focusing on the distance-decay relationship that is well known from the biogeography of macroorganisms. We used the *pmoA* gene as marker for methanotroph diversity in T-RFLP, microarray and pyrosequencing approaches. We could observe a significant increase of β-diversity with geographical distance at the global (12,000 km) and regional scale (20 km). Measured environmental parameters explained only a small amount of data variation and we found no evidence for dispersal limitation. Thus, we propose historical contingencies being responsible for the observed patterns. Furthermore, we performed an in-depth analysis of type II *pmoA* distribution at the sequence level. We used a novel approach to illustrate sequence diversity by projecting sequence dissimilarities into a 2-dimensional ordination space (non-metric multidimensional scaling). The ordination suggest that type II methanotrophs in paddy fields can be divided into four major groups, however, sequences from the different soils were widely distributed independent of the geographic origin. No distance-decay relationship was observed at sequence level. By including tropical field sites (Indonesia and Vietnam) into the analysis, we further observed the first paddy fields harboring a methanotroph community depleted in type II methanotrophs.

The influence of naturally associated microbes on *Caenorhabditis elegans* life history

Philipp Dirksen, Philip Rosenstiel and Hinrich Schulenburg
University of Kiel, Kiel, Germany

More or less all organisms, ranging from sponges to humans, associate with an often extremely diverse microflora. Microbial associations are known to be of immense importance for a host's development, immunity, and life history - all biological fields where the bacteriovorus nematode *Caenorhabditis elegans* has been studied with great success. On the other hand, laboratory cultures of the worm are axenic or monoxenic by default and we lack basic knowledge about its natural ecology; we even do not know what the nematode feeds on in nature. As a result, complex microbial interactions have mostly been created artificially by studying human pathogens or simply ignored. To rectify this discrepancy, we here used 16S rDNA deep sequencing of natural samples in combination with 16S rDNA Sanger sequencing of culturable bacteria to demonstrate a rich and diverse microflora associated with *C. elegans*. The prominent identified orders included Bacteroidetes (Flavobacteriales, Sphingobacteriales), Proteobacteria (Rhizobiales, Pseudomonadales, Enterobacteriales) and Firmicutes (Lactobacillales). We furthermore characterized the exact relationship between individual bacterial strains and the nematode host, using fitness assays, behavioral tests, differential interference contrast microscopy, and fluorescence *in situ* hybridization. Our analysis revealed high nematode fitness on Gammaproteobacteria, whereas Bacteroidetes and Proteobacteria were over time generally more attractive than Actinobacteria and Firmicutes. Our project combines the power of *C. elegans* as a model organism with its natural ecology to establish a tractable genetic model system for the in-depth analysis of naturally occurring host-microbiota interactions.

Metagenomic insights into an uncultivated group of ubiquitous oceanic Thaumarchaeota

Pierre Offre [1], Thomas Weinmaier [2], Thomas Rattei [2], Gerhard Herndl [3] and **Christa Schleper** [1]
[1]*Department of Genetics in Ecology, University of Vienna, Vienna, Austria*
[2]*Department of Computational Systems Biology, University of Vienna, Vienna, Austria*
[3]*Department of Marine Biology, University of Vienna, Vienna, Austria*

The Thaumarchaeota are ubiquitous and represent one of the most abundant groups of microorganisms in the biosphere. Few thaumarchaeal organisms have been cultivated so far, all of which grow autotrophically by oxidizing ammonia to nitrite. Since ammonia-oxidizing thaumarchaea represent only few lineages most of the diversity of this phylum as predicted by 16S rRNA gene surveys remains uncharacterized. Among these are the uncultivated thaumarchaeal lineages ALOHA, pSL12 and 1.1c group ("marine benthic group A-related"). Members of this cluster are ubiquitous in marine and freshwaters, acidic forest soils, sediments and hot springs but their physiology and ecological function remain currently unknown.
The aim of this study is to determine the metabolic potential of members of uncultivated thaumarchaeal groups. A metagenomic approach based on Illumina sequencing was used to reconstruct the genomes of the ALOHA group thriving in northern Atlantic waters. A total of four samples, each from a different depth, i.e. 100, 776, 2750 and 5000m, were collected from three different geographic locations in the eastern part of the subtropical Northern Atlantic. Representatives of the ALOHA group were present at all sites and depths and three almost complete genomes were reconstructed from approx. 120 Gb of paired-end sequence data. Ongoing genome annotation indicates that these archaea are most probably not ammonia oxidizers. Genomic features and key metabolic processes of these enigmatic organisms will be discussed.

The genetic architecture of natural variation in pathogen avoidance behaviour of the nematode *Caenorhabditis elegans*

Rania Nakad [1], L. Basten Snoek [2], Sunna Ellendt [1], Katja Dierking [1], Jan E. Kammenga [2] and Hinrich Schulenburg [1]
[1] *Department of Evolutionary Ecology and Genetics, Zoological Institute Christian-Albrechts University, Kiel, Germany*
[2] *Laboratory of Nematology, Wageningen University, Wageningen, The Netherlands*

The nematode *Caenorhabditis elegans* feeds on microbes in its natural environment. Some of these microbes are

pathogenic and thus harmful to *C. elegans*. To minimize resulting fitness reductions, *C. elegans* has evolved various defence mechanisms including behavioural responses (e.g. avoidance behaviour) that reduce contact with the infectious microbes. In this study, we characterized the genetic architecture of natural variation in *C. elegans* avoidance behaviour against the infectious stages of the Gram-positive bacterium *Bacillus thuringiensis*. We performed an analysis of quantitative trait loci (QTLs) using recombinant inbred lines (RILs) and isogenic lines (NILs) generated from a cross of two genetically as well as phenotypically distinct natural isolates. The analysis identified several QTLs that underlie variation in the behavioural response to pathogenic and/or non-pathogenic bacteria. One of the candidates is the *npr-1* gene. This gene encodes a homolog of the mammalian neuropeptide receptor. *Npr-1* was previously indicated to fully contribute to behavioural defence against the Gram-negative bacterium *Pseudomonas aeruginosa* and food patch-leaving behaviour on *Escherichia coli*. Interestingly, in our study, *npr-1* is not the only gene mediating avoidance behaviour toward *Bacillus thuringiensis*. Moreover, our functional analyses show that *npr-1* alleles appear to influence survival and avoidance behaviour toward *Bacillus thuringiensis* in exactly the opposite way than toward *Pseudomonas aeruginosa*. Our findings highlight the role of *npr-1* in fine-tuning nematode behaviour in an ecological context depending on the microbe to which *C. elegans* is exposed.

Host allelic diversity drives long-term host-parasite coevolutionary dynamics

Ellen Decaestecker
Aquatic Biology, IRF Life Sciences, Science & Technology, KULeuven-Kulak, Kortrijk, Belgium

Hosts and parasites are involved in a coevolutionary interaction in which hosts do not evolve as fast as their parasites. Yet, fast adaptive genetic changes occur upon infection, especially if host-parasite interactions are characterized by Red Queen dynamics. Red Queen dynamics between both antagonists are caused by negative frequency-dependent selection and are assumed to have constant amplitudes. Here, a long-term time shift experiment, based on a unique historical reconstruction of a *Daphnia*-parasite coevolution, reveals that infectivity cycles with a smaller amplitude in experienced than in naive hosts. Experienced hosts were isolated from recent time periods, naive hosts from past time periods. A coevolution model, incorporating an increase in allelic diversity over time in the host confirmed the asymmetry in the infectivity cycles. In contrast, increased virulence over time did not confirm the observed experimental results. The accumulation of resistance alleles affects long-term Red Queen dynamics. Long-term effects in host-parasite coevolution have so far been neglected, but this reconstruction in combination with a theoretical study on long-term time shifts between a host and a parasite extends current insight into the dynamics of co-evolutionary antagonistic interactions.

Genomic variations in European Arabis alpina natural populations: A Re-sequencing Study

Thomas Odong [1], Yiannis Kourmpetis [1], Jörg Wunder [2], George Coupland [2], Per Toräng [3], Tanja Slotte [3], Jon Ågren [3], Michel Herzog [4], José Ramón Obeso [5] and Marco Bink [1]
[1] *Wageningen University and Research, Lab of Bioinformatics & Biometris, Wageningen, The Netherlands*
[2] *Max Planck Institute for Plant Breeding Research, Cologne, Germany*
[3] *Evolutionary Biology Center, University of Uppsala, Uppsala, Sweden*
[4] *Centre National de la Recherche Scientifique, CNRSUniversité Joseph FourierLaboratoire d'Ecologie Alpine, LECA, Grenoble, France*
[5] *University of Oviedo, Ecology Unit, Oviedo, Spain*

Arabis alpina, a relative of a well-studied *Arabidopsis thaliana* provides an excellent model organism for understanding molecular and genetic mechanisms of perennial plants. Recent developments in next generation sequencing technology offer us with opportunity to explore genomic variation in many model organisms such as *Arabis alpina*. In this re-sequencing study, we provide an extensive genome-wide variation data from 23 *Arabis alpina* individual accessions. The overall aim of this project is to unravel the molecular and genetic basis of the perennial life strategies of *Arabis alpina.* For our study, nine natural populations were selected from different geographic locations (populations) across Europe (Sweden(4), Spain(2) and France(3)). The genomes of the 23 individuals were sequenced using Hiseq 2000 and mapped to the reference genome of *Arabis alpina*, which was assembled (yet unpublished) at the Max Planck Institute in Cologne, Germany. Using the assembled sequences, we discovered 5.2 million variant positions(SNPs and short indels) distributed across the genome of *Arabis alpina*. In this talk, we present the result of in-depth analysis of genome-wide variation in *Arabis alpina.* We

will analyze the relative abundance and distributions of the different types of variations along the different segments of the genome (genes, coding regions, exons, introns). We will also present the impact of these variation on the genes expression and protein coding. To understanding the adaptive relevance of these variants we will explore the relationship the spatial distributions of these variations and their predicted impact on genes.

Strong adaptive differentiation between populations of the alpine perennial herb *Arabis alpina* in northern and southern Europe

Per Toräng [1], Jörg Wunder [2], José Ramón Obeso [3], Michel Herzog [4], Stefan Wötzel [2], George Coupland [2], Marco Bink [5], Thomas Odong [5] and Jon Ågren [1]

[1] *Department of Ecology and Genetics, EBC, Uppsala University Uppsala, Sweden*

[2] *Department of Plant Developmental Biology, Max-Planck-Institute for Plant Breeding Research, Cologne, Germany*

[3]*Unidad Mixta de Investigación en Biodiversidad, Universidad de Oviedo - Campus de Mieres, Mieres, Spain*

[4] *Laboratoire d'Ecologie Alpine (LECA), Université Joseph Fourier, Grenoble, France*

[5] *Biometris, Wageningen Laboratory of Bioinformatics, Wageningen University and Research Centre, Wageningen, the Netherlands*

Reciprocal transplant experiments can be used to determine the magnitude of adaptive differentiation among natural populations and to help identify putative adaptive traits and selective agents. In reciprocal transplant experiments, we quantified local adaptation between Scandinavian and Spanish populations of the alpine perennial herb *Arabis alpina*. At the Scandinavian field site, survival and fruit production of Scandinavian populations were higher than those of Spanish populations, while the opposite was true in Spain. The magnitude of the home advantage varied among years and was highest in a drought year in Spain and after a cold winter in Sweden. The results suggest that differences in tolerance to drought and cold contribute to adaptive differentiation between populations from the two regions. They further suggest that these *A. alpina* populations represent a highly suitable model system for examining the functional and genetic basis of plant adaptation in alpine environments.

Local adaptation of sex-indiction in a facultative sexual crusacean: insights from QTL mapping and natural population of *D. magna*

Anne Roulin

Institute of Zoology, Basel, Switzerland

Diapause is a common adaptation in invertebrates to survive harsh conditions following the production of resting eggs. Their production is triggered by environmental cues, and allows populations to survive temporally unsuitable conditions. Daphnia magna is a crustacean reproducing by cyclical parthenogenesis alternating between production of asexual offspring and production of diapausing eggs (ephippia) by sexual reproduction. Prior to ephippia production, males (necessary to ensure ephippia fertilization) are produced parthenogenetically. Sex of parthenogenetic offspring and ephippia production is environmentally induced. Here, we tested the hypothesis that the induction of Daphnia magna resting egg production shows a signature of local adaptation. We postulated that Daphnia from permanent ponds produce less ephippia and males than Daphnia from intermittent ponds, and that the frequency and season of intermittence correlates with the timing and amount of male and ephippia production. We quantified males and ephippia production of clonal D. magna populations in different controlled environments to test for local adaptation in these traits. We found that both the production of ephippia and males varies strongly among populations in a manner suggesting that both traits are locally adapted. By performing a QTL mapping with parent clones from contrasting pond environments, we identified non-overlapping genomic regions associated with male and ephippia production. Since both traits are influenced by two distinct genomic regions and both are necessary for successful resting egg production, we suggest that the genes for their induction co-evolves.

