Swiss Mycology Symposium 16th June 2023

University of Zürich, Vetsuisse Faculty, Winterthurerstrasse 260, 8057 Zürich, building TFA, room 00.44 (see here for a map and for directions)

Program

09:00 – 09:30	Arrival & Registration
09:30	Welcome address
09:35 - 09:55	**Danaé Bregnard** (Uni Neuchâtel): Assessment of fungal diversity in deep geothermal fluids: accessing DNA from all cell types
09:55 - 10:15	**Johanna Mayerhofer** (Agroscope Reckenholz): Biogeography of soil fungi and their correlations to plants
10:15 - 10:35	**Claude Müller** (ETH Zürich): Diversity of the subsoil fungi under organic and conventional farming
10:35 - 11:10	Coffee break
11:10 - 11:30	**Derek Troiano** (BFH-HAFL): Artificial microbial consortia for the conversion of lignocellulosic biomass into fuels and chemicals
11:30 - 11:50	**Natacha Bodenhausen** (FiBL Frick): Successful prediction of crop yield increases after inoculation with arbuscular mycorrhizal fungi
11:50 - 12:40	**Keynote by Colin Averill** (ETH Zürich): Restoring the forest fungal microbiome
12:40 - 15:00	Lunch & Poster viewing
15:00 - 15:20	**Guido Puccetti** (University Neuchâtel): The complex genetic landscape of fungicide resistance evolution in *Zymoseptoria tritici*
15:20 - 15:40	**Chen Chen** (ETH Zürich): Gene-for-gene relationship: Detection of effector-gene virulence in the fungal pathogen of sugar beet, *Cercospora beticola*
15:40 - 16:00	**Julia Lagler** (University of Zürich): The role of the fungal virulence factor Ece1 in homeostatic immunity towards *Candida albicans*
16:00 - 16:20	**Gabriel Giger** (ETH Zürich): Expanding the Host Range for a Fungal Endosymbiont Through Implantation by FluidFM
16:20 - 16:40	**Maxime Staedler** (University of Fribourg): Bacterial volatiles and their impact on *Botrytis cinerea* spore production
16:45	Closure note & farewell

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ABSTRACTS OF THE ORAL PRESENTATIONS

Assessment of fungal diversity in deep geothermal fluids: accessing DNA from all cell types

Danaé Bregnard (University of Neuchâtel)

Deep geothermal fluids used for electricity production present several environmental conditions that are considered extreme for life such as a high temperature and high pressure. As a result, the ability of microorganisms to form resistant structures likes spores will allow them to withstand these extreme conditions. Thus, when assessing microbial diversity in these extreme environments, being able to access the DNA within spores or any resistant cells, is mandatory. In this study, a method based on vegetative cell lysis to enrich for lysis-resistant structures, previously developed for bacterial spores or spore-like cells, was validated for fungi. Different types of fungal cells (mycelium, spores, yeast cells) were assessed. By comparing a usual DNA extraction and the combination of an enrichment of lysis-resistant structures prior to DNA extraction, we showed that DNA extraction needs to be combined with the enrichment of lysis-resistant structures to fully recover fungal DNA from both vegetative and resistant cells. Thus, this approach needs to be applied to assess more thoroughly the diversity of fungi in the environment, especially in extreme environments where resistant structures are expected to be abundant. We applied this method to samples originating from deep geothermal fluids of different power plants around Europe to assess the still unknown fungal diversity in these extreme environments. Between power plants, the impact of the enrichment of lysis-resistant cells treatment varies. In some cases, such as the power plants with the most extreme abiotic conditions, the fungal communities detected with one or the other method are highly similar, suggesting that all organisms present have to form resistant cell structures to survive in such environments. In other power plants, with less extreme conditions, the communities detected with the two methods are different and combining both allowed to have a broader knowledge on the total community present in these fluids.

Biogeography of soil fungi and their correlations to plants

Johanna Mayerhofer (Agoscope Reckenholz, Zürich)

Fungi are part of the hidden majority of soil organisms and important for soil functions. For assessing soil fungi, their environmental drivers and cooccurrence with plants, we used 255 sites representing four land-use types (LUT), i.e., arable land, meadow, alpine grassland and forest from the regular grid of the Swiss Biodiversity Monitoring (BDM). Fungal communities were determined using metabarcoding and plant presences were recorded at each site. In total, 28,088 fungal amplicon sequence variants (ASVs), i.e., operational taxa, were detected with a mean of 310 ASVs per site. About half of the ASVs was assignable to genus-level. Fungal and plant communities correlated significantly within LUTs, while no significant correlation of fungal ASV and plant species richness was found. Environmental factors explained between 7 and 13% of fungal and between 13 and 17% of plant community variation. The most important factors that significantly shaped fungal communities were soil pH and depending on the LUT also elevation and variation of precipitation. Similarly, variation in plant communities was significantly explained by elevation, soil pH, precipitation and temperature. In alpine grassland, abundance of 201 fungal ASVs correlated with presence of plant species and only 13 with environmental factors, while in forest and meadows 21 of 46 and 58 of 85 fungal ASV abundances correlated with environmental factors. In conclusion, the currently most comprehensive assessment of soil fungal communities across Switzerland revealed an unexpected high diversity and several drivers of their communities. A large portion of 82% unknown species indicated the need for extending sequence databases, specimen collections and integration of sequence-based species descriptions. Fungi and plants were correlated at the level of communities and single taxa. Further analyses of rhizosphere and root samples are required to resolve whether the correlations between fungal ASVs and plant species represent interactions or similar environmental preferences.

Diversity of The Subsoil Fungi Under Organic and Conventional Farming

Claude Müller (ETH Zürich)

One of the aims of sustainable nutrient management in agricultural soils is to preserve soil organic matter (SOM) which influences important ecosystem services such as carbon storage and soil fertility. As most

biogeochemical processes in soils are driven by microorganisms, understanding the impact of soil nutrient management on microbial diversity is essential to select appropriate agricultural practices that optimally use soil nutrients. The aim of this study is to gain insights into how agricultural nutrient management alters the structure of the soil fungal diversity down to 90 cm. Soil samples were collected at the DOK long-term agricultural field experiment (Therwil, Switzerland). The trial was established in 1978, and the experimental treatments include the application of different fertilizer types and crop protection regimes representing typical conventional and organic agricultural practices in Switzerland. Soil samples have been collected down to 90 cm depth in increments of 5 cm. Fungal diversity and taxonomic community composition were assessed using a DNA metabarcoding approach targeting ribosomal markers. We hypothesize that (1) shifts in fungal community composition are mainly driven by the type of organic residues added, and that (2) the effect of nutrient management on the fungal diversity decreases with depth. Also, (3) that the subsoil fungal community structure changes compared to the topsoil towards more specialist organisms that grow slow and have a higher carbon use efficiency (i.e., oligotrophs). Preliminary results show that the effect of agricultural practices on fungal beta diversity is significant above the plough layer (i.e, 25-30 cm depth), but not below. They also indicate that the effect of depth on fungal beta diversity can be observed all along the soil profile and that sampling in increments of 5 cm enables to account for differences that are neglected when samples are collected with a lower resolution.

