

Neuchâtel Interuniversity Doctoral Program in Organismal Biology, Meeting 2019 Wednesday, May 29th 2019

9.00 – 9.30	Arrival, registration and hanging of posters
9.30 – 9.45	Welcome session by Prof Sergio Rasmann (Head of the doctoral program)
9.45 – 10.30	Professor Rosmarie Honegger University of Zurich <i>The private life of lichens - recollections of a happy professional life</i>
10.30 – 10.45	Students short presentations session 1
10.45 – 11.30	Coffee break and poster session
11.30 – 12.15	Student presentations 1 Luca Grandi, Vinciane Monod, Vinciane Mossion
12.15 – 13.30	Lunch break (Group picture at 13.15) poster session
13.30 – 14.15	Professor Jan Roelof van der Meer University of Lausanne <i>Evolution of evolvability: genetic adaptations in a bacterial integrative- and-conjugative element relationship</i>
14.15 – 14.45	Student presentations session 2 Pamela Bruno, Nikhil Kumar Singh
14.45 – 15.15	Coffee break and poster session
15.15 – 15.45	Student presentations session 3 Moe Bakhtiari, Hamed Vayghan Sattari
15.45 – 16.30	Nicola Genovese ZHdK/Artists in labs <i>Displacements - Art, Science and the DNA of Ibex</i>
16.30 – 16.45	Votation of best talk and best poster Prize giving
16.45 - open	Short movies Artists in labs, Social Apero

ABSTRACTS TALKS

Communication among Cotton Plants

Luca Grandi*, W.Ye, L.Abdala-Roberts, T.Brévault, A.Vallat, G.Glauser, B.Benrey, T.Turlings

*Presenting author

Cotton (*Gossypium* spp) is involved in several beneficial and antagonistic interactions and has evolved several direct and indirect defenses to ward off antagonists and to facilitate beneficial interactions. Studies on indirect plants defenses showed that volatile organic compounds (VOCs) emitted by an attacked plant, are able to attract the natural enemies of pests and repel pests, as well as serve as signals between leaves and neighboring plants and prime them for enhanced defense induction. Recent studies suggest that the priming of neighbors has potential for application in pest resistance, but the underlying mechanisms are still poorly understood. In order to better understand communication among cotton plants, we exposed intact receiver cotton plants to VOCs from either *Spodoptera* ssp-infested or control intact cotton and performed several analyses. In plants that had been exposed to VOCs from infested plants, we found general upregulation of defense genes, increased direct defenses (i.e. gossypol) and lower caterpillar preference compared to controls. Our results provide evidence of beneficial VOCs-mediated communication among cotton plants.

In follow-up experiments we also screened several wild populations (Yucatan, Mexico) for high defense traits. Further experiments may lead to the identification of responsible VOCs that trigger defense improvement in neighboring plants. To this intent, we are currently assessing differences between exposure of receiver cotton plants to groups of constitutive or inducible VOCs. In case of confirmed increase of cotton defenses, specific VOCs could be applied on cotton cultivars, providing a novel sustainable strategy to enhance the resistance of cotton plants to pests.

« Terroir »'s influence on the physiological state of vine plants of Gamaret and on the fungal community of vineyards

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Grapevine Trunk Disease (GTD) is a newly emerging disease that has been observed for two decades and is causing important decay of the vine in Switzerland and worldwide. Although considerable work has been done to monitor and identify the various associated fungi, the causes and dynamics of the disease are still poorly understood. GTD is a complex disease due to many fungal species acting together or in succession. The same fungal species were repeatedly isolated from necrotic wood, which lead to the assumption that GTD is caused by fungi. However, these fungi have been isolated both from healthy and symptomatic plants and were then considered to be latent pathogens. Inconstancies of leaf symptoms and latency of disease outbreak made even more complicated the monitoring, forecast and research on GTD. In Switzerland, Gamaret, a wine variety widely planted since the late 1990, shows highly unequal sensitivity to GTD. The objective of this project is to explain why the same vine variety show high dieback rate in certain locations and no impact in others. Three main aspects will be investigated in various Swiss locations: the plants physiological state, including sanitary state of the vineyards; the terroir aspect (pedological, climatic and hydrological properties of each location) and the structure of the fungal community. These three dimensions of the patho-system will help to determine if there is any link between pedo-climatic situation, fungal community and dieback rate. This study will create a basis to understand why a unique vine variety is unequally affected by GTD and will provide information about the suitable location to plant it.