Photosynthesis genes in pelagic freshwater bacteria affiliated with the genus *Polynucleobacter* (*Burkholderiaceae, Betaproteobacteria*)

Martin W. Hahn
Research Institute for Limnology, University of Innsbruck, Austria

A variety of aerobic anoxygenic phototrophic bacteria (AAPB) was isolated from freshwater systems and phenotypically and taxonomically characterized previously. These taxa seem to occur with rather low abundance in the water columns of freshwater habitats. On the other hand, previous cultivation-independent investigations suggested that some abundant groups of pelagic freshwater bacteria include AAPB. Salka et al. (2011) suggested that bacteria related to *Rhodoferax* dominate the aerobic anoxygenic phototrophic communities in German freshwater lakes, and Martinez-Garcia et al., (2011) suggested that "members of the ubiquitous betaproteobacteria *Polynucleobacter* spp. are the dominant AAPs in temperate freshwater lakes".
We screened eleven genomes of free-living *Polynucleobacter* strains for the presence of genes encoding anoxygenic photosynthesis and found in six strains complete photosynthetic gene clusters, while the other strains completely lack photosynthesis genes. Genes contained in these clusters differ from the majority of photosynthesis genes of known AAPB in GC content. Photosynthesis genes of *Polynucleobacter* are characterized by GC values < 50% but in other organisms these genes usually have values > 50%. PCR primers commonly used for detection of genes encoding the photosynthetic reaction center subunit M (PufM) have mismatches to pufM sequences obtained from *Polynucleobacter* genomes. We used PCR primers adjusted to the *Polynucleobacter* genes for screening of a large culture collection of more than 200 *Polynucleobacter* strains for the presence of pufM genes, and obtained from 30% of strains partial gene sequences. Strains isolated from a broad variety of freshwater habitats encode this key gene of anoxygenic photosynthesis. This includes, for instance, strains from acidic and alkaline habitats, systems rich in humic substances, as well as clear water lakes. None of the pufM encoding strains differs from strains lacking this genotypic trait in pigmentation or other phenotypic characteristics indicating capability of photosynthesis. Batch experiments with one strain did not result in higher biomass yield in light exposed treatments compared to treatments grown in the dark. This was observed in the presence and absence of organic substrates. Furthermore, in none of the treatments a change in pigmentation of the investigated *Polynucleobacter* strain was observed. Our results suggest that *Polynucleobacter* bacteria could really represent, at least in some freshwater systems, the most abundant pelagic bacteria encoding genes of anoxygenic photosynthesis, however hints on a significant role of *Polynucleobacter* as AAPB were not obtained so far.

Effect of energy flow on the susceptibility of aerobic methanotrophic communities to disturbance

Andreas Reim and Peter Frenzel
Max Planck Institute for Terrestrial Microbiology, Marburg, Germany

At the surface of flooded soils and sediments, methanotrophic bacteria thrive on the oxidation of methane. Methane is supplied from the anoxic bulk soil below, and oxygen from the overlying water body. The spatial separation of the two substrate sources leads to the formation of overlapping gradients of methane and oxygen, with minima where the methanotrophs are active. Irrespective of methane source strength, the substrate concentrations at the interface are comparably low. Only the location of the oxic-anoxic interface and, hence, the flux at the interface is affected. The energy flow as a potential factor affecting the methanotroph community was neglected so far. Here we demonstrate that the energy flow is influencing methanotrophic community's structure as well as population dynamics. By diluting a native into a g-ray sterilized soil (1:40) we simulated a severe die-off of the microbial community, including methanotrophs. This disturbance allowed us to follow the re-establishment of a methanotroph community as a function of energy flow. Community structure was analyzed by T-RFLP, a diagnostic microarray, and by competitive RT-PCR targeting the *pmoA* gene, a functional and phylogenetic marker for methanotrophs. *pmoA* transcripts served as a proxy for species-specific activity. In general, *Methylobacter* related methanotrophs (type I) recovered faster under high energy flow. *Methylocystis* and *Methylosinus* methanotrophs (type II) were not significantly affected by the energy flow, but rather by disturbance in general. Hence, higher energy flows seem to select for a more resilient type I dominated community. However, in the undisturbed control incubations, we observed a shift from type I to type II methanotrophs under high energy flows. We hypothesize this to be the result of a higher resistance of type II methanotrophs to grazing. This is consistent with earlier experiments on the susceptibility of methanotrophs to grazing, where type I methanotrophs were shown to be stronger affected by protozoan grazers.

Functional comparison of freshwater SAR11 (LD12) and Actinobacteria ac1 using single cell genomics

Stefan Bertilsson [1], Alexander Eiler [1], Trevor Ghylin [2], Siv Andersson [3], Ramunas Stepanauskas [4], Katherine D. McMahon [2]

[1] *Department of Ecology & Genetics, Limnology, Uppsala University, Uppsala, Sweden*

[2]*Departments of Civil and Environmental Engineering, and Bacteriology, University of Wisconsin-Madison, Madison WI, USA*

[3]*Department of Molecular Evolution, Uppsala University, Uppsala, Sweden*

[4]*Bigelow Laboratory for Ocean Sciences, West Boothbay Harbour, Maine, USA*

Freshwater lakes play critical roles in global biogeochemical cycling of carbon and many other bioactive elements while also representing a valuable natural resource. Aquatic bacteria mediate much of the transformation and partitioning of elements in these systems and also control water quality by means of their metabolic activities. Recent cultivation-independent studies have demonstrated that bacterial communities in lakes are distinct from marine systems and soils, with a few, globally distributed bacterial lineages being quantitatively dominant in most freshwater systems. Freshwater SAR11 (LD12) and Actinobacteria ac1 are two examples of such globally successful freshwater lineages that have thus far resisted any cultivation attempts and hence their metabolism and physiology is largely unknown. Both groups feature small cell size and can at least occasionally make up nearly half of the total bacterioplankton in lakes. We compared the functional potential of these two lineages by sequencing of single cell bacterioplankton genomes from different lakes. Ten single amplified genomes from each of the two lineages were identified by their 16S rRNA sequence and then subjected to genome sequencing and annotation using IMG/M. For LD12, partial genome assemblies ranged in size from 0.3-0.9 Mb and feature both low GC content (< 30%) and genome streamlining similar to marine SAR11. Actinobacteria ac1 genome assemblies ranged from 0.7 to 1.2 Mb with a GC content below 50%. Focusing on genome-encoded metabolic traits, there are clear indications of organic substrate resource use partitioning between LD12 and Actinobacteria ac1, with the former capable of active uptake of carboxylic acids and lipids while genes coding for transporters involved in e.g. sugar uptake are essentially absent. In contrast, Actinobacteria acI members seem to be specialized in use of monomeric sugars, oligo-peptides and polyamines. Analogously, LD12 feature several COGs implied in transport of compatible solutes, while such systems appear to be largely missing in Actinobacteria acI. Both groups feature genes putatively involved in phototrophy (Actinorhodopsin or Proteorhodopsin), but phototrophic potential seems more widespread in ac1. Actinobacteria and LD12 also differ with regards to the central metabolism and pathways to fix inorganic carbon. These and other differences in genome encoded traits will be discussed to illustrate the genomic background to metabolic and ecological differentiation in freshwater ultramicrobacteria.

Assessing Marine Protistan Grazing on Marine Prokaryotes in the Bathypelagic Realm of the eastern Mediterranean Sea

Thorsten Stoeck [1], Maria Pachiadaki [1, 2], Andreas Oikonomou [1] and Michail Yakimov [3]

[1] *University of Kaiserslautern, School of Biology, Kaiserslautern, Germany*

[2] *Department of Geology and Geophysics, Woods Hole Oceanographic Institution, Woods Hole, USA*

[3] *Institute for Coastal Marine Environment, IAMC-CNR, Spianata S. Raineri, 86, 98122 Messina, Italy*

Protistan assemblages are essential components of food webs in the vast majority of aquatic ecosystems that have been examined to date. Despite the pivotal roles that these organisms play in carbon/elemental cycling and energy utilization in marine ecosystems, no information exists about the *in situ* activities of these communities in the deep ocean. The impact of protistan phagotrophy was estimated using fluorescently labelled prokaryotes (FLPs) as a prey analogue. During the R/V Urania cruise in September 2012, long- and short-term grazing experiments were conducted *in situ* with the use of SID-ISMS sampler (Woods Hole Oceanographic Institution) at two deep sea water masses with distinct characteristics: (a) the oxic seawater layer at 3000m and (b) the suboxic halocline interface of the deep hypersaline basin Urania (~3460m). Water sample was collected (in each respective depth) and the tracer was injected. The FLPs-seawater incubation was subsampled at selected time points ranging from minutes (for the short-term incubations) up to 12h (for the long-term incubations), and mixed with fixative. Total prokaryotic and eukaryotic abundances and the number of ingested FLPs per eukaryote were counted by epifluorescence microscopy and estimators of phagotrophy were calculated. These estimators are in close agreement for both short- and long- term experiments and indicate that protists have a major impact in the prokaryotic abundance in the deep sea. Almost 10% of the standing prokaryotic abundance was found to be consumed daily from protistan grazers, while the same estimator reached up to 20% at the interface of the anoxic basin. The suboxic, hypersaline interface layer exhibited higher abundances of prokaryotes and microscopic eukaryotes, (as well as microbial productivity) indicating that niches like that can

be hot-spot microbial ecosystems in an oligotrophic environment. Prokaryotic populations are able to support a secondary food web in specific deep-sea habitats.

Trait-based approaches in microbial ecology: A case study on methane oxidizing bacteria testing the phylogenetic signal on functional traits

Sascha Krause, Peter van Bodegom, Paul L.E. Bodelier
Netherlands Institute of Ecology, Wageningen, Netherlands

Microbes form a major part of earth biomass and biodiversity and have important impacts on biogeochemistry and ecosystem processes. Since microbial communities and their functioning can be sensitive to anthropogenic and non-anthropogenic disturbances, efforts have been made to understand the relation between microbial diversity and ecosystem functioning. An ultimate goal is to predict how microbial communities and their functioning respond to environmental changes. Trait-based approaches have been used by ecologists to make these predictions for animals and plants. Hence, this concept may be also applicable to microbes. A first step is to assess the relationship between phylogeny and functional traits. Here we focus on aerobic methane oxidizing bacteria (MOB) as a model system because they have a well-characterized physiology, can be targeted specifically with molecular tools and catalyze an important ecosystem function. We investigated the relationship between MOB phylogeny and functional traits related to physiology (pH, temperature) and a biochemical trait widely used in taxonomy (GC content). However, we were particularly interested in functional traits related to methane oxidation kinetics (Km, specific affinity, methane concentration for maintenance). If a link between phylogeny and functional traits is present, this would increase the predictability of microbial processes substantially. We calculated the phylogenetic signal using Blomberg's *K*, which describes the statistical dependence among species traits through their phylogenetic relationship. We applied the software package Picante, Ape, and Phangorn as implemented in the statistical software R. First, a literature study was performed to generate a representative data set of biochemical and physiological traits. Second, the only known comprehensive data set on methane oxidation kinetics from Knief and colleagues (2005) has been applied to identify the degree of phylogenetic signal in functional traits related to methane oxidation kinetics. All analyses were performed both on the *16s rRNA* gene and the *pmoA* gene level. The *pmoA* gene encodes a subunit of methane monooxygenases which are involved in the first step of methane oxidation. Results demonstrated indeed that functional traits displayed phylogenetic signals, albeit much weaker than assumed under a Brownian motion model of trait evolution. Based on the *16s rRNA* phylogeny, however, phylogenetic signals for functional traits related to methane oxidation kinetics were virtually absent. In conclusion, these results indicated that functional traits related to methane oxidation kinetics are connected to phylogeny and can thus be considered in trait-based biodiversity ecosystem functioning models. However, more experimental data is necessary to verify these initial results.

