FRANCE GENOMIQUE

EAGS2024

GDR GÉNOMIQUE ENVIRONNEMENTALE

Environmental and Agronomical Genomics Symposium

February 14-16, 2024

BOOK OF ABSTRACTS

UNIVERSITÉ TOULOUSE III PAUL SABATIER Université de Toulouse

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Table of contents

Monitoring of ecosystems functioning and health / Eco-exposome

01 - Applications of environmental DNA & RNA in freshwaters ecosystems: recent advances, expectations for operational biomonitoring, challenges and prospects

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eDNA has increased in popularity in the last decade for the monitoring of aquatic biota across a broad taxonomic spectrum. There is clearly a growing potential for information derived from eDNA to be generated more extensively and used in decision making in aquatic systems The presentation aims to illustrate (i) the levels of advancement of these eDNA/eRNA applications for different freshwater biological groups used to assess the ecological status of lakes or rivers, (ii) the remaining -general or specific- challenges to get eDNA tools into practice for operational routine biomonitoring (iii) the expectations related to the integration of omics methods other than metabarcoding for biodiveristy characterisation and ecological diagnoses of freshwater ecosystems.

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02 - Meet the bushmeat pathogens: A metabarcoding snapshot of the bacterial diversity in the bushmeat sold in West Africa

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Bushmeat hunting and consumption expose African populations to zoonotic pathogens and food-borne diseases. So far, a narrow set of targeted detection assays has been applied to bushmeat pathogen diversity, limiting our understanding of the sanitary risks associated with this broadly consumed food resource.
We used 16S rRNA metabarcoding to screen the bacterial spectrum from 183 bushmeat carcasses belonging to mammals sold in the main bushmeat market of Benin (West Africa).

We generated c. 4.2 Gb of data from which we could delineate c. 118,000 MOTUs of bacteria. The diversity of bacterial communities was both driven by sample type (oral *vs.* rectal microbiomes) and host taxonomy (orders). A specific search for bacterial pathogen species ($\geq$99% similarity threshold with SILVA SSU database) allowed the identification of 163 species. The presence of several bacteria involved in serious human diseases such as *Clostridium perfringens, Campylobacter jejuni, Escherichia coli, Haemophilus influenzae, Helicobacter pullorum, Mycobacterium tuberculosis, Neisseria meningitidis, Pasteurella multocida,* highlighted the health risks related to bushmeat consumption and trade.
Bushmeat surveillance in Africa -and more generally in the tropics- is crucial to prevent future epidemics. The "untargeted" screening of pathogens through the metabarcoding approach shows great potential for an upstream, exhaustive assessment of pathogen communities. In conjunction with denser sampling and interdisciplinary investigations at the human-ecosystem interface, metabarcoding shall significantly contribute to the One Health toolkit applied to the surveillance of emerging zoonotic diseases.

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Keywords: 16S rRNA, metabarcoding, bushmeat, pathogens, Benin

03 - What is the importance of genetic load in successful biological invasions of pests?

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Biological invasions, and more specifically those enhanced by anthropogenic activities, are becoming more and more frequent. They in turn have a huge impact on these activities, but also on biodiversity. During biological invasions, we generally observe a decrease in genetic diversity in the introduced populations compared to the native populations. This is basically explained by a reduced number of individuals at the origin of the settlement of the new population. To explain the success of an invasion despite low genetic diversity, one hypothesis proposes a purging of the genetic load (i.e., a loss of deleterious mutations) in the introduced populations. We are currently testing the purge hypothesis on a large taxonomic scale – around ten invasive insect species from five orders. Using whole-genome pool-sequencing and a reproductible Nextflow pipeline, we are quantifying and comparing the deleterious mutations of native and invasive populations. Here, we will present a first round of results on the evolution of the genetic load in invasive insect populations.

Keywords: Genetic load, Invasive Insects, Pool sequencing, Population genomics

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Exploring ecosystems using metagenomics

04 - Revealing the potential for lignin degradation by the termite gut microbiome

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Lignocellulose is the most abundant renewable resource to produce energy and commodity chemicals to replace fossil resources. Among the polymers constituting the lignocellulose, lignin is the most difficult to convert into valuable products.
Termites are known as the most efficient lignocellulose degraders in nature, producing acetate and hydrogen used by the termite host. Nevertheless, the exact role of the termite gut microbiome in lignin conversion has not been fully characterized. Therefore, we studied the capacity of termite gut microbiome to degrade lignin in controlled bioreactors useful for biotechnological purposes.By combining shotgun metagenomics and function-driven metagenomic screening, and using state-of-the-art analytical technics, including 2D HSQC NMR and 13C-IS py-GC-MS, this work brings new information on the enzymes and microbial species present in the termite microbiome involved in the degradation of lignin and lignin-related aromatics.
Termite gut microbiomes removed up to 20% lignin, although hemicellulose and cellulose were degraded more efficiently. Important structural differences such as a decrease of C-C lignin linkages and production of HPV/HPS units, indicative of ligninolytic action, were discerned in the residual lignin. Genome- resolved metagenomics revealed 173 metagenomic species derived from the termite gut microbiome, crevealing species and genes involved in the degradation of polysaccharides, lignin and lignin-derived aromatics. In addition to lignolytic microbial consortia, our results reveal putative genes involved in Kraft lignin degradation and phenol oxidation that are promising catalyst for lignin modification that can be potentially applied to a biorefinery.

Keywords: Lignocellulose, lignin, termite gut, metagenomics

*Speaker

05 - Exploring microbial disease dynamics in natural phyllospheres

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Despite the rich knowledge on composition of plant-associated microbial communities, little attention has been paid to how microbial communities and environmental cues correlate with microbial disease incidence in natural conditions. Therefore, in the context of the PATHOCOM project, we surveyed *Arabidopsis thaliana* populations over multiple seasons and years in the South West of France and observed different levels of disease symptoms across time and locations. Interestingly, environmental variables such as temperature and rainfall correlated with the occurrence of disease symptoms in *A. thaliana* leaves. In order to more closely track microbial fluctuations which may lead to disease in *A. thaliana* plants, we set up a common garden experiment with natural *A. thaliana* accessions, and we collected entire rosettes every week for the whole life cycle of the plant. By sequencing the 16S rRNA marker gene, we observed that while most bacterial groups remained stable over the course of the experiment, the abundance of certain bacterial taxa, including potential pathogenic groups, fluctuated over the life cycle of *A. thaliana*, and this was soil- and accession-dependent. Furthermore, the strongest microbial community changes were observed after a rainfall, highlighting this factor as one of the major drivers of bacterial community composition in plant leaves. To explore this specific hypothesis, we set up another common garden experiment, and harvested leaf and soil samples before and after a rain event. Microbial community profiles on these samples and the additional assessment of absolute microbial abundances will allow us to investigate the interaction between rain, host genotype and soil composition and their effect on host disease.

Keywords: plant microbiota, environmental cues, plant, microbe interactions, commongarden experiments, amplicon sequencing

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35 - Metagenomics reveal contrasted and dynamic responses of microbial soil communities to in situ wheat straw amendment in croplands or grasslands

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Soil microbial communities respond quickly to agricultural land uses and management practices. To assess the effects of contrasted land-use history on microbial diversity dynamics induced by a wheat straw amendment, an *in situ* experiment was conducted on adjacent plots with different land use-history (20 years croplands or 17 years grasslands in Lusignan, France). A metabarcoding study conducted previously1 highlighted the compositional differences in bacterial, archaeal and fungal successions in the two types of soils. To explore the whole microbial communities' responses (especially viral and eukaryotic dynamics), a global metagenomic approach was used on the same samples. About 2 billion paired-ends reads (Illumina 2*150bp) were obtained for a total of 48 samples. After quality control steps, Kaiju v.1.7.0 was used to taxonomically assign the reads. These taxonomic counts were then used to follow the dynamics of the total microbial communities. Metagenomics and metabarcoding results were concordant, with similar dynamics for bacterial and fungal genera, which confirms the validity of both approaches. Then, we defined clusters of genera: (i) specific to either grassland or cropland systems, and/or (ii) responding at different time points after the amendment. A high increase of viral sequences shortly after amendment, alongside stimulation of particular bacterial and fungal fast-growing populations, might suggest a regulatory role of viruses in soils. Differential responses of microbial groups provide new insights into the ecology of the communities involved in C turnover in soil. This metagenomic dataset will also be used to study the functional potential of these communities in carbon cycling.

Keywords: agroecology, metagenomics, organic carbon turnover, microbial communities, soil

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36 - Nitrogen metabolism in the picoalgae Pelagomonas calceolata: disentangling cyanate lyase function under nutrient stress

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Global ocean warming could lead to a decrease of nutrient export from the deep ocean, contributing to the increase of oligotrophic areas and the decline of global phytoplanktonic biomass. The lack of nitrate and ammonium can lead phytoplankton to use alternative organic nitrogen (N) sources. Among nitrogenous compounds in the ocean, cyanate is a potentially important N source, given the activity and prevalence of cyanate lyase genes in microalgae. However, their capacity to assimilate cyanate and the conditions activating this gene remain unexplored. We studied the N metabolism of the abundant and cosmopolite ochrophyte *Pelagomonas calceolata* with environmental metatranscriptomes and culture experiments under changing N sources and concentrations. In nitrate-poor oceanic regions, we observed that the cyanate lyase gene is one of the most differentially expressed genes, suggesting that cyanate is essential to *P. calceolata* persistence in oligotrophic environments. In the lab, we confirmed that this gene is overexpressed in low-nitrate media alongside several genes involved in N recycling from endogenous molecules (purines and amino acids). *P. calceolata* can grow on cyanate as the sole N source, but surprisingly, the cyanate lyase gene is underexpressed in cyanate. Taken together, these results reveal that if the cyanate lyase is essential in nitrate-poor environments, it is probably to reduce the toxicity of cyanate produced due to endogenous nitrogenous compound recycling rather than using extracellular cyanate to produce ammonium. With a better understanding of microalgae nutrient metabolism in oligotrophic conditions, we will be able to predict how they will acclimate to environmental changes.

Keywords: picoeukaryote, cyanate, nitrate, nitrogen, pelagomonas calceolata, microalgae, ocean, metatranscriptomic, transcriptomic

*Speaker

37 - Combined metabarcoding and metatranscriptomic approaches unravel taxonomic and functional diversities of a coastal sediment microbiome and its reaction to hypoxia

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Aiming to decipher how hypoxia events affect biogeochemical processes at the water-sediment interface in the Berre lagoon (France). We collected sediment cores that were incubated for one month under controlled oxygenation conditions. Sediment cores were sliced in the upper 3 cm part and subsamples were used for nucleic acid extractions. We developed joint metabarcoding and metatranscriptomic approaches using ILLUMINA sequençing to explore microbial and functional diversities of the sediment microbiome. SSU sequences were analysed with the bioinformatic pipeline DADA2 and the SILVA SSU dataset. Complemented reverse transcripts of environmental RNA (cDNA) data were submitted to the ENA database and the EMBL-EBI MGnify platform provided services of transcript assembly and annotation. Comparing metabar-

[*]Speaker

coding with environmental DNA (eDNA) to that with cDNA, showed that 75% of the whole sediment microbiome was metabolically active. However, the microbial turnover observed during incubation was higher in anoxygenic than in oxygenic condition. Differential abundance analysis (DAA) across incubation conditions and across metabarcoding approaches allowed (i) to identify stimulated or inhibited prokaryotic taxa according to oxygenation and (ii) to distinguish active taxa from the whole microbiome. Functional diversity analysis based on Gene Ontology (GO), InterPro (IPR) and KEGG pathway databases showed functional changes were limited to the upper sediment-water interface slice (0-5 mm). However, DAA allowed identifying cellular components, molecular functions, biological processes, protein domains and metabolic pathways that were specifically affected during incubation in either oxygenation conditions.

Keywords: Hypoxia, Sediment microbiome, Metabarcoding, Metatranscriptomic

38 - Genomic basis of temporally persistent and narrow prokaryotes in a coastal marine ecosystem

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Planktonic bacteria play an essential role in marine ecosystems. In the coastal environment, they face numerous variations in physical, chemical and biological parameters. Studying how they have adapted to these temporal changes is essential to better understand the mechanisms underlying microbial community dynamics and how they could face putative perturbations. To answer these questions, we have compared the genomic and functional characteristics of "temporal persistent" bacteria, i.e. those that remain abundant throughout the year, with "temporal narrow" bacteria, which will be abundant only briefly at one point in the year. To do this, we analyzed a dataset of 515 prokaryotic MAGs reconstructed at the Brest SOMLIT station over a 7-year period. The results show that temporal persistent and narrow bacteria differ markedly not only in their taxonomy, but also in their genomic and functional characteristics. But first, we observed two distinct types of "temporal persistent", depending on their versatility in acquiring energy, which will present very different genomic characteristics. While not-versatile persistent present streamlined genomes and multiple auxotrophy, versatile ones present characteristics similar to temporal narrow MAGs. Some key functions were separated between the last two groups, with an unexpected separation in the biosynthesis of two essential vitamins that are cobalamin and biotin. Overall, our work highlights mechanisms that underlie survival strategies of coastal marine bacteria.

