

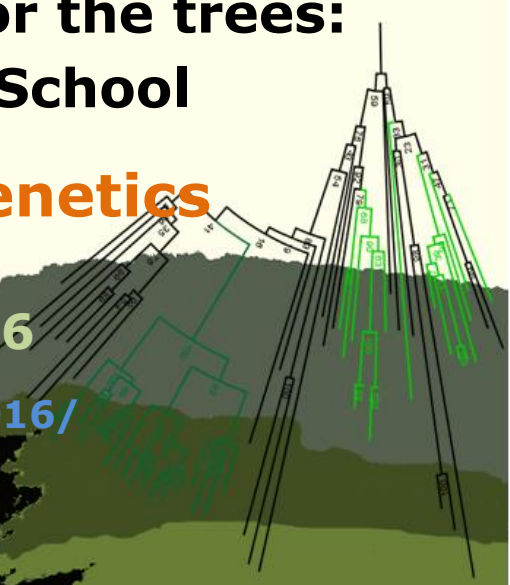
# To see the (Black) Forest for the trees: Black Forest Summer School

on

## NGS data for phylogenetics

Sep 13<sup>th</sup> – 16<sup>th</sup> 2016

<http://plantco.de/BFSS2016/>



# Abstract Book

Edited by Stefan A. Rensing  
Marburg, Germany, August 2016

*Venue: Leistungszentrum Herzogenhorn (Black Forest Highlands, Germany)  
~1,300 mtrs above sea level*



## Acknowledgements

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Support by the institutions and companies shown below is gratefully acknowledged.



Thank you very much!

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## **Program**

*Venue: Leistungszentrum Herzogenhorn*

All workshops and talks take place in the hall (gymnasium). Poster sessions and industry exhibit take place in the gymnasium and the seminar room. Breakfast, lunch, supper, coffee breaks and evening entertainment are located in the dining rooms.

### **Tuesday Sep 13<sup>th</sup>**

15:15, 16:15, 17:15 Bus shuttles from Feldberg-Bärental train station to venue

19:00 Welcome reception with food and beverages

20:15 Opening remarks

Welcome lecture: "**Plant Phylogenomics: Comparative Analyses of Plant Genes and Genomes**" (James Leebens-Mack, University of Georgia)

*later* "*Moss cocktail workshop*"

### **Wednesday Sep 14<sup>th</sup>**

9:00 – 9:45 Lecture 2: NGS data generation: tools, terms and pitfalls  
(Stefan Rensing, University of Marburg)

9:45 – 10:30 Workshop 1: NGS data processing and transcriptome assembly  
(Fabian Haas & Eva Neumann, University of Marburg)

10:30	Coffee break								
10:45 – 11:30	...continuation of workshop 1								
11:30 – 12:00	<u>Lecture 3: Introduction to Galaxy</u> (Anika Erxleben, University of Freiburg)								
12:00	Lunch								
13:00 – 14:00	<i>Poster session I</i> (with coffee) <b>EVEN</b> numbered posters								
14:00 – 15:30	<u>Workshop 2: Mapping: short read alignment to a reference</u> (Eva Maria Willing, MPIZ Cologne)								
15:30	Short break								
15:45 – 16:30	<u>Lecture 4: Principles of phylogenetics</u> (Stefan Rensing, University of Marburg)								
17:00 – 22:00	<u>Excursion</u> <table> <tr> <td>17:00</td> <td><i>Departure to Rothaus</i></td> </tr> <tr> <td>18:00</td> <td><i>Brewery excursion</i></td> </tr> <tr> <td>19:30</td> <td><i>Black Forest Food and Rothaus beer</i></td> </tr> <tr> <td>22:00</td> <td><i>Return to Herzogenhorn</i></td> </tr> </table>	17:00	<i>Departure to Rothaus</i>	18:00	<i>Brewery excursion</i>	19:30	<i>Black Forest Food and Rothaus beer</i>	22:00	<i>Return to Herzogenhorn</i>
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19:30	<i>Black Forest Food and Rothaus beer</i>								
22:00	<i>Return to Herzogenhorn</i>								
18:00	Supper (for those not on excursion)								

### **Thursday Sep 15<sup>th</sup>**

9:00 – 9:45	<i>Oral session 1</i> T1     Andrea Soelinger "The complex rumen microbiota - Structural and functional dynamics during ruminal plant biomass degradation revealed by integrated quantitative metatranscriptomics" T2     Jutta Baldauf "Transcriptomic patterns underlying heterosis manifestation in primary roots of maize ( <i>Zea mays</i> L.) at the interface of genotype and development" T3     Michael Ignatz "Combined priming and ageing treatments of sugar beet seeds and their impact on the transcriptome and hormone levels"
9:45 – 10:30	<u>Workshop 3: Assembly and annotation of bacterial genomes</u> (Oliver Rupp, University of Gießen)
10:30	Coffee break
10:45 – 11:30	<u>Workshop 4: From NGS data to phylogenetics</u> (Daniel Lang, Helmholtz Center Munich)
11:30 – 12:00	<i>Oral session 2</i> T4     Lindsey Dougherty "Phylogenetic analysis of a unique flashing display in the 'disco' clam, <i>Ctenoides ales</i> " T5     Metha Klock "Provenance of rhizobial symbionts is similar for invasive and non-invasive acacias"
12:00	Lunch

13:00 – 15:00	<i>Poster session 2</i> (with coffee & <b>industry exhibits</b> ) 13:00 – 14:00 <b>ODD</b> numbered posters 14:00 – 15:00 <b>ALL</b> posters
15:00 – 15:30	<u>Lecture 5: The advantages of long reads</u> (Bruno Huettel, MPIZ Cologne)
15:30 – 16:45	<u>Workshop 5: RNA-seq analyses (normalisation, differential expression, DEseq)</u> (Bernd Klaus, EMBL Heidelberg)
16:45 – 17:30	<u>Workshop 6: How to infer my own phylogeny?</u> (Stefan Rensing, University of Marburg)
18:00	Supper
19:00 – 20:00	<u>Workshop 7: Coalescence-based species tree estimation using ASTRAL</u> (James Leebens-Mack, University of Georgia)
19:00 – 21:00	<u>PHYCOMORPH round table</u> : specific problems of your work with macroalgae (Daniel Lang, Helmholtz Center Munich)
<i>later</i>	<i>Farewell party</i>

### **Friday Sep 16<sup>th</sup>**

9:00 – 10:30	<u>Workshop 8: SNPs &amp; Co.: Variant Calling</u> (Micha Bayer, The James Hutton Institute)
10:30	Coffee break
10:45 – 11:45	<u>General round table: Annotation: GO, RBH and more</u>
11:45	Prizes and concluding remarks
12:00	Lunch, end of summer school
13:30, 14:30	Bus shuttles to Feldberg-Bärental train station