Feeding on their competitor's remains: Grazing resistant freshwater bacteria profit from organic carbon possibly released through protistan foraging

Ester M. Eckert, Michael Baumgartner, Iris M. Huber and Jakob Pernthaler
Limnological Station, Institute of Plant Biology, University of Zurich, Kilchberg, Switzerland

The rise of grazing resistant planktonic bacteria in freshwater lakes during vernal phytoplankton blooms is favoured by the high predation pressure from heterotrophic nanoflagellates (HNF). At the same time, the spring period is also characterized by the availability of organic carbon species that are in parts derived from cellular debris generated during bacterivory or viral lysis, such as peptidoglycan, chitin and their subunit N-acetylglucosamine (NAG). We tested the hypothesis that two dominant groups of grazing resistant bacteria, ac1 tribe of Actinobacteria (ac1) and filamentous bacteria from the LD2 lineage (Saprospiraceae), would profit from such carbon sources during periods of intense HNF predation. The abundances of ac1 and LD2 rose in parallel with the HNF population, and disproportionally high fractions of cells from both bacterial lineages were involved in NAG uptake. Members of ac1 and LD2 were significantly (albeit transiently) more enriched after

NAG addition than in control incubations. However, highest growth rates of both bacterial lineages were found on the amino sugar containing polymers chitin and peptidoglycan. Moreover, the direct or indirect transfer of organic carbon from peptidoglycan to LD2 filaments could be demonstrated. We thus provide evidence that these taxa may benefit twofold from HNF predation: by direct removal of their competitors, and by specific physiological adaptations to utilize carbon sources that are released during protistan grazing or viral lysis.

Rapid epigenetically-based evolution in experimental plant populations

Vit Latzel [1], Yuanye Zhang [1], Vincent Colot [2], Markus Fischer [1] and Oliver Bossdorf [1,3]
[1] *Institute of Plant Sciences, University of Bern, Bern, Switzerland*
[2] *Institut de Biologie de l'Ecole Normale Supérieure, CNRS UMR 8197, INSERM U1024, Paris Cedex 05, France.*
[3] *Evolutionary Plant Ecology, University of Tübingen, Tübingen, Germany*

Epigenetic variation can cause heritable variation in ecologically important traits, and it should therefore allow rapid epigenetically-based evolution, even in the absence of DNA sequence variation. However, so far this possibility was never experimentally tested. Here we provide, for the first time, experimental proof of epigenetically-based rapid evolution in plant populations. We used epigenetic recombinant inbred lines (epiRILs = nearly isogenic lines that are highly variable in DNA methylation) of *Arabidopsis thaliana* to construct 100 experimental plant populations that were initially all composed of the same 30 epiRILs. We subjected these populations to four generations of quasi-natural selection imposed by four different environmental conditions: drought, increased nutrient availability, competition by other weeds, and a control environment. To test whether rapid evolutionary divergence had occurred among these different experimental environments, we grew offspring of the fourth generation in a common environment. We found significant divergence of phenotypes among the different environments: In the competition environment, taller plants had been selected, and in the drought environment, earlier-flowering epigenotypes had been selected. Molecular epigenotyping of the same offspring plants confirmed significant and non-random shifts in epigenotype frequencies among the different environments. Our results demonstrate that heritable epigenetic variation alone can provide the raw material for rapid evolution of plant phenotypes in changing environments.

New challenges in ecological epigenetics: epigenetics linked to inbreeding depression

Philippine Vergeer [1,2], Niels Wagemaker [1] and Joop Ouborg [1]
[1] *Section of Molecular Ecology, Institute for Water and Wetland Research, Radboud University Nijmegen, the Netherlands*
[2] *Nature Conservation and Plant Ecology group, Wageningen University, the Netherlands*

One of the most intriguing current puzzles of evolutionary plant biology is to understand the role of epigenetic processes in ecology and evolution: How do plants respond to habitat fragmentation and environmental change and what is the role of epigenetic processes in these evolutionary processes? Habitat fragmentation results in smaller populations with decreased genetic variation and increased risk of inbreeding. Inbreeding depression, the reduced performance as a result of inbreeding, is known to be environmentally dependent with inbred individuals responding differently to environmental change than outbred individuals. We hypothesize that epigenetic mechanisms, which can be triggered by the environment and inherited across generations, may contribute to inbreeding depression. We studied phenotypic and epigenetic variation, measured as DNA methylation, of the perennial plant *Scabiosa columbaria* in response to different environmental factors. Substantial differences in phenotypic as well as epigenetic variation were observed in response to the different environments. In addition, methylation levels were found to be affected by inbreeding and different epigenotypes were observed for inbred and outbred plants. Moreover, after the addition of a demethylation agent (i.e., 5-azacytidine), phenotypic differences between outbred and inbred plants were nullified. These results show that epigenetic processes are affected by inbreeding and suggest that inbreeding depression has an epigenetic component, as was hypothesised. The ecological consequences of these results are discussed.

Environmental and ecological genomics of *Daphnia magna*: identifying processes and mechanisms of adaptation to stress

Luisa Orsini [1], Joachim Mergeay [2], Katina I. Spanier [1], Joost Vanoverbeke [1] and Luc De Meester [1]
[1] *Laboratory of Aquatic Ecology, Evolution and Conservation, University of Leuven, Leuven, Belgium*
[2] *Research Institute for Nature and Forest, Geraardsbergen, Belgium*

Natural populations are confronted with multiple selection pressures resulting in a mosaic of environmental stressors at the landscape level. Identifying the genetic underpinning of adaptation to these complex selection environments and assigning causes of natural selection within multidimensional selection regimes is challenging, because of the interaction among neutral and selective processes in the wild. Overcoming this level of complexity requires an unbiased search for the evidence of selection in the genomes of populations sampled from their natural habitats and the identification of past demographic processes that lead to present-day populations' genetic structure. Identifying these past evolutionary processes requires to monitor evolution through time and to unravel the mechanisms underlying adaptive responses. A temporal perspective is thus crucial to produce important insights into the dynamics and processes. Understanding how evolution shapes the genetic structures of populations has principally relied on investigations through space, in lieu of time, because long-term phenotypic and molecular data are scarce. Dormant propagules in sediments, soils and permafrost are convenient natural archives of population-histories from which to trace adaptive trajectories along extended time periods. The water flea *Daphnia magna* is a renowned ecological model system with its well-documented ecology, the possibility to analyze dormant egg banks and the short generation time allowing an experimental evolution approach. Capitalizing on the strengths of this model system we identify selective and demographic processes driving present-day population genetic structure by analyzing dormant eggs in space and time. We identify the types of genetic variation that matter for adaptation, and discover the sources of genetic variation driving adaptive responses to environmental changes. Using a genome scan approach in space, time and experimental evolution trials, we provide solid evidence of differential selection at the genome level under well-characterized environmental gradients in populations distributed at regional scale and identify candidate genes linked to three environmental stressors. We validate these spatial patterns in "time" on populations hatched from sediment cores with known history for the same three stressors and in experimental evolution trials. Building on these results, we study how the complex mosaic of biotic and abiotic variables naturally occurring in the landscape, in addition to the already studied gradients, contributes to driving neutral and adaptive genomic variation. We infer past and recent demographic processes by contrasting patterns of local and regional neutral genetic diversity at markers with different mutation rates. This genome-based analysis reveals that selection plays a major role in determining the population genetic structure of *D. magna*. On one hand, environmental selection directly impacts genetic variation at loci hitchhiking with genes under selection. On the other, priority effects enhanced by local genetic adaptation (cf. monopolization) affect neutral genetic variation by reducing gene flow among populations and genetic diversity within populations. To unravel mechanisms underlying adaptive responses to environmental stress and identify the types of genetic variation driving adaptive responses, we are now analyzing full transcriptome data. The parallel analysis of transcriptome and genome data will allow us to gain unprecedented insights into processes and mechanisms of adaptation to environmental stress.

Poster session

1A Unraveling mode of actions and toxin profiles with high throughput microarrays: a case study in Daphnia exposed to different cyanobacterial stressors

Jana Asselman [1], Michael Pfrender [2], Jacqueline Lopez [2], Joseph Shaw [3] and Karel De Schamphelaere [1]

[1] *Laboratory of Environmental Toxicology and Aquatic Ecology, Ghent University (UGent), Ghent, Belgium*
[2] *Department of Biological Sciences, University of Notre Dame, Notre Dame, USA*
[3] *The School of Public and Environmental Affairs, Indiana University, Bloomington, USA*

Over the last decade, molecular technologies have evolved into robust high throughput platforms available for many scientists in a wide variety of disciplines. Yet, knowledge of stress responses and mechanisms of toxicity across stressors remains limited. Therefore, we have studied stress response to 6 distinct cyanobacterial species in Daphnia pulex. By studying effects on gene level with high throughput microarray technology and effects on organism level through standard ecotoxicology methods for a group of stressors simultaneously, this study aimed

at identifying and unraveling stress response patterns and mechanisms of toxicity. Furthermore, by first considering all 6 species as cyanobacterial stress in general without distinction, it was possible to identify mechanisms involved in general cyanobacterial stress response. Four day old Juveniles of *Daphnia pulex* were exposed to a diet contaminated with cyanobacteria for a period of ten days. The experiment consisted of six different treatments, representing six different species of cyanobacteria. Afterwards, RNA was extracted, reverse transcribed to cDNA and hybridized to a two color whole transcriptome microarray. Image data was processed and normalized in R using Limma. Data analysis revealed six pathways involved in a general cyanobacterial stress response. These could be divided in three groups of mechanisms. First, pathways involving detoxification (Cytochrome P450 and Glutathione) were significantly induced in organisms exposed to cyanobacterial stress. This could potentially be a response to the toxins produced by the cyanobacteria as these pathways are known detoxification steps for the cyanobacterial toxins. Second, pathways involved in steroid and poly unsaturated fatty acid synthesis, were also significantly induced. This may potentially be linked to the lack of steroid and PUFA's in cyanobacteria in contrast to green algae as a nutrient source. Third, we observed a significant inducement of genes in pathways related to the energy metabolism (ubiquinone cofactor and carbohydrate metabolism). By combining expression of exposures to different cyanobacteria, it was possible to gain insights in mechanisms involved in cyanobacterial stress response that could be potentially related to effects at the organism level. Finally, species specific stress response for each cyanobacteria can be identified by contrasting the expression profiles with these mechanisms of general cyanobacterial stress.

2A Ecophysiological features of ammonia oxidizing thaumarchaeota from hypoxic waters of the Baltic Sea

Carlo Berg [1], Luisa Listmann [1], Verona Vandieken [1, 2] and Klaus Jürgens [1]
[1]*Leibniz Institute for Baltic Sea Research Warnemünde (IOW), Rostock, Germany*
[2]*Institute for Chemistry and Biology of the Marine Environment (ICBM), Carl von Ossietzky University of Oldenburg, Oldenburg, Germany*

Within nitrification, ammonia oxidation is a process relevant to the biogeochemical cycling of nitrogen and carbon and - in the Baltic Sea - mainly initiated by members of the *Thaumarchaeota*, playing a pivotal role in this ecosystem. To better characterize physiology, enrichment cultures can be used to infer features which otherwise are less well accessible from environmental studies. We studied growth, autotrophy and phylogeny of ammonia oxidizing archaea obtained from pelagic hypoxic waters of the Gotland Basin, central Baltic Sea. From this site, an enrichment culture was obtained under oxic conditions in cell-free natural redox-zone water as a medium. Ammonium, total and archaeal cell numbers and CO_2 fixation were determined to relate biomass production to growth rates. Further, CO_2 fixation served as indicator to elucidate the efficiency of the archaea-specific hypusination inhibitor GC_7. The highly enriched culture consisted of up to 97% *Archaea*, reaching up to 2.9×10^7 archaeal cells mL^{-1}. Phylogenetically, the enrichment fell within the *Nitrosopumilus* cluster of *Thaumarchaeota* group I.1a and was closely related to *Nitrosoarchaeum limnia* SFB1 with 99% similarity based on both 16S rRNA gene and *amoA* gene sequences. Growth correlated with consumption of ammonium while CO_2 was used to generate biomass. The ratio of N oxidized per C fixed was 10:1, respectively. Based on the amount of inorganic carbon that was fixed during exponential phase, the cells were growing autotrophically and accounted for approximately 10 fg C per cell. Sensitivity to the domain-specific archaeal inhibitor GC_7 was expressed by a decrease in autotrophic activity by up to 82% within 24h. Our results highlight the biogeochemical role of ammonia oxidizing thaumarchaeota for Baltic Sea hypoxic waters via the coupling of N and C cycles through ammonia oxidation and autotrophy, respectively. The applicability of the specific inhibitor GC_7 in short-term experiments allows targeted environmental studies on processes mediated by thaumarchaeota.