Keywords: Marine procaryotes, time, serie, niche breadth, MAGs, genome size, metabolisms

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Exploring diversity and evolution of Life

06 - The ecology of bacterial immune systems

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Bacteria have developed a range of distinct immune strategies that provide protection against bacteriophage infections. Such pan-immune system encompasses a growing number of defense lines that include innate and adaptive systems such as abortive infection, CRISPR-Cas and restriction-modification. While much has been learned about the mechanism of action of these defense strategies (1-4), it is less clear the extent of their diversity across distinct ecological settings, and why such diversity has evolved. Here we discuss recent and ongoing findings from our group on the multiple immune barriers present in environmentally distinct bacterial communities (5). We also use statistical modeling to pinpoint fundamental tradeoffs between the amount of immune memory and effectiveness of response to a given threat. Finally, we discuss some of the broader consequences, beyond resistance to phages and other mobile genetic elements, resulting from the operation of different immune strategies. (1) Oliveira et al. Nucleic Acids Res. (2014). 42: 10618-10631; (2) Oliveira et al. PNAS (2016). 113: 5658-5663; (3) Bernheim A and Sorek R. Nat. Rev. Microbiol. (2020). 18: 113-119; (4) Oliveira PH and Fang G. Trends in Microbiol (2021). 29: 28-40; (5) Beavogui et al. BioRxiv (2023). DOI: 10.1101/2023.08.12.553040

Keywords: defense systems, phage, bacteria arms race, metagenome assembled genomes, defense islands, environmental defensomes

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07 - Unravelling the molecular mechanisms underlying nutritional polyphenism in the bird cherry-oat aphid Rhopalosiphum padi

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Polyphenism is a special case of phenotypic plasticity described as the capability of a single genotype to produce varying phenotypes in response to changes in the environment. Such plasticity requires underlying gene regulatory networks. However, little is known about the molecular cascade regulating polyphenisms. Here, we address this issue in *Rhopalosiphum padi* which displays a nutritional polyphenism i.e the ability to produce specialized morphs able to feed on very distinct host plants. The annual life cycle of *R. padi* involves an obligatory migration between a woody host, *Prunus padus* and grasses as secondary hosts. This alternation is achieved by migrant morphs. We have determined that migrant production is triggered by plant cues and identified two critical periods of nutritional polyphenism. The first is the induction of migrant morph development which occurs in the first larval instar, and the second is a gradual acclimation to the secondary host. In this study we aimed to characterize the underlying genetic programs that are turned on/off to regulate nutritional polyphenism. We explored it through a gene expression analysis by generating 109 mRNA-seq libraries from age-standardized samples at all stages for morphs feeding either on *P. padus* or grasses. Transcriptomes comparison between first or second larval instar reared under either inductive or non-inductive conditions allowed us to identify genes involved in the migrant development induction. We also compared migrants still feeding on *P. padus* and migrants after migration allowing us to highlight differentially expressed genes involved in host plant exploitation. This study provides new clues to unravel the molecular mechanisms involved in nutritional polyphenism.

Keywords: Polyphenism, Rhopalosiphum padi, transcriptomic analysis

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08 - Phylogeny-guided environmental genomics of giant viruses

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DNA viruses have a major influence on the ecology and evolution of cellular organisms, but their overall diversity and evolutionary trajectories remain elusive. Recently, we performed a phylogeny-guided genome-resolved metagenomic survey of the sunlit oceans and discovered plankton-infecting relatives of herpesviruses that form a putative new phylum dubbed '*Mirusviricota*'. The virion morphogenesis module of this large monophyletic clade is typical of viruses from the realm *Duplodnaviria*, with multiple components strongly indicating a common ancestry with animal-infecting *Herpesvirales*. Yet, a substantial fraction of mirusvirus genes, including hallmark transcription machinery genes missing in herpesviruses, are closely related homologs of giant eukaryotic DNA viruses from another viral realm. These remarkable chimeric attributes connecting '*Mirusviricota*' to herpesviruses and giant eukaryotic viruses are supported by a fast-growing number of environmental mirusvirus genomes as well as one culture. Moreover, mirusviruses are among the most abundant and active eukaryotic viruses characterized in the sunlit oceans, encoding a diverse array of functions used during the infection of microbial eukaryotes from pole to pole. The prevalence, functional activity, diversification, and atypical chimeric attributes of mirusviruses point to a lasting role of '*Mirusviricota*' in the ecology of marine ecosystems and in the evolution of eukaryotic DNA viruses.

Keywords: Genome, resolved metagenomics, giant viruses, mirusviruses, herpesvirus, anvi'o

*Speaker

09 - Disparate genetic divergence patterns in three corals across a pan-Pacific environmental gradient highlight species-specific adaptation trajectories.

Christian Voolstra [1], Benjamin C.c. Hume [1], Eric J Armstrong [2], Guinther Mitusashi [3], Barbara Porro [4], Nicolas Oury [5], Sylvain Agostini [3], Emilie Boissin [2], Julie Poulain [6], Quentin Carradec [6], David A Paz-Garcia [7], Didier Zoccola [8], Hélène Magalon [5], Clémentine Moulin [9], Guillaume Bourdin [10], Guillaume Iwankow [2], Sarah Romac [11], Coordinators Tara Pacific [12], Serge Planes [2], Denis Allemand [8], Didier Forcioli * [13]

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We quantified the standing genetic diversity of three coral morphospecies - *Porites lobata, Pocillopora meandrina*, and *Millepora platyphylla* - in relation to past sea temperature variations, as part of the Tara Pacific Expedition. Coral specimens with the targeted colony morphologies

*Speaker

were sampled across 11 Pacific Ocean, and either genome-wide (*Porites*, *Pocillopora*) or targeted (*Millepora*) SNPs were obtained for 109, 103, and 57 colonies, respectively.
Within the targeted morphologies, 3 and 5 independent genetic lineages/species were identified in *Porites* and *Pocillopora* respectively, highlighting the difficulty of morphology-based sampling in corals. Contrastingly, all *Millepora* samples belonged to the targeted species.

The biogeographical distribution of these lineages in the same oceanographical context was distinct for each genus, which do question the relation of these genera to the environment. We showed that less past temperature outlier SNPs were included in genomic islands of differentiation and under divergent selection among species in *Porites* than in *Pocillopora.*
This lesser genomic signature to prevailing environments in *Porites* argues for a built-in stress tolerance, whereas the stronger genomic imprint by the environment on *Pocillopora* may point to a stronger adaptation to different niches, and a higher sensibility to climate perturbations. Within the context of conservation, *Millepora*, with its high within species biogeographic structuring, will require more reefs to be preserved to protect its extant diversity. These recently published results (https://www.nature.com/articles/s44185-023-00020-8) show that reef conservation will require species-specific strategies.

Keywords: coral reefs, climate change, population genomics, biogeography, adaptive evolution

10 - A global view of the biocomplexity underlying coral holobiont diversity and climate change resilience

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Productivity and biodiversity of coral reef ecosystems depend on healthy corals, sessile cnidarians of the class Anthozoa that engage in symbioses with microalgae of the family Symbiodiniaceae and a suite of other microbes (bacteria, archaea, etc.) that all contribute critically to the resilience and functioning of the emerging coral metaorganism or holobiont. This notion necessitates that one must examine the biocomplexity of the coral holobiont (i.e., the interplay between metaorganism biodiversity, ecological function, and the emergent phenotype) to better understand how identity and functional diversity of associated microbes contribute to the health and resilience of coral metaorganisms. At present, we lack an integrative understanding of how holobiont diversity and the implied functional differences scale over geographical and environmental regimes, and how this in turn affects coral metaorganism phenotypes. Current efforts towards setting up standardized analytical frameworks are presented that integrate holobiont biodiversity, stress tolerance, and environmental setting to elucidate coral holobiont assemblages that underlie metaorganism resilience.

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27 - Introducing an integrative and generalizable approach to elucidate cryptic diversifications using mouse lemurs as a model system.

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Madagascar has long been recognized as a model for diversification research and a major conservation priority given the high levels of endemism and critically endangered status of its biodiversity. Yet, the study of the island's biodiversity and the implementation of effective conservation strategies have likely been impeded by an inflation of its taxonomic diversity as a consequence of excessive over-splitting of phenotypically similar lineages when DNA sequencing first became readily available. We address this issue by providing an integrative framework to delimit species and understand cryptic diversification using mouse lemurs (genus *Microcebus*) as a model system. We collected and integratively analysed genomic, morphological, acoustic, behavioural and geographical occurrence datasets to address major outstanding evolutionary questions about the extent to which geographically limited gene-flow and incomplete sampling of cryptic lineages leads to spurious taxonomic delimitations. Using a comprehensive approach, we significantly deflate the taxonomy of mouse lemurs resulting in a potential decrease in the conservation status of these lineages. To mitigate this effect, we define intraspecific conservation units, which can thus guide conservation of both species and population genetic diversity. We show that the cryptic nature of mouse lemurs results from morphological stasis and niche conservatism, making this the first case study comprehensively assessing crypsis at the level of an entire primate genus. Our work results from the efforts of more than 50 collaborators, through more than 30 years of field research, across the still largely unexplored and inaccessible forests of Madagascar.

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28 - Exploring Horizontal Gene Transfers in Phytoparasitic Nematodes through Soil Metagenomes

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Plant-parasitic nematodes (PPN) cause substantial annual losses to edible crops, estimated to 11% globally (1). A deeper comprehension of the evolutionary dynamics of these plant pests is crucial for mitigating their impact. PPN have acquired fungal and bacterial genes pivotal to parasitic functions, including the production of enzymes facilitating plant cell wall degradation (2). Transcriptomic, biochemical, and proteomic evidence underscores the 'domestication' of microbial genes in these pathogens (3). However, key questions persist such as the total rate of horizontal gene transfer (HGT) in host genomes and the identity of donor organisms. Hypothesizing soil-dwelling microorganisms as gene sources, our current understanding was hindered by their lack of representation in public databases. We overcame these limitations by curating a comprehensive protein library from 6,800 publicly available soil metagenomes (4). This improved library, twice the size of NCBI-nr, better mirrors soil biodiversity. Using this soil-enriched library, we conducted a broad-scale HGT detection supported by phylogenetic validation for 18 PPN proteomes (5). We detected 0.5-1.9% protein-coding genes acquired by HGT mainly from bacteria and fungi, protists and plants. Metagenomic data clarified known HGTs and uncovered novel events. Integrating environmental data in our reference library has allowed us to extend the detection of HGTs and to complete the catalog of potential donor-related extended species.
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Keywords: Horizontal gene transferts, Root, knot nematodes, Metagenomic, Parasitism, Evolution

Ancient DNA and paleo-environments

11 - Microbial time travellers: preserving the past, shaping the future

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Ancient environmental DNA offers a unique window into past ecosystems, providing insights into the dynamics of those environments. By analyzing the microbial communities from Kap København in Northern Greenland, dating back two million years, we shed light on what can be described as 'microbial time travellers'. Our findings indicate that microbial responses to environmental changes are not uniform across geological timescales. Instead, specific microbial taxa have shown remarkable adaptability and resilience, remaining consistent over extended periods. Understanding the dynamics of these ancient microbial communities is crucial, providing essential context for deciphering and addressing the environmental challenges we face in the present day.

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12 - Diversity of ancient rhizosphere microbial communities preserved in herbaria, a paleomicrobiological approach

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The current biodiversity crisis is mainly documented for plants and animals, for which exist temporal records of populations and communities that are sometimes more than a century old. But what about microorganisms, and more specifically soil microorganisms? In the absence of archives from the past, the only available data of current environmental changes on microbial diversity come mainly from short-term impact studies comparing soils subjected to different management practices. We have observed that plant roots preserved in herbariums are occasionally surrounded by a sheath of rhizospheric soil that may have preserved traces of ancient microbial communities. From this unique material, we are developing a paleomicrobiological approach based on the extraction of ancient DNA from this matrix and its systematic sequencing. Annotation of the resulting sequences illustrate the taxonomic and functional diversity of ancient rhizosphere microbial communities associated to several crop species (different cereals and lettuce). This approach will allow us to assess the long-term impact on these communities of global changes such as the intensification of agricultural practices. In future, de novo assembly of the sequences could allow reconstruction of parts of the genome and genes of the main microbial species that dominated ancient soil microbial communities.