# The Black Forest Summer School Concept

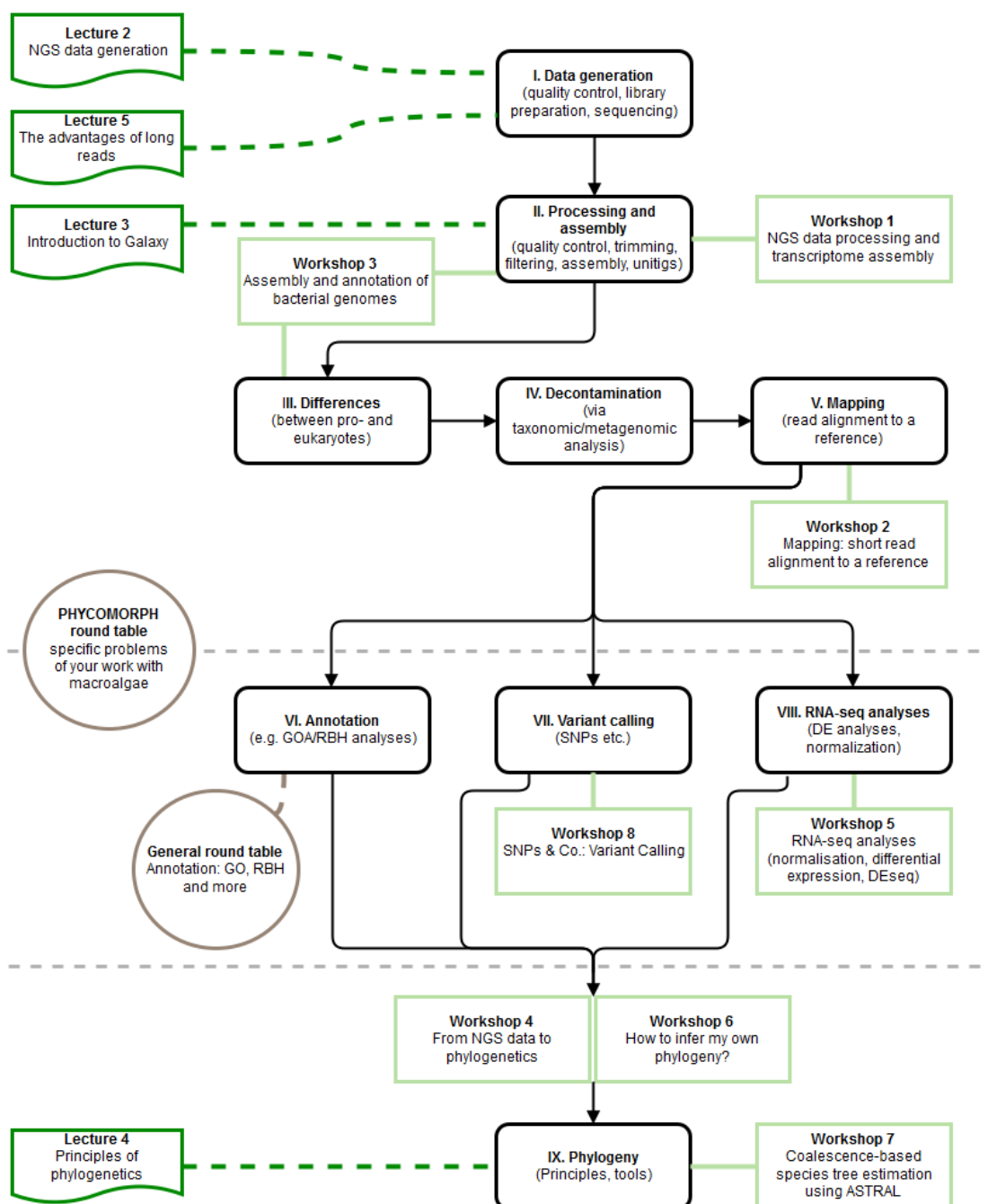
We will not teach you how to program – we will teach you which existing tools are out there to process NGS data and to infer phylogenies.

**BFSS workshops are interactive lectures**, in which the participants are encouraged to ask questions and will be charged with questions / exercises by the lecturer.

Both lectures and workshops often include **life demo sessions** (but typically no hands-on part).

**You will receive digital materials** that will enable you to repeat what was demonstrated at the school, as well as helpful lists of software applications.

Please note that **no computers are available**. While we encourage you to bring your own laptop/tablet, computer use is not necessary to follow the schools' program.



# Lecture Abstracts

## L1 Plant Phylogenomics: Comparative Analyses of Plant Genes and Genomes

*James Leebens-Mack*

University of Georgia, USA

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Phylogenetic frameworks for species relationships and gene families are becoming increasingly essential for organization and analysis of the avalanche of sequence data that has come with development of massively parallel sequencing technologies. Sequence clustering approaches such as OrthoFinder are providing approximations of gene families. Phylogenetic analyses of these gene family clusters are yielding improved understanding of species relationships and new insights into gene, genome and organismal evolution. I will illustrate the utility of low copy gene families for estimating species relationships and how resulting species tree inferences are foundational for inferring the evolution of multi-copy gene families and whole genomes. For example, I will discuss how phylogenomic frameworks are elucidating the timing of paleopolyploidy events in angiosperm history and their impact on the evolution of key innovations. At the same time, species-level phylogenomic analyses are aiding mechanistic investigation of the molecular and genomic bases for phenotypic variation among and within species. I will illustrate this last point by presenting some of our work on the origin of sex chromosomes within the genus *Asparagus*.

## L2 NGS data generation: tools, terms and pitfalls

*Stefan Rensing*

Plant Cell Biology, University of Marburg, Germany

stefan.rensing@biologie.uni-marburg.de

In this lecture, I shall first compare highly parallel (“NGS”) sequencing technologies with Sanger and each other and talk about pros and cons of the different technologies.

I will then define technical terms that will be needed for comprehension of the summer schools’ topics, and will talk about some methodological details like fastq format, phred scores, fragment sizes, regional bias, multiplexing, paired ends, mate pairs etc.

Some potential pitfalls will be highlighted and finally I will introduce some tools that might be useful but are not covered by individual workshops.

### **L3 The Galaxy framework as a unifying bioinformatics solution for HTS data analysis**

*Anika Erxleben*

University of Freiburg, Germany

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The Freiburg Galaxy Team is member of the German Network for Bioinformatics Infrastructure (de.NBI) and aims to provide comprehensive bioinformatics services to users in life sciences research, industry and medicine. Within this network, we are part of the RNA Bioinformatics Center (RBC) and we are responsible for supporting RNA related research in Germany. In this talk we will present our analysis platform Galaxy which makes advanced bioinformatics software accessible to biologists directly by providing an intuitive web interface to these applications while fostering reproducibility through the automatic creation of re-runnable protocols of each analysis. We describe the use of Galaxy for HTS data analysis in genomics, proteomics, imaging and metabolomics. Focusing on the perspective of a biological user, we will demonstrate the benefits of Galaxy for these analyses, as well as its value for software developers seeking to publish new software.

### **L4 Principles of phylogenetics**

*Stefan Rensing*

Plant Cell Biology, University of Marburg, Germany

stefan.rensing@biologie.uni-marburg.de

How does sequence evolution occur and why does it allow to infer gene and species phylogenies? What are substitution matrices and why do we need them all over the place?

Everybody knows BLAST, everybody uses it. But how do you define homology from a BLAST result? By E-value? By bit score? By alignment length? Or by % identity?

Once you have determined homology, how do you go on? How do you generate an alignment and how do you visualize and curate it?

Based on an alignment, which methods are there to infer phylogenetic trees and what are their pros and cons? Finally, how do you interpret a phylogenetic tree?



## **L5 The advantages of long reads**

*Bruno Huettel, Janina Fuss*

MPI for Plant Breeding Research, Cologne, Germany

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The Max Planck Genome Centre Cologne is a central core facility of the Max Planck Society, with a focus on Next Generation Sequencing using short (Illumina) and long read technology (Pacific Bioscience). In the presentation, the focus will be on the aim of very long reads in *de novo* genome sequencing of prokaryotic and eukaryotic species as well as on full-length cDNA transcriptome sequencing of model species, crop species and species from which no transcriptome data have been available yet. The data provided will demonstrate the straight forward help of very long reads in any genomic project, reducing the bioinformatic reconstruction effort to an unexpected minimum and therefore mainly concentrate on answering biological questions. An overview of current technology as well as developments in the near future will be shown.

# Workshop Abstracts

## W1 NGS data processing and transcriptome assembly

*Fabian Haas & Eva Neumann*

Plant Cell Biology, University of Marburg, Germany

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Next Generation Sequencing (NGS) has become the method of choice to address a multitude of biological questions in all areas of natural sciences from ecology to biotechnology. NGS datasets are used to reconstruct genomes, unravel population and evolutionary relationships, build expression profiles and many more applications. This workshop provides a detailed introduction how to process NGS datasets by using state of the art programs and methods. Short tutorials will cover all necessary steps from raw sequencing data to cleaned good quality reads which are mandatory for follow-up applications. Further, the focus of this workshop will be on one of these applications, transcriptome assembly. All relevant key points and key terms will be highlighted to discuss how to assemble contigs and to do further analysis steps such as transcript prediction.