Artificial microbial consortia for the conversion of lignocellulosic biomass into fuels and chemicals

Derek Troiano (University for Applied Sciences, Bern)

Microbial consortia are a promising alternative to monocultures for complex biotransformations due to their inherent advantages which include the distribution of metabolic burden by division of labor, the ability to convert complex substrates more efficiently, and their modularity. Previously, our group has engineered a biofilm reactor which enables control of spatial organization along several different gradients of abiotic factors within a biofilm. This reactor harbored an artificial cross-kingdom microbial consortia with oxygen-replete and anoxic niches and was employed in the consolidated bioprocessing of lignocellulose to valuable chemicals such as ethanol, lactic acid, or short chain fatty acids. Ongoing research in our group seeks to expand synthetic niche engineering to other types of spatial niches (e.g., light, carbon dioxide, etc.) for application in microalgal bioprocesses. Microalgal biomass represents a promising feedstock for the sustainable production of, among other things, biofuels, but its use is hindered by high water and energy requirements for growth and harvesting as well as nutrient cost. Growing microalgae in a biofilm may represent one solution to the high water and energy consumption associated with more commonly employed suspended growth approaches. Concerning nutrient cost, which is high due to the requirement for supplemental organic carbon (i.e., for mixotrophic growth) to promote sufficiently rapid microalgal growth, process economics may be improved by replacing typical sources of supplemental organic carbon (e.g., acetate) with cheap and abundant lignocellulosic biomass. While microalgae do not possess the capacity for metabolizing the complex biopolymers available in lignocellulosic biomass (e.g., cellulose), filamentous fungi are excellent at degrading components of lignocellulose into simple molecules. Here, Chlamydomonas reinhardtii was co-cultured with the cellulolytic filamentous fungus Trichoderma reesei in cellulose-based media within the biofilm bioreactor. Ultimately, the engineered symbiosis in cellulose-based media yielded significantly more algal biomass vis-a-vis anexic microalgal cultures grown in typical growth media.

Successful prediction of crop yield increases after inoculation with arbuscular mycorrhizal fungi

Natacha Bodenhausen (FiBL, Frick)

Mineral fertilizers and pesticides pose significant challenges due to their detrimental effects on the environment and human health. Alternative solutions are needed and include inoculation with arbuscular mycorrhizal fungi (AMF). In pot experiments, AMF can increase plant nutrient uptake and growth; however, successful inoculation with AMF depends on the soil properties and the soil microbiome. To solve the context dependency, we conducted on-farm experiments in 54 fields in Northern Switzerland and quantified the effects on maize growth. We calculated the mycorrhizal growth response (MGR) and found MGR to be greater than 10% in one quarter of the fields. We developed a predictive model combining selected soil parameters and soil fungal OTUs. Interestingly, the fungal OTUs had a greater impact than the soil parameters in predicting MGR. Finally, we analyzed the root fungal communities and observed that the

introduced mycorrhizal strain replaced mostly native arbuscular mycorrhizal fungi in low MGR fields, while in high MGR fields, the relative abundances of several plant pathogenic fungi were significantly reduced. These findings suggested that the introduced mycorrhizal fungi outcompeted pathogenic fungi in the roots, leading to improved maize growth. Overall, the research highlights the importance of considering soil fungal communities and specific pathogenic fungi when predicting the success of mycorrhizal inoculation and subsequent crop growth. By assessing soil parameters and fungal composition prior to inoculation, it becomes possible to anticipate the effectiveness of mycorrhizal treatments and enhance sustainable agricultural practices.

The complex genetic landscape of fungicide resistance evolution in *Zymoseptoria tritici*

Guido Puccetti (University of Neuchâtel)

The evolution of fungicide resistance in fungal pathogens poses a threat to the health of animal and plant species. The repeated emergence of pathogens with reduced sensitivity to specific fungicides illustrates the rapid process of resistance evolution. The widespread application of target-site fungicides in European agricultural fields over the last three decades makes the continent an excellent geographic range to identify the rise of specific resistance mutations in space and time. Target-site fungicides such as demethylation inhibitors (DMIs), succinate dehydrogenase inhibitor (SDHI) and quinone outside inhibitors (Qoi) target enzymes involved in essential cellular processes. The most common mechanism leading to insensitivity are structural changes in the target protein. There is strong evidence that additional loci are also contributing to overall resistance. Here, we aimed to unravel the evolutionary trajectories driving the genetic architecture of fungicide resistance in Europe for the major wheat pathogen, *Zymoseptoria tritici*, towards different fungicide classes. Using a panel of 1420 whole-genome sequenced isolates spanning 27 European countries and a period of 15 years, we performed genome-wide association mapping for resistance using genotyping methods. We identified dozens of previously unknown loci contributing to resistance in addition to mutations in the gene encoding the target. Our study highlights the power of microbial genome-wide association studies to retrace rapid evolutionary processes.

Gene-for-gene relationship: Detection of effector-gene virulence in the fungal pathogen of sugar beet, *Cercospora beticola*

Chen Chen (ETH Zürich)

Fungal plant pathogens are a major threat to agriculture and food security. Although, cultivars with resistance genes are a major part of disease control strategies, pathogens can evolve virulence – eventually overcoming resistance. This is a natural process of co-evolution between cultivars and pathogens. A well-known pathway to virulence is via mutations in pathogen effector genes, which encode proteins that modulate plant immune response. Recently, a cultivar of sugar beet with a previously unmatched resistance level to *Cercospora beticola*, the causal agent of *Cercospora* Leaf Spot (CLS) was developed. This was possible through the combination of a newly identified resistance gene conferring a high phenotypic effect with the widely deployed polygenic resistance used traditionally. Field sampling and genotyping at two sites in Switzerland identified single isolates that were potentially virulent to this new resistance gene. To validate the virulence of these isolates and search for candidate virulence genes, we performed a large-scale field trial and GWAS. Virulence could be confirmed for single isolates and was most pronounced on a sugar beet hybrid without additional background resistance. Using GWAS, we identified a single locus associated with virulence containing a single effector gene. Virulent isolates were either lacking the effector gene or had non-synonymous mutations within the gene. This study is a perfect example of the classic gene-for-gene model. To slow down further evolution of virulence, new resistance needs to be embedded in a genetic background with polygenic resistance. In addition, the cultivation of resistant hybrids needs to be accompanied by a proper fungicide application strategy to reduce selection pressure on the CLS population.

The role of the fungal virulence factor Ece1 in homeostatic immunity towards *Candida albicans*

Julia Lagler (University of Zürich)

The peptide toxin candidalysin is a key virulence factor of *C. albicans* inducing damage in host cells and promoting inflammation in infected tissues. Candidalysin is also promoting IL-17 production in an acute oral candidiasis model. The role of the toxin during commensalism however, where it is expressed at low levels, remains unclear. Here, we investigated whether candidalysin is involved in driving homeostatic Th17 immunity in the *C. albicans*-colonized oral mucosa, which is required for long-term maintenance of commensalism. For this, we assessed the fungus-specific Th17 response of mice that were experimentally colonized with a commensal strain of *C. albicans* deficient or sufficient for the candidalysin-encoding gene *ECE1*. Th17 immunity in the oral cavity and in the draining lymph nodes (drLNs) was strongly decreased in mice colonized with an ECE1-deficient commensal isolate of *C. albicans* in comparison to the wildtype control strain. This contrasted with the Th17 response to a high-virulent and rapidly cleared strain of *C. albicans*, which was found to be *ECE1*-independent, indicating that *ECE1*-dependence differs between high- and low-virulent strains. The observation that dendritic cell (DC) maturation and migration from the mucosal tissue to drLNs was also found reduced during commensal colonization, although independently of direct recognition of candidalysin by DCs, provide insights into the mechanisms of *ECE1*-dependent Th17 priming. In conclusion, Ece1 is required for driving homeostatic Th17 immunity during commensal *C. albicans* colonization while Ece1-independent factors can compensate for the induction of Th17 cells during acute OPC with the high-virulent strain

Expanding the Host Range for a Fungal Endosymbiont Through Implantation by FluidFM

Gabriel Giger (ETH Zürich)

The mucoromycete *Rhizopus microsporus*, which harbors rhizoxin-producing *Mycetohabitans rhizoxinica* as a bacterial endosymbiont, has become a model system to study mutually beneficial symbioses. Strict vertical transmission of the endosymbiont is ensured because sporangiospore formation occurs only in the presence of the endosymbiont. This dependence on the bacterial endosymbiont does not occur in related *R. microsporus* strains that are not infected by *M. rhizoxinica*. To explore the constraints of bacterial host range expansion to other fungi, we adapted the FluidFM and injected GFP-labeled *M. rhizoxinica* directly into a non-host *R. microsporus* strain. Upon injection, *M. rhizoxinica* cells divided and were motile within the mycelium, and the fungal host continued growth. FACS analysis and subsequent microscopy of spores harvested from the injected fungus showed that bacteria were present in a subset of spores. The germination rate of these spores containing bacteria was reduced. However, we could demonstrate the propagation of this induced endosymbiosis to the next generation. The novel adaptation of the FluidFM for the targeted implantation of bacteria into *R. microsporus* constitutes a powerful tool to investigate novel host-endosymbiont pairings.