Keywords: Ancient DNA, Herbaria, Plant microbiome, Museomics

13 - Reconstruction of the past evolution of plant biodiversity through paleogenomic studies.

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Paleogenomics consists of the study of the evolution of modern species by deciphering the genome of their extinct founding ancestors, by either a synchronic approach (that is to say the modeling of evolution by comparing genomes of modern species) or an allochronic approach (that is to say the investigation of ancient DNA from fossil remains).
On the synchronic approach, we describe the reconstruction of the genome of the most recent common ancestor (MRCA) of modern angiosperms (including cereals) as a starting point for deciphering the reticulated evolutionary plasticity between species (rapidly *vs.* slowly evolving lineages), subgenomes (pre- *vs.* post-duplication blocks), genomic compartments (stable *vs.* labile loci), genes (ancestral *vs.* species-specific genes) and functions (gained *vs.* lost ontologies), the key mutational forces driving the evolutionary success of plants.

On the allochronic approach, we redraw wheat cultivation origin and expansion during the holocene, using genome sequencing of a worldwide panel of almost 500 wheat genotypes compared to ancient DNA of wheat remains to explore how 10,000 years of hybridization, selection, adaptation and plant breeding has shaped the genetic makeup of modern bread wheats.
Overall, both complementary approaches, synchronic and allochronic, deliver genomic signatures of species' evolution and adaptation allowing the identification of key genes/alleles that could be used in modern plant conservation and breeding programmes in the context of the ongoing and future climate change.

Keywords: Paleogenomics, Plant, Evolution, Adaptation

*Speaker

Environnemental genomics and participatory science, openness to society

14 - Climate change microbiology: novel insights into methane cycling archaea

Cornelia Welte *† [1]

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Methane is a potent greenhouse gas that is produced by microorganisms living in the absence of oxygen, the methanogenic archaea. The amount of emitted methane is controlled by a powerful biological filter where aerobic and anaerobic methane oxidizing microorganisms thrive. While aerobic methanotrophic bacteria are already relatively well understood, the physiology of anaerobic methanotrophic (ANME) archaea is still enigmatic, in part due to the inability to cultivate them axenically. In this project, we cultivated ANME archaea in bioelectrochemical systems with methane as electron donor and an anode as electron acceptor. We observed stable and reproducible methane-dependent electrical current generation at the anode poised at 0V vs. SHE. Depending on the cultivation conditions, up to 91% of the current was derived from methane oxidation. Metagenomic and metatranscriptomic analyses indicated the selective enrichment of ANME archaea at the anode where they comprised ca. 80% of the entire microbial community. Scanning electron microscopy as well as fluorescence in situ hybridization labelled confocal laser scanning microscopy confirmed this observation and provided insights into the spatial organization of the biofilm. Finally, metatranscriptomics yielded insight into the mechanism of extracellular electron transport. These exciting insights elucidate previously unknown physiological properties of ANME archaea.

*Speaker

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15 - The french molecular inventory of soil fungi

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The fungal kingdom has been diversifying for more than 800 million years and has colonized a large number of habitats on Earth. With 2 to 12 million estimated species mostly found in soils, this heterotrophic kingdom is probably the second most diverse one among *Eukaryota* after animals. Fungi are major ecological players that support various ecosystem functions across trophic levels. Based on a unique dataset in the world (2,200 soil samples) from the French Soil Quality Monitoring Network and using a metabarcoding approach (18S rDNA marker), we described the abundance, diversity, and composition of soil fungal communities on a large scale and identify the environmental filters (e.g., soil parameters, land uses, climate types) which condition these distributions. We also addressed more precisely the distribution of major fungal taxa (phylum and class level) present in French soils, allowing a better understanding of their ecology, in terms of sensitivity to certain environmental conditions and involvement in the major functions and services provided by soils. We also identified a complex mosaic of 10 distinct terrestrial habitats, based on soil type, land uses and climate type. All these information have been synthetized in the French Atlas of Soil Fungi (2024, Biotope editions), a unique work both in terms of the subject matter and original representations, allowing us to understand the mysterious and incredible world of this invisible kingdom. Moreover, this Atlas concludes with operational demonstrations on fungi as bioindicators of soil quality to assess the impact and sustainability of certain agricultural or urban practices. Release scheduled during 2024.

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39 - FungiSol: A citizen science project to investigate environmental yeasts in urban soils.

Isabelle Ourliac-Garnier *† [1], Raquel Supo-Santillan [1], Manon Cadeau [1], Marjorie Albassier [1], Estelle Robert [1], Julien Auclaire [1], Patrice Le Pape [1], Florent Morio [1]

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A citizen science project on the fungal biodiversity of yeasts in urban soils has been set up in order to explore its role as a reservoir of pathogenic yeasts.
In 2023, 128 children from 4 schools of Nantes, each collected two separate soil samples in their school (playgrounds, vegetable garden, potted plants, flowerbed). 2 g of each of the 256 soil samples were put into YPD medium with antibiotics and incubated at 30∘C. After three days, dilutions were inoculated onto YPD agar plates for yeasts isolation. For each sample growing yeasts, up to 24 colonies were subcultured on both Sabouraud and chromogenic media plates to screen for distinct morphotypes (by Maldi-TOF mass spectrometry, or ITS rDNA barcoding when necessary).

Under our conditions, yeasts were isolated in 62 % of the samples (n = 159). Among the 2042 yeasts colonies screened, 216 morphotypes were distinguished among all the samples. Of these, 28 distinct yeast species, belonging to 16 different genera, have been identified by Maldi-TOF mass spectrometry. Species distribution and diversity differed between schools although *Torulaspora delbrueckii* was the main isolated species (20-38%, n = 81) followed by *Hanseniaspora uvarum* (3-17%, n = 25). Beyond *C. tropicalis* which appeared to be the more prevalent *Candida* species (n = 13), several other human opportunistic yeasts were identified. Some isolates remain to be identified by ITS rDNA barcoding.
As part of the One Health initiative, our first findings underline the role of urban soils as potentiel reservoir of opportunistic yeast. Further experiments are underway to assess the *in vitro* susceptibility of these environmental yeasts to medical antifungals.

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Pangenome and structural variants

16 - Reference-free pangenomics and other large indexes

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The field of eukaryote pangenomics has predominantly utilized variation graphs to understand genomic diversity and structure. However, a wealth of alternative approaches exists within less accessible scientific literature, often overlooked by the bioinformatics community. In this talk, I will present an overview of these lesser-known techniques, highlighting their properties and potential advantages over conventional methods. I will discuss the current promises and limitations of these approaches. Additionally, I will review various applications of these novel structures, notably in transcriptomics.

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17 - High-quality genome assembly to identify structural variations in the quarantine plant parasitic nematode Meloidogyne enterolobii

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Root-knot nematodes of the genus *Meloidogyne* are obligatory plant endoparasites that threaten the global food supply. The preferred non-chemical control method consists in deploying plant-resistant genes against *Meloidogyne* species. However, most European vegetable plant resistance genes are inefficient against *M. enterolobii,* which was recently declared a quarantine pest. To unravel the molecular characteristics underlying its parasitic success, a thorough exploration of the genomic plasticity of *M. enterolobii* is essential. In this study, we report PacBio high-fidelity long-read genome data for distinct geographical isolates of *M. enterolobii,* exhibiting different ranges of compatible hosts. Using the gap-aware sequence transformer, DeepConsensus, we have further improved reads quality and assembled each isolate genome. We selected as a reference genome, the assembly that returned the best contiguity, which yielded a 273 Mbp genome with 556 contigs and a N50 value of 2.11Mb, the highest so far for a polyploid parthenogenetic root-knot nematode at the contig level. Combined analysis of k-mers and distribution of gene copies indicate the genome is triploid with diverged AAB sub-genomes. By aligning PacBio Hi-Fi long reads from the different geographical isolates to the reference genome, we have detected and genotyped genomic structural variants (SVs). We also propose a novel framework for the detection of SVs in complex organisms. Our findings indicate on average 7053 SVs per sample spanning 2.96% of the reference genome. The most represented are deletions (56%) and insertions (38%). We will next investigate whether SVs patterns correlate with differences in the ranges of compatible hosts and geographical distribution.

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Keywords: Root knot nematodes, Structural variants, PacBio HiFi

18 - Overdominance likely maintained large structural variants polymorphism in pearl millet

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With the development of high throughput sequencing, new and effective approaches to study inversions have emerged recently. This has led to a renewed interest to study the role of inversions in the evolution and the adaptation of species. We used a population genomic approach to analyze 126 cultivated samples of the African staple cereal pearl millet. We identified six large putative inversions ranging from 5 to 88 Mb, and which covered a total of 308 Mb, corresponding to 17% of the genome. We conducted a more in-depth study of the largest candidate region of 88 Mb on chromosome 3. We validated the presence of inverted segments within the region with genome assembly. One haplotype spanning the whole 88 Mb region is only found at the heterozygous state, suggesting a strong heterozygote advantage. Study of segregation of this variant confirmed the absence of the homozygous state in the offspring. To rule out a very recent occurrence, we showed its maintenance over 40 generations. We further showed that the variant is slightly associated with phenotypic traits including an earlier flowering time and a greater number of productive tillers, which could lead to a greater fitness. Additionally, we showed that the putative inversion likely originates from introgression with wild relatives. Reduced recombination has likely maintained large introgressed fragments in the cultivated population. Association with traits related to fitness and phenotypic values closer to those of wild relatives may indicate an adaptive role of the introgression in the cultivated population.

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Keywords: structural variant, overdominance, low recombination, heterozygotes excess, inversion, introgression, pearl millet

19 - Ten years of pangenomics in rices, lessons and knowledge

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Ten years of pangenomics in rices, lessons and knowledge

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20 - The pangenome of the cosmopolitan picophytoplankton Bathycoccus prasinos: Understanding latitudinal and seasonal adaptation

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Phytoplankton are central to marine ecosystems and rival terrestrial plants in primary production. Their genomic characterization mostly relies on metagenomic datasets, such as those from the Tara Ocean cruise, and limited reference genomes, providing only a partial view of their genetic diversity and the underlying adaptation mechanisms. The acquisition of genome sequences of phytoplankton strains from contrasting environments is therefore necessary to complement the available datasets and provides a robust framework to study adaptation. *Bathycoccus prasinos* is a cosmopolitan and abundant eukaryotic phytoplankton showing seasonal blooms. With a small and compact genome, *Bathycoccus* makes for an interesting model to study genetic diversity and the genetic basis of adaptation to seasonal and latitudinal changes in photoperiod and temperature.

To describe *Bathycoccus*' genetic landscape, we constructed a genomic resource consisting of 31 strains isolated along a latitudinal gradient from the Austral to the Arctic oceans. Leveraging a combination of Oxford Nanopore Technology and Illumina, we produced high-quality *de novo* assembled genomes and built the first pangenome of *Bathycoccus prasinos*. With access to sequence and function diversities, we revealed unexpected subpopulation structures and identified population-specific genetic variations potentially involved in latitudinal and seasonal adaptation.

Keywords: Phytoplankton, Natural diversity, Whole genome sequencing, Pangenome, Adaptation mechanisms

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21 - Understanding the limits of pangenome graphs for the analysis of large inversions in a complex of butterfly species

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Among structural variants, inversions are of particular interest in ecology studies as they can introduce phenotypic diversity and have a lasting impact on population genetics by locally impairing recombination. Pangenome graphs (PG) are a way to represent all scales of genomic diversity in a species. Several tools have been developed to construct PG from genome alignments and to detect variants from the graph topology. PG were shown to be particularly efficient for identifying and genotyping deletions and insertions in model organisms. However they have not yet been thoroughly assessed on inversion polymorphism. We propose here to evaluate the ability of PG tools to represent and detect inversions in the case of a complex of butterfly species (Coenonympha genus, 9% nt divergence) for which we already detected a dozen of large ($>$ 100 kb) inversions. To compare the tools, we selected a chromosome with 2 large, 6 medium sized ($>$ 1 kb), and 9 small ($<$ 1 kb) inversions, and built PG with 4 state of the art tools. Minigraph and minigraph-Cactus failed to build an accurate PG for such a degree of sequence divergence, while PGGB and Cactus found at most two inversions. In order to understand how PG handle inversions, we simulated the 17 known inversions in several synthetic chromosomes with increasing levels of single nucleotide divergence. We found that in such simplified graphs, most large simulated inversions are well represented with most tools. However, for smaller inversions and when the sequence divergence is higher, there is a significant variability in how the inversions are represented between PG tools. We analyzed the various obtained topological motifs, leading to methodological avenues for improving the detection of inversions in PG.