## W2 Mapping: short read alignment to a reference

*Eva-Maria Willing*

Genome Plasticity and Computational Genetics, MPI for Plant Breeding Research, Cologne, Germany

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Read alignments are the starting point for most re-sequencing projects using next generation sequencing technologies. No matter if you want to identify single nucleotide polymorphisms between your reference and another individual/population of the same species, find structural rearrangements or do transcriptome/expression analyses between individuals, you always have to start with mapping the reads against an available reference. This workshop aims at giving a brief introduction to the underlying principle of establishing short read alignments and discuss in this context problems that usually occur during the alignment process. We will also discuss what kind of information we can extract from different read alignment patterns in both whole genome and transcriptome short read data and introduce different tools commonly used in the different analyses. In the end we will look at the limits of short read alignment analysis and give an outlook on future perspectives in next generation sequencing.

### **W3 Assembly and annotation of bacterial genomes**

*Oliver Rupp*

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Next-generation high throughput sequencing has enabled extensive and enhanced genomic studies. The first step in most of the genomic analyses of bacteria is the assembly and functional annotation of the genome. Assembly describes the method to reconstruct the complete genome sequence from small sequence fragments created by the next-generation sequencing technology. In the next step, the content of the genome, like protein-coding genes, tRNAs or ribosomal RNAs, need to be identified and the function of these features need to be inferred. In this workshop the theory of the main assembly and annotation methods will be explained. Furthermore, current bioinformatic tools will be introduced and limitations or pitfalls will be addressed. The usage of the tools on the Linux command-line and as part of a Galaxy platform will be shown on example data.

### **W4 From NGS data to phylogenetics**

*Daniel Lang*

Plant Genome and Systems Biology, Helmholtz Center Munich, Germany

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The prevalence, speed and relatively low cost of NGS technologies provides us with the unparalleled opportunity to basically sequence any genome or transcriptome we can get our hands on. As impressively demonstrated by the success of the 1kp project (keynote lecture), NGS-driven transcriptomics and phylogenomics are revolutionizing our approach to the phylogenetic study of gene and species evolution at increasing taxonomic resolution. Genome and transcriptome assembly tools (workshops I and III) enable the identification of coding regions on genomic contigs derived by shotgun sequencing and allow the reconstruction of (virtual) transcripts from RNASeq libraries.

Like in any high-throughput approach, the sequences produced by these methods are not devoid of artifacts and errors. Problems arising from fragmented transcripts, remnant adapter or barcode sequences, frameshift errors and chimeric assemblies etc. affect the success of any downstream (phylogenetic) analysis. In my workshop I will highlight some of the pitfalls in the reconstruction of geneic regions from genome and transcriptome assemblies.

Depending on the aimed taxonomic resolution, for phylogenetic inference we are frequently focusing our analysis on the conserved open reading frames (ORFs) of protein-coding genes. The completeness and correctness of the chosen ORF limits the reliability of the insights we can obtain from the sampled species or gene family. Transcriptome assemblies provide us with the additional complexity introduced by alternative splicing, which not always results in mature mRNAs that are translated into functional (and conserved) proteins. Thus, a crucial task in NGS-based phylogenomics is isoform selection and ORF prediction. I will present strategies and methods that can be used to identify the major, conserved isoform and reliably infer an open reading frame for phylogenetic analysis.

## **W5 Differential expression analysis of RNA-Seq data with DESeq2**

*Bernd Klaus*

Centre for Statistical Data Analysis (CSDA), European Molecular Biology Laboratory, Heidelberg, Germany  
bernd.klaus@embl.de

In this workshop we introduce a workflow for a typical RNA-Seq data analysis. We start from a “count table”, which summarizes the number of sequence reads mapping to each of the genes and discuss quality control, differential expression (using DESeq2) and GO enrichment analysis of the data. Important aspects of the R language and Bioconductor data structures for high-dimensional genomic data are discussed along the way. We re-analyze RNA-Seq data obtained by comparing the expression profiles of WT mice to mice harboring a deletion that is associated with a MYC enhancer which is required for proper lip/palate formation. The data was published along with the following paper:

Uslu et. al. – Long-range enhancers regulating Myc expression are required for normal facial morphogenesis, 2014 [<http://dx.doi.org/10.1038/ng.2971>]

All the material presented will be made available for public download so that the participants can easily reproduce the analysis steps.

We will also briefly discuss recent trends in the alignment of reads to the reference genome and gene level quantification using transcript level estimates [<http://f1000research.com/articles/4-1521/v2>]

## **W6 How to infer my own phylogeny?**

*Stefan Rensing*

Plant Cell Biology, University of Marburg, Germany  
stefan.rensing@biologie.uni-marburg.de

This workshop will essentially cover the same topics as lecture 4, namely the principles of phylogenetics. We shall talk about why and how we can infer molecular phylogenies, look at the term homology in detail, and aim to understand duplication events and what the difference between gene and species trees is.

The workshop will present you with conceptual questions with regard to phylogeny and will mainly be a discussion forum. I will also introduce useful tools that you can use for retrieving homologs, for aligning and visualizing them, for tree inference, model selection and tree visualization.

Finally, we will leave space for your specific questions – phylogenetic methods are an expected aim of many or most of your projects (given the topic of the summer school), so we will try and come up with helpful suggestions for your work.

## **W7 Coalescence-based species tree estimation using ASTRAL**

*James Leebens-Mack*

University of Georgia, USA

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There is growing appreciation that incomplete lineage sorting in the face of rapid speciation and its impact on supermatrix analyses. A hands-on demonstration and tutorial will guide participants through species tree estimation using the coalescence-based gene tree summary approach implemented in ASTRAL (Mirarab & Warnow 2015 *Bioinformatics* 31:i44). Participants might want to download ASTRAL in advance - <https://github.com/smirarab/ASTRAL> (will also be provided on memory stick). The tutorial will include analysis of pre-calculated gene trees. Participants wanting to analyze their own data should estimate gene trees before the tutorial.

## **W8 SNPs & Co.: Variant Calling**

*Micha Bayer*

Information and Computational Sciences, The James Hutton Institute, Scotland UK

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Molecular markers are rapidly becoming the data type of choice for many phylogenies. In the age of Next Generation Sequencing (NGS), we can generate molecular markers in vast numbers and at very low cost per marker. The most abundant type of molecular marker are SNPs (single nucleotide polymorphisms), followed by small insertions and deletions (collectively known as indels). In this workshop we will look at how we discover new SNP/indel markers using NGS data. In most cases, variant discovery in NGS data is based on mapping short reads to a reference sequence and then using dedicated variant calling tools to scan the mapping for positions where there are alleles that disagree with the reference sequence. This process is potentially error-prone at a number of levels, and we will look closely at the suite of options available for keeping our error rate as low as possible.