Bacterial volatiles and their impact on *Botrytis cinerea* spore production

Maxime Staedler (University of Fribourg)

Grapevine (*Vitis vinifera L.*) is a very important horticultural crop, with a long domestication history. It is subject to a high number of fungal and viral diseases, which are responsible for important economic losses in the global wine sector every year. One of those diseases – grey mould – is caused by *Botrytis cinerea*, a necrotrophic ascomycete. This fungus has a broad range of possible plant hosts, with over 200 different host species known, including grapevine. The most widespread way to fight such pathogens is through heavy use of synthetic fungicides or copper-based products, which have numerous negative impacts, both for the environment and for human health. In this regard, biocontrol agents offer a promising alternative crop protection strategy considering the increasingly important need to reduce the use of pesticides. Our group formerly isolated ca. 200 bacterial strains from the grapevine phyllosphere in order to assess their biocontrol potential. Many of those bacterial strains showed activity against *Botrytis cinerea*, either through reduction of mycelial growth, or by negatively affecting spore germination of this fungus. We have assessed the ability of the volatile organic compounds (VOCs) emitted by a selection of those bacteria to reduce spore production, which would be a promising way to fight this disease-causing agent. Volatiles emitted by this bacterial selection were harvested, as well as VOCs emitted by *Botrytis cinerea* and by sterile in vitro grapevine plants. Some plants were infected by *Botrytis cinerea* or the obligate biotroph *Plasmopara viticola*, with or without the presence of the bacterium. Collected volatiles were then analyzed with Gas Chromatography –

Mass Spectrometry (GC-MS) in the hope of elucidating which chemical cues are responsible for the communication between the different organisms.

POSTER ABSTRACTS (IN ALPHABETICAL ORDER)

A new automated method for real-time fungal spore quantification.

Julien Alassimone, Bruce A. McDonald (ETH Zürich)

Experimental designs aiming to decipher plant-pathogen interactions often time entail the production of normalized inoculums. Depending on their shape and morphological characteristics, quantification of fungal pathogen spores could be challenging. For instance, automated cell counting devices or flux cytometry techniques are optimized for round-shaped cells. Quantification of *Zymoseptoria tritici*'s spores, the causal agent of *Septoria Tritici* Blotch, is essentially achieved manually using counting chambers with hematocytometer-type grids and clicker counters. This technique is time-consuming, low throughput and does not preserve raw data for later use or data validation. The amount of counted spores per sample is limited and accuracy decrease with operator exhaustion. We developed a new automated method for spore quantification based on unbiased automated image analysis. Spore suspensions are loaded in counting chambers and placed on a microscope equipped with a camera. Our developed ImageJ script is accessing the camera live feed, the user will then adjust focus and selects the area to be counted. Images automatically undergo background removal and non-overlapping spores detection (based on colour thresholding techniques and independent of their shape or size). Spore detection can be refined using the size and roundness exclusion settings. Large spore counts (i.e 200-300 spores) can be processed per image. In addition to the quantification results, statistics, settings, image acquisitions, detection overlays and outputs (ROI) are saved for quality control and traceability. Also, those data can be used to re-run the analysis or perform downstream analysis. For instance, we developed a phenotyping ImageJ script that utilizes the generated outputs to extract phenotyping traits such as spore length and branching. In our hands, quantification results benefited from the increase in the counted spores numbers and results were not only generated faster but gained in robustness. One thing is clear, we are not manually counting spores again.

Antibacterial defense in model mushroom *Coprinopsis cinerea*

Emma Alessandri, M. Stöckli, Marks Kuenzler (ETH Zürich)

Lactone rings are common structural features of natural products. In bacteria and fungi, lactone-based natural products, or lactones, can serve as signaling mediators of intra-species communication, e.g. quorum sensing in Gram-negative bacteria, but also as antimicrobial agents and virulence-promoting toxins. Lactones can be inactivated by enzymes that hydrolyze the ester bond of the lactone ring. These enzymes are commonly referred to as lactonases. The saprophytic mushroom *Coprinopsis cinerea* dwells in herbivore dung and competes with several bacterial and fungal species for this ecological niche. Hence, *C. cinerea* is a model system to study fungal antagonism and defense. *C. cinerea* possesses two intracellular lactonases, which are known to be active against the lactones of Gram-negative bacteria. In this study, we aim to establish whether the same enzymes are relevant to the interactions of *C. cinerea* with other microbes, including gram-positive bacteria and fungal competitors. To address this question, we will test the activity of *C. cinerea* lactonases against lactones with antifungal activity of different origins. These will include the antifungal agent rapamycin, produced by the Gram-positive bacterium *Streptomyces hygroscopicus*, and the mycotoxins patulin and zearalenone produced by plant-pathogenic fungi. The model yeast *Saccharomyces cerevisiae* will be used for assaying the activity of the *C. cinerea* lactonases against the selected lactones. Firstly, we will determine the working concentrations of the componds against *S. cerevisiae*. Secondly, *S. cerevisiae* will be transformed to express *C. cinerea* lactonases and tested for growth at inhibitory lactone concentrations. Lactonase activity will be revealed by the ability of transformants to grow.

Transposon activity shapes short-term evolution of an important fungal pathogen of wheat

Thomas Badet, Ursula Oggenfuss, Simone Fouché, Marcello Zala, Bruce A. McDonald, Daniel Croll (University of Neuchâtel)

Structural variation is a common source of intraspecific genetic variation. Human diseases, crop improvement traits and pesticide resistance have all been associated with such structural rearrangements. Understanding the mechanisms promoting genomic instability is therefore essential for the study of rapid

evolution. In this work, we show that transposable elements are major drivers of the pangenome architecture of the fungal wheat pathogen *Zymoseptoria tritici*. We find that specific transposon families and chromosomal sequence characteristics are tightly correlated with the occurrence of specific genomic rearrangements. Using machine-learning, we were able to predict the emergence of spontaneous insertion-deletion variants produced during meiosis in a four-generations pedigree. Some of the most expansive structural variants generated in the pedigree were tied to a single highly active transposon. Retracing the activity of the element in the *Zymoseptoria* genus, we identified multiple independent reactivation events generating a complex set of transposon copies. Within the focal species *Z. tritici*, we identified recent reactivation of the transposon with a geographically localized rapid expansion in copy numbers. Our results retrace the recent origin of a transposon that successfully evaded host control to promote major structural rearrangements within a species.