Keywords: pangenome graphs, structural variants, inversions, comparative genomics, method benchmark

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Technological advances: producing and analyzing genomic data

22 - Statistical methods for the exploration and the integration of multi-omics datasets

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The high-throughput data generated by new biotechnologies used in biological studies require specific and adapted statistical treatments. The methodologies presented start from multivariate exploratory statistics with Principal Component Analysis and lead to multi-block and multi-group integration analysis with PLS-related based methods. Each method, either unsupervised or supervised, has to be used in order to address a specific biological problem:
- explore a single dataset (e.g. Transcriptomics) and identify the trends or patterns in the data, experimental bias or identify if the samples 'naturally' cluster according to the biological conditions: an unsupervised factorial analysis such as Principal Component Analysis (PCA) provides such information about one dataset without any a priori.

- classify samples into known classes based on a single dataset: supervised classification methods such as Partial Least Squares Discriminant Analysis (PLS-DA) assess how informative the data are to correctly classify the samples, as well as to predict the class of new samples.

- unravel the information contained in two datasets, where two types of variables are measured on the same samples: using PLS-related methods enable knowing if common information can be extracted from the two datasets (or highlight the relationships between them).

- the same as above but considering more than two datasets: multi-block PLS-related methods.

- the same as above, but in a supervised context: multi-block PLS-DA (referred as DIABLO for Data Integration Analysis for Biomarker discovery using Latent variable approaches for Omics studies)
- investigate the relationships among individuals within several known groups: multi-group analyses.

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23 - RdRp-scan: A bioinformatic resource to identify and annotate divergent RNA viruses in metagenomic sequence data

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High-throughput sequencing technologies are greatly revolutionising virus discovery. Without a priori detection of viruses present in a sample is a major step in revealing the virosphere but the commonly-used sequence-based workflows still suffer from a limited power of detection, especially when it comes to the fastest evolving entities on Earth. Protein structures are 3 to 10 times more conserved than primary amino acid sequences, such that structure-based comparisons provide an outstanding opportunity to reveal the viral 'dusk matter': viral sequences with low, but detectable, levels of sequence identity to known viruses with available protein structures. Here, we present a new open computational resource-RdRp-scan*-that contains a standardized bioinformatic toolkit to identify and annotate divergent RNA viruses in metagenomic sequence data based on the detection of RNA-dependent RNA polymerase (RdRp) sequences. By combining new RdRp-specific hidden Markov models (HMMs) and structural comparisons, we show that RdRp-scan can efficiently detect RdRp sequences with identity levels as low as 10per cent to those from known RdRp structures. In addition, to facilitate the annotation and placement of newly detected and divergent virus-like sequences into the diversity of RNA viruses, RdRp-scan provides new custom and curated databases of viral RdRp sequences and core motifs, as well as pre-built RdRp multiple sequence alignments. The astonishing advances in ab initio protein structure predictions is expected to enlarge even more the scale of detection across the RNA virus phylogeny. Time has come to integrate structures in our metagenomic pipelines ! ** Charon et al (2022) Virus Evolution, 8:veac082*

Keywords: HMM, based homology detection, RNA, dependent RNA polymerase, evolution, metagenomics, phylogeny, viral dusk matter.

*Speaker

24 - EPIK : Evolutionary Placement with Informative K-mers for scalable taxonomic identification

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Taxonomic identification of metabarcode or metagenome sequences can be performed with different approaches, namely OTU clustering, taxonomy-supervised classification or phylogenetic placement. The latter provides a classification on the branches of an pre-existing reference phylogeny (ex: large rRNA or organelle phylogenies). It offers two main advantages: the precision of Maximum Likelihood framework and measures of identification uncertainty that can be taken into account in post-analyses. See Czech et al, 2022 for a review of taxonomic and functional post-analyses opened by phylogenetic placement.
For many years phylogenetic placement was dependent to a preliminary step of query sequence alignment. Then, we developed a alignment-free method named RAPPAS, based on the original concept of "phylo-k-mers" (Linard et al, 2019). While faster, it required huge preprocessing times and lots of memory. More recently we improved this approach with the concept of "informative" phylo-k-mers, eg. filtering of phylogenetically informed k-mers that are the most informative for taxonomic classification given a phylogenetic context. This lead to the recent publication of EPIK (Romashchenko et al, 2023), a scalable and accurate tools for large-scale and routine phylogenetic placement.

During this talk, I will present briefly discuss the concept of phylogenetic placement, then explain how informative phylo-k-mers opened a new path to large-scale and routine phylogenetic placement. Finally, I will mention how tools for phylogenetic placement benchmarking can be used to evaluate putative marker genes accuracy, given a clade and its phylogeny. This preliminary analysis can help with metabarcoding experiment design.

Keywords: taxonomic identification, metabarcoding, phylogenetic placement

*Speaker

Genomics of plants and animals and their microbiota

25 - The Ocean and Coral Reefs as Microbial Treasure Troves

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Microbes that inhabit the ocean are phylogenetically and metabolically diverse. Exploring this diversity for yet unknown taxa and biochemistry, including for biotechnologically relevant enyzmes and therapeutic leads, has been greatly accelerated by cultivation-independent DNA sequencing of microbial communities (metagenomics). However, the requirement for genome-resolved information has limited the identification and host assignment of biosynthetic gene clusters (BGCs) that encode such enzymes and the synthesis of bioactive, chemical compounds. Here, we analyzed metagenomic data from decades of ocean sampling efforts as well as a recent expedition across coral reefs of the Pacific Ocean to reconstruct at large scale microbial genomes with the goal to explore the discovery potential of these resources for novel microbial taxa, enzymes and biosynthetic products. Our results show that prior to our efforts, thousands of ocean microbial species had been genomically uncharacterized. By compiling these data together with publicly available reference and single-cell genomes into interactive web databases, facilitated the discovery of *Ca.* Eudoremicrobiaceae as a new family of bacteria with an unparalleled biosynthetic diversity in the open ocean and Acidobacteriota spp. as BGC-rich lineages to be hosted by reef-building corals. By biochemically characterizing BGCs, we revealed an unusual bioactive chemical structure and an enzymatic activity, which was unexpected based on bioinformatic prediction. Together, this work highlights the promise of environmental genomics for discovering new microbial products and the value of conserving coral reefs by adding a microbial perspective to the loss of biodiversity.

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26 - From the diversity of the diploid progenitors to a new diversity in a polyploidy crop, oilseed rape

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A fine understanding of the amazing intra and interspecific genetic diversity is being provided by the constant improvement of sequencing and assembling technologies, but also by the recent efforts to collect still uncharacterized populations. In the genus *Brassica*, notably comprising the allotetraploid oilseed rape crop (AACC, $2n$=$4x$=38), deriving from the two diploid species *B. rapa* (AA, $2n$=$2x$=20) and *B. oleracea* (CC, $2n$=$2x$=18), it is known that its origin and human selection of few agronomical traits led to a severe erosion of its genetic diversity. Fortunately, an important genetic and phenotypic diversity exist in its parental diploid species. However, numerous wild populations and landraces had neither been collected nor investigated. To that purpose, we collected 102 *B. oleracea* and 146 *B. rapa* populations on large climatic gradient surrounding their center of origin, the Mediterranean Basin, multiplied this material and sequenced each population in bulk. In the same time, some genome assemblies have and are currently being produced, revealing the presence of large structural variations. Using the closest reference genome to the populations we collected, we then analyzed their genetic variability and are currently investigating the regions that may have played a major role in their adaptation

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in very contrasted environments. In parallel, we also found a natural method to rapidly and efficiently introduce this diversity in oilseed rape varieties; allotriploid hybrids (AAC) produced by direct crosses between oilseed rape and one of its diploid progenitors, *B. rapa*, present on average 3.6 times more crossovers than the AA or AACC hybrids, with crossovers present in the normally non-recombining pericentromeric regions. We used this strategy for introducing small genomic regions all along the chromosomes from the diploids into oilseed rape. International core collections of both diploid species were crossed with the same *B. napus* variety, which was *de novo* assembled. After two backcrosses, with the same *B. napus* variety, and intercrosses, 453 doubled haploid lines were produced. Their resequencing allowed a precise identification of the genomic regions introduced from each diploid. Their phenotypic characterization of all this material and *de novo* assembly of several diploid parental lines are in progress. All these data will allow us to target the diversity of interest to be introduced in oilseed rape, but will also provide a tremendous help towards identifying genes of agronomical interests.

Genomics of biological interactions: holobionts, pathogens, symbionts

29 - Mosquito-microbe symbiosis: an ecogenomic perspective for novel control strategies of infectious diseases

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The global burden of mosquito-transmitted diseases, including Plasmodium, Dengue, West Nile, Zika, Usutu, and yellow fever, continues to increase, posing a significant public health threat. With the rise of insecticide resistance and the absence of an effective vaccine, new strategies are emerging that focus on the mosquito's microbiota. Among these, the intracellular bacterium *Wolbachia* which can interfere with pathogen transmission and manipulate host reproduction, stands out. However, despite its promise, the genomic variability of *Wolbachia* and its mobilome, along with its impact on pathogen blocking in interaction with other symbiotic life, particularly in naturally infected vectors like *Culex* mosquitoes, remains poorly understood. Leveraging shotgun metagenomes, the team reconstructs bacterial genomes in *Culex* specimens at the organ level. This enables us to investigate *Wolbachia*'s genomic variability and examine how it contributes to distinct phenotypes of density and protection against viruses. Simultaneously, we identify and analyse other symbiotic genomes that could be exploited to fight vector-borne diseases. Moreover, we study the complex interaction of these newly reconstructed symbiotic genomes with arboviruses through differential expression analyses in infection conditions. Lastly, we explore the diversity, biogeography, and transformation capacity of the recently discovered *Wolbachia* plasmid element, revealing its critical role in the endosymbiont biology. These findings could pave the way for the development of novel vector biocontrol strategies.

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30 - A genomic map of local adaptation in Arabidopsis thaliana to native non-pathogenic bacteria: from mono-infections to complex communities.

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There is growing interest in the potential of harnessing the microbiome towards the improvement of plant health to achieve agricultural goals. To do so through plant breeding, requires a better understanding of the role of the host genome in modulating microbiota variation. In particular, there is a need to overcome the current limits on the description of host-microbiota interactions at the genomic and molecular levels. However, the host genetic architecture structuring microbiota is only partly described in plants. To dissect the genetic architecture driving adaptive plant-microbiota interactions, I will present the results of complementary approaches in association genetics applied on *Arabidopsis thaliana*: (i) a Genome-Environment Association (GEA) analysis on 141 whole-genome sequenced natural populations of *A. thaliana* characterized *in situ* for their leaf and root bacterial communities and a large set of non-microbial ecological factors (i.e., climate, soil, and plant communities), and (ii) a Genome-Wide Association study conducted in field conditions on 162 whole-genome sequenced accessions of *A. thaliana* inoculated with 13 native Plant Growth-Promoting Bacteria (PGPB) isolated from these populations. By combining these two approaches, we established a genomic map of local adaptation in *A. thaliana* to its native bacterial microbiota. Plant immunity appears as a major source of adaptive genetic variation structuring beneficial interactions between *A. thaliana* and the main members of its microbiota.

Keywords: microbiota, pathobiota, Plant Growth, Promoting Bacteria, GWAS, native interactions

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31 - Comparative genomics of Xylella fastidiosa subsp. multiplex strains from France reveals pathogen dynamics after its introduction

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Xylella fastidiosa is a plant pathogen responsible for numerous crop diseases worldwide. It specifically colonizes the xylem of plants and is transmitted exclusively by sap-feeding insects. *X. fastidiosa* has a significant adaptive capacity, as evidenced by its great genetic diversity and frequent recombination events between subspecies. Originating from the Americas, *X. fastidiosa* is now present in several European countries (Italy, France, Spain and Portugal) due to accidental introductions of contaminated plant material. This situation calls for a better understanding of the evolutionary dynamics of the pathogen in its new areas of distribution. Herein, we present a comparative genomics analysis of strains belonging to the subspecies *multiplex* that have been isolated from various host plants in France since the first detection of the pathogen in 2015. High-quality genome sequences were obtained using both PacBio and Oxford Nanopore MinION sequencing technologies. We further employed a metagenomic approach to sequence additional genomes directly from infected plants, thereby eliminating the time-consuming isolation step. Comparative genomics analyses identified genes that occur exclusively in the strains isolated in France. In addition, several strains harboured plasmids, in contrast to American strains of this subspecies which rarely possess plasmids. We will discuss the putative functions and acquisition routes of these genes and plasmids as well as their potential role in the adaptation to new host plants after pathogen introduction.