3A Host phylogeny and microenvironment are major drivers of methanogenic community structure in arthropod guts

Carsten Dietrich, James O. Nonoh, Kristina Paul, Lena Mikulski and Andreas Brune
Max Planck Institute for Terrestrial Microbiology, Marburg, Germany

Termites, cockroaches, scarab beetle larvae, and millipedes are the only arthropods known to harbor methanogens in their intestinal tracts. Particularly the termites, whose methane emission rates rank second only after the ruminants, contribute substantially to the global budget of this greenhouse gas. However, only little is

known about the diversity of methanogens and their community structure in their sometimes highly compartmented guts. We analyzed the archaeal communities in a wide range of host species, using a combination of cloning and pyrotag sequencing of the 16S rRNA gene. The assemblages of methanogenic archaea in different hosts showed distinct phylogenetic patterns. Highest diversity was found in soil-feeding termites, which are the highest methane emitters and harbor methanogens of several orders, including the recently discovered 'Methanoplasmatales'. To identify the environmental drivers responsible for the differences in community structure observed between the consecutive gut compartments and the functional roles of the different populations, we investigated the stimulation of methanogenesis by the addition of potential substrates. Our results indicate that the structure of the methanogenic communities in arthropod guts are shaped by a combination of host phylogeny and exogenous factors, including pH, oxygen status, and the availability of methanogenic substrates.

4A Microbial eukaryote plankton community structure in the Matapan Deep (Mediterranean Sea)

Sabine Filker [1], Maria Pachiadaki [1,2], Michail Yakimov [3], Francesco Smedile [3], Thorsten Stoeck [1]
[1] *University of Kaiserslautern, School of Biology, Kaiserslautern, Germany*
[2] *Department of Geology and Geophysics, Woods Hole Oceanographic Institution, Woods Hole, USA*
[3] *Institute for Coastal Marine Environment, IAMC-CNR, Messina, Italy*

The marine pelagic zone situated >200 meters below sea level is the largest marine subsystem comprising more than two-thirds of the oceanic volume but at the same time it is one of the least explored ecosystems on Earth. Here, the community structure of microbial eukaryotes (protists and fungi) was investigated in the deepest site of the Mediterranean Sea, the Matapan Deep. The diversity of microbial eukaryotes was examined through the construction and analyses of V4 SSU rDNA pyrosequenced amplicon libraries at six different depths (200 m, 500 m, 1250 m, 2222 m, 3500 m and 4500 m). We found varying diversity patterns throughout the different sampling depths, with the highest eukaryotic diversity being encountered in 200 m and the lowest in 1250 m. Down to a depth of 2222 m, alveolates were identified as the most abundant protistan group, being replaced by the stramenopiles in the deeper regions. Interestingly, groups like the choanoflagellida, green alga, centroheliozoa, cryptophyta and rhodophyta cannot be observed in the mean depths (1250 m, 2222 m), while amoebozoa appear in low abundances. A hierarchical UPGMA cluster analysis identified two clusters, separating the eukaryotic communities of 200, 500 and 1250 m depths from the other communities. The data indicate depth-related community structures, possibly being influenced by nutrient availability, increasing pressure and present water currents.

5A Succession of the prokaryotic community in the North Atlantic's deep water masses

Alexander H. Frank, Juan A. Garcia, Gerhard J. Herndl and Thomas Reinthaler
University of Vienna, Department of Limnology and Oceanography, Vienna, Austria

The horizontal and vertical changes of bacterial and archaeal community composition and beta-diversity were determined in the eastern North Atlantic's dark realm. Following a 3000 km long latitudinal transect (49ºN-26ºN), the North Atlantic Drift province and the Gyre province were sampled from the base of the euphotic layer to the abyssopelagic realm. We used tag encoded FLX amplicon pyrosequencing with primer sets targeting the V1-V3 region to obtain ~1500 sequences per sample of an average read length of ~370 bp. Microbial beta-diversity was assessed using several estimators. Microbial diversity in the mesopelagic water masses was variable, reflecting three different biogeographic provinces of the North Atlantic, namely the drift, the subtropical, and tropical region. In the bathypelagic waters, however, microbial community composition clustered according to water masses and a clear succession of the major microbial groups was found from the north toward the south of the transect. Taken together, our data suggest that in surface waters, the prokaryotic community composition follows the Longhurst biogeographic provinces while in bathypelagic waters, it gradually changes due to the aging of the deep water masses.

6A Eco-evolutionary dynamics of antipredator defenses in temporally variable environments- an evolving metacommunity perspective

Hendrik Frey, Aline Waterkeyn, Luc De Meester and Luc Brendonck
Laboratory of Aquatic Ecology, Evolution and Conservation, KU Leuven, Leuven, Belgium

There is growing awareness that ecological and evolutionary processes can occur at the same time scale and can strongly interact. Ignoring these interactions may distort our view on population, community and ecosystem responses to environmental change. The evolving metacommunity framework integrates both ecological and evolutionary processes. It focuses on diverse communities and includes responses to all kinds of environmental gradients (such as predation). Thus, it can help to improve our understanding of the impact that keystone predator species exhibit on aquatic ecosystems on the (meta-)community level. In this project we will study the impact of a key invertebrate predator, the tadpole shrimp *Triops cancriformis*, inhabiting isolated pond habitats as a model of a selection pressure that impacts an evolving metacommunity of zooplankton prey (including *Daphnia magna* as dominant grazer species and *Daphnia atkinsoni* as a competitor of *D. magna*).
In the course of this PhD project we want to integrate data concerning five different endpoints to generate new insights in the structure and dynamics of evolving metacommunities. To investigate the different endpoints we will use a combination of field sampling- and experimental approach. First, we will investigate the (1) genetic metapopulation structure of both the predator *T. cancriformis* and its focal prey species *D. magna* in relation to predation pressure and in presence and absence of *D. atkinsoni.* Then, we will analyze patterns of (2) genetic variation in *D. magna* antipredator traits (in the presence of *T. cancriformis* some clones of *D. magna* show an increase in body length and shoulder shield width as well as an increase in carapace rigidity). Additionally, we will explore (3) zooplankton community structure and associated (4) community trait values in relation to predation gradients and presence/ absence of competition, both experimentally and in the field. Finally we want to (5) link the defensive traits of induced *D. magna* to genomic variation in the studied populations using a candidate gene approach focusing on genes identified by the STRESSFLEA consortium. Integrating these data will allow us to analyze the degree to which genetic variation in traits of a key species may impact species sorting or community trait responses to environmental gradients (such as predation and competition).

7A Microbial community composition and functional gene variation with depth in the deep-water masses from the Atlantic

Juan A. Garcia [1], Kristin Bergauer [1], Pierre Offre [2], Thomas Weinmaier [3], Christa Schleper [2], Gerhard Herndl [1,4]
[1] *Department of Limnology and Oceanography, University of Vienna, Vienna, Austria.*
[2] *Department of Genetics in Ecology, University of Vienna, Vienna, Austria*
[3] *Department of Computational Systems Biology, University of Vienna, Vienna, Austria*
[4] *Department of Biological Oceanography, Royal Netherlands for Sea Research (NIOZ), Den Burg, The Netherlands*

The phylogenetic but particularly the functional diversity of prokaryotes in the deep-water masses of the Atlantic are poorly characterized. To address this lack of knowledge, metagenomic analyses using single-end pyrosequencing and paired-end Illumina sequencing were performed on eight stations in the North Atlantic gyre at six different depths, ranging from the deep euphotic (100 m) to the abyssal zone (5000 m). Distinct water masses were sampled such as the oxygen minimum zone, the North Atlantic Deep Water and the Antarctic Bottom Water. Phylogenetic analyses showed that the microbial assemblages of all metagenomic libraries were dominated mainly by Proteobacteria. Moreover, Firmicutes, Cyanobacteria and Thaumarchaeota contributed to a substantial fraction in surface waters whereas the presence of Actinobacteria, Thaumarchaeota and Crenarchaeota was substantial in deeper waters. Despite these taxonomic differences, each of the communities shared a similar metabolic composition as indicated by the similar percentage of COGs and ORFs involved in the main metabolic pathways. However, substantial differences were detectable in the percentage of some functional genes, mainly related to energy acquisition and membrane transporters among the different depth layers. The percentage of transporters was significantly higher at the base of the euphotic zone than in deeper

layers. From the meso- to the abyssopelagic layers, the percentage of transporters to the total number of proteins was rather constant. The number of ORFs related to phosphorus metabolism decreased with depth as well as the number of carboxylases. The percentage of ORFs involved in the nitrogen and sulfur metabolism decreased with depth down to the bathypelagic zone but increased again in the abyssal possibly reflecting the influence of resuspension of bottom sediments or outwelling of N- and S-compounds from the deep sea floor.

8A Identification of the gammaproteobacterial sulfur-oxidizer SUP05 as a potential key organism in Baltic Sea pelagic redoxzones

Sabine Glaubitz, Katrin Kießlich, Christian Meeske, Matthias Labrenz and Klaus Jürgens
Leibniz Institute for Baltic Sea Research Warnemünde, Rostock-Warnemünde, Germany

Gammaproteobacterial sulfur oxidizers (GSO), particularly SUP05-related sequences, have been found worldwide in numerous marine oxygen-deficient environments. However, knowledge is scarce regarding their abundance, distribution, and potential ecological role. In the present study, we identified by 16S cloning gammaproteobacterial sulfur oxidizers in Baltic Sea pelagic redoxclines. A specific oligonucleotide probe was designed to quantify their abundance throughout the oxygen gradient in different years and sampling stations by CARD-FISH. By this, we succeeded to identify a SUP05 related phylotype in Baltic Sea redoxclines, which affiliated with sequences generated in numerous marine oxygen minimum zones. Quantification of the *in situ* population of SUP05 with the newly designed oligonucleotide probe revealed that this phylogenetic group occurred in high numbers, reaching 15 -30 % of total prokaryotes (~ 10^5 cells mL^{-1}) in depths where both oxygen and sulfide concentrations were minimal (below 5 µmol L^{-1}). The vertical distribution of SUP05 cells resembles that of the epsilonproteobacterial subgroup GD17, which dominates chemolithoautotrophic denitrification around the sulfide-nitrate interface. In order to elucidate the specific ecological niche of SUP05 we conducted stimulation experiments with different electron donor and acceptor combinations. Preliminary results revealed that cell abundance was positively affected by manganese oxide, nitrate and dithionite. Moreover, *cbbM* transcripts potentially originating from SUP05 cells support previous evidence for the chemolithoautotrophic activity of this taxon in Black Sea redoxclines. Our results on the vertical distribution and high abundance of SUP05 suggest that this group plays an important role in marine redoxcline biogeochemistry, probably as anaerobic or aerobic sulfur-oxidizers. Future studies should aim to resolve the niche differentiation of SUP05 with the epsilonproteobacteria which inhabit the same habitat and harbor similar metabolic pathways.

9A Transgenerational effects of stress on plant performance in Arabidopsis

Maartje Groot [1], Rik Kooke [2], Saskia van Wees [3], Joost Keurentjes [2], Philippine Vergeer [4], Joop Ouborg [1] and Koen Verhoeven [5]
[1] *Molecular Ecology, Radboud University Nijmegen, the Netherlands*
[2] *Laboratory of Plant Physiology, WU Plant Sciences, Wageningen, the Netherlands*
[3] *Plant Microbe interactions, Department of Biology, Faculty of Science, University of Utrecht, Utrecht, the Netherlands*
[4] *Nature Conservation and Plant Ecology Group, Wageningen University, Wageningen, the Netherlands*
[5] *Department of Terrestrial Ecology, Netherlands Institute of Ecology, Wageningen, the Netherlands*

Transgenerational stress memory allows offspring of exposed plants to react more quickly and adequately when they encounter the same stress. Growing evidence suggests that such effects may be mediated through epigenetic mechanisms and can persist for multiple generations. However, it is largely unknown how common transgenerational effects are and whether they play a relevant role under natural conditions. In our research we exposed Arabidopsis Col-0 plants for three consecutive generations to salt stress or control environments, in a factorial design with all possible combinations of G1, G2 and G3 environments. Offspring was evaluated in a common garden field experiment. Offspring of salt-stress plants had smaller rosettes in the field experiment (maternal effect), and this effect was also observed when grandparents but not parents were salt-stressed (transgenerational effect). Strikingly, a maternal effect on offspring rosette diameter was not expressed after 2 or 3 consecutive generations of salt exposure, suggesting gradual acclimatization to the effects of salt stress over multiple generations. Similar results were observed for silique number. These results indicate that Arabidopsis

phenotypes under field growing conditions are affected by parental and also grandparental environmental conditions. Ongoing work is aimed at evaluating offspring performance under controlled salt stress and control environments. Similarly we exposed Arabidopsis Col-0 plants for three consecutive generations to Jasmonic acid or control environments, in the same factorial design mentioned above. Offspring was exposed to either *Pseudomonas syringae* or *Botrytis cinerea* and the disease symptoms were scored. Results of both treatments showed stochastic patterns. This could indicate, without direct selection pressures, the importance of chance in epigenetic processes.