Reduction of the post-harvest losses in organic beetroot production

Alessio Bernasconi, Tobias Härri, Carlo Gamper Cardinali, Martin Koller, Lucius Tamm, Nadine Peter, Pascale Flury, Hans-Jakob Schärer (FiBL Frick)

The market for organic agriculture is rapidly growing. In Switzerland, the production of organic Beetroot is particularly renowned. However, their storage until spring has become increasingly difficult in recent years, and losses due to post-harvest rots can lead to over 50 % by March. Therefore, most organic beetroots sold in spring need to be imported. The causes for the various storage rots in beetroot are currently unclear, and therefore there are few measures to prevent them in organic production. Pathogen infections causing storage rots in beetroot, but also in other long-stored vegetables, can occur via the seed, in the field, or post-harvest. Understanding the process of infection is, therefore, critical to find preventive solutions. Here, we present the results of a two-year project aiming at reducing post-harvest losses in organic beetroot production. In a combination of on-farm field experiments and laboratory analyses, we aim to elucidate the causes of storage rots in organic beetroot and develop measures to improve storability. Analysis of stored beetroot in February 2021 revealed Fusarium species and *Phoma betae* as predominant pathogens in Switzerland. *Botrytis cinerea*, *Rhizoctonia solani*, and *Pythium sp*. were found as additional causative agents of storage rots. In summer 2021, a field trial in cooperation with four producers of organic beetroot was started, where the production from sowing to storage was monitored. Different measures, such as steam sterilization of the seed, the use of biocontrol products in the field and before storage, or processing and cooling methods after harvest, as well as cultivar differences were investigated. The various measures were found to affect seed health, seedling emergence, leaf health, and the quality of beetroot after storage.

Using a Centroid-based approach for a reliable identification of morels (*Morchella* spp.): a case-of-study for food authentication

Melissa Cravero, Jean Ruelle, Saskia Bindschedler, Stefan Emler and Pilar Junier (University of Neuchâtel)

Food fraud is a problematic but common phenomenon occurring in the food industry. It impacts numerous sectors, including mushrooms. Multiple analyses exist to evaluate food frauds. One of them is the Centroid-based approach, a gene sequencing method previously developed to identify medically important microorganisms and that has been expanded to authentify samples of meat and fish. In this study, the method was tested on a genus of high taxonomic complexity, namely *Morchella* (true morels). Morels are mushrooms that are prized worldwide for culinary and medicinal purposes, and in which food fraud has already been reported. Based on the single internal transcribed spacer (ITS) genetic marker, the Centroid-based approach was able to identify *Morchella spp*. at species level in 83.64%, which is higher of about 30% compared to other methods based on the single ITS. This method was made reliable to be used for non-specialists of the genus (e.g., actors of the food sector), but it is also a useful tool for taxonomists. Indeed, the Centroid-based approach allowed to clarify the status of different (invalid) morel taxa, namely *Morchella conica* inv., *Morchella crassipes* inv., *Morchella elata* inv., *Morchella costata* inv. and *Morchella vulgaris*.

Wheat breeding against the most common fungal diseases in Switzerland landscape

Kevin Gauthier, Alain Handley-Cornillet, Amandine Fasel, Elodie Isoz, Rachid Majdi, Pierre Stevenel, Boulos Chalhoub (Agroscope Changins, Nyon)

Wheat followed a long breeding path which led to tremendous improvements of the grain yield and quality. Both traits are affected in Switzerland by several fungal diseases that affect wheat, notably *Fusarium* head blight, *Septoria* blotch, rusts, powdery mildew and bunts. These pathogens were controlled by fungicides but their negative impacts on the environment urged the development of more sustainable solutions. Therefore, the aims of wheat breeding are focused to develop more resistant wheat cultivars. Pre-breeding for resistance, defined here as the identification of resistance genes and QTL in wheat germplasm collections and their introgression into elite cultivars, is a main prerequisite to any breeding program for disease resistance in wheat. Genericity and stability of the genes and QTL of resistance, accuracy and speed of their identification and size of the introgression into wheat elite lines are key success parameters of disease resistance pre-breeding processes. In our work, the monitoring of the diversity and dynamics of fungi pathogens within Switzerland and the use of the most representative pathotypes is very important to insure an efficient screening of wheat resistances in the field. This characterization is complemented with greenhouse trials involving inoculum with other virulence to find new resistance donors. Thereafter, we use mapping populations (generated by double haploid or recombinant inbred lines approaches) and genome wide association studies to identify genes and QTLs of resistance within the genome of the donor. Once mapped, resistance genes and QTLs are introgressed into elite lines using the most recent technologies (e.g., speed breeding, backcross assisted by markers and genomic selection) to insure a fast availability of the resistance in breeding programs. Our wheat disease resistance pre-breeding and breeding strategy deployed here is ensuring development of resilient and robust wheat cultivars, with the best durable resistance, contributing to sustainable agriculture.

Interaction of rhizosphere fungal and prokaryotic communities with root phenotypes in maize under nitrogen and water limitation

Elena Giuliano, Tania Galindo-Castañeda, Jagdeep Singh Sidhu, Ivan Lopez Valdivia, Cody L. DePew, Rafaela Feola Conz, Alexander Strigens, Mark Mescher, Jonathan Lynch, Johan Six, Martin Hartmann (ETH Z)

The interaction between rhizosphere microbiomes and roots can affect plant responses to resource limitation. It is known that specific root phenotypes (i.e., root architecture and anatomy) can influence resource uptake in soil. However, little is known about the interplay between microbiomes and root phenotypes under stress. Moreover, the effect of plant domestication and breeding on rhizosphere interactions remains still underexplored. The aim of this study was to identify combinations of resource-efficient root phenotypes and microbiomes in maize (*Zea mays* L.) that can potentially favour resource uptake and plant growth under nitrogen and water limitation. We characterized plant performance, root phenotypes, and fungal and prokaryotic community composition of 28 maize inbred lines and 6 landraces under nitrogen and water deficiency in the field. Microbial community composition was evaluated with metabarcoding of ribosomal markers. Root images for anatomical characterization were obtained by laser ablation. Soil properties, such as nitrogen and water content, pH and cation exchange capacity were measured. Preliminary results show that rhizosphere fungal and prokaryotic alpha and beta diversity were significantly affected by the treatment and the genotype. For fungal beta diversity in particular, the treatment and the genotype explained between 13-18% and 17-20% of the variance, respectively. Fungal alpha diversity increased under nitrogen deficiency compared to the optimally fertilized plots, and decreased under water deficiency compared to the respective control conditions. Moreover, rhizosphere fungal communities separated between inbred lines and landraces under both nitrogen and water limitation. The next steps aim to link microbiomes, root phenotypes, soil nutritional properties, and plant agronomic data to have a better understanding of the role played by microbial and root traits in the rhizosphere of maize. This study will provide novel insights about root phenotype-microbiome associations as potential target to develop crops that grow better under resource limitations.

Functional characterization of a recent loss of a de novo DNA methyltransferase in a major wheat pathogen

Ivona Glavincheska, Bruce McDonald and Cecile Lorrain (ETH Zürich)

Epigenetic modifications, including DNA methylation, regulate genome stability in eukaryotes. In fungi, cytosine DNA methylations are primarily found in transposable elements (TEs) and repetitive sequences, which repress their transcription and are associated with repeat-induced point mutations (RIP). Cytosine DNA methylations and RIP mechanism help maintain genomic integrity by limiting the rearrangements caused by TE proliferation and movement. However, the strong repression of TEs and gene duplication could impede the rise of beneficial genetic variation. In the case of the wheat fungal pathogen *Zymoseptoria tritici*, the inactivation of the DNA methyltransferase, Dim2, has resulted in a near-complete loss of cytosine DNA methylation in TEs. Interestingly, related species and *Z. tritici* strains from the pathogen center of origin still possess an intact copy of the Dim2 gene. We aim to understand how the dynamics between TE mobility and dim2-mediated regulatory mechanisms affect the evolutionary potential in *Z. tritici*. We selected four *Z. tritici* strains naturally containing an active dim2 gene copy and four containing an inactive dim2 gene copy to generate Dim2 deletion and complementation transformants, respectively. We will employ nanopore long-read sequencing to quantify cytosine DNA methylation in active dim2 and inactive dim2 strains. Finally, we will explore if the loss of dim2 confers a fitness advantage through TE de-repression in *Z. tritici* strains using an evolve-and-sequence approach. The combination of experimental evolution, genomics, and epigenomics will highlight the significance of TE mobility in the evolvability of a wheat pathogen and facilitate further exploration of the genetic factors influencing the adaptability of pathogens to stressful environmental conditions.