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32 - Hologenome 2.0: taking the genomes of social partners into account

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Genetics is traditionally understood as the study of how an individual's genotype affects the individual's phenotype. However, in recent years it has become clear that genes in the environment are also important. Indeed, the genotype of a social partner (e.g. family member, peer) can affect the phenotype of a focal individual by determining traits of the social partner that influence the focal indidual's phenotype. Similarly, genes of the microbiota can impact the focal individual (host) through host/microbiome interactions. Finally, there may be exchanges of microbiota between socially interacting individuals, creating a complex (genetic) ecosystem that can contribute to health or disease. We are developing experimental and computational methods to study this ecosystem in outbred laboratory rodents. We have quantified social genetic effects from cage mates (peers) on behavioural, physiological and morphological phenotypes in several mouse and rat populations, and have started to uncover specific genes and mechanisms involved. We have also studied host/microbiome interactions in a sample of 4,000 outbred rats spread across three different locations (research institutes) to identify host/microbiome interactions that replicate across locations. Finally, we have started investigating microbiota transfer between cage mates, which in rodents occurs at high volume through allo-coprophagy.

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33 - Genomics of palm tree-palm weevil interactions

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The Red Palm Weevil (RPW) *Rhynchophorus ferrugineus* (Olivier) is the most destructive and invasive insect pest of palm tree. However, the molecular bases of palm tree-palm weevil interactions are still poorly understood. As valuable resources, three genomes have been published, with contrasting ecological interpretations based solely on automatic annotations. Such automatic annotations usually miss genes poorly conserved and can identify artefactual duplications through heterozygosity. More, functional genomics require perfectly annotated genes since slight variation in protein-coding sequences can lead to protein miss function or inefficient gene editing.

To fulfill this gap, we have sequenced a new RPW genome, generated multiple RNAseq data from key tissues, and manually curated gene families involved in important functions, including immunity, detoxification, digestion, and chemoreception. This allowed us to 1) correct previous gene numbers and sequences, 2) evidence expansions of CYPs trough alternative splicing, and duplication of digestion enzymes, 3) identify the highest number of ionotropic chemosensory receptors in Coleoptera, 4) reveal tandem duplication of key odorant receptors whose function could be addressed.

Our results suggest that the RPW metabolic gene repertoire is adapted to tree feeding and xenobiotic resistance, while its chemosensory repertoire is adapted to its interaction with palm trees. Our collection of curated genes constitutes a valuable resource for functional genomics,

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and our functional data suggest that plant odor receptors specialized to detect pheromone cues. Volatile screens also pinpoint interesting volatiles to be included in olfactory-based RPW control strategies.

Keywords: Rhynchophorus ferrugineus, genome, functional genomics, chemosensory receptors, plant insect interaction

34 - Signals of generalist plant species adaptation to local pollinator communities and abiotic factors

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[1] Déterminismes génétiques et environnementaux de l'adaptation des plantes à des systèmes de cultures innovants – Agroécologie [Dijon] – France

The combined effect of changes in pollinator communities, and the direct impact of soil and climate variation on plant-pollinator interactions can strongly affect the reproductive success of flowering plants. However, knowledge of the adaptative potential of plants to complex ecological networks and the underlying genetic mechanisms is still limited. Based on a pool-sequencing approach of 21 natural populations of *Brassica incana* in Southern Italy, we combined a genome-environmental association (GEA) analysis with a genome scan for signature of selection to discover genetic variants associated with pollinator communities, edaphic and climatic variation. We identified genomic regions involved in adaptive response of *B. incana* to both single pollinator species and the overall pollinator interactions. Interestingly, we observed a significant number of genetic variants shared between the soil texture (fine silt) and the visits of bumblebees and hoverflies, while few genetic variants involved in both pollinator and climate variation were identified. Our results highlight the adaptive potential of generalist species to complex biotic interactions, and the importance of considering multiple environmental factors to describe their adaptive landscape.

Keywords: Ecological genomics, plant, pollinator interactions, natural populations, genome, environmental association, biotic factors

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Poster - Monitoring of ecosystems functioning and health / Eco-exposome

01 - Advancing Freshwater Biomonitoring with Environmental DNA: a case study targeting algae, macroinvertebrates and fish in water and biofilm

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Freshwater ecosystems face escalating threats due to climate change and human activities, underscoring the need for effective biomonitoring approaches. Traditional methods for biomonitoring have intrinsic limitations, prompting the exploration of molecular techniques like DNA metabarcoding. In this year-long investigation, we sought to evaluate the complementarity of environmental DNA (eDNA) signals in both water and biofilms concerning algae (23S), macroinvertebrates (COI), and fish (12S). The survey was conducted in a near-shore zone of Geneva lake submitted to erosion. Our primary objective was to provide valuable insights into optimizing sampling strategies for eDNA biomonitoring purposes. This investigation underscores the complementarity of biofilm and water matrices in providing a comprehensive perspective on biodiversity for algae and macroinvertebrates. For fish, the choice of sampling matrix can be influenced by field convenience since both matrices provide robust inventories of fish diversity. Our study offers valuable insights into the challenges and advantages associated with utilizing eDNA for multi-taxonomic surveys in aquatic environments, and allow drawing practical recommendations for the design of future sampling strategies for biomonitoring.

Keywords: eDNA, biomonitoring, multi, taxa, lake, water, biofilm

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02 - Metabarcoding and metabolomics reveal the effect of the invasive alien tree Miconia calvescens DC. on soil diversity on the tropical island of Mo'orea (French Polynesia)

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Miconia calvescens is a dominant invasive alien tree species that threatens several endemic plants in French Polynesia (South Pacific). While most analyses have been performed at the scale of plant communities, the effects on the rhizosphere have not been described so far. However, this compartment can be involved in plant fitness through inhibitory activities, nutritive exchanges, and communication with other organisms. In particular, it was not known whether M. calvescens forms specific associations with soil organisms or has a specific chemical composition of secondary metabolites. To tackle these issues, the rhizosphere of six plant species was sampled on the tropical island of Mo'orea in French Polynesia at both the seedling and tree stages. The diversity of soil organisms (bacteria, microeukaryotes, and metazoa) and of secondary metabolites was studied using high-throughput technologies (metabarcoding and metabolomics, respectively). We found that trees had higher effects on soil diversity than seedlings. Moreover, M. calvescens showed a specific association with microeukaryotes of the Cryptomycota family at the tree stage. This family was positively correlated with the terpenoids found in the soil. Many terpenoids were also found within the roots of M. calvescens, suggesting that these molecules were probably produced by the plant and favored the presence of Cryptomycota. Both terpenoids and Cryptomycota were thus specific chemicals and biomarkers of M. calvescens. Additional studies must be performed in the future to better understand if they contribute to the success of this invasive tree.

Keywords: bacteria, biological invasion, metabolites, metazoans, microeukaryotes

*Speaker

03 - The sea urchin, Paracentrotus lividus, serves as a model organism for studying the cocktail effects of chlordecone and the main pesticides present in the islands of Martinique

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In the Anthropocene era, organisms face escalating exposure to synthetic molecules, particularly endocrine disrupting chemicals (EDCs), which, even at low chronic doses, have marked biological effects. Chlordecone (CLD) is an EDC heavily utilized in Martinique from 1973-1993 as a banana crop insecticide. Characterized as a persistent organic pollutant (POP) due to its high chemical stability, chlordecone persists in Martinique today. CLD readily bioaccumulates, leading to chronic exposure for Martinique citizens. The 2023 OPALE report identified CLD, AMPA (glyphosate degradation product), and azoxystrobin (fungicide) as primary pesticides in Martinique. CLD exposure is associated with higher risks of cancers, developmental and gestative impairments, and endocrine disorders. However, the cocktail effects of CLD and Martinique's main pesticides remain unknown. Using the sea urchin model *Paracentrotus lividus,* the LiCOCO project aims to elucidate the cocktail effects of CLD and primary pesticides in Martinique. *P. lividus* has a sequenced genome and human orthologs. *P. lividus* adults will be exposed to chlordecone alone and to a pesticide cocktail representing those found in Martinique. Using RNA-Seq analysis, the impact of cocktail pesticides on gene expression will be assessed. This evaluation will focus on genes related to oxidative stress, detoxification, endocrine response, inflammation, cellular proliferation, and cell death. The results of this study will unveil the risks of cocktail pesticide exposure, including CLD, for both human and environmental health.

Keywords: Chlordecone, RNASeq, Cocktail pesticides, Paracentrotus lividus, Endocrine disruptors

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04 - Variation in mosquito microbiota composition in different habitat types on the Mediterranean coast

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Vectorial capacity depends on numerous factors such as the pathogen community (richness, abundance), the vertebrate host community (e.g. richness, abundance), the vector ecological and behavioural traits (e.g. life cycle, biting rate, aggressiveness), and the environment (e.g. temperature, water quality). Endosymbiotic bacteria communities also play a major role in vector biology and affect their vectorial capacity. Under changing environmental conditions, changes in the diversity and abundance of microorganisms can therefore have a direct impact on the dynamics of host-vector-parasite interactions. The aim of the study was to evaluate how anthropogenic pressures such as urbanisation can affect mosquito microbiota. In this exploratory project, the microbiota of 58 mosquitoes from two species collected from June to October 2020 was analysed individually using 16S metabarcoding. Mosquitoes were collected at nine sites spread over the Mediterranean coast, which present variation in artificial surface from 0 to 51%. We found that the microbiota composition depends not only on the mosquito species but also on habitat characteristics.

Keywords: microbiota, mosquitoes, vectorial capacity, artificial surface

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Poster - Exploring ecosystems using metagenomics

05 - CRISPR-Cas9 enrichment for microbial metagenomics

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Environmental integrons (EI) are genetic elements ubiquitous in natural microbial communities suspected to play a role in bacterial adaptation. Yet, due to methodological limitations, they are poorly characterized hindering their eco-evolutionary significance. Here, we describe an innovative approach combining CRISPR-Cas9 enrichment with long-read nanopore sequencing to target a putative adaptive EI, InOPS, from a microbial metagenome of oil-contaminated coastal sediments. A contig of 20 kb was recovered allowing to unveil InOPS complete structure and genetic context. The integrase, closely related to integrases of marine Desulfobacterota, harboured the canonical features of a functional integron integrase. The gene cassettes have mostly unknown functions hampering inferences about their ecological importance. Moreover, the putative InOPS host, likely a hydrocarbonoclastic marine bacteria, raises questions as to the adaptive potential of InOPS in response to oil contamination. Finally, mobile genetic elements within InOPS region highlights likely genomic plasticity, source of genetic novelty. This case study showed the power of CRISPR-Cas9 enrichment to elucidate the structure and context of specific DNA regions for which only a short sequence is known. This method is a new tool for environmental microbiologists working with complex microbial communities to target low abundant, large or repetitive genetic structures hardly recover by classical metagenomics. More precisely, here, it offers new perspectives to comprehensively assess the eco-evolutionary significance of EI.

Keywords: complex genomic regions, CRISPR, Cas9 enrichment, environmental integrons, microbial communities, microbial metagenomics, mobile genetic elements

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06 - Deciphering environmental integrons in pristine environment reveals their significance for ecosystem functioning and health

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Environmental integrons (EI) are genetic elements requiring in-depth study in a One Health perspective, due to their capacity to disseminate resistance genes at risk for human and animals. Yet, contrary to clinical integrons, the eco-evolutionary significance of EI and their role in bacterial adaptation are still elusive. Here, we investigate the diversity and spatio-temporal dynamics of EI in a French peatland to decipher their ecology and evolution in poorly-anthropized ecosystems. Untargeted metagenomic combining short- and long-read sequencing over 24 samples revealed 2875 EI elements including complete integrons, CALINs (gene cassette arrays) and In0 (integron integrase genes). Over the 515 MAGs, 35 harboured complete EI. A wide range of elements were hosted by Acidobacteriota and Nitrospirota, both key phyla in peatland functioning. As well, poorly characterized phyla such as Binatota and Gemmatimonadota exhibited EI while Archaea harboured only CALINs. Moreover, in agreement with previous studies, Alphaproteobacteria and Actinobacteriota recurrently lacked EI. Integron integrase genes distribution evidenced structuring along the environmental gradient. Importantly, no clinical integrons were detected suggesting environmental filtering and/or poor anthropization. However, several gene cassettes being resistance determinants may be at risk. The recovery of integron integrase and gene cassette transcripts suggested that integrase might trigger the dynamics of some elements while certain gene cassettes could play a role in the peatland functioning. Together, our results show that EI do play a role in natural context and may serve as reservoir of resistant genes.