A reference transcriptome for *Botrychium lunaria* reveals insights into the biology and evolution of the Lunaria clade of ferns

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Ferns, the sister clade of seed plants, were the dominant group of plants prior to the radiation of flowering plants in early Cretaceous ~125 Myr ago. Understanding the unique adaptations of ferns to the environment requires genomic resources and knowledge of protein functions. Despite the importance of this group of plants, full genome sequences are only available for two species and transcriptomic resources use is limited by the deep divergences within ferns. Here, we established a reference transcriptome for *Botrychium lunaria*, a diploid species. The *Botrychium* genus belongs to one of the earliest divergent lineages of vascular plants and has a global distribution. Currently, 35 species are recognized of which half are polyploids. We assembled 27'945 high-quality transcripts with an average length of 1'274 bp. We sequenced an additional 11 transcriptomes of individuals from two populations in Switzerland including the population of the reference individual. We identified 567'290 single nucleotide polymorphisms (SNP) segregating among individuals for an average density of 19 SNPs per kb. Those transcriptome-wide markers provide unprecedented resolution of the population structure and revealed that individuals vary substantially in levels of heterozygosity. The transcriptomic resources for the Lunaria clade will enable high-resolution population and phylogenetic studies of this species complex.

Sequestration of cucurbitacins by *Diabrotica balteata* larvae and their possible role in providing protection against entomopathogenic nematodes

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Cucurbitacins are bitter tetracyclic triterpenoids commonly produced by Cucurbitaceae plants. These defense compounds protect the plants against several insects and pathogens. Interestingly, cucurbitacins are sequestered by adult Diabroticina beetles for their own protection against natural enemies. It has been speculated that the larvae of these beetles can also sequester and use the cucurbitacins as a defense. We study the sequestration of cucurbitacins from cucumber plants (*Cucumis sativus* L.) by larvae of *Diabrotica balteata* LeConte and their effects on entomopathogenic nematodes. As a first step, we identified and quantified the main cucurbitacins present in leaves, cotyledons, stems and roots of seven commercial varieties at their vegetative stage. We then fed *D. balteata* larvae on cucumber plants with different levels of cucurbitacins, removed their guts and profiled the cucurbitacins present in the rest of the larvae after different periods of feeding to confirm sequestration. We found tremendous qualitative and quantitative variation in cucurbitacin contents among the tissues of commercial cucumber varieties, with surprisingly low levels in the roots, as well as in the larval tissues. The results confirm, for the first time, that *D. balteata* larvae actively sequester cucurbitacins and reveal the metabolic transformations of the main compounds sequestered. We also observed an unusual feeding behavior by the larvae: these presumably soil dwelling herbivores do not only feed on roots, but actually also come to the surface where they feed on aboveground tissues such as stems, cotyledons and leaves. To study the effects of sequestered cucurbitacins on entomopathogenic nematodes (EPN) and potential adaptations of nematodes to these compounds, we are using 24 *Heterorhabditis bacteriophora* isolates collected in Mexico, where *D. balteata* originates from. We hypothesize that entomopathogenic nematodes will be negatively affected by sequestered cucurbitacins and we are currently testing this with *D. balteata* larvae fed on several cucumber varieties that quantitatively and qualitatively differ in their cucurbitacin content. A good understanding of this complex defense mechanism may provide new insights on the ecological interactions between plants, herbivores and their natural enemies, with implications toward the development of sustainable pest control strategies.