10A Copper-containing monooxygenases in type I methanotrophs: environmental distribution and function

Stephanie Hainbuch [1], Claudia Lüke [2] and Peter Frenzel [1]
[1] *Max Planck Institute for Terrestrial Microbiology, Marburg, Germany*
[2] *Radboud University Nijmegen, Nijmegen, The Netherlands*

The copper containing particulate methane monooxygenase (pMMO) is the key enzyme of the aerobic methane oxidizing pathway performed, among others, by proteobacterial methanotrophic bacteria (MOB). Alphaproteobacterial (Type II) MOB contain a second pMMO isoenzyme with a high substrate affinity allowing the oxidation of methane down to atmospheric concentrations. Very recently, a monooxygenase homologue (pXMO) was found in several gammaproteobacterial (Type I) MOB. Sequences of the pXMO subunit (*pxmA*) form a deep branching lineage (M84_P105) distinct from all known *pmoA* sequences. Here, we designed a novel primer set based on aligned *pmoA*-like environmental sequences of the M84_P105 cluster. Type I and Type II MOB pure cultures were scanned with these primers for additional pMMO isoenzymes. We could detect *pxmA* gene sequences in Type Ia *Methylomonas spec*., *Methylobacter luteus*, *Methylosarcina fibrata* and *Methylosarcina quisquilarum* that cluster within the M84_P105 group. To predict the physiological function of the pXMO, pure cultures of *Methylobacter luteus* were incubated with different substrates. Due to the close relationship of the M84_P105 cluster to homologue sequences of ethane and ammonium oxidizers the substrates methane, ethane or ammonium were chosen. Substrate consumption was followed by gas chromatography. Population growth and transcriptional levels of *pxmA* were analyzed by competitive PCR. Methane was rapidly consumed, but neither ethane nor ammonium decreased within 28 days of incubation. However, in the presence of methane, ethane was rapidly co-oxidized. The expression of the *pxmA* gene was low and only detectable in samples incubated with methane. The results indicate that the pXMO isoenzyme is rather a methane monooxygenase with an alternative enzyme kinetic than a monooxygenase used for the oxidation of short chained alkanes or ammonium. To get an insight into the distribution and the abundance of the *pxmA* gene pyrosequencing analyses of three rice field soils and two freshwater sediments were performed with the newly designed primer set. A great number of sequences clustered within M84_P105 cluster. Further analyses with three additional novel primer sets showed that the diversity of the *pmoA*-like lineages is much higher than expected so far and widespread in different environments.

11A Plant adaptation to frost. A systemic approach in *Arabis alpina*

Frédéric LaPorte [1], Emil Ganchev [1,2], Evgueni Ananiev [1,2], Marie-Françoise Niogret [3], Alain Boucherau [3] Jenny Renaut [4], Karl Nordström [5], George Coupland [5] and **Michel Herzog**[1]
[1] *Laboratoire d'Ecologie Alpine, Université Joseph Fourier and CNRS, Grenoble, France*
[2] *Department of Plant Physiology, University of Sofia, Bulgaria*
[3] *Amélioration des Plantes et Biotechnologies Végétales, Université de Rennes 1, INRA-Agrocampus Ouest, Rennes, France*
[4] *EVA, Centre de Recherche Public – Gabriel Lippmann, Luxembourg*
[5] *Max-Planck-Institute for Plant Breeding Research, Cologne, Germany*

One of the aspects of global warming is the occurrence, at local or regional scales, of severe temperature inversions with cold or freezing episodes, which possibly lead to important economic consequences. Growing interest is focussed on *Arabis alpina*, a perennial *Brassicaceae* closely related to *Brassica* sp. and *Arabidopsis* sp. and widely spread in the arctic zone and in mountainous areas of the northern hemisphere, at elevations ranging from 500 to 3200 m depending on latitude. The present pioneer collaborative

project aims to integrate multi-level system biology technologies to map and dissect complex regulatory mechanisms into characterized gene, protein and metabolite networks that underlie the complex phenotypic trait of adaptation to cold and freezing stress. Genes, proteins and metabolites differentially expressed or accumulated between frost tolerant (T) and non-tolerant (NT) genotypes of A. alpina are identified in various chilling and/or freezing conditions through high-throughput methods. Next-generation sequencing and comparative bioinformatics are used to identify differentially expressed genes in chilling and freezing conditions. Proteome investigations using 2D-DIGE and LC-MS are performed to identify differentially accumulated proteins. Finally, metabolites profiling is used to analyse variations in response to temperature treatments with the aim of identifying metabolites more strictly related to chilling and/or freezing. The results will be integrated and combined with data from the literature so as to identify a short list of candidate genes, proteins and metabolites responsible for the freezing tolerance of adapted genotype. The work should provide novel information on how plants cope with their environment and how they can respond to global change. As far as economic fallouts are concerned, the project also aims at producing reliable molecular markers to be used for polymorphism exploration and genetic improvement of cultivated species like *Brassica* crops.

12A The more, the merrier: High heterotroph richness enhances methanotrophic activity

Adrian Ho and Nico Boon
Laboratory for Microbial Ecology and Technology, Faculty of Bioscience Engineering, Ghent University, Belgium

Methanotrophs, autotrophic bacteria, represent a unique microbial guild, possessing the ability to oxidize and assimilate methane. Methanotrophs co-exist with other microorganisms in the environment and they are known to sustain whole communities in methane-driven ecosystems.
However, little is known of the microbial interaction, more specifically on the methanotrophheterotroph interaction. Here, co-cultures of *Methylomonas methanica* (type I methanotroph) and ten heterotrophs covering diverse classes (*Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, and *Firmicutes*) were artificially assembled in a 1:1 ratio. Further, co-cultures of *M.methanica* and a combination of ten heterotrophs were constructed in equal total starting cell numbers. Incubations of only *M.methanica* serve as a control. Incubations were performed in nitrate mineral salts (NMS) media in 120 ml bottles (working volume: 10 ml) under 20 vol.% methane in air at 28°C in the dark. Methane depletion was monitored over three days, including pre-incubation (1 day). Results showed an enhanced to significant stimulation of methanotrophic activity in incubations with high heterotroph richness, while activity in co-cultures with one single heterotroph was similar to the control. Micro-nutrients and other essential elements may become available in the co-culture from lysed heterotroph cells. However, incubations of methanotroph in heterotroph lysate showed decreased and variable activity, suggesting a more direct methanotroph-heterotroph interaction than an uptake of lysed cell material. The total cell numbers after incubation were similar in all co-cultures even in the incubation with high heterotroph richness, suggesting an increase of the specific activity per methanotroph cell; incubation of heterotrophs in methanotroph lysate generally yield appreciable cell growth. Overall, we show that heterotroph richness stimulates methanotrophic activity, presumably by increasing the apparent cell-specific activity *via* a yet unknown mechanism. Future research involving proteomic analysis will assist to reveal the underlying mechanism of the present observation.

13A Micro-evolutionary response in a natural *Daphnia magna* population under Cu and Zn stress

Jennifer Hochmuth, Colin Janssen and Karel De Schamphelaere
Laboratory of Environmental Toxicology and Aquatic Ecology, Ghent University (UGent), Gent, Belgium

A 10 week experimental evolution study was carried out under semi-field conditions to test for micro-evolutionary effects in a natural *Daphnia magna* population exposed to a control, 2 Cu (45µgCu/L and 180µg Cu/L), and 3 Zn (240µg Zn/L, 428µg Zn/L, and 760µg Zn/L) concentrations. We investigated if the long-term exposures to Cu or Zn resulted in higher organism fitness compared to that in the control evolution treatment and in the original (= start) population. At the end of the natural selection experiment a life-table experiment was initiated with clones from the start population, the control evolved population and the metal evolved populations to test for a combined effect of metal acclimation and adaptation in the metal evolved treatments. At this point in

time we could not disentangle short term acclimation effects (acting on individuals) from adaptation at the population level. The populations experimentally evolved at 180µg Cu/L and 760µg Zn /L had significantly higher reproduction at the corresponding concentrations than the lower metal or control treatments, i.e. evidence that metal acclimation and/or adaptation had occurred. After 4 months of culturing under control conditions, thus eliminating any acclimation history, an additional life-table experiment was conducted to determine any changes in fitness solely due to adaptation, measured as an increase in mean population fitness. We observed a significantly higher total reproduction at 760µg Zn /L and at 180µg Cu/L in the respective metal evolved populations compared to the long-term control evolved population and the start population. In the population evolved at 760µg Zn/L acclimation and adaptation to the metal had enabled the population density to recover, matching that of the control, despite an initial reduction of 75% of the clones. Under long-term exposure to 180µg Cu/L, however, despite lower initial mortality (50%), acclimation and adaptation effects were not sufficient to lead to a full recovery of the population density. Our results confirm that micro-evolution can occur after only a few generations but that adaptation in itself is not a guarantee for a complete recovery of the population density.

14A *Daphnia magna* in a world of stress

Mieke Jansen and Luc De Meester
Laboratory of Aquatic Ecology, Evolution and Conservation, KU Leuven, Leuven, Belgium

In their natural habitat *Daphnia* are confronted with different stressors, making their life complicated. Not only natural stressors like temperature and parasites, but also chemical stressors like pesticides could drastically change life quality of *Daphnia*. A selection experiment was performed to quantify the capacity of natural populations to locally adapt to pesticide exposure. In this experiment, *Daphnia* populations from ponds differing in land use in their surroundings were exposed to presence and absence of standardized carbaryl pulses. Genetic changes in carbaryl tolerance were assessed by comparing acute toxicity values of populations before and after the selection experiment. While carbaryl tolerance after carbaryl selection did not increase significantly compared to the tolerance present in the original populations, there was a significant decrease in carbaryl tolerance in the populations of the control treatment compared to the original populations. The magnitude of the decrease in carbaryl tolerance between the original populations and the populations in the control treatment increased with land use in the neighbourhood of the ponds from which the original populations were sampled. These responses in the control rather than the carbaryl exposure treatment provide strong support for selection for pesticide tolerance in the field, and indicate that this evolution comes at a cost. Our data point to a widespread selection for pesticide tolerance in Flemish farmland ponds and illustrate the overwhelming impact of agricultural land use on natural populations living in non-target habitats.

15A Spatiotemporal succession of different lineages of *Limnohabitans* bacteria in a canyon-shaped reservoir

Jan Jezbera [1], Jitka Jezberová [1], Petr Znachor [1,2], Jiří Nedoma [1], Vojtěch Kasalický [1,2], and Karel Šimek [1,2]
[1] *Biology Centre of the ASCR, v.v.i., Institute of Hydrobiology, České Budějovice, Czech Republic*
[2] *Faculty of Sciences, University of South Bohemia, České Budějovice, Czech Republic*

Limnohabitans genus is nowadays widely accepted as one of the key members of freshwater *Betaproteobacteria*. Recently designed *Limnohabitans* lineage-specific Reverse Line Blot Hybridization (RLBH) probes enabled to examine the spatiotemporal succession of distinct lineages affiliated with this genus in two independent studies: (i) during the intensive spring period sampling program at the lacustrine part of the Římov reservoir (from ice melt through onset of a phytoplankton bloom to the clear-water phase), and (ii) during the whole-year study when occurrence of distinct *Limnohabitans* lineages was related to inherent longitudinal variability of this canyon-shaped reservoir. Significant spatiotemporal changes in the composition of distinct *Limnohabitans* lineages have been recorded in both studies. We were able to identify 'generalists' *Limnohabitans* lineages that were always present throughout the whole season as well as 'specialists' that appeared in the reservoir only for short periods of time or irregularly. Several groups of algae, such as *Cryptophytes*, were proposed to be potentially affecting the distribution and dynamics of distinct *Limnohabitans* lineages. Other parameters, such as

pH, chl-A, and primary production were also found to be theoretically responsible for occurrence of different *Limnohabitans* lineages. Revealed diversity, in terms of *Limnohabitans* lineages recorded, changed downstream in the Římov reservoir, with most significant differences found between the inflow and dam parts of the reservoir. Highest diversity of *Limnohabitans* lineages, recorded at the inflow part, hints on important influence of watershed on *Limnohabitans* microdiversity.

16A Ethogenomics: *arabidopsis* genes affecting aphid behaviour

Karen J. Kloth [1,2,3], Manus P.M. Thoen [1,2,3], Cindy J.M. ten Broeke [1], Olga E. Krips [4], Maarten A. Jongsma [3] and Marcel Dicke [1]

[1] *Lab. of Entomology, Wageningen University, Wageningen, the Netherlands*

[2] *Lab. of Plant Physiology, Wageningen University, Wageningen, the Netherlands*

[3] *Business Unit Bioscience, Plant Research International, Wageningen University and Research Centre, the Netherlands*

[4] *Noldus Information Technology BV, the Netherlands*

Aphids are phloem feeding insects, they use their stylets to probe intercellularly between plant cells to reach a sieve element and feed on nutritious plant sap. Although these insects inflict little tissue damage, they reduce plant growth and transmit plant viruses. Previous studies showed that natural plant populations often display exceptional levels of polymorphisms in defence genes, but so far little is known about how plants identify and counter-act aphid feeding. In this study we explore natural variation in resistance of *Arabidopsis* to the generalist aphid *Myzus persicae*. We developed an automated video-tracking system to screen aphid probing behaviour on 350 natural Arabidopsis accessions and validated this high-throughput phenotyping platform with Electrical Penetration Graphs. Several plant lines showed higher levels of resistance than others. On these resistant plants aphids performed more short, epidermal probes and less long, feeding probes. In a Genome-Wide Association study we mapped the behavioural data of all 350 plant lines against a 250.000 SNP haplotype map. We could identify two major genomic regions of interest, containing genes putatively involved in different resistance mechanisms.