Keywords: environmental integrons, peatlands, metagenomics, metatranscriptomics, metagenome assembled genomes

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07- Evaluation of the environmental fate and impact of biopesticides using an innovative approach coupling high-throughput methods

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Biopesticides are complex substances that are derived from natural sources (e.g., plants, microorganisms), and recently offer a promising alternative to traditional pesticides, but it is still unknown how long biopesticides and their residues remain in the environment, then how long they impact organisms living in soil (prokaryotic and microeukaryotic species).
In this context, we conducted an experiment to evaluate the environmental fate and impact of Beloukha, a bioherbicide containing Pelargonic acid as active substance. A kinetics study was performed over 56 days in soil microcosms comparing treated and non-treated conditions. The samples were analyzed using high-throughput omics techniques (16S and 18S rRNA gene metabarcoding and metabolomics UHPLC-HRMS).

Thanks to the metabolomics data, we could determine the dissipation time. It corresponds to the time required for the dissipation of the biopesticides compounds (i.e. the Beloukha extract with the formulating agents). Bioinformatics approaches allowed us to separate the soil metabolites and the degradation products from the pesticide components. Subsequent kinetic modeling revealed that 99% of the biopesticide compounds have half-life below 30 days.
Metabarcoding analyses revealed biodiversity changes over time (with alpha and beta diversity) as well as the impact of the biopesticide and its degradation products on bacteria. In particular, we found that bacterial assemblages were only impacted at the first-time steps of the experiment. Moreover, correlation analyses revealed high correlations between biopesticide components and three bacterial genera (Anaeromyxobacter, Mycobacterium and Rhodococcus).

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Keywords: Metabarcoding metabolomics bioinformatics statistical analysis genomics analytical chemistry

08 - Efficient Multiplexing of Pollinator Metabarcodes Using Nanopore Sequencing: Insights for Meadow Management from Floral Environmental DNA

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Many pollinating species are declining globally, making effective, fast, and portable pollinator monitoring methods very important. Pollinators can leave DNA on flowers, and metabarcoding of this environmental DNA (eDNA) provides an opportunity to detect the presence of flower visitors. This study introduces an efficient, cost-effective workflow for utilizing DNA barcoding to monitor biodiversity through environmental DNA (eDNA) left on flowers from pollinators, employing Nanopore sequencing on the MinION. The method employs multiplexing with dual molecular tags on universal cytochrome oxidase 1 (COI) barcode primers. We used this approach to compare the arthropod diversity present in three meadows with different vegetation at three time points during the flowering season. We describe the presence and interactions of 65 species from 30 families. We multiplexed individual eDNA samples from hundreds of flowers and found plant-pollinator dynamics that showed differences in species richness between sampling times and meadow diversity. Comparative analyses with conventional methods showed eDNA metabarcoding's ability to identify diverse species and ecological interactions compared to field sampling. While some DNA likely came from eggs or microscopic insects difficult to remove from flowers, traces of eDNA from various arthropods on multiple plant species confirmed the method's applicability, promising robust ecological monitoring and research potential in the wake of global pollinator declines. This is the first reported use of MinION based nanopore sequencing to detect arthropod species from eDNA samples collected from flowers using the described affordable multiplexing method.

Keywords: environmental DNA, metabarcoding, nanopore sequencing, pollinator diversity, meadow management

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09 - Fine-scale congruence in bacterial community structure from marine sediments sequenced by short-reads on Illumina and long-reads on Nanopore

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3

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Following the development of high-throughput sequencers, environmental prokaryotic communities are usually described by metabarcoding with genetic markers on the 16S domain. However, short-read sequencing encounters a limitation in phylogenetic coverage and taxonomic resolution, due to the primers choice and read length. On these critical points, nanopore sequencing was much undervalued because of its relatively higher error rate per read. Here we compared the prokaryotic community structure in a mock community and 52 sediment samples from two contrasted mangrove sites, described by short-reads on 16SV4-V5 marker (ca. 0.4kpb) analyzed by Illumina sequencing (MiSeq, V3), with those described by long-reads on bacterial nearly complete 16S (ca. 1.5 kpb) analyzed by Oxford Nanopore (MinION, R9.2). Short- and long-reads retrieved all the bacterial genera from the mock, although both showing similar deviations from the awaited proportions. From the sediment samples, with a coverage-based rarefaction of reads and after singletons filtering, co-inertia and Procrustean tests showed that bacterial community structures inferred from short- and long-reads were significantly similar, showing both a comparable contrast between sites and a coherent sea-land orientation within sites. In our dataset, 84.7 and 98.8% of the short-reads were assigned strictly to the same species and genus, respectively, than those detected by long-reads. Primer specificities of long-16S allowed it to detect 92.2% of the 309 families and 87.7% of the 448 genera that were detected by the short 16SV4-V5. Long-reads recorded 973 additional taxa, some belonging to 11 exclusive phyla, albeit accounting for only 0.2% of total long-reads.

Keywords: microbial metabarcoding, environmental DNA, methods, primers, diversity

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10 - MetaGenomic-based Quantification of Plankton

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Marine plankton are crucial actors in major oceanic biogeochemical cycles and climate regulation. To gain a better understanding of plankton communities composition and functions, biogeography or evolution – especially in the context of climate change – it is necessary to acquire data that combine genomic resolution with quantitative measurement. Current metagenomic analyses can address ecological questions with high resolution regarding taxonomic composition and population or functional diversity at genomic level. But most data remain compositional, providing relative abundances of taxa or genes that are sample related. Omic relative abundances limit intersample comparisons and do not refer to absolute in situ quantities. Previous studies have also demonstrated that quantitative omic approaches could supplement or even rectify results based on compositions.
Here, we propose a DNA based quantification of marine plankton at both size community and genome levels. Using metagenomic and imagery data from *Tara Oceans* plankton samples, we show that the quantitative values we obtain could be proxies for the biovolume or the biomass of planktonic communities. Based on this, we estimate cell concentrations for almost 700 eukaryotic and 8000 prokaryotic environmental genomes that are among the most abundant in *Tara Oceans* metagenomes. This resource gives a new quantitative vision of plankton biogeography and will be of interest for biogeochemical modeling.

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11 - Monitorization of the community structure and diversity of soil microbiome in a young forest

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This project aims to profile and monitor the soil microbiome of a reforested area, spanning from initial planting to a five-year period. The main objective is to discern potential correlations between tree species and the extent of microbiome diversity, with a critical evaluation of their combined role in carbon sequestration.
Our study analysed 80 soil samples, systematically drawn from 64 soil cores from a reforested plot (experimental group) and 16 cores from an adjacent non-reforested site (control group) in central Portugal. The sampling, conducted in 2021, and revisit in 2022, encompassing consociations of Pine sp. and Quercus sp. (48 soil cores), and a solely Pine or Quercus (8 soil cores each) reforested zone across two discrete depths (10-20 and 20-30 cm). Metagenomic sequencing was used to amplify 16S, 18S, and ITS rRNA genes for discrimination of archaea, bacteria, eukaryota and fungi.

The analysis of the microbial diversity among samples revealed a profound divergence in bacterial microbiome composition within the reforest groups across the two sampling years, indicating significantly higher diversity in 2021. *Conexibacter* emerged as the predominant bacterial genus, while *Penicillium* held precedence within the fungal community. Intriguingly, the counts of ASVs within eukaryotes revealed a pronounced year-on-year variation.
The initial data analysis presented reveals stark differences between the microbiomes of non-reforested and reforested soils. It emphasizes noticeable changes in microbiome diversity, especially at greater depths (10cm-30cm), over time. This study offers insights into how the microbial ecosystem changes as years pass since tree planting and the impact of tree species and their combinations.

Keywords: soil microbiome, metagenomics, biodiversity, pine, quercus

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12 - Plankton Communities and Marine Ecosystems at the Scale of Protein Folds

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Although significant progress has been made in understanding gene repertoires and functional diversity of oceanic plankton in recent years, the distribution and diversity of the structures of the protein domains coded by those repertoires remains poorly understood. However, they represent a highly informational biological level intermediate between genotype and phenotype and are necessarily subject on some level to selective pressures. 3071510 proteins from the eukaryote MAGs from the Tara Oceans expedition (Delmont *et al.*, 2022) were annotated structurally using CATH (Sillitoe *et al.*, 2021). This allowed for an estimation of the distribution of the abundances of 910 folds across natural eukaryotic diversity and the oceanic basins. It was found that these distributions follow a Pareto law, a mathematical law that describes well systems operating with preferential attachment processes. The analysis of the parameters of this law at the plankton sampling sites resulted in a classification of the folds into three categories carrying different levels of biogeographic information. As a result, novel patterns of organization were observed at the level of the fold communities. Eventually, biogeographies at the scale of each fold were estimated to identify folds which spatial distribution in the oceans is linked to environmental context rather than the community of organisms. These folds represent good candidate for further structural analysis, especially experimental determination of thermodynamic properties.

Keywords: protein fold, biogeography, eukaryote, model

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13 - ROSKO-GO : A Genomic observatory at the Roscoff Marine Station

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Understanding the changes of marine biodiversity and ecosystem functioning are widely relies on long-term observation at different spatial and temporal scales. Observation activities at the Roscoff marine station, implementing long-term series initiated over 20 years ago for physical, chemical and biological variables, now widely integrates -omic data to broaden the scale and scope of marine environment monitoring. Activities of the Roscoff genomic observatory occur in the context of four interacting initiatives: the local genomic observation program GENOBS, the national Augmented Observatories of the European Marine Biological Resource Centre AO-EMBRC, the European Marine Omics Biodiversity Observation Network EMO-BON and the Future-Obs project. GENOBS aims to monitor changes in plankton communities using genomic data (metabarcoding, metagenomics and metatranscriptomics) from the water column of the Bay of Morlaix. EMO-BON is the first coordinated long-term biodiversity observatory based on -omics at the European scale. Its major goal is to collect samples from a variety of habitats (water column, soft sediments and hard substrates), with habitat-specific frequencies, to analyze the marine biodiversity from Norway to the Red Sea, using standardized operating procedures for metabarcoding and metagenomics, and continuously feeding biodiversity databases. GENOBS and EMO-BON contribute to the development of a French genomic observatory as supported by the AO-EMBRC and Future-Obs project in which data from environmental genomics, automated imaging technologies, and physico-chemical and biogeochemical sensors will be integrated

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through long-term time series at fixed stations.

Keywords: eDNA, genomic observation, long time series, marine biodiversity

14 - The anti-MGE defensome of complex microbial communities

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Bacteria have developed various defense mechanisms to avoid infection and killing in response to the fast evolution and turnover of viruses and other genetic parasites. Such pan-immune system (or *defensome*) encompasses a growing number of defense lines that include well-studied innate and adaptive systems such as abortive infection, CRISPR-Cas and restriction-modification, but also newly found ones whose mechanisms are still poorly understood. While the abundance and distribution of defense systems is well-known in complete and culturable genomes, there is a void in our understanding of their diversity and richness in complex microbial communities. Here we performed a large-scale in-depth analysis of the defensomes of 7,759 high-quality bacterial population genomes reconstructed from soil, marine, and human gut environments. We observed a wide variation in the frequency and nature of the defensome among large phyla, which correlated with lifestyle, genome size, habitat, and geographic background. The defensome's genetic mobility, its clustering in defense islands, and genetic variability was found to be system-specific and shaped by the bacterial environment. Hence, our results provide a detailed picture of the multiple immune barriers present in environmentally distinct bacterial communities and set the stage for subsequent identification of novel and ingenious strategies of diversification among uncultivated microbes.