Dissecting the genetic architecture of complex pathogenicity traits in a fungal pathogen of wheat

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In the arms race between host and pathogen, plants evolve to maintain their ability to recognize virulence proteins and pathogens evolve to escape this recognition. This results in accelerated evolution of virulence genes in pathogens and resistance genes in plants. The genetic architecture behind the emergence of new pathogenicity mechanisms remains largely unknown. The fungal pathogen *Zymoseptoria tritici* causes the wheat disease Septoria tritici Blotch (STB). STB poses a serious challenge to global wheat production. Previous genome-wide association mapping studies (GWAS) on a global collection of *Z. tritici* showed genetic variations like presence-absence polymorphism of genes to severely affect the pathogenicity. However, these studies were performed on fungal isolates and wheat cultivars that may not have shared a common evolutionary history. Here, we performed GWAS to retrace adaptation to cultivar that is commonly grown in Switzerland using a *Z. tritici* mapping population collected from single experimental field. The virulence on the Swiss cultivar was mapped to a transposable element with gene presence-absence polymorphism. Since pathogenicity likely requires a complex set of adaptations to biotic and abiotic stress, we also explored genetic architecture of additional traits like adaptation to the thermal environment, pH and fungicide application. We found polymorphisms very close to genes related to metabolism and DNA repair polymerase which helped the pathogen adapt to change in temperature. Thus, this study helped in understanding difference in genetic architecture for adaptive traits that shape the evolution of the pathogenicity.

Variation in Below-to Aboveground Systemic Induction of Specific Glucosinolates Mediates Plant Fitness Consequences under Herbivore Attack

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To date we still lack evidence for the fitness benefits of across-organ systemic induction of secondary metabolites in plants. We measured variation in aboveground (AG) resistance against generalist herbivore *Spodoptera littoralis* and specialist herbivore *Pieris brassicae* among 26 maternal plant families in the widespread natural system, *Cardamine hirsuta*, in response to induction with jasmonic acid (JA) belowground (BG). We showed that root induction increased AG resistance against the generalist herbivore, *Spodoptera littoralis* and found substantial family variation for resistance against generalist herbivory, while specialist herbivores were indifferent to induction. We also found that the systemic induction of several glucosinolates increased plant seed production under the generalist herbivore attack, among which we detected family level variation. Particularly, a single indolic metabolite, neoglucobrassicin, was significantly induced in the leaves, and correlated negatively with *S. littoralis* weight gain. While we show that such genetic variation in systemic induction is in part dictated by allocation trade-offs, the varying response of individual glucosinolates within plants in response to root induction, and the differential effect of each glucosinolate on seed production suggests that diffuse selection is at play for maintaining variability in BG-AG systemic induction in nature.

Key Words: Brassicaceae, fitness impact of herbivory, generalist herbivore, glucosinolates, induced systemic resistance, plant-herbivore interaction

Engineering the Light Harvesting Complex II

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To sustain the life on Earth the solar energy has to be converted into chemical energy, this requires a complex process called photosynthesis. Through a set of interconnected pigment-binding proteins, called light harvesting complex (LHC), photons energy is collected and funneled to the reaction centre (RC) of the photosystems. The latter feeds a series of redox reactions that ultimately allow the production of reducing power and ATP for the synthesis of organic molecules. Despite having a highly conserved core structure, the light harvesting complex II (LHCII) evolution resulted in a wide range of acclimation strategies, which permitted the photosynthetic organisms to colonize almost every ecosystem on Earth. In fact, LHCII connectivity to the photosystems is dynamic allowing to increase light use efficiency and to respond quickly to changes in light quality and quantity. This process, known as state transitions, relies on the activity of a kinase (STN7) and phosphatase (PPH1). The major LHCII is composed by trimers that can be associated to other minor antennae or directly to the photosystems (photosystem I and photosystem II). These dynamic interactions allows the plants to cope with different light intensities and spectra, thus equilibrating photochemistry and allowing the dissipation of excess light. In *Arabidopsis thaliana*, these trimers are made of different combinations of three LHC isoforms: Lhcb1, Lhcb2 and Lhcb3. The first two, through the regulation of a critical N-terminal phosphorylation, are crucial for the regulation of the dynamics of the LHCII network. In particular, Lhcb2 phosphorylation plays a central role in LHCII association^{1,2}.