## Contributed Talk Abstracts

### **T1 The complex rumen microbiota - Structural and functional dynamics during ruminal plant biomass degradation revealed by integrated quantitative metatranscriptomics**

Andrea Söllinger<sup>1</sup>, Morten Poulsen<sup>2</sup>, Samantha Joan Noel<sup>2</sup>, Jörg Bernhardt<sup>3</sup>, Thomas Rattei<sup>4</sup>, Ole Højberg<sup>2</sup>, Christa Schleper<sup>1</sup>, Tim Urich<sup>3</sup>

<sup>1</sup> Department of Ecogenomics and Systems Biology, University of Vienna, Austria; <sup>2</sup> Department of Animal Science, Aarhus University, Denmark; <sup>3</sup> Institute of Microbiology, University of Greifswald, Germany; <sup>4</sup> Department of Microbiology and Ecosystem Science, Vienna, Austria  
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Ruminant livestock is the major anthropogenic methane (CH<sub>4</sub>) source. A complex rumen microbiota (Bacteria, Eukarya and Archaea) accomplishes the anaerobic plant biomass degradation. Understanding their activities and tight interactions is essential to develop sustainable CH<sub>4</sub> mitigation strategies. Using an integrated approach (quantitative metatranscriptomics, gas-, short chain fatty acid (SCFA) profiling) we investigated the temporal rumen microbiome dynamics during feed degradation in lactating cows. The rumen microbiome (including viruses) was highly individual and remarkably stable within each cow, suggesting a high functional redundancy. Nevertheless, a consistent successional pattern could be observed, with bacterial and eukaryotic taxa significantly more abundant in earlier and later time-points, respectively. Gene expression profiles revealed a fast microbial growth response, reflected by a drastic increase of rumen microbial biomass, CH<sub>4</sub> emissions and SCFA concentration. Accordingly, transcript numbers of glycoside hydrolases (quantified per gram rumen fluid) increased steeply upon feed intake but returned to low before-feeding abundances after five hours. Functional transcripts of the dominant methanogens showed a similar trend. This first comprehensive longitudinal study of the complex rumen microbiome during feed degradation revealed a defined sequence of events and interactions between microbiota members that might be exploited to mitigate ruminal CH<sub>4</sub> emissions.

### **T2 Transcriptomic patterns underlying heterosis manifestation in primary roots of maize (*Zea mays* L.) at the interface of genotype and development**

Jutta Baldauf, Carolin Marcon, Frank Hochholdinger

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Distantly related maize (*Zea mays* L.) inbred lines exhibit an exceptional degree of structural genomic diversity, which is likely unique among plants. Heterozygous F1-hybrid progeny of such inbred lines are often more vigorous than their homozygous parents, a phenomenon known as heterosis. In this study, we investigate how the genetic divergence of seven selected parental inbred lines is reflected in the transcriptomic landscape of their hybrid primary roots during development. The design of the RNA-seq experiments was developed to maximize the number of direct comparisons among the parent-hybrid pairs, and at the same time to ensure a very good precision for indirect comparisons. The quality trimmed paired-end sequencing reads were aligned to version 3 of the maize reference genome using TopHat2. For subsequent data analysis in R, the primary aligned reads were counted by the python-based package HTSeq. Previously, duplicates were marked and removed by the java-based tool picard. The normalized read counts were then projected to an analysis pipeline within the Bioconductor package limma to determine differentially and nonadditively expressed genes. Furthermore, SNPs will be called in the genetically diverse inbred lines to determine allele-specific expression patterns in the hybrids. With this project we aim to generalize previously observed transcriptomic patterns in reciprocal hybrids of the inbred lines B73 and Mo17 to a more diverse panel of maize inbred lines.

### **T3 Combined priming and ageing treatments of sugar beet seeds and their impact on the transcriptome and hormone levels**

Michael Ignatz<sup>1</sup>, Juliane Meinhard<sup>2</sup>, Annette Büttner-Mainik<sup>1</sup>, Fridtjof Weltmeier<sup>2</sup>, Veronika Tureckova<sup>3</sup>, Mirek Strnad<sup>3</sup>, Uwe Fischer<sup>2</sup>, Gerhard Leubner-Metzger<sup>1</sup>

<sup>1</sup> RHUL, School of Biological Sciences, Royal Holloway, University of London, UK; <sup>2</sup> KWS SAAT AG, Einbeck, Germany; <sup>3</sup> Laboratory of Growth Regulators, Palacky University, Czech Republic  
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Sugar beet seed quality improvement by priming leads to uniform germination but also to accelerated ageing during seed storage. We investigated the molecular mechanisms of sugar beet seed ageing during storage by comparing ageing models differing in the three factors temperature, relative air humidity and duration. Comparative population-based modelling of seed ageing of primed versus unprimed seeds delivered storage conditions with defined stress responses for which we conducted transcriptome and hormone analysis. Abscisic acid (ABA) contents decreased in imbibed sugar beet seeds with a distinct pattern affected by the priming and ageing treatments. While ABA inhibits sugar beet germination, 1-aminocyclopropane-1-carboxylic acid (ACC), the biosynthetic precursor of ethylene, is known to promote it. In agreement with this, our transcriptome analysis of sugar beet seeds exhibited decisive differences in the transcript abundance patterns for ABA degrading enzymes and ACC oxidases. For some transcripts prior priming is affecting how severe the response to ageing is. The identification of differentially regulated genes of the sugar beet seed transcriptome will support our knowledge of the impact of the priming and ageing treatments.

### **T4 Phylogenetic analysis of a unique flashing display in the ‘disco’ clam, *Ctenoides ales***

Lindsey Dougherty, Jingchun Li

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The ‘disco’ clam *Ctenoides ales* (Limidae) is the only bivalve known to have a behaviorally mediated photic display. The white flashing on the clam’s mantle lip was initially confused for bioluminescence because it is so vivid. It is actually the result of light scattering by silica nanospheres. The flashing tissue has two sides: one highly scattering (white) that contains silica nanospheres, and one highly absorbing (red) that does not. High-speed video shows the two sides quickly alternate (2-3Hz) to create the flashing. Optical modelling suggests the sphere size is nearly optimal for scattering visible light, especially at shorter wavelengths which predominate in the ocean. Conspecific recruitment and prey luring were ruled out as potential functions of the flashing, but preliminary evidence shows that the flashing may act as an aposematic predator deterrent. As the only bivalve with a flashing display, the evolutionary history of *C. ales* is of interest. We want to establish a phylogeny within the family Limidae to compare the use of flashing in *C. ales* to the evolution of various other defenses within the clade, including tentacle autotomy (Limaria), aposematism (Ctenoides), and swimming (Lima). Preliminary Bayesian analysis was conducted using three genetic markers (16S, 28S and COI) that all place *C. ales* at the base of the Ctenoides genus (four *Ctenoides* species examined). Transcriptomic analysis will be conducted to identify candidate genes for the flashing.

## **T5 Provenance of rhizobial symbionts is similar for invasive and non-invasive acacias**

Metha M. Klock, Hector Urbina, Luke G. Barrett, Peter H. Thrall, Kyle E. Harms

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klockmm@gmail.com

Mutualistic interactions can be important drivers of species invasions. In particular, the mutualism between legumes and nitrogen-fixing soil bacteria (i.e., rhizobia) may be influential in plant invasion success. Acacias, which are legumes native to Australia, have been introduced around the world, with certain species becoming invasive. We examined the acacia-rhizobia symbiosis to determine whether this mutualism plays a role in invasiveness of introduced acacias. Specifically, we assessed whether acacias varying in invasiveness were co-introduced abroad with their native rhizobial symbionts. We predicted that invasive acacias would be found associating with rhizobia from their native range, whereas non-invasive acacias would not. To determine whether acacias were co-introduced with their native rhizobia, we selected four *Acacia* species occurring in California (two invasive and two non-invasive) and identified rhizobial strains associating with each species, sequencing the 16S rRNA, *nifD*, and *nodC* genes. We found that all *Acacia* species, regardless of invasive status, associated with rhizobia of Australian origin in their introduced range, suggesting that concurrent acacia-rhizobia introductions have occurred for all species tested. These findings clarify the role that the co-introduction of rhizobial mutualists plays in the invasion of acacias abroad, aid in winnowing out mechanisms of species invasions, and shed light on an important aspect of legume biology.