Giant Starship elements are engines of adaptive variation in fungal pathogens

Emile Gluck-Thaler, Daniel Croll (University of Neuchâtel)

Accessory genes are variably present among members of a species and are a reservoir of adaptive functions. In fungal pathogens, accessory genes contributing to pathogenicity represent significant fractions of genome content and differences in accessory genes between individuals accumulate rapidly. However, we often lack a mechanistic understanding of how genes become accessory and why variation in accessory genes exists. Here, we demonstrate that differences in accessory gene content in many fungal pathogens is attributable to Starships, a newly described group of giant mobile elements that have evolved mechanisms to transpose fungal genes as genetic cargo. By systematically annotating Starships and classifying them into families, we found that individual fungal species harbour complex communities of distinct elements ranging from 60-600kb in length and differing in their activity. Active Starships represent between 1-5% of any given genome and carry diverse genes implicated in host- and environment- adaptation, including metabolic gene clusters and candidate virulence factors. Starship-associated genes typically make up around 10% of the overall accessory genome, implicating Starship activity as a direct mechanism generating variation in accessory gene content. However, we found that most Starship insertions are under strong purifying selection, suggesting there are intrinsic costs to maintaining Starships despite any benefits their cargo may confer. Our results shed light on the origins of accessory variation in fungi, and reveal a novel mechanism for eukaryotic pathogen adaptation.

Independent massive retrotransposon activity underpins repeated genome expansions in species of the *Pseudocercospora* genus

Sandra González Sáyer, S. Oggenfuss, T. Baril, A. Zaccaron, A. Stergiopoulos, Daniel Croll (University of Neuchâtel)

Fungi show extraordinary potential to adapt and survive in detrimental environmental conditions. The adaptive potential is often associated with the plasticity of the genome to generate genetic variation. Transposable elements (TEs) are critical for restructuring the host genome architecture. TEs can jump into new genomic locations, trigger chromosome rearrangements, and affect gene content and expression. Fungi vary considerably in genome size, with recent expansions most prominently observed in some mycorrhizal and rust fungi. TE proliferation is the most likely trigger of genome size increases, but the mechanisms that govern this phenomenon are largely unknown. The ascomycete genus *Pseudocercospora* comprises host-specific pathogens that have experienced genome size increases. We aimed to analyze mechanisms at the origin of the genome size evolution within the genus. The *Pseudocercospora* spp presents variable genome sizes and TE content. The *P. ulei* and *P. fijiensis* genomes are the most expanded genomes, with 93.8 and 74 Mb, respectively, and 79% and 50% TE content, respectively. In contrast, the closely related species *P.*

macadamiae retained a compact genome of 40 Mb and less than 2% TEs. The expanded *Pseudocercospora* spp genomes are dominated by long-terminal directed repeat (LTR) TEs, including the Gypsy superfamily in two independent recent genome expansions. We analyzed the evolutionary history of LTRs in the two expanded genomes and contrasted this with LTR content in non-expanded genomes. Our analyses provide mechanistic insights into recent genome expansion dynamics among species.

Rooting for fungi: Influence of prolonged water limitation on soil fungal communities in Scots pine mesocosms

Astrid C. H. Jaeger, Martin Hartmann, Rafaela Feola Conz, Johan Six, Emily F. Solly (ETH Zürich)

Reductions in soil moisture due to prolonged episodes of drought can potentially affect whole forest ecosystems, including soil fungi and their functions. However, little is known about the effect of prolonged water deficit on soil fungal communities during the development of saplings. Therefore, a greenhouse experiment was conducted with mesocosms of natural forest soil and young Scots pine saplings (*Pinus Sylvestris* L.). These mesocosms were adjusted to three soil moisture levels – control, intermediate, and severe water deficit (40% and 75% reduction compared to control, respectively) – and maintained over two years. We used DNA metabarcoding of fungal and prokaryotic ribosomal markers to evaluate changes in the soil microbiome on a seasonal basis. We related alterations in the structure of soil microbial communities to changes in plant development and soil properties. Prolonged water limitation induced progressive changes in soil microbial community composition regardless of the recurrent seasons, while fungal communities were less affected than prokaryotic communities by reduced soil water contents. Although water stress did not alter the total abundance and biomass of soil microorganisms, it shifted the composition of microbial communities towards desiccation-tolerant groups that outcompete less adapted groups. Specifically, the abundance of saprotrophic and ligninolytic groups increased alongside an accumulation of dead plant material. In contrast, the abundance of symbiotic taxa decreased, likely impairing the development of the young trees. Overall, prolonged episodes of drought appeared to continuously alter the structure of microbial communities, pointing to a potential loss of critical functions provided by soil fungi.

The impact of drought on the wheat microbiome and its consequences for soil functioning

Elena Kost, Dominika Kundel, Rafaela Feola Conz, Paul Mäder, Jochen Mayer, Johan Six, Martin Hartmann (ETH Zürich)

Soil microbes are essential for soil functioning and crop production. Their diversity and functions can be impacted by drought events that are projected to increase in frequency, severity, and duration. Since organic and conventional farming systems are known to promote contrasting soil microbiomes, they may have different abilities to maintain soil functions under drought. This project aims to better understand the effects of drought on the soil microbiome and the consequences for microbial-mediated soil functions in different farming systems to improve the drought resilience of cropping systems. An on-field drought simulation was performed in the DOK long-term field trial, which is comparing organic and conventional farming systems since 1978. Rainout-shelters were installed to study the drought response of soil and root-associated microbes in contrasting agricultural systems. Microbial abundance, activity and taxonomic diversity were assessed using in-situ soil respiration, quantitative PCR, and metabarcoding. Early-summer drought altered soil fungal and bacterial communities in organically and conventionally managed systems. Fungi were more strongly influenced by drought compared to bacteria, since drought stress explained around 2.5% and 1.7% of the variation over all sample types, respectively. Microbial activity (soil respiration) and abundance (copy numbers of fungal and bacterial ribosomal markers) were 25% and 27% lower under drought compared to the control. Root-associated (e.g., root and rhizosphere) microbial communities were more heavily affected by drought compared to bulk soil communities, as 3-11% of the variation was explained for root-associated compared to 1-2% for bulk soil. Preliminarily results show that drought affects soil microbial biomass, activity, and diversity, but these responses were largely independent from the agricultural practices. The project results will help to elucidate if certain agricultural systems foster soil microbes that have the capabilities to increase the resilience towards drought.

Deciphering the dynamics of 3D genome organization in *Zymoseptoria tritici*

Alice Laigle, Victor Mac, Stefan Grob, Daniel Croll

In Eukaryotes, the 3D genome organization is involved in major biological processes, such as gene expression and DNA replication. However, the genomic organization remains poorly understood in fungi. *Zymoseptoria tritici*, the fungal pathogen leading to the *Septoria tritici* leaf blotch of wheat, presents a massive intra-species diversity in terms of genomic content, accessory chromosomes, and transposable elements . Interestingly, core and accessory chromosomes show similar genomic content, but are not enriched with the same histone post-translational modifications. At the core of my PhD project, I want to understand the general 3D features of the species' genome by performing a high-throughput sequencing coupled with chromosome conformation capture (Hi-C) method. Based on these investigations, I will be able to link the chromosomal conformation at different genomic scales with histone modifications, trans-expression quantitative trait loci, structural variants, and gene expression. Doing Hi-C with different strains will allow me to investigate the role of the 3D structure linked to the high genomic variability of the species.