Thanks to the CRISPR/Cas9 technique, it is possible to produce multiple mutants also for clustered genes, thus allowing the production of complete knock out mutants for multi genic families such as those coding for Lhcb1 and Lhcb2. These mutant lines constitute an ideal platform to study the impact of Lhcb modifications on the LHCII network organization via the production of complemented lines.

Modification of the N-terminal Threonine of the phosphorylation site to Alanine (non phosphorylable) or Aspartate (constitutive negative charge "phospho-mimic") reveals the impact of such an irreversible modification on photosynthetic acclimation and on the dynamics of the photosynthetic complexes.

¹ Longoni, P., Douchi, D., Cariti, F., Fucile, G., & Goldschmidt-Clermont, M. (2015). Phosphorylation of the Lhcb2 isoform of Light Harvesting Complex II is central to state transitions. *Plant physiology*, pp-01498.

² Pan, X., Ma, J., Su, X., Cao, P., Chang, W., Liu, Z., ... & Li, M. (2018). Structure of the maize photosystem I supercomplex with light-harvesting complexes I and II. *Science*, 360(6393), 1109-1113.

ABSTRACTS POSTER SESSION/1 minute presentations

Genome-wide expression analyses to dissect the genetic basis of gene regulation in a major fungal pathogen

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In agricultural ecosystems, outbreaks of diseases are frequent and pose a significant threat to food security. What enables pathogens to overcome host resistance and cause damage is poorly understood. A key evolutionary step for pathogens is to evolve effector proteins that specifically target and disable the plant immune system. Maladaptive expression of effector proteins recognized by the host triggers defences and prevents further infection. A successful pathogen undergoes a complex and well-timed sequence of regulatory changes to avoid detection by the host immune system. Hence, transcriptional control of effectors must be under strong selection. Previous studies showed that many effector genes are not uniformly up-regulated during infection. However, the nature and evolvability of effector gene regulation in plant pathogens is essentially unknown. In this project, we will assess genome-wide transcription levels of 150 pure culture strains of *Zymoseptoria tritici*, the most important pathogen of wheat. Individual gene transcription levels will be considered as a quantitative trait for association mapping to identify expression quantitative trait loci (eQTLs). We will partition genetic control of transcription into *cis*-acting (e.g. promoter region mutations) and *trans*-acting elements (e.g. major regulators). In the second stage, we will assess genome-wide transcription levels at the onset of infection on wheat. We will use the transcription fold-change between the pure culture and leaf infection for an additional association mapping study. This analysis will identify eQTLs linked specifically to transcriptional control during infection. We expect that the dynamics of the transposable element landscape plays a major role in population-level variation in transcriptional control. The mechanistic basis of regulatory evolution is a major unexplored area of rapid pathogen evolution.

Biodiversity and biogeography of soil protists in continental and oceanic islands Euglyphida under the spotlight

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Global patterns of plant and animal diversity have been known for more than two centuries. However, the diversity and the distribution of soil microorganisms, especially of protists, as well as the factors driving their biogeography, are still poorly understood. Protists are all eukaryotes excluding plants, animals and fungi. They are mostly unicellular and found in all ecosystems where they play major roles as primary producers, microbial grazers, parasites and symbionts. Despite recent improvements of high throughput sequencing (HTS) methods coupled with bioinformatics tools for environmental DNA (eDNA) studies, a high proportion of the huge diversity of terrestrial protists remains unknown or unidentified. Thus, information about the global distribution of soil protists is scarce.

The main goal of my project is to test how far basic biogeographical rules developed for macroscopic organisms like plants or animals apply to Euglyphida (Rhizaria), an abundant and diverse group of testate amoebae. The aims of my thesis are to 1) increase the knowledge on the diversity, the taxonomy and the phylogeny of soil Euglyphida, 2) improve reference databases by describing new species, 3) characterize the niche partitioning of Euglyphida along elevation gradients, 3) assess the influence of seasonality on the community composition of Euglyphida, 4) analyse the biogeographical patterns of Euglyphida at a large scale, and 5) write a review on the biogeography of testate amoebae.