## Poster Abstracts

### **P1 Dimorphic fruits, seeds and seedlings in *Aethionema arabicum* as adaptation mechanisms to abiotic stress in unpredictable environments**

Waheed Arshad<sup>1</sup>, Kai Graeber<sup>1</sup>, Margaret Collinson<sup>2</sup>, Wolfgang Stuppy<sup>3</sup>, Gerhard Leubner-Metzger<sup>1</sup>

<sup>1</sup> Royal Holloway University of London, School of Biological Sciences, Egham, UK; <sup>2</sup> Royal Holloway University of London, Department of Earth Sciences, Egham, UK; <sup>3</sup> Millennium Seed Bank Partnership, Royal Botanic Gardens, Kew, UK

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Diaspore heteromorphism occurs in a number of angiosperm families. One species exhibiting this phenomenon is *Aethionema arabicum*, an annual belonging to the most basal lineage of the Brassicaceae family, which exhibits true diaspore dimorphism with no intermediate morphs. It has the remarkable ability to produce two distinct fruits on the same inflorescence. These two types of fruits harbour two different seed types, denoted as M+ (mucilaginous) and M– (non-mucilaginous). This project will elucidate the morphological, biomechanical and molecular mechanisms of dimorphic traits, as well as the evolutionary diversification of heterodiaspory in other extant members of the Brassicaceae and in the fossil record. *Ae. arabicum* M+ and M– seed development will be investigated using modern imaging, molecular, and genetic approaches. Seed and seedling growth will be physiologically modelled under a range of abiotic stresses, and the genetic adaptations underlying the natural variation of ecotype-specific seedling traits to those stresses will be explored. The work will build on resources and knowledge from the ERA-CAPS SeedAdapt project ([www.seedadapt.eu](http://www.seedadapt.eu)), and will establish how heterodiaspory functions as a “bet-hedging” strategy in variable and unpredictable environments. Together with existing data, new findings will make *Ae. arabicum* an attractive model species for future research on diaspore dimorphism.

### **P2 Isolation and characterization of mutants impaired in excess energy dissipation in the Eustigmatophyceae alga *Nannochloropsis gaditana***

Michela Cecchin, Silvia Berteotti, Alessandro Alboresi, Massimo Delledonne, Roberto Bassi, Matteo Ballottari

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*Nannochloropsis gaditana* is a unicellular microalgae considered one of the most interesting marine algae for the production of biofuels due to its rapid growth rate and high lipids accumulation. Although the microalgae are attractive biomass and biofuel producers, the overall efficiency of photosynthesis is reduced due to the onset of photoprotection processes which dissipate as heat a portion of light energy absorbed. In order to improve biofuel phenotypes, in this work a biotechnological approach was directed for the isolation of strains with a reduction in heat dissipation (NPQ) induction. We produced a library of *N. gaditana* strains by chemical mutagenesis that were screened for reduction of NPQ induction. We selected three mutants called npq3, npq7 and npq21 with reduced level of NPQ respect to WT. Mutants selected present a higher lipid content respect to WT. In order to correlate the phenotype with the genotypes, the selected mutants were sequenced with Illumina to identify SNPs present in each strain. About 200 mutated genes were identified in npq3 and about 45 in npq7 and npq21, among which only one gene was found to be mutated in all mutants. This gene encodes for a protein phosphatase that is a good candidate for biotechnological engineering in microalgae in order to manipulate the photoprotection properties toward an increase photosynthetic efficiency.

### **P3 Root and cell-type specific transcriptome responses to water deficit during early barley development**

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Drought is among the most devastating of natural hazards – crippling food production, depleting pastures, disrupting markets, and, at its most extreme, causing widespread famine. Understanding of the molecular mechanisms involved in drought response of agronomically important cereals is the key for plant improvement. In the present study, we investigate the early transcriptomic responses of barley (*Hordeum vulgare* L.) seminal roots to water deficit conditions. The transcriptomic response of barley seminal roots of different genotypes with contrasting sensitivity to drought stress are compared after 6 hours and 24 hours of severe (-0.8 Mpa) water deficit conditions. Subsequently, the transcriptome response of pericycle cells from which lateral roots are initiated is studied in more detail. This is of relevance because under drought conditions pericycle cells initiate less lateral roots compared to control conditions. The transcriptomic responses of pericycle cells of the differentiation zone of barley seminal roots are compared under the same drought conditions as whole seminal roots.

### **P4 Metabolism of defensive compounds by phloem feeding insects**

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Many insects of the order Hemiptera are worldwide crop pests which cause severe agricultural loss through their phloem feeding behaviours. The best studied members of the order are whiteflies and aphids. Metabolic analyses of honeydew from insects fed on different plants allowed the identification of modified plant defensive chemicals in the honeydew of both whiteflies and aphids. These modified metabolites are stable to plant-derived enzymes responsible for activation of these toxic defense compounds. The identities and quantities of these products are now being determined. Furthermore, the importance of these modified metabolites as detoxification products will be investigated using artificial diet feedings to determine toxicity *in vitro*. Through transcriptomic mining and sequence annotation, we began identifying potential gene targets from different classes (GSTs, UGTs, sulfo- and phospho-transferases, sulfatases and P450s) which may be responsible for the observed metabolic activities for cloning and recombinant expression. Ultimately, we hope to determine reasons for phloem-feeding insect adaptation and super-abundance through qualitative and quantitative analysis of metabolism of ingested plant compounds in various plant-insect relationships.

## **P5 Differential expressions of *anfH* and *nifH* of *Kosakonia radicincitans* DSM16656T in nitrogen enriched media**

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*Kosakonia radicincitans* DSM16656T is a plant growth promoting associative diazotrophic bacterial strain with two gene clusters for Biological Nitrogen Fixation (BNF): the *anf* and the *nif* operon. The *nif* operon is found in all diazotrophic prokaryotes while the *anf* operon is additionally found in some diazotrophs that already have *nif* genes. On the *nif* operon *nifHDK* encode for the synthesis of the main iron-molybdenum nitrogenase while *anf* operon *anfHDK* encodes for the alternative iron-nitrogenase. Because of the triple bond of the dinitrogen gas, BNF is an energetically expensive process. For this reason nitrogenase activities are greatly controlled by mineral nitrogen availability through a negative feedback system and most diazotrophs express this through what is termed “ammonia switch off”. To investigate the impact of mineral nitrogen availability on *K. radicincitans* nitrogenases, we measured its *nifH* and *anfH* expression in liquid nitrogen free media enriched with either 50ppm or 100ppm N of both  $\text{NH}_4\text{Cl}$  and  $\text{CH}_4\text{N}_2\text{O}$ , at 12, 24 and 36 hours by qPCR measurements. Both  $\text{NH}_4\text{Cl}$  and  $\text{CH}_4\text{N}_2\text{O}$  inhibited *nifH* and *anfH* expression within the first 12 hours of incubation. After 24 hours of incubation, *nifH* expression was higher than *anfH* expression in all treatments, with the highest *nifH* expression in  $\text{NH}_4\text{Cl}$  100ppm.

## **P6 Transcriptome profiling coupled with physiological response during drought stress and recovery in *Pinus halepensis***

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Climate change, which is leading to increased mean temperatures and is expected to decrease annual precipitation, may be too rapid to allow for adaptation to stress in long-lived forest trees. *Pinus halepensis* (Aleppo pine), which is widespread in the Mediterranean basin and is one of the most drought-tolerant pine species, has been subjected to drought stress and recovery. Total RNA extraction and cDNA libraries were prepared from drought-stressed and well-watered clones from 6 time points according to the physiological stage, i.e. baseline, stomatal closure, maximum drought, post-irrigation partial recovery and recovery. A 'drought-stressed *P. halepensis* transcriptome' was *de novo* assembled and was compared to the published *P. halepensis* transcriptome. There were ~4,000 differentially expressed non-redundant transcripts. Gene Ontology Enrichment results suggest that drought response involves down-regulation of cell cycle, cell growth, transcription, response to endogenous stimuli, RNA metabolism and biosynthesis, and up-regulation of localization, chloroplast-related and homeostasis processes. This multi-disciplinary approach utilizing clones from mature trees to achieve high-throughput physiological measurements and RNA-seq offers a better opportunity to identify drought-related genes in forest trees.