Host-specific genomic trait associations reveal potential new virulence factors in the natural infection of a major wheat pathogen

Cécile Lorrain, Alice Feurtey and Bruce A. McDonald (ETH Zürich)

Various pathogens, including the fungal pathogen *Zymoseptoria tritici*, threaten wheat production. Despite extensive research efforts, the mechanisms of pathogenesis in this pathogen remain largely unknown. The main limitation of large-scale genome-wide association studies (GWAS) remains the phenotyping of several hundred to thousands of strains in many hosts. Since the concept of environmental GWAS involves looking for associations between specific genetic variations and particular environmental factors and since the host represents an essential factor driving the evolution of pathogens, we propose to investigate host-associated GWAS in *Z. tritici*-wheat natural infections data. We thereby sampled and sequenced nearly a thousand *Z. tritici* strains from twelve naturally infected wheat cultivars growing simultaneously in the same field to identify evolutionary responses to the host and potentially new virulence loci. We employed a "one-versus-all" approach using the host cultivar as a phenotype to perform iterative GWAS for host-associated genomic traits. Our preliminary results from five cultivars identified seventeen candidate genes associated with specific wheat cultivars. Among these, we highlighted the effector Avr3D1, one of the few *Z. tritici* characterized effectors. We used this finding as a proof-of-concept for our host-associated GWAS approach as our approach detected associations specifically on samples from the cultivar Runal – from which Avr3D1 was initially characterized. Additionally, we identified two candidate effector genes associated with two different cultivars, highlighting the power of our approach for high-throughput de novo identification of candidate effectors. Overall, host-specific genomic trait association is a powerful approach to understanding the genetic basis of pathogenesis in natural infections.

How is pre-mRNA leakage regulated in stress and aging?

Jordan McCarthy, Mihailo Mirkovic, Yves Barral (ETH Zürich)

Pre-mRNA retention in the nucleus is essential for proper RNA processing, and defects in this process have been associated with nucleopore dysfunction. However, recent findings suggest that pre-mRNA leakage can also occur in response to stress or aging, and may have important regulatory roles in these contexts. In heat stress, the nuclear basket dissociates, forming a condensate with GBP2 and other components, suggesting that pre-mRNA leakage may be involved in regulating cellular aging and stress. Further research in budding yeast has shown that Glc7 levels increase specifically in response to aging or pre-mRNA export defects, but not in splicing defect strains. This highlights the need to distinguish between splicing and pre-mRNA export defects and emphasizes the significance of investigating the regulation of pre-mRNA leakage in RNA processing and aging. Understanding the regulation of pre-mRNA leakage is crucial for advancing our understanding of RNA processing and cellular aging, and could lead to new therapeutic targets for age-

related diseases. By shedding light on the roles that pre-mRNA leakage plays in cellular processes, this research has the potential to uncover new mechanisms and pathways involved in these processes.

Discrimination of exogenous DNA from chromosomal DNA in budding yeast

Rachel Mellon, Hassan Mustapha, Yves Barral (ETH Zürich)

How cells differentiate chromosomal DNA from exogenous DNA in the nucleus is poorly understood across eukaryotic organisms. In mammalian cells, we know that after transfection, exogenous DNA can be expressed. However, a majority of the DNA is lost from the population. In unicellular organisms such as the budding yeast *Saccharomyces cerevisiae*, it is poorly reported if such transient transfection events take place. Therefore, we aim to understand if S. cerevisiae cells are able to discriminate chromosomal DNA from exogenous DNA in the nucleus and how this may function. To investigate this, we established a method using the Cre/loxP system to assay the frequency of which plasmid DNA transiently enters the nucleus. We observe that plasmid DNA which enters the nucleus and is expressed is only heritably retained in ~50% of the cells. These results not only indicate that transient expression of exogenous DNA is not sufficient for heritable retention in *S. cerevisiae*, but also support the existence of mechanisms governing chromosomal-exogenous DNA discrimination in the nucleus. Which begs the question, what mechanisms are at play? In order to identify players involved in this process, a genetic screen using transposon-based mutagenesis was carried out to identify mutations affecting transformation efficiency. The screen revealed that multiple processes seem to restrict transformation of exogenous DNA, such as the cell wall, the endocytic machinery, the nuclear import machinery, chromatin remodelling, DNA transcription, replication and repair. Building on this, next steps involve using the developed Cre/loxP system along with mutations affecting transformation efficiency such as those involved in DNA repair. This will allow us to gain a mechanistic understanding of chromosomal-exogenous DNA discrimination within the nucleus of S. cerevisiae.

Local adaptation to temperature in a genetically diverse world-wide collection of a major plant pathogen

Silvia Minana-Posada, Alice Feurtey, Bruce A. McDonald (ETH Zürich)

Environmental factors, such as temperature, significantly affect the growth and fitness of plant pathogen throughout their life cycles. In light of climate change, understanding the mechanisms underlying temperature adaptation has become crucial. *Zymoseptoria tritici*, one of the most damaging wheat pathogens in the world, is a compelling model to study temperature adaptation. While previous studies have identified genomic regions involved with the response to temperature, evidence for adaptation remains limited, mostly focusing on European locations. In this study, we aimed to evaluate the local adaptation of *Z. tritici* to temperature by using population and individual response of 420 world-wide distributed isolates to 5 different temperatures using in-vitro phenotyping. Our investigation reveals substantial variability in thermal performance and high genomic diversity at both individual and population levels. Leveraging this diverse global collection, we identified novel genetic variants involved in adaptation to temperature through genome wide association analyses (GWAS). By providing insights into the mechanism governing local adaptation in *Z. tritici*, our research enhances our understanding of how emerging pathogens could access ecological niches that were not previously favorable.

Single-cell analysis of the switch from vegetative to filamentous growth in *S. cerevisiae*

Shashank Pandey, Valentin Borgeat, Serge Pelet (University of Lausanne)

Opportunistic pathogenic fungi pose a great threat to human health, particularly to immune compromised individuals. In the pathogenic fungi like *C. albicans*, filamentation is associated with host-cell attachment, tissue invasion and virulence. Thus, there is a need to understand the molecular machinery causing the switch from vegetative to filamentous growth. In the model organism *S. cerevisiae*, exposure to low-nutrients

can trigger evolutionarily conserved signaling pathways that lead to the formation of filament-like structures called pseudohyphae. This growth pattern characterized by unipolar attached growth of elongated cells can be compared to filamentation in other fungi. Most of our knowledge on this process comes from agar invasion assays and colony morphology analysis. These studies allowed to identify the main players that control this morphological transition. However, we still lack a clear understanding of how the different signaling pathways interact to promote this cell fate transition. Therefore, our objective is to develop a microscopy assay where we can follow the dynamic transition from vegetative to filamentous growth. We have established fluorescent protein reporters to quantify the induction of theFLO11 gene to monitor the temporal dynamics of filamentation in individual cells. When combined with specific gene deletions, these reporters will help us to understand the contribution of various signalling pathways to cell fate decision.