1B Deep-sea sampling with Niskin bottles – how strong this common sampling procedure biases the analysis of *in situ* microbial activity?

Violetta La Cono [1], Sebastiaan Rampen [2], Erika Arcadi [1], Gina La Spada [1], Francesco Smedile [1], Maria Pachiadaki [3], Thorsten Stoeck [3], Michail M. Yakimov [1] and DEEP_C Consortium

[1] *Institute for Coastal Marine Environment, IAMC-CNR, Messina, Italy*

[2] *Royal Netherlands Institute for Sea Research, NIOZ, Texel, The Netherland.*

[3] *Technical University of Kaiserslautern, Germany*

A challenge for investigating microbes and especially their activities is that most of all environmental prokaryotes have yet not been cultured. Consequently, the study of microbial assemblages largely depends on cultivation-independent methods. Concerning the linkage of microbial activity and identity, one of the most promising approaches is the investigation of functional mRNA transcripts isolated from an environmental sample. Study of mRNAs is very useful approach because their relative concentration reflects not only the gene expression but also provide insights into the metabolic pathways operative at the time of sampling. The last statement is very important regarding the study of active microbial communities thriving in deep sea.
Common deep seawater sampling is performed with a rosette equipped with battery of Niskin bottles. The time required to analyze deep-sea sample includes recovery of the cast, bringing the sample to the laboratory, filtration of required volume and, finally, its fixation. For the sample collected from 3,000 meters depth this operation from sampling to fixation usually takes at least two-three hours. Unfortunately, since mRNA molecules are extremely labile, it is unclear whether the abundance patterns detected in recovered sample truly reflects the situation *in situ*. To estimate how strong the different water-sampling procedures (classical Niskin bottle, *in situ* filtartion, etc.) affect the relative abundance of transcripts we sampled water column of the central Ionian Sea at the depth of 2,222 m. This zone has been reasonably well studied and is known to have a relatively high aerobic ammonium oxidation rates accompanied by high concentrations of thaumarchaeal *amoA* transcripts.

The results on recovery of these transcripts obtained by different sampling procedures, their phylogenetic affiliation and following analysis of the obtained biases are present in a corresponding poster.

2B Effects of parental environment on fitness and methylation variation

Christian Lampei and Karl Schmid
Crop Biodiversity and Breeding Informatics *University Hohenheim, Germany*

Recent investigations in *Arabidopsis thaliana* showed that epigenetic variation may be responsible for phenotypic variation in potentially adaptive traits. Furthermore, it was suggested that some of this variation may be heritable over several generations. These findings offer the possibility that environmentally induced epigenetic changes may accelerate adaptation to changing climatic conditions. However, most experiments were conducted in highly controlled environments that makes it difficult to judge the fitness relevance of epigenetic variation under natural conditions. We conducted a field experiment that utilized climatic differentiation along an altitudinal gradient. Offspring of several inbred *A. thaliana* accessions were cultivated in locations at 140 m, 400 m and 700 m altitude that differ by climatic conditions (e.g. minimum temperature) for one or two seasons. In the third season, a reciprocal transplant experiment between the three sites was conducted. For comparison, offspring of all accessions and sites were cultivated under controlled conditions at three different temperatures (17°, 23°, 28° C). We expect that offspring grew best in the maternal environment suggesting local adaptation in form of a "home site" advantage. Similarly, we expect a fitness advantage of offspring from the lowest altitude side at highest controlled cultivation temperature. We will present first results of parental effects on offspring fitness. It is further planned to re-sequence the methylome of the offspring of some chosen accessions to test for systematic methylation changes that correlate with maternal environment and fitness effects.

3B Linking growth phase dependent thiosulfate oxidation with anaplerotic gene expression in *the heterotrophic marine Roseobacter sp. MED193*

Saraladevi Muthusamy [1], *Federico Baltar* [1], José M. González [2] and Jarone Pinhassi [1]
[1] *Centre for Ecology and Evolution in Microbial model Systems – EEMiS, Linnaeus University, Kalmar, Sweden*
[2] *Department of Microbiology, University of La Laguna, La Laguna, Tenerife, Spain*

Chemolithotrophs have the potential to oxidize various inorganic sulfur compounds for energy. Although marine bacteria are generally recognized to be heterotrophs, metagenomic analysis of the ocean water column reveal that nearly 10% of the bacterioplankton communities possess the gene for oxidation of sulfur compounds. However, little is known about the molecular mechanisms involved in gaining energy from these alternative metabolisms. To address this issue, *we investigated the growth response, heterotrophic production, CO_2 fixation and gene expression profile of Roseobacter strain MED193 in acetate minimal medium with and without thiosulfate.* The addition of thiosulphate enhanced the bacterial growth yield and biomass production by approximately 40%, consistent with an observed 3-fold upregulation of sulfur oxidation (sox) genes. During exponential growth, the rates of bacterial production and bicarbonate uptake were high and rapidly decreased in stationary phase. Nevertheless, in stationary phase, these rates were 10-fold higher with thiosulfate compared to controls. The higher rate of bicarbonate fixation during exponential growth was linked with an upregulation of anaplerotic CO_2 fixation genes (i.e, pyruvate carboxylase, propionyl CoA and crotonyl CoA carboxylase), and could contribute around 10% to the cellular carbon budget. The expression of crotonyl CoA and Propionyl CoA suggest that the ethlmalonyl CoA pathway for acetate assimilation is a particularly relevant anaplerotic pathway for this heterotrophic bacterium. Overall, our studies revealed that Roseobacter MED193 has the chemolithotrophic potential to oxidize inorganic sulfur compounds to gain additional energy that can be used to improve heterotrophic efficiency and allow this bacterium to use organic carbon more efficiently for biomass production.

4B Local adaptations more important than genetic diversity for salinity tolerance in three rock pool *Daphnia* species

Yi-Fan Liao, Leanne K. Faulks, **Örjan Östman**
Uppsala University, Sweden

Fragmentation can cause populations to be small and isolated, which can render a loss of genetic variability due to genetic drift. This may reduce population viability through inbreeding depression and loss of adaptive potential for populations to cope with changing environments, especially under stressful conditions. Local genetic or phenotypic adaptations to previous and current environmental conditions can in addition to genetic diversity be important for tolerance to different stress factors. But most studies consider the effect of one of these aspects on stress tolerance at a time. Here we study how salinity stress affects fecundity and population growth of a rock pool *Daphnia* (*D. longispina*, *D. magna*, and *D. pulex*) metacommunity with varying degrees of population isolation and salinity conditions between populations. First, all populations survived at higher salinity levels when salinity was gradually increased than at a shock increase, indicating gen regulation adjustments are important for salinity tolerance. Second, *in situ* salinity condition was more important for their tolerance of high salinity conditions than genetic diversity *per se*. There was furthermore no correlation between the salinity conditions of the rock pool and population level genetic diversity, if anything it was a negative relationship. This indicates that the natural environmental conditions that a population experiences influences that population's response to induced stress through local genetic adaptations and can increase their potential to tolerate such stresses. The results were rather similar for all three species but *D. magna* was the only species showing indication of population isolation and low genetic diversity had a negative impact on fecundity and population abundances in ambient conditions. The results imply that variation in environmental disturbances can be important to consider in the conservation of fragmented populations in addition to genetic variability.

5B News on the natural ecology and evolution of the model nematode *C. elegans*

Carola Petersen and Hinrich Schulenburg
Department of Evolutionary Ecology and Genetics, Zoological Institute Christian-Albrechts University, Kiel, Germany

Although the nematode *Caenorhabditis elegans* is a major model organism in diverse biological areas and well studied under laboratory conditions, little is known about its ecology and evolutionary history. Therefore, characterization of the species' natural habitats should provide a new perspective on the otherwise well-characterized biology and life-history of this nematode. *C. elegans* was for a long time thought to be a soil nematode, but actually seems to prefer nutrient- and microorganism-rich substrates. In order to extend these findings, this project focuses on a continuous long term sampling of *C. elegans* in rotting apples and compost heaps. Since these habitats degrade rapidly and are only available temporarily, nematodes need to escape harsh conditions and food limitation. We observed that slugs and isopods are likely vectors for transport to new environments. Moreover, *C. elegans* was found to share its habitat with the related nematode species *Caenorhabditis remanei*, which could thus represent an important competitor for a similar ecological niche. Microsatellite markers are currently used to characterize population genetic differentiation of the recently isolated *C. elegans* and those isolated in 2002 from the same location.

6B Assessing population structure of a *Daphnia*-infecting microparasite: spatial and temporal patterns in *Caullerya mesnili* revealed by ITS sequencing

Sabine Giessler [1], **Adam Petrusek** [2], Enrique Gonzalez-Tortuero [1], Jakub Rusek [1], Henrike Koerner [3], Mingbo Yin [1,3], Jaromir Seda [4] and Justyna Wolinska [1]
[1] *Department of Biology II, Ludwig-Maximilians-University Munich, Planegg-Martinsried, Germany*
[2] *Department of Ecology, Faculty of Science, Charles University in Prague, Prague, Czech Republic*
[3] *Department of Neuroimmunologie, Institut für Multiple Sklerose Forschung, Georg-August Universität, Göttingen, Germany*
[4] *Biological Centre AS CR, Institute of Hydrobiology, Ceske Budejovice, Czech Republic*

Microparasites are important stressors affecting microevolutionary dynamics of *Daphnia* populations. Until recently, little information was available on their population structure, in contrast with their intensively studied hosts. We investigated temporal and spatial variation of *Caullerya mesnili* (Ichthyosporea), an endoparasite infecting guts of planktonic *Daphnia longispina* complex. This protozoan can reach prevalences of up to 40% within a host population, has high virulence and strong specificity for infection of certain genotypes, and thus it seems to be a good model to study host-parasite coevolution dynamics. By evaluating sequence variation of highly variable internal transcribed spacer in ribosomal DNA (ITS), we analysed spatial and temporal differences among seven geographically isolated populations of *C. mesnili* in reservoirs in the Czech Republic, some of them sampled repeatedly in multiple years. Two methodological approaches were applied, traditional cloning followed by Sanger sequencing, and 454 amplicon pyrosequencing. Analysis of molecular variance confirmed that both spatial and temporal components were significant, suggesting that population structure of this microparasite dynamically changes, and may strongly impact the dynamics of host clones. However, the distribution of ITS variants were similar in infected individuals of two or even three different host species coexisting in the same reservoirs, indicating that the parasite genotypes are apparently able to infect susceptible clones of multiple taxa within the host species complex. Our approach combined with genotyping of host individuals allows simultaneous analyses of population structures of both hosts and microparasites, assessing changes of frequencies in genotypes of both, and also studying genotype x genotype associations at individual level. Thus, it will be useful in further coevolutionary studies of *Daphnia*-microparasite systems.

7B Flow-sorting of uncultivable ultramicrobacteria according to their taxonomic affiliation

Stefan M. Neuenschwander, Jakob Pernthaler, Thomas Posch and Michaela M. Salcher
Limnological Station, Institute of Plant Biology, University of Zurich, Kilchberg, Switzerland

Ultramicrobacteria numerically dominate the bacterioplankton in many natural aquatic systems. Understanding their genetic properties is therefore of high interest, however, cultivation of those microbes is still very challenging. Separating phylogenetically defined populations from their natural communities prior to (meta-) genome analysis could therefore be a promising alternative. On way to achieve such a physical separation is to combine fluorescence *in situ* hybridisation (FISH) and flow cytometry. Such protocols are available since the nineties and have been successfully applied to samples originating from nutrient rich environments. Limited signal intensities however hindered a broad application to samples from natural aquatic environments. The introduction of CARD-FISH (Catalyzed reporter deposition-FISH) based protocols partly solved this problem, but when applied to ultramicrobacteria, insufficient signal to noise ratios are still an issue. We aimed to improve the sensitivity of such a protocol by complementing it with antibody mediated secondary tyramide signal amplification. For evaluation we used samples from the oligo-mesotrophic Lake Zurich and a oligonucleotide probe specific for the highly abundant but so far uncultivable LD12 (SAR11 IV) cluster of *Alphaproteobacteria*. Compared to previously existing protocols we observed superior signal to noise ratios which allows high purity sorting. It is however not practicable to sort sufficient numbers of cells for direct sequencing. We are therefore working on a protocol combining CARD-FISH, flow-sorting, and multiple displacement amplification (MDA) to obtain sufficient yields of genomic DNA for subsequent genomic analysis.