Keywords: defense systems, phage, bacteria arms race, metagenome assembled genomes, defense islands, environmental defensomes

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15 - The relative effects of abiotic and biotic factors in explaining the structure of soil bacterial communities at diverse taxonomic levels in natural sites inhabited by Arabidopsis thaliana

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Soil bacterial communities contribute to diverse ecosystem processes, including nutrient and carbon cycling, plant productivity, pathogen management, greenhouse gas emissions and bioremediation. Consequently, environmental drivers of soil bacterial structure have been extensively investigated at diverse geographical scales. While informative, most of these studies focused on identifying abiotic factors explaining variation of the soil bacterial structure at a low taxonomic resolution (*e.g.* phylum). In this study, we aimed at estimating the relative effects of abiotic and biotic factors in explaining the structure of soil bacterial communities at diverse taxonomic levels. To do so, we characterized the soil bacterial communities of 160 natural populations of *Arabidopsis thaliana* located south-west of France, with a *gyrB* marker that has a deeper taxonomic resolution (*e.g.* species level) than the traditional 16S marker. These natural populations have been previously characterized for a large set of ecological factors, including climate, soil physico-chemical properties, descriptors of plant communities and spatial descriptors. We observed a switch in the main category of explanatory ecological factors according to the taxonomic level, with the identification of soil properties and descriptors of plant communities as the main factors shaping bacterial communities at the low and high taxonomic resolution, respectively. Interestingly, most of the associations with plant communities were positive. Altogether, our results suggest that plant species could be used to shape soil microbiota at a functional level.

Keywords: soil, microbiota, plant, metagenomics, sequencing, ecology, climate, soil properties

*Speaker

16 - Unlocking the Soil Microbiome: Unraveling Soil Microbial Complexity using Long-Read Metagenomics

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The soil microbiome remains poorly understood, but unraveling its genetic diversity is essential, given the pivotal functions primarily mediated through their protein arsenal (1). Although short-read (SR) shotgun metagenomics provided interesting insights into microbiome gene diversity, it fell short in delivering comprehensive microbial genome reconstructions. Metagenome-assembled genomes (MAGs) obtained from SR often yield fragmented assemblies and incomplete gene sets (over 90% contigs

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2. Belliardo, C., et al., 2022, Scientific Data 9, 311
3. Gaëtan, B., et al., 2023. BioRxiv

Keywords: Metagenomics, Soil Microbiome, Genome assembly, HiFi sequencing, method benchmark

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17 - Using DNA from digestive contents to highlight the dietary diversity of an invasive alien species feeding on soil macrofauna

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Invasive alien species (IAS) can be responsible for many ecological disturbances particularly through their predatory behavior. Identifying consumed prey is then a key step to characterize the impacts of an IAS on indigenous biodiversity. Due to the cryptic behavior of some predators, it is not always possible to observe predation directly. Diet identification can then be carried out through metagenomic approaches, using high-throughput sequencing of DNA from digestive contents.

Our study model is a terrestrial flatworm native to Argentina, *Obama nungara*, which has recently invaded Europe and is now reported in more than 70 departments in metropolitan France thanks to citizen science programs. Mostly found in anthropized environments, this species is known to feed on earthworms, gastropods, and other flatworms, but field data are still missing. DNA metabarcoding, a powerful approach for the taxonomic identification of consumed species, was then used to characterize the diversity of earthworms consumed by *O. nungara* and potential diet preferences.

In a first study based on samples collected through a citizen science program, high-throughput sequencing of an earthworm-specific fragment of the 16S rRNA gene allowed us to analyze digestive contents from 35 samples and to validate our metabarcoding approach. In a second ongoing study, flatworms are collected twice a year for three years and earthworms' communities are analyzed, allowing us to compare data obtained from the sequencing of digestive contents with prey diversity on the field. Results for the first year of sampling are presented here and we discuss how soil ecosystems may be impacted by *O. nungara*'s predation on different ecological categories of earthworms.

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Keywords: Invasive flatworm, metabarcoding, predation, earthworms' communities

Poster - Exploring diversity and evolution of Life

18 - Bioinformatic study of the genomes evolution and their epigenomes of different Rosaceae

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1 Introduction

The apple tribe is native to the Tian Shan mountains in central Asia. The common ancestor of apple and pear underwent a whole genome duplication (WGD) that occurred during Himalayan chain formation. This WGD, dated to 27 Mya, resulted in the development autopolyploid plants containing two identical subgenomes. Duplicated genes originating from WGD are named ohnologous genes, from Ohno.

WGD is considered a genomic shock, which added to the environmental changes due to ground elevation (temperature, UV...), may have led to a burst of transposable elements (TE) 21 Mya. In a previous study we identified that QTLs in apple are not evenly distributed among ohnologous chromosomes. This imbalance has been primarily associated with significant differences in the expression level of ohnologous genes M. domestica.

2 Apple genome evolution

In all the available public 149 RNA-seq experiences derived from a wide array of apple cultivars (Pink Lady, gala, Honeycrisp, Fuji, Golden Delicious, hybrids and more) we performed differential expression analysis comparing expression of ohnologous genes within the same experimental condition. Surprisingly, we found 828 ohnologous genes pairs for which one gene of the pair systematically overexpressed relative to the other, in all the RNA-seq experiences. These genes are called non switching.

In this project we focus on these 828 non switching ohnologous gene pairs. We compare them to the 808 ohnologous gene pairs for which differential expression level varies throughout the

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RNA-seq experiences (called swiching genes). Our objective is to identify the epi/genetic mechanisms, including TE composition and DNA methylation, that can explain the expression of these striking non switching genes.

Keywords: apple evolution, genomics, transcriptomics, transposables elements, epigenomics

19 - Environmental genomics of honey bees through the direct sequencing of hive products

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Performing whole-genome resequencing in honey bees is facilitated by the relatively small genome size of the species (225 Mb) and the haploidy in males, allowing to generate high quality phased data at a reasonable cost. Over the last decade, our research team located at INRAE in Toulouse, France, has put considerable effort into collecting and sequencing hundreds of haploid males to describe the population structure and levels of diversity of honey bees in Europe. Collecting drones however requires regular visits to the hives, which can be an issue for large regional sampling. Our "Mallaurie" project aims at testing a potential alternative for future population genomic analyses, namely the direct shotgun sequencing of honey samples, as well as other beehive products (e.g. beeswax, propolis). Sequencing DNA from beehive products instead of individuals would have several advantages associated with our research: (i) non-lethal sampling, (ii) lower carbon footprints (iii) easy access to a large diversity given the importance of the market around the globe and (iv) access to metagenomic data, such as microbiome, parasites, plants etc. We detail here results obtained as part of a pilot study based on 30 honey samples, especially focusing on the endogenous content, i.e. proportion of reads corresponding to the bee DNA. We then open up some prospects at the interface between individual sequencing and environmental DNA.

Keywords: Genomics, metagenomics, Honey bee, Honey, beehive products

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20 - Exploration of the protein reservoir of unknown functions of the Ocean

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Unicellular eukaryotes are highly diverse and abundant in the sunlit ocean, where they play major roles in the marine food web and climate, but are understudied compared to their bacterial counterparts due to the complexity of their genomes. Recently, hundreds of environmental genomes covering abundant marine unicellular eukaryotes were characterized from the Tara Oceans metagenomic legacy. We found that genes of unknown function (42% total gene pool) represented 46% of the total expression levels based on metatranscriptomics, underlining the role of unknowns on the functioning of unicellular eukaryotes. To further explore this pool of unknown, we are developing a gene-centric multi-layer approach that includes protein clusters, predicted 3D structures, gene expression levels and environmental metadata. As a first insight, our results show that most genes of unknown function highly expressed in situ correspond to singletons with no close relative in our millions proteins database. This contrasts with the highly expressed genes with known functions that often connect multiple environmental genomes. Nevertheless, we also found many unknown genes commonly shared and highly expressed among these unicellular eukaryotes. In those cases, predicted 3D structures are essential, enabling us to expand the scope of these protein clusters and provided functional insights by comparing them with known protein structures. Thus, our preliminary results made it possible to contextualize key genes of unknown functions. Overall, our research into eukaryotic unknowns offers opportunities to interrogate genomes, metagenomes, assign biological context to genes without known annotations and potentially discover novel functions.

Keywords: Unicellular eukaryotic plankton, Environmental genomes with Tara Oceans, Unknown functions, Multi, layer approach

*Speaker

21 - From corals to plankton: Unraveling parasitic diversity across marine environments

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Parasitism is a far-reaching way of life in nature. Yet we don't know much about this trophic mode in marine ecosystems, either in terms of its global distribution patterns or the evolutionary processes behind them. A biogeographical study was conducted using metabarcoding data from the Tara Pacific expedition to better characterize the underlying patterns and forces governing the diversity of ocean parasitism within two major clades. The apicomplexans, major references for parasites responsible for human and veterinary diseases but with only few marine representatives described, and the Syndiniales, a clade of early-branching parasitic dinoflagellates highly abundant in marine ecosystems but little described. Tara Pacific's data enabled us to explore a wide range of environments, from plankton in oceanic and coastal waters to sediments and corals. Overall patterns of Apicomplexan and Syndiniales diversity varied between and within the 3 biomes studied (coral, fish and plankton), as well as geographically. Corallicolid apicomplexans are widespread within coral tissues. A transcriptomic approach allowed us to study both its genetic content and its interaction with the coral host. Planktonic environments are richer and more diverse in parasites than coral and fish samples, with a high abundance of MALVs and a dominance of crustacean-parasitic gregarines for Apicomplexa. The impact of environmental factors on the distribution of these clades in the various samples will be shown.

Keywords: Parasitism, Biogeographical, Metabarcoding, Apicomplexans, Syndiniales, Marine ecosystems

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22 - Full annotation of IG loci in Lemur catta and evolution

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The immune system provides innate and adaptive immunity. The innate immune system protects against infection during the initial exposure to a pathogen, while the adaptive immune system remembers previous encounters with specific pathogens and can respond quickly in the case of repeated exposure. Immunoglobulins (IG) and T cell receptors (TR) are crucial in identifying antigens and eliminating threats. An IG is a heterodimer composed of two heavy chains (IGH) and two light chains produced by B cells and can be found on the membrane or in the soluble form known as the antibody. Studying immune responses in different species is essential for developing vaccines and therapeutic and diagnostic tools.
Lemurians are early-branching organisms in primate evolution, and they have been isolated in Madagascar. Different species of Lemorians exhibit different types of social behavior. Lemur catta is a species living in groups. Studying Lemur catta's adaptive immune response enables a better understanding of the evolutionary history of immune response in primates.

The Lemur Catta genome is currently being analyzed. Sequences coding for key proteins in the acquired immune response have been retrieved from databases and annotated. Semi-automated annotation of the IG regions has identified 221 functional genes, 71 pseudogenes, and 24 ORFs.

In this work, the annotation of the three loci of IG (IGH, IGL, and IGK) is presented after following the IMGT-ONTOLOGY. The V-genes at the IGH and IGL loci are mainly of subgroup 3, which can be part of antibodies in humans. IGK locus has subgroup 1 and 2 V-genes. Most paralogues have a single lemurian origin.
The high quality of the annotation enables us to study V(D)J recombination by RNAseq analysis.

Keywords: immunogenetics, immunoglobulin (IG), biocuration, adaptive immune system, T cell receptors (TR), Lemur catta

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23 - Genomic analyses of light-receptors in the open-ocean microalgae Pelagomonas

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Phytoplankton are aquatic micro-organisms generating energy through photosynthesis. In oceans, they live close enough to the surface to benefit from sunlight and deep enough to access nutrients brought up by water column mixing. Climate change will warm ocean surfaces and create oligotrophic conditions. Only adaptable phytoplankton species surviving in oligotrophic conditions or accessing nutrients at depth are expected to persist.
Pelagomonas calceolata, a cosmopolite photosynthetic pico-eukaryote (PPE), is abundant in oligotrophic oceans thanks to its small size and effective adaptation capacities. We observed that *P. calceolata* genome carry a large number of genes coding Light-Harvesting Complexes (LHC) similarly to its closest relative, the low-light bloom-forming *A. anophagefferens*. Among LHC, the subfamily LHCy is particularly amplified, suggesting a particular role of these proteins for the adaptation to low-light conditions.

In this project, we investigate how *P. calceolata* thrives in low light and what are the genes supporting this adaptation. Starting with environmental metatranscriptomes collected during *Tara* expeditions, we will search for differentially expressed genes in varying light environments, focusing on LHC and different families of light-sensitive proteins. We will then cultivate *P. calceolata* under different light intensities and wavelengths, extract mRNAs and sequence them to carry out differential gene expression analyses, to identify expression differences in known light-sensitive genes and potential genes of unknown functions.
Overall, we believe that this project will highlight adaptive mechanisms of microalgae to low-light conditions in the context of global ocean disturbance.