## **P7 *Physcomitrella patens* RNA-seq big data analysis**

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We contribute to the US Department of Energy Gene Atlas project that aims at generating transcription atlas data for seven plant model (“flagship”) species. Such projects generate plenty of new data. To manage the large amount of sequences, new approaches are required. Additionally, analytical methods for comparison need to be standardised. Within our cooperation 4,2 billion *Physcomitrella patens* Illumina paired-end RNA-seq reads were created. Overall we analyse 99 libraries from 34 different developmental stages or perturbations. Here, I will show the design of our pipeline, from raw data handling to differentially expressed gene (DEG) calling. This includes quality control steps with trimming and filtering as well as merging overlapping paired end reads, and subsequent mapping to the reference genome. An important step during RNA-seq data analysis usually is the detection of DEGs, in this case of 42 pairwise comparisons based on the 34 experiments. From mapped read counts we inferred DEGs with three published tools (edgeR, NOISeq and DESeq2). A high confidence set was generated by using the strict consensus of those. Cross-experiment normalization is difficult for any expression profiling methodology and not well developed for RNA-seq data yet. We are developing a methodology to make use of External RNA Controls Consortium (ERCC) spike-in RNA, that were added to the RNA pools before sequencing, for normalization.

## **P8 A closer look at the *Physcomitrella patens* ‘Reute’ strain.**

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The comparison at genome level of different accessions/ecotypes of plant species is a powerful tool to describe and understand species diversity. Nowadays reference genomes provide a good framework to easily perform such analysis. The moss *Physcomitrella patens* reference genome is an important comparator genome for plant evo-devo and comparative genomics, and is based on the accession collected in Gransden Wood, Great Britain (Gd) in 1958. In this study we compare Gd with accessions collected from three different locations: Villersexel, France (Vx strain), Reute, Germany (Rt strain) and Kaskaskia, USA (Ka strain). Genome-wide Single Nucleotide Polymorphism (SNP) analysis revealed low levels of diversity for the Rt strain and higher diversity for the Vx and Ka strains. Despite low amounts of polymorphisms, experiments on sporophyte development find variations between the accession with Gd developing lower amounts of sporophytes. Additionally, whole transcriptome analysis performed with microarray showed differences in gene expression profiles for the Rt strain. Thus, we show based on genetic and phenotypic data that the different accessions are *P. patens* ecotypes. Future work will involve comparative epigenetic analysis that may help to understand the relationship between (epi)genome, transcriptome and phenotype.

## **P9 Ammonium Starvation Responses of the Ammonia Oxidizing Archaea *Nitrososphaera viennensis***

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Ammonia oxidizing archaea are ubiquitous and abundant in terrestrial environments and contribute considerably to nitrification in soils. However, little is known about their central energy and carbon metabolism and their responses to environmental changes. The first and only available organism from soil that can be grown in pure culture is *Nitrososphaera viennensis*, isolated from the departmental garden at the University of Vienna. The annotated genome of *N. viennensis* contains an abundance of genes involved in the regulation of carbon and nitrogen metabolism. Understanding the interplay and regulation of these metabolic modules in response to environmental cues will help determine how the physiology of *N. viennensis* has adapted to a complex soil environment. In this context, we applied transcriptomics to gain insight into the ammonium-starvation response of *N. viennensis*. The experimental setup included starving cultures of ammonium and extracting RNA in a time series upon re-addition of ammonium. The extracted RNA was sequenced on an Illumina HiSeq 2000 and the results are being analyzed to determine the differential expression of genes during culture recovery. The results of this experiment will demonstrate how *N. viennensis* can respond to varied nitrogen concentrations and limitations found in its natural environment. This will give insights into the role this microorganism plays in the biological turnover of nitrogen in the environment.

## **P10 Heat-resistant *Escherichia coli* as a potential persistent reservoir of plasmids encoding extended-spectrum $\beta$ -lactamases and Shiga toxin-encoding phages in dairy**

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Heat-resistant *Escherichia coli* were isolated from raw milk cheeses. Detection of the heat-resistance marker *clpK* was followed by phenotypical confirmation. These strains are Shiga toxin-negative and no plasmids encoding extended-spectrum  $\beta$ -lactamases (ESBL) were found. The aim of this study was to assess the potential of these strains to acquire ESBL and Shiga toxin-encoding phages. Only four ESBL-encoding, heat-sensitive *E. coli* strains were isolated from 1,251 samples. One IncFII plasmid (CTX-M-14) and three IncI1 plasmids (CTX-M-15) were fully sequenced and *de novo* assembled. All four plasmids are readily transferred to heat-resistant *E. coli* isolates in plate matings ( $9.7 \times 10^{-5}$  to  $3.7 \times 10^{-1}$  exconjugants per recipient) and milk (up to  $7.4 \times 10^{-5}$  exconjugants per recipient), and are maintained during passaging in liquid media without antimicrobial pressure. The heat-resistant strain FAM21805 was also shown to be capable of acting as donor of all four plasmids. Also, three of 11 tested ESBL-exconjugants of heat-resistant strains were lysogenized by the modified Shiga toxin-encoding phage 933W  $\Delta$ stx::gfp::cat. Isolation of heat-resistant *E. coli* seems to indicate a selection advantage in the raw milk cheese production environment. Should these strains acquire ESBL-encoding plasmids and Shiga toxin-encoding phages, these genetic elements would profit from the selection advantage of their host, which could lead to an increased threat to consumers of raw milk products.

## **P1 Living with oxygen: Evolution of the unconventional O<sub>2</sub>-scavenging system in diplomonads**

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Diplomonads are a group of unicellular, parasite or free-living, heterotrophic protist. They live in anaerobic or microaerophilic environments, although parasite species may experience increasing level of O<sub>2</sub> during infection. Diplomonads lack the traditional aerobic mitochondria. Instead, they contain either hydrogenosomes (*Spironucleus salmonicida*) or mitosomes (*Giardia intestinalis*) and energy is produced by fermentation. Most of the enzymes involved in this process are O<sub>2</sub>-labile. Previous studies showed that diplomonads have enzymes similar to bacterial homologous, indicative of a possible prokaryotic origin by lateral gene transfer (LGT). Here, we perform a bioinformatics study of the oxygen stress response genes in this group, with the goal to understand the evolutionary adaptation to increasing O<sub>2</sub>-levels coupled to pathogenicity. A total of 24 enzymes involved directly or indirectly in the O<sub>2</sub>-scavenging system are targeted. We use a phylogenetic approach to systematically investigate the origin of these enzymes. Our results show the existence of a central eukaryotic core where different enzymes were added via LGT during the evolution of this lineage. These enzymes of different origins interact to create a redox pathway well-adapted for coping with changing O<sub>2</sub>-levels. Synthesis of different thiols show different processes of LGT and gene losses in diplomonads. These processes might have played an important role in the adaptation of diplomonads to parasitic lifestyle.

## **P12 The World of Amaranthus and Beyond**

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The Caryophyllids clade of the core Eudicots includes the Amaranthaceae family with the highly diverse genus *Amaranthus*. The majority of *Amaranthus* species are classified as annual summer weeds, commonly referred to as pigweed. Several weed species such as *A. palmeri* and *A. tamariscinus* are dominant in the agricultural landscape of the Americas and Mexico. Distinctive properties of these species is their ability to germinate at temperature as high as 35°C and reproduce to generate high-volume of seeds from an individual plant which are disperse in the surrounding environment. The aim of this project is to gain a greater understanding of mechanism of *Amaranthus* seed persistence, dormancy release, longevity and germination. Several approaches have been applied including Thermal-Time Modelling, seed ageing assays, as well as methods to investigate the physiological, morphological and molecular changes during germination. The findings from these initial studies will provide a framework to understand the underpinning mechanisms and develop future germination promoting compounds for a 'flush-and-kill' strategy to combat weeds in the arable soil. This project is funded by the BBSRC and is in collaboration with Syngenta, UK.