8B The ecological role of pelagic methylotrophic ultramicrobacteria assessed by high-resolution *in situ* analysis and studies on isolated strains

Michaela M. Salcher, Thomas Posch and Jakob Pernthaler
Limnological Station, University of Zurich, Kilchberg, Switzerland

Pelagic freshwater bacteria are important sources but also sinks for major nutrients and substrates in lakes. Methylotrophic bacteria can be key players in the carbon cycle, as C1-compounds are used as sole sources of carbon and energy gain. Members of the ubiquitous betaproteobacterial tribe LD28 seem to be highly relevant for the turnover of methanol, a degradation product of pectin and lignin. We followed the spatio-temporal distribution of these microbes in Lake Zurich, Switzerland, in a high-resolution sampling campaign during 4 consecutive years (992 individual samples). Members of the LD28 tribe were present in variable abundances (up to 1.1 x 10^5 cells ml^{-1}, up to 11.4 % of all *Bacteria*) with pronounced peaks after phytoplankton blooms in spring and at the onset of mixis in late autumn. Interestingly, these microbes were rare in the warm stratified water layers during summer, but colonized the deep and cold hypolimnion, hinting at cold-stenothermic growth.

More than 90 strains of these so far uncultivated bacteria were isolated from the pelagial of Lake Zurich by dilution to extinction. The isolates showed very slow growth (0.4 d^{-1} maximum growth rate) and were of conspicuous small cell size (0.05 μm^3, i.e., ultramicrobacteria). They did not incorporate amino acids or sugars, but showed enhanced growth after the addition of methanol to sterile lake water, reflecting an obligate methylotrophic lifestyle. Phylogenetic analysis of the 16S rDNA proved a close relation to the ubiquitous marine OM43 lineage and the separation into 2 distinct phylotypes, both of which seem to be species of the same genus. As the closest relative (*Methylotenera mobilis*) has sequence similarities of <95%, we propose the establishment of a new genus with 2 species, i.e., *Candidatus* Methylopumilus planktonicus and *Candidatus* Methylopumilus turicensis.

9B A field experiment testing the importance of lineage sorting and priority effects in freshwater bacterioplankton

Caroline Souffreau, Bert Peccue, Tomas Verbeeck and Luc De Meester
Laboratory of Aquatic Ecology, Evolution and Conservation, KU Leuven, Leuven, Belgium

Field studies show that freshwater bacterioplankton communities are predominantly shaped by the local environmental conditions, a process called species sorting (or lineage sorting in bacteria). However, there is rising evidence for different organism groups including (cyano)bacteria that priority effects could have an underestimated importance on the observed community structure. Priority effects are lasting effects of the colonization order and timing of organisms on community structure. We tested the importance of lineage soring versus priority effects in a field experiment on three locations in Belgium (Leuven, Genk, and Ghent). Two water types (eutrophic and dystrophic) were inoculated with the indigenous microbial community, the opposite community, or were left un-inoculated. After 30 days, the bacterial communities were sampled and characterized using 454-pyrosequencing. First analyses using perMANOVA and redundancy analysis show that significant regional effects were present for the un-inoculated treatment, indicating that colonization of the medium was affected by local conditions (e.g. weather conditions, local soil communities) or that dispersal limitation or priority effects are important. However, perMANOVAs and redundancy analyses on the inoculated samples show that there were no significant differences in the final communities when inoculating the indigenous or non-indigenous bacterial community in the same water type, indicating that lineage sorting is very strong, while priority effects are less important. Also, when comparing the samples inoculated with the non-indigenous communities with the un-inoculated samples, no significant differences were found within water type, again indicating that priority effects are unimportant in shaping the bacterial communities. In total, these first results suggest that priority effects are not important at the longer term (30 days), while lineage sorting is strong and results in highly similar communities in similar water types. However, regional effects are present indicating that external environmental conditions can affect the bacterial communities to different extent.

10B Finding genes related to fish predation in the water flea *Daphnia magna*

Katina I. Spanier, Luisa Orsini and Luc De Meester
Laboratory of Aquatic Ecology, Evolution and Conservation, University of Leuven, Leuven, Belgium

Evolutionary and Ecological Functional Genomics seek to understand the link between genes and ecologically relevant traits underlying adaptive responses to biotic and abiotic factors. The water flea *Daphnia* is a keystone species in lakes and ponds, with a wealth of data on adaptive responses to its environment and increasing availability of genomic tools. We aim at identifying candidate genes underlying adaptive responses of *D. magna*, focusing on anti-predator responses to fish, one of the most important and best-studied *Daphnia* stressors. We used 36 clones that were hatched from a sediment core and originate from a population with known and changing fish-predation history over the past 30 years. Those clones are phenotypically well-characterised and rapid evolution of fish-responsive traits in this population was shown in a previous study. We genotyped about 800 SNP loci in those 36 clones, using a recently developed SNP-chip. We used those data for a genome-wide association study to scan for genes which are potentially involved in the evolution of adaptive traits in response to fish predation. This study is a first important step toward the understanding of molecular mechanisms underlying ecological responses and adaptation to predation in *Daphnia*.

11B Characterization of exopolyphosphatase in *Actinobacteria* ac1

Abhishek Srivastava and Hans-Peter Grossart
Leibniz-Institute for Freshwater Ecology and Inland Fisheries, Department 3: Limnology of Stratified Lakes, Stechlin, Germany

Limnic *Actinobacteria* of the acI cluster generally dominates the bacterioplankton in temperate lakes reaching up to ~60% of the entire epilimnic bacterial population. In Lake Stechlin, an oligotrophic lake in North-East Germany they contribute to ~30% of total bacteria in average. This lake is poor in phosphate content with an average of 2µg SRP l^{-1}, indicating that P limits production during the growing season. Some organisms, e.g. the cyanobacterium *Aphanizomenon flos-aquae* and the giant sulfur bacterium *Achromatium oxaliferum* contain high amounts of polyphosphate as a phosphorus storage component. However, it is largely unknown whether ultramicrobacteria such as the acI *Actinobacteria* are able to store polyphosphate or whether and how they utilize it. Single cell genome analysis of an *Actinobacteria* belonging to the acI lineage from Lake Stechlin and Lake Mendota has revealed several polyphosphatases, polyphosphate kinases, ABC type polyphosphate transporter system, phosphate-starvation inducible protein PhoH and phosphohydrolase -coding genes present in the genome. This indicates that these bacteria potentially store and also degrade polyphosphorous compounds in oligotrophic Lake Stechlin. Therefore, we have targeted an exopoly-phosphatase-coding gene (*exoP*) to investigate its role in phosphate degradation. Successful, heterologous overexpression and functional analysis of *exoP* gene in *E. coli* has suggested a mode of phosphate utilization in a phosphorus poor lake where phosphorus availability is dependent upon lysis and waste products of other organisms, e.g. *A. flos-aquae*.

12B Investigation into population genetic structure of Woodpigeon (*Columba palumbus*) in Europe based on D-loop sequencing data

Aniolas Sruoga [1,2], Dalius Butkauskas [2], Saulius Svazas [2] and Antonio Bea [3]
[1]*Vytautas Magnus University, Kaunas, Lithuania*
[2]*Nature Research Centre, Vilnius, Lithuania*
[3]*Ekos Estudios Ambientales, Donostia Etorbidea, Lasarte, Spain*

The population genetic structure of Woodpigeon (*Columba palumbus*) in Europe was studied analyzing the distribution of different D-loop haplotypes among bird samples, collected in various breeding and wintering sites of the species (in Russia, Belarus, Lithuania, Sweden, Hungary, France, Spain and Portugal). Totally 89 different haplotypes ascertained as belonging to 5 haplo-groups were identified after examination of partial D-loop sequences consisting of 359 bp mtDNA fragments derived from tissue specimens of 360 harvested birds. Significant differences in frequency of five haplogroups A, B, C, D and G were found after comparison of Woodpigeon samples attributed to migratory and sedentary populations. Based on relatively high proportion of representatives of haplogroup A in the Baltic region including Sweden, Russia, Lithuania and Belarus and absence of representatives of this haplogroup in sedentary populations of Woodpigeons originating from Spain and Portugal it could be suggested that earlier proposed hypothesis of panmictic European population of the species should be rejected. Specific population genetic structure of wintering in the Pyrenean Peninsula Woodpigeon populations partially consisting of migratory individuals of the Baltic region origin is clearly distinguished among samples collected in Spain and Portugal. A distinct population genetic structure is characteristic also of Woodpigeons breeding in Hungary and using the Mediterranean Flyway.

13B Evolutionary consequences of polyploidization: The example of *Vicia cracca*

Maria Šurinová [1,2], Kirsten Bomblies [3] and Zuzana Münzbergová [1,2]
[1] *Department of Botany, Faculty of Science, Charles University in Prague, Prague, Czech Republic*
[2] *Institute of Botany, Academy of Sciences, Průhonice, Czech Republic*
[3] *Department of Organismic & Evolutionary Biology, Harvard University, Cambridge, USA*

In this study, we investigate the evolutionary consequences of polyploidization, using *Vicia cracca* as a model system. For this purpose we use natural diploid and tetraploid populations of *Vicia cracca* and synthetic neo-tetraploids derived from natural diploid population by using colchicin. All the tested samples originated from populations in the Czech Republic. A first step is to ensure that we will compare plants from the same lineage, so we will identify the natural tetraploid most closely related to the diploid and the neopolyploid derived from the diploid. This will be done using several markers: (1) the sequences of chloroplast DNA regions *atpI-atpH* and *trnL-trnF*, (2) and several nuclear regions: sequences of the nuclear ribosomal internal transcribed spacer (ITS1-5.8S-ITS2), cyclic nucleotide-gated ion channel 4 (CNGC4), glutamine synthetase (GLNA), ferredoxin- NADP reductase (FDXR), sulfate adenylyltransferase (SAT), 1-aminocyclopropane-1-carboxylic acid oxidase (ACCO) and disulfide isomerase P5 precursor (DSI). After the characterization of the populations and the determination of structure of populations we will obtain a reference transcriptome. We have chosen 454 sequencing methods with coverage >30x. Obtained reads will be aligned to *Medicago truncatula* transcripts database. The following step will include quantification of the whole transcriptome of natural diploid, tetraploid and synthetic tetraploid individuals. For this part of the project we decided to use Illumina sequencing. For the mapping at reference transcriptome from the previous step we will use Burrows- Wheeler Aligner. To confirm changed levels of expresion, qPCR will be used. For functional identification of selected genes SwissProt and NCBI- non redundant protein database will be searched by using Blast X. The results will be processed with Blast2GO software. The last step will be to identify methylated nucleotides for genes with changed expression by using McrBC enzyme. This enzyme acts on purine-$C_{methyl.}$ If PCR of the investigated gene is successful, this indicates that the investigated gene was not methylated. As the result of this project we will obtain a list of genes with different expression levels for each ploidy level and the origin of *Vicia cracca* individuals, including evidence of methylation. We will be able to determine which genes are more affected by process polyploidization itself, and for which genes evolution had a bigger impact (which genes are more influenced by evolution). These data will be complemented with data comparing growth of these cytotypes in a wide range of conditions.