To this end, soil samples from ca. 500 sites along 26 elevation gradients located in both southern and northern hemispheres will be collected. Four islands/archipelagos (Canary < Reunion < Azores < Hawaii), two archipelagos of large islands/subcontinent (Japan < New Zealand) with contrasted distances to the nearest continent, and two distant continents of different paleogeographic origin (Laurasia: Western Europe, Gondwana: Chile) form the set of sampling locations. We will extract eDNA from all soil samples. By using different HTS methods (e.g. Illumina, PacBio, Sanger sequencing) and different genetic markers, we will identify and study the taxonomy, the phylogeny, phylogeography and ecology of soil protists (overall project), and especially of Euglyphida (my PhD project). Euglyphida will be isolated, described morphologically, and sequenced using a DNA barcoding approach to describe new protist species and fill gaps in reference databases. The integration of vegetation and soil physico-chemical data will allow us to compare biodiversity and biogeography patterns between soil protists and plants and assess to what extent they are determined by local conditions vs. large scale and historical processes.

Bacterial Spore Biodiversity in the Environment

Andrea Corona

Since the discovery of bacterial spores, research has been mainly focused on spores produced by members of four bacterial clades: Firmicutes, Actinomycetes, Cyanobacteria and Myxococcales. However, it is possible that sporulation or a similar morphogenetic process resulting in a highly resistant cell is more widely spread in nature, in which this survival strategy could be an adaptive response of microbial communities to adverse environmental conditions. This is suggested by the use of metagenomic tools using lysis-resistance to target these populations. Nevertheless, biases in the steps leading to metagenomics can result in low coverage of the less abundant taxa or underrepresentation of certain taxa. The utilization of a DNA extraction protocol with increased physical force to enrich the endospore fraction in combination with sequencing has enabled us to obtain genetic information about the most abundant spore-forming clades present in environmental samples, but more rare species might be overlooked. One approach to address this problem is the use of single cell Raman spectroscopy or Atomic Force Microscopy to sort individual spores. The aim of this study is to develop a method to generate single-spore genomic information to characterize the diversity of spore-forming clades from environmental samples. First, we will investigate the existence of a distinctive signal for bacterial spores by analyzing pure cultures containing spores and vegetative cells in model organisms. Once we have a specific Raman signal for bacterial spores, we will assess the suitability of Raman to sort bacterial spores from synthetic communities and later from natural communities.

Neuchâtel Interuniversity Doctoral Program in Organismal Biology

Pragmatic Inference in Wild Vervet Monkeys

Adwait Deshpande

Interactions between temperature, immunity and microbiota in vector mosquitoes

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¹Laboratory of ecology and epidemiology of parasites

The influence of temperature on interactions with pathogenic or symbiotic microbes is a driving force behind the survival of insects under climate change. However, little is known about how insects physiologically respond to these environmental pressures.

Beyond survival of the mosquitoes, shift in temperature also affect their capacity to transmit pathogens such as viruses. As ectotermes, the physiological response of mosquitoes to temperature follows a Jensen inequality curve. Small changes toward warmer temperature can have dramatic effects on their development, survival, fertility and immunity. During this thesis, I will explore the thermal biology of the mosquito immune system and its interaction with their capacity to transmit diseases. First, I will measure the immune gene expression of the genes implicated in all known immune response pathways of the dengue (DENV) vector, *Aedes aegypti*. Humoral immune pathways play an essential role in the defenses mounted against bacterial and viral pathogens. A change in the humoral immune response can also modify the microbiota of the vector mosquito. Further investigating the effect of temperature on the vector's microbiota is the second objective of this thesis. As current vector control methods using the *Wolbachia* symbiont have shown, the mosquito's microbiota are central in its ability to fight or transmit a pathogen.