Phyllosphere bacteria as biocontrol solution against grapevine pathogens

Nicolas David Rappo, Sébastien Bruisson, Laure Weisskopf, Katia Gindro, Sylvain Schnée (University of Fribourg)

Grapevine cultivation is strongly dependent on the use of pesticides. Many biocontrol solutions have been tested in order to reduce the use of conventional pesticides, but none of them was able to achieve the same efficiency. This work focuses on the use of bacteria collected from the phyllosphere as biocontrol agents to protect the aerial parts of the plant. With the use of bacteria naturally adapted to the organs to protect, we believe that our solution will achieve a higher level of protection over the existing biocontrol solutions based on microorganisms isolated from the soil that most likely lack the adaptations to reach their full potential or even to persist above ground. We tested the protective abilities of bacterial isolates used alone and as consortia against the economically devastating pathogens, *Plasmopara viticola* and *Botrytis cinerea* responsible for downy mildew and grey mold respectively. Most of our strains were able to induce a significant reduction of symptoms in planta and to trigger an impairment in spore fitness in both pathogens. In addition, some strains are also able to elicit significant defense response by upregulating several defense genes and by increasing the production of stilbenic compounds, adding another layer of protection for the plant. We are currently investigating the efficiency of combining different strains together to cover a wider array of protection for the plant. We are confident our approach will provide a more sustainable and environmentally friendly solution for the future of viticulture.

The plasticity of Mycorrhizal Interactions in the Mixed Mediterranean Forest

Ido Rog, Stav Livne-Luzon, Shifra Avital, Tamir Klein (Agroscope Reckenholz, Zürich)

The symbiosis between mycorrhizal fungi and forest tree roots influences forest structure and function. These fungi can be divided into two main functional groups: arbuscular and ectomycorrhizal fungi (AMF and EMF, respectively). A longstanding dogma is that respective guilds of symbiotic mycorrhiza occupy certain guilds of trees, i.e. EMF host trees vs. AMF host trees. Most biomes are dominated by either AMF or EMF hosts. However, the mixed Mediterranean forest is one of the few places where AMF and EMF hosts have evolved to live in proximity for many years. The ecological and evolutionary mechanisms driving host specificity and host expansion are not well understood. In this study, we examined the level of host expansion and the proportion of shared mycorrhizal species in three experimental designs: (1) among five mature tree species in a diverse mixed forest, (2) among saplings of these same species growing in communal containers, or (3) among saplings growing in individual pots using soil from the mixed forest. The fungal communities of the different tree hosts were highly diverse, with higher diversity in the known EMF host species, Pinus and Quercus. Surprisingly, some assumed AMF-host species, like Cupressus and Pistacia, also associated with several EMF species (e.g., Inocybe and Tuber species) in the forest and in the mixed potted saplings. However, saplings potted individually associated only with the presumed compatible functional mycorrhizal group. The ecology of tree-mycorrhiza interaction complicates our assumptions about the plasticity of this mutualistic interaction and the effect of neighbor tree. These novel belowground interactions, specifically host expansion, can potentially influence forest biodiversity and connectivity.

Gains, diversifications and losses in time and space of a major fungal effector

Ana Margarida Sampaio, Sabina Moser Tralamazza, Alice Feurtey, Daniel Croll (University of Neuchâtel)

Interaction between plant pathogens and their hosts is highly dynamic and mainly driven by pathogen effectors (e. g. Avr) and plant resistance (R) genes. Despite their importance, how effectors evolve to escape host recognition encoded by R genes is still poorly understood. *Zymoseptoria tritici* is one of the major fungal foliar diseases in wheat-growing areas worldwide. The most well-known *Z. tritici* effector is AvrStb6, an effector that is being maintained by the pathogen over time. AvrStb6 is recognized by the wheat R gene Stb6 widely deployed in wheat cultivars. In this study, we want to investigate how effectors respond to selection promoted by host resistance. We analyze a global thousand-genome panel comprising of the pathogen to assess the AvrStb6 evolutionary origins and diversification. The effector gene was missing from ~3 % from all analyzed strains contrary to previous expectations of conservation. AvrStb6 DNA haplotypes and protein isoforms are highly diverse with most of being of low frequency or unique. In contrast, the most frequent isoform was shared by almost half of the global collection of strains. Phylogenetic and residue change analyses suggest though that the dominant haplotype is not the ancestral isoform but may have emerged more recently. As a continuation, we will analyze associations between the deployment of resistant cultivars and effector evolution. In conjunction, our findings will elucidate principles of effector evolution governing plant disease emergence.

Evolution of mitochondrial genomes in the fungal kingdom

Ivan Skakov, Daniel Croll (University of Neuchâtel)

Mitochondria are important organelles in eukaryotic cells, responsible for the production of ATP. As a result of endosymbiosis with the bacteria, mitochondria contain their own DNA, separate from the nuclear genome. Fungal mitochondria may be inherited both uni- or biparentally raising the possibility of a heteroplasmic state. Fungal mitochondrial genomes are highly variable in size compared to the animal kingdom with large differences in gene content and gene order. Variation in mitochondrial genome size may arise from various factors including recombination in the heteroplasmic state, high mutation rates, and rearrangements during the replication of selfish elements. My PhD project aims to unravel fungal mitochondrial genome evolution across different scales from kingdom-wide dynamics to individual species. How often do the bursts in mitochondrial genome sizes occur, what is the nature of such bursts? What selfish elements contribute to such expansions? Can mitochondrial genome size expansions be recapitulated at the within species and population scale? What singular events of selfish element transposition or relocation contribute to mitochondrial genome size variation? I am planning to focus on two main approaches: (1) to investigate the more than a thousand available fungal mitochondrial genomes across the fungal kingdom and (2) on mitochondrial genomes of *Zymoseptoria trittici* and three other fungal crop pathogens at a populational level. The first part will provide a broad picture of genome size expansions. The second part will reveal singular events of expansions on short evolutionary time scales.

Heterologous production of ribosomal backbone N-methylated macrocyclic peptides

Lukas Sonderegger, Olivia Bossert, Emmanuel Matabaro, Markus Künzler (ETH Zürich)

Peptide backbone N-methylations and macrocyclization are desired properties for the development of peptide therapeutics, as these modifications can improve cell permeability, target selectivity and proteolytic stability. One famous example of a macrocyclic, backbone N-methylated peptide is the immunosuppressive agent cyclosporin A, a fungal non-ribosomal peptide natural product. Omphalotin A on the other hand is a ribosomally produced and post-translationally modified peptide (RiPP) that consists of a 12-amino acid macrocycle with 9 backbone N-methylations. This compound, produced by the mushroom *Omphalotus olearius*, exhibits strong toxicity against nematodes by an unknown mechanism. Also, the biosynthesis of omphalotin A is still not understood completely. Its precursor protein contains a SAM-dependent α-N-methyltransferase domain, which iteratively methylates the core peptide located at the C-terminus. After

complete methylation, the core peptide is cleaved off by an unidentified protease and subsequently macrocyclized by a prolyl oligopeptidase. Heterologous production of omphalotin A by expression of the precursor protein and prolyl oligopeptidase has so far only been successful in yeast, but not in E. coli, suggesting that E. coli lacks the protease responsible for the initial cleavage of the precursor protein. We aim at establishing the production of omphalotin A or related backbone N-methylated macrocyclic peptides in E. coli by engineering the precursor protein via introduction of specific amino acid sequences that are recognized and cleaved by co-expressed proteases. This heterologous production platform will enable the biosynthesis of diverse novel backbone N-methylated macrocyclic peptides with potentially promising pharmacological properties. In addition, efficient production of omphalotin A in E. coli will allow us to screen for the still unknown molecular target of omphalotin A.