14B High-throughput phenotyping of thrips resistance in *Arabidopsis thaliana*. A genome-wide association study

Manus Thoen [1,2,3], Karen Kloth [1,2,3], Marcel Dicke [1] and Maarten Jongsma [3]
[1] *Laboratory of Entomology, Wageningen University*
[2] *Laboratory of Plant Physiology, Wageningen University*
[3] *Business Unit Bioscience, Plant Research International, Wageningen University and Research Centre, the Netherlands*

The Western Flower thrips (*Frankliniella occidentalis*) is a major agricultural pest. We have developed a novel automated video-monitoring method to screen insect behavior and performance. In addition to classical end-point measurements, we have used this novel method to screen 350 wild type accessions of *Arabidopsis thaliana* (HapMap collection) for thrips resistance. These accessions have been collected globally and genotyped for 250,000 SNPs . The tremendous source of phenotypic data obtained by our assays combined with the high resolution haplotype map of *A. thaliana* allow for multiple genome wide association mappings. Here we will present our first mapping results after five replicates of the HapMap collection screening, and bring forward some candidate genes for thrips resistance in *Arabidopsis thaliana.*

15B Freshwater acI Actinobacteria: single cell genomics and genome-informed cultivation efforts

Sarahi L. Garcia [1], Abhishek Srivastava [2], Hans-Peter Grossart [2], Trina McMahon [3], Ramunas Stepanauskas [4], Alex Sczyrba [5,6], Tanja Woyke [5], Sandra Barchmann [1] and **Falk Warnecke** [1]
[1] *Friedrich Schiller University, Jena School for Microbial Communication, Jena, Germany*
[2] *Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Department for Limnology of Stratified Lakes, Neuglobsow, Germany*
[3] *University of Wisconsin – Madison, Department of Civil & Environmental Engineering, Madison/WI, USA*

[4] *Bigelow Laboratory for Ocean Sciences, Single Cell Genomics Center, West Boothbay Harbor/ME, USA*

[5] *DOE Joint Genome Institute, Microbial Program, Walnut Creek/CA, USA*
[6] *Bielefeld University, Center for Biotechnology, Computational Metagenomics, Bielefeld, Germany*

Actinobacteria of the phylogenetic clade acI are ubiquitous in lake bacterioplankton and often numerically dominate temperate freshwater ecosystems where they can contribute >50% of the bacteria in the surface water. However and as often with environmentally important species they are uncultured to date. That is why we set out to study their genomic information in order to learn about their physiology and ecological niche. We used a single cell genomics approach which consisted of the following steps: (1) single cell sorting by Fluorescence-activated cell sorting (FACS), (2) whole genome amplification (WGA) using Phi29 DNA polymerase, (3) screening of SAG (Single cell amplified genome) DNA by 16S rRNA sequencing, (4) shotgun genomic sequencing followed by (5) genome assembly, annotation and data analysis using The Joint Genome Institute's (JGI) Integrated Microbial Genomes (IMG) analysis platform. We obtained a draft genomic sequence in 75 larger contigs (sum = 1.16 Mbp) and with an unusual low genomic G+C mol% (i.e. ~42%). Single copy gene analysis suggests an almost complete genome recovery. We also noticed a rather low percentage of genes with no predicted functions (i.e. ~15%) as compared to other cultured and genome-sequenced microbial species. Our metabolic reconstruction hints at the degradation of pentoses (e.g. xylose) instead of hexoses. We also found an actinorhodopsin gene that may contribute to energy conservation under unfavorable conditions. This project reveals the possibilities and limitations of single cell genomics for microbial species that defy cultivation to date. Moreover we used genomic information to attempt cultivation and in fact we obtained several stable co-cultures that were dominated by acI Actinobacteria. One of those was partially characterized in the mean time and altogether six enrichment cultures will undergo metagenomic sequencing at the Joint Genome Institute in the near future.

16B Genome sequences of novel isolates of limnic aerobic anoxygenic phototrophic bacteria suggest a complex evolution pattern of photosynthesis gene cluster among Proteobacteria

Yonghui Zeng [1] and Michal Koblížek [1, 2]
[1] *Institute of Microbiology CAS, Opatovický mlýn, Czech Republic*
[2] *University of South Bohemia, Faculty of Science, České Budějovice, Czech Republic*

Aerobic anoxygenic phototrophs (AAPs) are prokaryotic organisms containing reaction centers composed of bacteriochlorophyll *a* (BChl a). AAPs represent an important part of microbial communities inhabiting the euphotic zone of world oceans and lakes. In contrast to the extensive studies of marine AAPs, only little is known about their presence and diversity in limnic habitats. A recent study of the diversity of AAP in a German lake suggested that freshwater species are formed mostly by Alpha- and Betaproteobacteria. So far only a limited number of limnic AAPs exist in pure cultures, which constrains our knowledge on their metabolism, physiology and their role in biogeochemical fluxes.
For this reason we attempted to isolate representative AAP species from various limnic environments. We developed a rapid isolation protocol which employs fluorescence screening of AAP colonies directly on agar plates using an infrared CCD camera. We successfully isolated over 200 AAP strains from different types of freshwater lakes located in Austria, Czech Republic and China (a fishpond, a low-elevation volcanic lake, a highly contaminated lake, and a clear Tibetan plateau lake). Analyses of 16S rRNA gene sequences suggest most of the strains were affiliated with Alphaproteobacteria belonging to genera *Afipia*, *Agrobacterium*, *Bosea*, *Brevundimonas*, *Methylobacterium*, *Novosphingobium*, *Phyllobacterium*, *Porphyrobacter*, *Rhizobium*, *Rhodocista*, *Rhodopseudomonas*, *Sandarakinorhabdus*, and *Sphingomonas*. Nine isolates were Betaproteobacteria belonging to genera *Caenimonas*, *Ideonella*, *Leptothrix*, and *Methylibium*. We also isolated one Gammaproteobacterial AAP strain from the Huguangyan Maar Lake, South China, which has not been reported from a freshwater lake before. An analysis of *puf*L, *puf*M, and *bch*Y genes sequence suggests a complex phylogenetic history of phototrophic genes in AAP species. Furthermore, by means of next generation sequencing (NGS) technology, we carried out whole genome sequencing on 27 representative strains. Complete photosynthesis gene clusters (PGC) were successfully assembled directly from NGS data in most genomes. Together with available AAP genomes from some marine isolates in public database, these data for the first time

enable us to make an insightful comparison of PGC composition and their possible evolution history among Proteobacteria.

Annex 2: Participant and attendance list general conference

Last Name	University/Institute	Country	Group
Ågren	Uppsala University	Sweden	A. alpina perenniality
Coupland	Max Planck Institute for Plant Breeding Research	Germany	A. alpina perenniality
Herzog	Université Joseph Fourier, Grenoble	France	A. alpina perenniality
Laporte	Universite Joseph Fourier, Grenoble 1	France	A. alpina perenniality
Odong	Wageningen University and Research, Lab of Bioinformatics	Netherlands	A. alpina perenniality
Slotte	Uppsala University	Sweden	A. alpina perenniality
Toräng	Uppsala University	Sweden	A. alpina perenniality
Vass	Uppsala universitet	Sweden	A. alpina perenniality
Wötzel	MPI for PLant Breeding Research	Germany	A. alpina perenniality
Wunder	Max Planck Institute for Plant Breeding Research	Germany	A. alpina perenniality
Filker	University of Kaiserslautern	Germany	DEEP_C
La Cono	Institute for Coastal Marine Environment, IAMC-CNR	Italy	DEEP_C
Stoeck	University of Kaiserslautern	Germany	DEEP_C
Yakimov	Institute for Coastal Marine Environment, IAMC-CNR	Italy	DEEP_C
Bossdorf	University of Bern, Institute of Plant Sciences	Switzerland	EPICOL
Groot	Nijmegen University	Netherlands	EPICOL
Lampei	University Hohenheim	Germany	EPICOL
Latzel	University of Bern, Institute of Plant Sciences	Switzerland	EPICOL
Schmid	University of Hohenheim	Germany	EPICOL
Vergeer	Radboud University Nijmegen	Netherlands	EPICOL
Zhang	Institute of Plant Sciences	Switzerland	EPICOL
Bertilsson	Uppsala University	Sweden	FREDI
Eckert	Univerisity of Zurich, Limnological Station	Switzerland	FREDI

Eiler	Uppsala University	Sweden	FREDI
Hahn	University of Innsbruck	Austria	FREDI
Jezbera	Biology Centre, v.v.i., Institute of Hydrobiology	Czech Republic	FREDI
Neuenschwander	University of Zurich	Switzerland	FREDI
Pernthaler	Univ. of Zurich, Inst Plant Biology, Limnological Station	Switzerland	FREDI
Salcher	Limnological Station, University of Zurich	Switzerland	FREDI
Sinclair	Uppsala University	Sweden	FREDI
Srivastava	Leibniz-Institute of Freshwater Ecology	Germany	FREDI
van het Groenewoud	NWO	Netherlands	MC
Bodelier	Netherlands Institute of Ecology (NIOO-KNAW)	Netherlands	MECOMECON
Frenzel	MPI for Terrestrial Microbiology	Germany	MECOMECON
Hainbuch	Max Planck Institute for Terrestrial Microbiology	Germany	MECOMECON
Henneberger	ETH Zürich	Switzerland	MECOMECON
Krause	Netherlands Institute of Ecology	Netherlands	MECOMECON
Lüke	Max Planck Institute for Terrestrial Microbiology	Germany	MECOMECON
Reim	MPI for terrestrial microbiology	Germany	MECOMECON
Svenning	University of Tromsø	Norway	MECOMECON
Berg	Leibniz Institut für Ostseeforschung (IOW)	Germany	MOCA
Frank	University of Vienna, Department of Marine Biology	Austria	MOCA
Garcia	University of Vienna	Austria	MOCA
Glaubitz	Leibniz-Institut für Ostseeforschung	Germany	MOCA
Gonzalez	University of La Laguna	Spain	MOCA
Herndl	University of Vienna	Austria	MOCA
Jürgens	Leibniz Institute for Baltic Sea Research	Germany	MOCA
Muthusamy	Linnaeus University	Sweden	MOCA
Offre	University of Vienna/ Department of Genetics in Ecology	Austria	MOCA
Pinhassi	Linnaeus University	Sweden	MOCA
Schleper	University of Vienna	Austria	MOCA
Dirksen	University of Kiel	Germany	NEMADAPT
Kammenga	Wageningen University	Netherlands	NEMADAPT

Nakad	Christian-Albrechts University, Zoological Institute	Germany	NEMADAPT
Petersen	Christian-Albrechts-Universität zu Kiel/Zoological Institute	Germany	NEMADAPT
Schulenburg	University of Kiel	Germany	NEMADAPT
Volkers	WUR	Netherlands	NEMADAPT
Hardtke	University of Lausanne	Switzerland	Review Panel
Hurka	Osnabrück, Botany, Faculty of Biology	Germany	Review Panel
Plohl	Ruder Boskovic Institute	Croatia	Review Panel
Sruoga	Institute of Ecology, Nature Research Centre	Lithuania	Review Panel
Asselman	University of Gent	Belgium	STRESSFLEA
De Meester	University of Leuven	Belgium	STRESSFLEA
Decaestecker	KU Leuven / KULAK	Belgium	STRESSFLEA
Frey	Katholieke Universiteit Leuven/ Laboratory of aquatic ecology, evolution and conservation	Belgium	STRESSFLEA
Haag	University of Fribourg	Switzerland	STRESSFLEA
Hochmuth	University of Gent	Belgium	STRESSFLEA
Jansen	KULeuven	Belgium	STRESSFLEA
Laforsch	University of Bayreuth	Germany	STRESSFLEA
Orsini	University of Leuven	Belgium	STRESSFLEA
Petrusek	Charles University in Prague, Faculty of Science	Czech Republic	STRESSFLEA
Roulin	Basel, institut of Zoology	Switzerland	STRESSFLEA
Souffreau	KU Leuven	Belgium	STRESSFLEA
Spanier	KU Leuven	Belgium	STRESSFLEA
Andersson	University of Uppsala	Sweden	Keynote
Bernatchez	Laval University	Canada	Keynote
Brune	Max Planck Institute for Terrestrial Microbiology	Germany	Keynote
Hallam	University of British Colombia	Canada	Keynote
Hancock	University of Vienna	Austria	Keynote
Hoffmann	University of Melbourne	Australia	Keynote

Jones	Max Planck Institute for Developmental Biology and Friedrick Miescher Laboratory	Germany	Keynote
Mitchell-Olds	Duke University	USA	Keynote
Moran	University of Georgia	USA	Keynote
Polz	MIT	USA	Keynote
Dietrich	Max Planck Institute for Terrestrial Microbiology Marburg	Germany	OPEN
Galand	Observatoire Océanologique de Banyuls	France	OPEN
Ho	LabMET, Ghent University.	Belgium	OPEN
Kloth	Wageningen University	Netherlands	OPEN
Lachmuth	Plant Ecology, Martin-Luther-University Halle	Germany	OPEN
Ley	University Halle-Wittenberg / Institut of Geobotany	Germany	OPEN
Östman	Uppsala University, Dept. Ecology & Genetics	Sweden	OPEN
Penny	Centre de Recherche Public - Gabriel Lippmann	Luxembourg	OPEN
Schöler	Helmholtz Zentrum München	Germany	OPEN
Surinova	Charles University in Prague	Czech Republic	OPEN
Thoen	Wageningen University and Research Centre	Netherlands	OPEN
van Straalen	VU University Amsterdam	Netherlands	OPEN
Verhoeven	Netherlands Institute of Ecology	Netherlands	OPEN
Vrieling	Leiden University	Netherlands	OPEN
Warnecke	Friedrich Schiller University Jena, Institute of Applied and Ecological Microbiology	Germany	OPEN
Zeng	Institute of Microbiology, Academy of Science	Czech Republic	OPEN
Zhao	Uppsala University	Sweden	OPEN