The overall goal of this thesis is to understand how warmer temperature can affect the physiology and the vectorial competence of *Aedes aegypti* to transmit arboviruses such as dengue (DENV). Through experimental and evolutionary experiments, I will try to assess the adaptation potential of mosquitoes to warmer temperature and the effects on pathogen transmission.

SOCIAL LEARNING IN SOOTY MANGABEYS (*CERCOCEBUS ATYS*) VOCAL COMMUNICATION

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Humans and most primate species rely on vocalizations as their principal mode of communication. Thus, their study is essential to elucidate the evolution, acquisition, and function of numerous and elaborate behaviors. Accordingly, in recent years significant progress has been made in the understanding of core aspects of primate vocal behavior such as functional reference, call combinations, and audience effect. However, one main aspect of vocal communication is still largely unexplored: the acquisition of communicative capacities and skills by non-human primates. We will carry out a set of studies from an observational and experimental approach on sooty mangabeys (*Cercocebus atys*) to make progress in this domain. We are particularly interested in how social learning is related to the ecological and social processes that influence the vocal behavior of this species. First, we will use algorithms recently developed in vision research for acoustic feature detection to target the full sooty mangabeys vocal repertoire, this will allow us to better identify their full acoustic variance and explore how and when their different call types emerge. Special attention will be given to sampling individuals of different age/sex classes, particularly young individuals, in order to unmask the ontogenetic effects that affect their repertoire. Afterwards, we will look for signs of social learning by analyzing the acoustic similarity across mangabeys vocal repertoire as a function of their social bonds. Finally, we will address the questions of how and when sound-meaning lineages are established. To this end, we will carry out playback experiments using intra- and interspecific alarm calls and the presentation of a novel danger represented by a “chimera” predator model. Our overall goal is to make progress in the understanding of the process by which sooty mangabeys socially learn, develop, and use their vocal communicative skills and the cognitive processes underlying those abilities.

Biological control of wireworms in cover crops

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Wireworms can cause substantial losses in marketable yield of potatoes by feeding on and tunneling through the tubers. Control options are limited, creating a demand for new alternatives, like the use of biocontrol organisms. Laboratory and semi-field trials revealed the potential of the entomopathogenic fungus *Metarhizium brunneum* isolate ART2825 against *Agriotes obscurus* and *A. lineatus*, two of the most detrimental wireworm species. In this study we integrate the fungus in the agricultural crop rotation and try to adapt the application method to its ecological and environmental requirements. Application precedes sowing of cover crops in late summer in order to enhance disease development through higher soil temperatures and extend effect duration by the absence of soil disturbance.

In the first year of field trials we were able to establish the fungus on site and demonstrate the infectivity of the treated soils in laboratory assays. Tendencies to lower potato damages were seen in the majority of locations but damage levels did not significantly differ from the control. In the ongoing season we aimed to improve plant protection efficacy by increasing the application rate and precise selection of application time. Reasons for the yet pending success will be further investigated and potential synergies with selected cover crop species explored.

The functional ecology of plant microbiome interactions between fungi and bacteria

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Plants are exposed to a wide range of pathogenic fungi and bacteria. It has been shown that the outcome of individual interactions between pathogen and plant cannot be understood in isolation, as the presence of other microorganisms can act synergistically or antagonistically in the disease progression. Yet, the understanding of complex, i.e. at least tripartite interactions is largely missing. Here, we will establish a new microbiome interaction model using tripartite interactions of bacteria, fungi and plants. For this, we focus on wheat, *Zymoseptoria tritici*, the major fungal pathogen of wheat, and the bacteria *Pseudomonas*, a dominant member of the phyllosphere. We will characterize how intra-specific variation in a fungal pathogen determines microbial activities in the phyllosphere using genome-wide association mapping. In addition, we will characterize how differential gene expression of the fungus and the bacteria influences the outcome of bacterial-fungal competition. Our results will provide insights into the mechanism of competitive exclusion in the phyllosphere microbiome. We will generate knowledge of the exact loci that fungi evolved as defenses against *Pseudomonas*. The identification of such previously unknown loci will likely promote the description of new antimicrobial compounds that could be assessed for agricultural and even human applications.