### **P13 Repeatome composition of the moss (*Physcomitrella patens*) genome**

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A huge part of the most of the plant genomes is occupied by different repetitive DNA families (repeatome). DNA repeats demonstrate huge variability in unit length, genome proportion, chromosomal organization and epigenetic modifications. For a long time to be considered as junk DNA, recent studies showed that repeatome is an important part of genome playing an essential role in the biology of heterochromatin, centromere, telomeres and gene expression regulation. While important insight into repetitive DNA evolution and organization has been made for embryophytes, our knowledge about repetitive DNAs in early diverged plant lineages are scarce. Moss, *Physcomitrella patens* ( $2n = 54$ ,  $1C = 500\text{Mb}$ ), is a model plant species having sequenced and annotated genome which was recently assembled into the pseudochromosomes. We used next generation sequencing data to annotate moss repeatome, classify repetitive DNA families and estimate genome portion occupied by each repeat family. We further performed total RNA-seq from protonemata and protoplasts to identify repeat-contained lncRNAs transcripts. The evolution and transcription of repetitive DNA in a moss cell as well as further perspectives will be discussed.

### **P14 Can herbivore-induced plant volatiles prime defenses in black poplar?**

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Upon attack, plants release VOCs, which are known to play an important role in direct and indirect defense. Recent studies with herbaceous plants have shown that HIPV can prime non-infested tissues to show a faster and stronger defense response upon subsequent attack. Evidence for defense priming in woody plant species and the consequences for insect herbivore behavior and performance are still scarce. The aim of this study was to investigate the effects of volatile mediated priming on the defense chemistry of the tree species *Populus nigra* and consequences for its natural herbivore *Lymantria dispar*. First trees were exposed to HIPVs and then herbivory treated while VOC sampling. Larval performance and behavior were monitored in follow-up experiment. The herbivore induced volatile emissions of the primed trees significantly differed from the emission of the non-primed control trees. The levels of defense hormones and phenolic compounds did not change significantly during the first hours but the salicinoids showed an induction after 5 days. Caterpillars performed worse when feeding on primed tissues and actively avoided primed leaves. The results from this study suggest that volatile-mediated priming significantly affects the defense chemistry of black poplar trees with negative effects on insect herbivore performance. Volatile priming can thus be considered an efficient defense strategy in woody plant species.

## **P15 The formation of herbivore-induced salicylaldehyde in poplar**

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In response to herbivory, many plants produce defense compounds which can either be toxic or repellent for the herbivore or attractive for natural enemies of the herbivore. These direct and indirect defense mechanisms often present strong barriers against various attackers. Such induced plant defense mechanisms have been intensively studied above-ground, but little is known about the induced defenses of roots, especially those of trees. In this regard, the herbivory-induced accumulation of chemical metabolites was investigated in roots of the Western balsam poplar (*Populus trichocarpa*). Feeding of cockchafer (*Melolontha melolontha*) larvae on poplar roots leads to a highly increased accumulation of salicylaldehyde. To investigate the biochemical mechanisms of the observed herbivory-induced root response, a transcriptome dataset was generated to identify all differentially expressed genes in poplar roots. Putative candidate genes of a  $\beta$ -oxidative biosynthetic pathway were identified and heterologously expressed in *Escherichia coli*. Six cinnamic acid-CoA ligases (CNL), three cinnamoyl-CoA hydratases/dehydrogenases (CHD) and three 3-ketoacyl-CoA-thiolases (KAT) were cloned and characterized. These enzymes showed enzymatic activities *in vitro* and might be responsible for the formation of salicylaldehyde in poplar roots.

## **P16 Looking into LUCA's cofactors**

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The last universal common ancestor (LUCA) is a biological entity thought to have existed sometime between the origin of life and the divergence of the bacterial and archaeal domains [1,2]. After identifying candidate protein families in LUCA by phylogenetic analysis, we focused on the protein cofactors it used in order to try to elucidate its biology and relation to the environment where it originated. According to our findings LUCA's proteome encompassed enzymes involved in the biosynthesis of coenzyme F420, the molybdenum and iron-molybdenum cofactors, tetrapyrroles, pterins, thiamine, glutathione and coenzyme M. The most commonly required small molecule was ATP. However, proteins requiring transition metal-based cofactors were present at a comparably high extent (FeS and NiFeS clusters, molybdenum, iron-molybdenum and tungsten molybdopterin, corrinoids, hemes and active site iron, ferredoxins). The need for environmentally supplied transition metal catalysts is also mirrored in the number of metal transporters present, reflecting an environment rich in these metals. S-adenosylmethionine dependent reactions, especially of the radical type, were not unusual, and selenium had also already found its place in the make-up of selenoproteins.

(references: 1. T.A. Williams, T.M. Embley, Changing ideas about eukaryotic origins, *Philos. Trans. R. Soc. Lond., B*, 370 (2015) 20140318; 2.E.G. Nisbet, N.H. Sleep, The habitat and nature of early life, *Nature* 409 (2001) 1083–1091)



## **P17 Towards an understanding of genetic diversity and gene expression in Zygnematophyceae**

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Zygnematophyceae make up a complex group of more than 3500 species. Recent studies on evolution of land plants showed that Zygnematophyceae are sister group to the Embryophyta, which has important implications for early land plant evolution. Despite the importance in terms of ecology and evolutionary processes, molecular studies on this group are still scarce. Due to elasticity in phenotypic traits, a determination on the species level is so far only possible for taxonomists. Hence, the first part of this project focuses on the development of a barcoding system. We extracted DNA of more than 500 culture strains of the Microalgae and Zygnematophyceae Collection Hamburg (MZCH), which comprises species from all over the world. The *rbcL* gene region is used as a pre-barcode, resulting in clades distinguishing on the genera level. The next step will include the development of clade-specific barcode genes. The second part of my PhD project focuses on gene expression of a polar isolate of *Cosmarium crenatum*, a cosmopolitan species, essentially arctic-alpine. So far few studies were conducted on the effects of abiotic stress on physiology, but no study is available on transcriptomic response. Thus, the aim of this study is to determine transcriptomic acclimation patterns to abiotic stressors. The first milestone is to establish a comprehensive reference library of *Cosmarium crenatum*. Subsequently, gene expression will be investigated by Illumina HiSeq Sequencing.

## **P18 Metabolic and evolutionary insight into regulation of diterpene resin acids in Norway Spruce**

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Plant diterpenoids are a large family of diverse bioactive compounds that are ubiquitous across the plant phylogeny. Excluding those committed to plant primary metabolism, many diterpenoids in secondary metabolism appear to be confined to single plant lineages. One example of lineage-specific diterpenoids is the diterpene resin acids (DRAs) in conifers such as *Picea abies* (Norway Spruce), which are implicated in defense against herbivores and pathogenic fungi in spruce and other conifers (Keeling and Bohlmann 2006). Although significant advancements in our understanding of DRA biosynthesis have been made in the past (Ro and Bohlmann 2006; Hall et al. 2011; Geisler et al. 2016), a comprehensive picture of regulation, resistance, and evolution of these ecologically-relevant compounds remains out of reach. Here we show through bark chemical and transcriptome analyses, coupled with molecular phylogenetics of DRA biosynthetic genes, that 1) DRAs are differentially regulated in pathogen-susceptible and resistant spruce lines, and 2) cytochrome P450 monooxygenases in conifers were likely recruited into DRA metabolism from ancient brassinosteroid pathways.