Ultra-high resolution amplicon sequencing reveals cross-kingdom antagonists and synergists driving fungal infections in the wheat phyllosphere

Luzia Stalder, Monika Maurhofer, Daniel Croll (University of Neuchâtel)

Plant-associated microbiomes promote plant health in natural environments and can confer resistance to pathogens. Within these microbial networks, plant-beneficial and plant pathogenic strains are often closely related. Hence, monitoring pathogenic and plant beneficial microbes at the strain level is critical for our understanding of microbiome functions. However, high-resolution strain level monitoring is hindered by the available barcoding loci. Here, we introduce multiple 3-kb highly polymorphic bacterial and fungal amplicons to be sequenced in 10,000-multiplex pools on the PacBio Sequel II system. We analyzed large sets of high-quality genomes covering the phylogenetic breadth of Pseudomonas bacteria and the major fungal wheat pathogen *Zymoseptoria tritici* to define highly robust amplicon sets. Pseudomonads include synergistic and antagonistic species of *Z. tritici* in the wheat phyllosphere. We complemented the sequencing with the full-length 16S and fungal ITS loci to generate deep insights into crop microbiomes. We apply our set of amplicons to a hierarchical set of 500 wheat samples spanning the growing season, different plant genotypes, as well as replicated leaf and root compartments. The deep sequencing revealed highly granular structures of both the focal pathogen and the co-existing Pseudomonas diversity. We used evidence for co-occurrence and exclusion of individual genotypes to investigate synergistic and antagonistic microbiome interactions. A comprehensive strain collection from the same field allowed us to validate the predicted interaction network under controlled conditions. We build a model of biotic and abiotic factors determining the ecological niche of the crop pathogen and reveal broad principles of competitive exclusion and persistence. Overall, our work introduces a powerful new approach for ultra-deep amplicon analyses to interrogate plant microbiome interactions.

QTL mapping for temperature tolerance the wheat pathogen *Zymoseptoria tritici*

Jessica Stapley and Bruce A. Mcdonald (ETH Zürich)

Global warming is expected to have adverse impacts on global agriculture, as it influences plant disease occurrence and severity. Understanding the genetic basis of adaptation to temperature in fungal plant pathogens is crucial to predict how pathogen populations will respond to warming climates and how they may impact agricultural systems in the future. Temperature can influence fungal fitness in multiple ways, by directly influencing their growth, virulence and reproduction, and also indirectly by influencing plant immune responses. In this study we combine QTL crosses and a large phenotypic and genomic dataset to identify large effect loci associated with tolerance to temperature stress in *Z. tritici* and investigate how variation in candidate genes is related to temperature tolerance in globally distributed isolates. QTL mapping was performed in crosses established between strains collected in Switzerland. Size and melanin were measured in vitro at 8 and 12 days post inoculation, at 10, 18 and 27 degrees Celsius in 259 and 265 offspring from two QTL crosses. We identified QTL peaks specific to temperature stress that contained several promising candidate genes, including a heat shock protein (Hsp90). We found that variation in these genes also explained temperature related phenotypic changes in globally distributed isolates.

Malassezia regulates the skin barrier function via AhR signaling

Michelle Stokmaier, Eduardo Gushiken-Ibañez, Salomé LeibundGut-Landmann (University of Zürich)

The skin acts as a primary defence to protect the body from desiccation and entry of harmful substances. Environmental signals are constantly sensed and integrated to tightly regulate cellular processes such as the maintenance of an intact epidermal barrier which is essential for skin homeostasis and host physiology. The aryl hydrocarbon receptor (AhR) plays an important role in orchestrating those effects. It has recently been shown that the skin microbiota act as an important source of ligands that trigger AhR signaling in keratinocytes. A prominent member of the microbiota is the abundant skin commensal yeast *Malassezia*. We found that *M. furfur*, a species converting tryptophan into brown-pigmented indoles, activates AhR signaling in human keratinocytes. In vitro experiments showed that indole-producing *M. furfur* regulates the transcription of structural genes involved in skin barrier function and homeostasis. Furthermore, in vivo administration of *Malassezia* revealed AhR-dependent limitation of skin inflammation by *M. furfur*. The characterization of the interaction between *Malassezia* and the host skin presents new opportunities for understanding skin health and disease.

Characterisation of a dim2-mediated Repeat-Induced point mutation-like (RIP-like) mechanism in *Zymoseptoria tritici*

Julia Thomann, Cécile Lorrain, Bruce McDonald, Julien P. L. Alassimone (ETH Zürich)

Repeat-Induced Point Mutation (RIP) is a fungal-specific mechanism discovered in Neurospora crassa which induces C to A transitions in duplicated sequences, including repeats such as transposable elements. RIP detects duplications of chromosomal DNA above a certain length threshold (≈ 0.4 kb), irrespective of their transcriptional status, origin, and absolute positions in the genome. RIP is thought to occur during meiosis as described in model fungal species harboring RIP. However, it has recently been suggested that there could be a mitotic RIP-like mechanism in the wheat pathogen *Zymoseptoria tritici*. This RIP-like mechanism was shown to depend on the activity of the de novo methyltransferase Dim2. In order to find evidence of mitotic Dim2-mediated RIP-like in *Z. tritici*, we inserted two different types of RIP reporter (RIPorter) probes into strains with and without a functional dim2 allele: i) A partial duplication of the zmr1 gene, an essential transcription factor for melanin synthesis, and ii) a retrotransposon from a closely related species of *Z. tritici*, *Zymoseptoria passerinii*, without any intact copies in *Z. tritici*. We successfully introduced the partial duplication RIPorter into four *Z. tritici* strains, both with and without functional Dim2. Currently, we are generating transformants with the retrotransposon RIPorter. To assess the impact of dim2 presence/absence on RIP, we will conduct a mitotic evolution assay by cultivating the RIPorter strains in serial cultures. We will utilize target sequencing to monitor any potential RIP signatures in the RIPorter probes. Furthermore, we will track the RIPorter retrotransposon copy number variation using qPCR in strains with and without a functional Dim2. We anticipate gaining insights into the activity and specificity of Dim2-mediated RIP in *Z. tritici* during the mitotic stages as well as shedding light on its potential effects on various sequence duplications and transposable elements.

Swordfish-a massive Starship mobile element -mobilizes gene cargo linked to thermal adaptation

Sabina Moser Tralamazza, Emile Glucker-Thaler, Alice Feurtey, Daniel Croll (University of Neuchâtel)

Copy number variation (CNV) can drive rapid evolution of numerous traits including fungicide resistance and pathogen virulence. Yet, the contribution of CNV to environmental adaptation of fungal populations remains poorly understood. Here, we systematically investigate CNVs in a large genome sequencing dataset of a fungal pathogen to assess the contribution of CNVs to trait architecture and climatic adaptation. We analyzed a worldwide collection of 1109 *Zymoseptoria tritici* isolates sampled in 42 different countries. The chromosome complements of this destructive wheat pathogen are highly plastic with accessory chromosome variation and core chromosome duplications. We found that most gene CNVs were very rare in the species (i.e. singletons) with only 3% of gene CNVs segregating at high frequency across populations. We found that

secondary metabolism functions were the main targets of gene CNV events and important factors of population differentiation. We used global environmental datasets to associate CNVs with climatic gradients across wheat production areas. We found multiple gene CNVs significantly associated with environmental gradients, including a gene of the NAD-dependent Sirtuin family, paramount for metabolism regulation and chromatin silencing in eukaryotes. The CNV locus is part of a larger region of ~76 kb encoding proteins highly expressed during infection. Furthermore, the structural variation of the locus was dominated by a Starship (dubbed Swordfish) mobile element unique to the species and with the capacity to mobilize gene content in the genome. Taken together, CNVs are likely a major factor driving the climatic and metabolic adaptation of the species. The presence of a massive mobile element governing metabolic capacity and climate adaptation opens new avenues to understanding the rapid evolution of fungal plant pathogens.