## **P19 Co-evolution of the holobiont *Anemonia viridis*, a symbiotic sea anemone**

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Most organisms are in fact holobionts i.e. associations of several symbiotic organisms. To understand how the holobiont can adapt and evolve, we need to know the history of the symbiotic partners' genomes. In Mediterranean Sea and North-eastern Atlantic Ocean, a peculiar example of the relationship Cnidarian–Dinoflagellate exists: the mutualistic symbiosis between *Anemonia viridis* and its Symbiodinium symbionts. The wide distribution of *A. viridis*, the diversity of habitats it colonizes, its morph diversity (5 morphs with putatively different adaptive strategies) and the genetic diversity of its symbiotic *Symbiodinium* sp. (possibly a major actor in the adaptation of the whole holobiont) make it an interesting model for the study of adaptation at the holobiont level. How are host and symbiont diversities correlated? Are there Genotype Environment Associations? To address these fundamental questions, we characterized the distribution of in hospite Symbiodinium genetic diversity by sampling 3 morphs of *A. viridis* (for a total of 384 individuals) in ecologically differentiated populations from the English Channel and the Mediterranean Sea. We assessed this genetic diversity by combining microsatellite and sequence markers (ITS2, cp23S, *psbA*), genotyped by NGS. As the host genetic diversity is assessed in parallel in the lab, with EPICs and RADseq markers, we plan to unravel the co-evolutionary dynamics of the two partners along a geographical and/or environmental continuum.

## **P20 Identification of novel components in MicroProtein signaling**

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MicroProteins (miPs) are small, usually single domain proteins. They function as negative regulators of protein complex formation, sequestering their targets by competitively binding to their interaction domains and rendering them non-functional. MiPs have been implicated in developmental processes in plants, where they regulate the activity of specific components in the pathway. One such process, floral transition, involves a complex network of genetic factors and regulatory circuits influencing the plant shift from vegetative growth to floral initiation. We recently described microProtein miP1a as an important player in the modulation of flowering time. MiP1a is a small B-Box domain containing protein. It targets the CONSTANS transcription factor, a major regulator of flowering time. When overexpressed, miP1a strongly influences the innate floral transition pathway- consequently plants are late flowering. The presence and function of associated factors enabling or influencing miP1a function are unknown. We investigated candidate proteins involved in the miP1a functional pathway using forward genetics. In an EMS suppressor screen of miP1a overexpressing plants, and subsequent mapping-by-sequencing of single suppressor candidates, we mapped the location of causal mutations, thereby identifying genes encoding components critical to the functioning of the microProtein pathway. I will present an overview of the screen and preliminary results for suppressors of miP1a function.

## **P21 Detection and identification of rare and invasive Ulvales by different molecular and morphological approaches**

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Several representatives of the order Ulvales are characteristic foulers and known for their ability to form massive blooms. Additionally, their opportunistic lifestyle provides them with a good basis to invade new ecosystems. These combined traits underline the importance of species knowledge but due to the extremely variable and overlapping morphology of Ulvales the taxonomic identification of genetically distinct species was largely hampered. Within a survey which took into account both genetic and morphological criteria we were able to describe the diversity of Ulvales of Northern Germany. Use of several barcode markers revealed that species which were assumed to be very abundant are rather rare or even absent. Alternatively, some highly abundant taxa were detected for the first time in the research area. Different approaches prove that *U. compressa* Linneus 1753 from Germany is conspecific with the type strain of the model organism *U. mutabilis* Föyn 1958, Faro Portugal. This could not only be demonstrated through genetic investigation, but also through interbreeding experiments. Nevertheless, the detection of rare or newly introduced species by Sanger Sequencing of single thalli is not only time consuming, but also costly and for long term studies just partly supportive. A new approach using Next Generation Sequencing methods for early detection of invasive species and simplified long term observations of diversity among populations of Ulvales will be discussed.

## **P22 LUCA's informational core**

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Central to the studies of early evolution and the origin of life is the concept of a last common ancestor, or Luca and its genomic content. To date, most comparative genomic analysis of Luca's gene content considered Luca as the last common ancestor of bacteria, archaea and eukaryotes. However, recent findings show that eukaryotic ribosomes branch within archaea, and in the more modern two domain trees, Luca is the last common ancestor of prokaryotes. In here we wish to investigate the nature of the original ancestor, specifically which genes it contained as inferred from the gene collection present in extant genomes. Based on stringent phylogenetic criteria, the gene families shared between 134 archaeal and 1847 bacterial organisms were investigated. Besides evidence for genes coding for ribosome biogenesis within Luca's informational core, of special interest are the 8 rRNA nucleoside modifications protein families found, most of which, coding for SAM-dependent nucleoside methylations. This suggests the antiquity of methyl-based biochemistry at the start of Life.

## **P23 What is the functional role of the core circadian clock component TOC1 in mediating whole-transcriptome stress responses in plant root and shoots?**

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Circadian regulation of daily rhythms provides adaptive benefits for many organisms, including plants, and control many aspects of Darwinian fitness. At the heart of these rhythms is the core plant circadian clock: a self-regulating loop of broad molecular repressors which modulate gene expression based on the time of day, thus contextualizing plants' decisions about stress responses. One core circadian clock gene, the evening-expressed TIMING OF CAB EXPRESSION 1 (TOC1), is known to be directly involved in the modulation of both biotic and abiotic stress responses. Recent evidence also supports a timekeeping function for TOC1 in shoots, but not in roots. This suggests different functional consequences for the plant when TOC1 is manipulated in roots and shoots separately. Given that coordination between roots and shoots is essential for growth and development, our goal is to investigate the functions of TOC1 in the root and shoot, and the influence of TOC1 on the stress response of plants in the context of root-shoot signal transduction. We are analyzing microarray data from grafted plants with RNAi-silenced TOC1 expression in roots, or both roots and shoots, in comparison to empty vector controls under both water-stressed and control conditions. By looking at expression profile changes, we hope to get an overview of TOC1-regulated traits at the transcriptomic level, and to dissect which of these traits are root TOC1-dependent and which are shoot-dependent.

## **P24 Molecular response of *Pinus halepensis* to the aphid *Matsucoccus josephi***

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The genus *Matsucoccus* commonly known as bast scale insects has a worldwide distribution in temperate, tropical, and subtropical regions. In Israel, *Matsucoccus josephi* has killed many Aleppo pine trees (*Pinus halepensis*) on large planted forest areas. *M. josephi* affects Aleppo pine but not Brutia pine (*P. brutia*). This evolutionary differences in the system presents a unique opportunity to elucidating constitutive (always present in plant) and induced (when plants are attacked by insects) defense mechanisms. Our research objective is to determine the molecular response of Aleppo and Brutia pines to *M. josephi*. For this purpose, we first characterized the typical injury to *Pinus halepensis* by analyzing the different infection stages by means of anatomical structure. Feeding by the larvae on Aleppo pine mainly results in pathological changes in the cortex, whereas *P. brutia* does not display any signs of injury. For the molecular response, stem and needle tissues samples where insects feed and don't feed will be collected at 3 different infection stages. Molecular responses of the two pines will be assessed in these stages via RNA-seq analysis. The transcriptome of the three different stages will be determined and the differentially expressed genes between the two species will be used in future research as to develop molecular markers for resistance or susceptibility in pine trees.

## **P25 Circular RNAs: identification, expression and potential function in plant development and stress responses**

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In all living organisms, regulation of gene expression is fundamental for survival and adaptation. Gene expression can be modulated at various steps, including at the level of RNA processing. During the last year, the importance of alternative splicing of mRNAs in controlling plant development and stress responses were emerged. Recently, a novel type of alternative splicing has been reported which leads to the generation of circular RNAs (circRNAs). Several functions of circular RNAs have been proven or proposed, including functioning as microRNA or RNA-binding protein decoys. Relatively little is known about circRNAs in plants and functions of circRNAs in plants are enigmatic. In order to detect and classify circRNAs in Arabidopsis, we created a work-flow that includes generation of Illumina library enriched for circRNAs and a combination of biocomputational tools to discover and analyze novel endogenous circular RNAs. High-fidelity candidates are subjected for an in-depth analysis of their functional role in plant development and stress-related response. The overall aim of this project is to identify and understand the functions of circRNAs in plants and explore their potential for crop improvement strategies.

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