Keywords: Pelagomonas, microalgae, oceans, transcriptomic, light, LHC

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24 - Taxonomic identification of plants using a new matK cocktail of primers

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Identifying plants at the species level and at any developmental stage can be a challenging task in ecology. It generally requires the help of experienced botanists and is still impossible at very early developmental stages. Moreover, results are still dependent on the botanist and are error-prone.
Another way, more reliable and reproducible, to do so is to use DNA barcoding which relies on the identification of a species using a short sequence of DNA. Many molecular markers have been developed in order to identify plants but none is universal with a high taxonomic resolution. The chloroplast maturase k gene (matK) provides such a high taxonomic resolution. However, in our hands, the best primers found in the literature amplified only 80% of plant samples collected in 160 *A. thaliana* populations from the midi Pyrénées region.

We first try to develop new degenerated primers but they fail to amplify, probably because of a loss of locus specificity. Therefore, we developed a cocktail of primers that enable to increase universality without decreasing the specificity for the matK gene.
In the present study, we assessed both the universality and the accuracy of this new cocktail of primers on a wide range of plant samples (tree, ornamental, vegetable plant...). To do so we compared the results obtained on one hand through sequencing of the matK marker gene and on the other hand determined by an experienced botanist.

Keywords: matK gene, ecology, plant identification, species

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25 - Unraveling the genetic basis of host-alternation in aphids using comparative genomics.

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Host-alternation or heteroecy in aphids involves a complex life cycle where an obligatory migration between botanically different host plants is needed. This migration is triggered by environmental signals that induce the production of specific winged morphs. Heteroecy is present in all 3 families of aphids but only 10% of the 4800 aphid species are heteroecious. The evolutionary history of heteroecy remains elusive due to unresolved aphid phylogeny. While several studies support the hypothesis that host-alternation is the ancestral state of the *Aphidinae* subfamily, representing half of the described aphid species, a comprehensive understanding is lacking. Additionally, the genetic basis of heteroecy is poorly understood. This study aims to address this gap using a comparative genomic approach to characterize global genomic patterns and candidate genes associated with heteroecy in the *Aphidinae* subfamily by comparing the genome and gene repertoire of host and non-host alternating species.
Assuming that heteroecy is the ancestral state of *Aphidinae*, we propose that non-alternation or monoecy is a derived state. This loss of function in monoecious *Aphidinae* species is expected to lead to reduced selection pressure on genes involved in heteroecy, potentially resulting in gene alterations. We compared 31 complete genomes of *Aphidinae* species (15 heteroecious and 18 monoecious species) to identify orthologous genes and assessed their integrity by analyzing evolutionary rates (dN/dS ratio), the occurence of stop-codons, and gene duplication events.
Subsequent work will test whether monoecious and dioecious species show genomic differences, and attempt to identify candidate genes associated with host-alternation in aphids.

Keywords: Comparative genomic, Polyphenism, Host, alternation, Aphid, Genetic basis.

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Poster - Ancient DNA and paleo-environments

26 - Advancing Responsible Genomic Analyses of Ancient Mollusc Shells

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The DNA entrapped in ancient mollusc shells has the potential to shed light on the evolution and ecology of this diverse phylum. It could help reconstruct responses to past climate, aquaculture practices, pollution and extinction events at unprecedented time resolution. Such studies are lacking partly due to our limited knowledge of DNA preservation in calcium carbonate shells and the need for optimizing methods for genomic data generation from ancient shells. Here, we applied high-throughput DNA sequencing to 27 mollusc shells dated to 111-6,500 years Before Present to investigate the impact of micro computed tomography (micro-CT) scanning on ancient shell DNA to preserve morphological information, the performance of commonly used silica-based DNA extraction methods and predigestion treatments, and DNA recovery from the organic (periostracum) and carbonated (aragonite, calcite) mollusc shell layers. We propose recommendations for efficient and responsible genomic analyses of ancient mollusc shells. Micro-CT scans can be used without harming their DNA. Ancient shells' DNA is protected in preservation niches and its recovery was improved by double-digestion and predigestion bleach wash of shell powder. The periostracum, the calcite and the aragonite layers are good DNA reservoirs, the latter appearing as the best substrate for genomic analyses. Our results also give insight on long-term molecular preservation in biominerals.

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Keywords: Ancient DNA, DNA extraction, genomics, high, throughput DNA sequencing, mollusc shell, responsible research

Poster - Environnemental genomics and participatory science, openness to society

27 - Egyptian experience in the development of novel local Egyptian broiler lines in sustainable breeding strategies for hot climate resilience

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The natural genomic biodiversity and adaptability of African chickens facilitates sustainability of their productivity, whereas the performance of broilers is reduced in hot climate. Egyptian poultry breeders, therefore, aimed at developing new local Egyptian lines of broilers, as a model for similar initiatives in low and middle income nations. At Cairo University, since 2003, we are practicing breeding schemes on adapted local chickens to generate local crossbred lines (Cairo and Giza) from the initial crosses between two commercial broiler dam and sire lines, that exhibit high growth, and two local Egyptian breeds, that have adapted to heatwaves; White Baladi and Bandara. Individual selection was then practiced for high growth at 6-wk. In the Giza line, we introduced the Na gene to enhance heat tolerance by crossing the Naked-Neck breed with Giza chickens. Three chicken lines were established; Cairo, exhibiting normal feathering; Giza, normal feathering; and Giza, naked-necks. Last year, we crossed Giza males with Cairo females to produce, for first time in Egypt, the Cairo-Mix broiler. Although it is not as fast-growing as the international commercial strain, it performed much better than the locals. It reached 1.3 kg by 56 days of age compared to 600 g for the locals. The feed convention ratio was 2.1. The carcass dressing percentage was 65%. This improves chicken welfare and production. In prospective work, genomic selection will be integrated to accelerate breeding strategies. In addition, understanding which genes are associated with heat tolerance in chickens, will inform and enable breeding programs for producing more resilient birds – not only in smallholder farms, but also in larger production enterprises, globally.

Keywords: global warming, food security, animal welfare, local Egyptian chickens, genomics, environment interaction, animal protein, smallholder farms, growing human population, natural biodiversity

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28 - Identification of wild and domestic bees by non-destructive molecular methods

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The study of insect pollinator communities is at the heart of a great paradox: today, characterizing the diversity of these species implies sacrificing them. Methods based on the amputation of a member have been used in honey bees (Madella et al., 2021) or bumblebees (Holehouse et al., 2003) leading to mortality of the specimens. Several new non-lethal identification methods are developed such as acoustics, deep learning: mathematical algorithms capable of identifying specimens from insect photos (Høye et al., 2020), and sampling from faeces (Scriven et al., 2013). Rapid advances in sequencing methods, environmental DNA-based approaches have opened up promising prospects for inventorying biodiversity while preserving the integrity of specimens . We proposed to develop a non-lethal protocol to identify wild bees from the traces they can leave while foraging on flowers. It is based on the enclosure or exposure to the open air of strawberry plants and the collection of flowers for the extraction of insect DNA traces. The experimental protocol is composed of 4 strawberry plants placed in 3 conditions: a cage with insects, a cage without insects and exposed to the open air. Initially we extracted DNA from insect tracks left on flowers only in the "bees introduced into the enclosure" condition and by testing two types of extraction kits. The extracted DNAs were then amplified by PCR with the insect 16S minibarcode (Clarke et al., 2014). Several PCR conditions allowed us to select the DNA Extraction Kit that yielded sequencable amplifiates. Sequencing of 4 samples (2 duplicates) and bioinformatics processing of the data allowed us to find the sequences of the bees that were introduced but also sequences corresponding to 16S DNA of strawberry plants.

Keywords: eDNA, sequencing, Pollinator

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29 - Monitoring biodiversity of an alpine watershed using eDNA metabarcoding and ecological surveys: a collaborative work between students, scientists and citizens

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During the new master of Environmental Genomics program in Lyon, students conducted a two-year research project in collaboration with the scientific NGO "Le CREA Mont Blanc". In their first year, students collected environmental DNA (eDNA) from water and sediment in a river stream of the French Alps to determine the diversity of plants and mammals. They also visited the CREA observation sites, including camera traps and quadrats. Subsequently, DNA was extracted and amplified using primers targeting a chloroplastic gene for plants and 16S mitochondrial gene for mammals. In their second year, students developed an analysis pipeline to identify the taxa obtained by eDNA, in comparison with the ones detected by traditional methods: mammals were identified using camera trap images analyzed by artificial intelligence, scientists and participatory science (CREA's Wild Mont-Blanc project), while plants identified by botanists (le CREA). Although the majority of the observed species were recovered by eDNA, some taxa were specifically detected by only one of the two methods. Additionally, the students found a significant effect of the matrix (water or sediment) on the community composition. Finally, they concluded that altitude plays a crucial role in species distribution. As both a pedagogical exercise and a means of communicating their findings, the students authored a scientific article aimed at contributing to the exploration of the impact of climate change on fragile Alpine biodiversity and sharing this knowledge with the public.

Keywords: metabarcoding, master students, alpine watershed

*Speaker

30 - Studying Lebanese sourdough by exploring their microbial diversity with metabarcoding and conducting a comparative study on the different bread-making practices

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Although industrialization has led to the selection and spread of specific fermenting microbial strains, humans still rely on microbial communities to make fermented products. Lebanon similarly to other Mediterranean countries, still uses sourdough to produce traditional leavened breads known as "Saj" from traditional household and bakery practices. A wide microbial species diversity and genetic diversity have been found in home-made and bakery sourdoughs all over the world. However, the microbial diversity present in "Saj"making and Lebanese sourdough in general remains largely unknown. This study investigated both the bread making practices and the bacterial and fungal species diversity in sourdoughs collected from 26 bakers in different Lebanese regions. Statistical analysis was conducted on the bread making practices collected through interviews and questions asked during sampling from the different producers. In parallel, metabarcoding analysis was done on a total of 50 sourdough samples using V3-V4 and ITS1 for bacterial (16S) and fungal (ITS) diversity respectively. *Fructilactobacillus* was the most frequent bacterial genus in our samples (37% of the total reads) followed by *Levilactobacillus* (16%), *Lactiplantibacillus* (9.2%), and *Pediococcus* (5.8%). For the fungi, *Alternaria* genus was the most frequent (22% of the total number of sequences), followed by *Saccharomyces* (20%), and *Kazachstania* (10.5%). The bread making practices groups significantly influenced the level of fungal and bacterial α-diversity as well as B-diversity. All together these results revealed the

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impact of bread-making practices on sourdough microbial diversity, thus several hypotheses to explain this finding will be discussed.

Keywords: metabarcoding, sourdough, 16S, ITS, fungal diversity, bacterial diversity

31 - Where is Who? Inhabitants of soil aggregates

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The soil is a dynamic matrix composed of mineral, organic phases, and microbiota ensuring all major biogeochemical cycles and soil aggregation.
The objective of this work was to characterize the diversity of the microbiota within aggregates and to identify bacterial populations for each fraction of aggregates in order to determine their role in soil aggregation. We used a calcareous silty-clay soil sampled in the Aix-en-Provence region. We sorted the soil aggregates based on their water stability to obtain five granulometric fractions: from microaggregates (diameter of 2-50 μm) to macroaggregates (400-2000 μm). After DNA extraction, we used a metagenomic workflow integrating Illumina sequencing. A PCoA analysis revealed that the differences in overall diversity between each granulometric fraction follow the logic of aggregate sizes in these fractions (e.g. proximity between the three fractions of mesoaggregates, and maximum distance between microaggregates and macroaggregates). A LefSe analysis revealed a large number of specific taxa in the macroaggregates and fewer in the other fractions. The genera *Actinoplanes*, *Bradyrhizobium*, *Pseudolabrys*, and *Steroidobacter* are statistically more abundant in the macroaggregates, with a progressive decrease of their abundance towards the microaggregates. Conversely, the genera *Blastococcus*, *Acidobacteria* and *Candidatus* (*Sulfopaludibacter*) are statistically more abundant in the microaggregates, with a progressive decrease towards the macroaggregates.
In conclusion, the different granulometric fractions host both common bacterial species and more specific species/genera, the functions of which related to aggregate size are yet to be determined.

Keywords: soil, aggregates, microbiota, biodiversity, bacterial population, metagenomics

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Poster - Pangenome and structural variants

32 - Benchmarking read mapping on pangenomic variation graphs

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A pangenome represents the total genetic diversity of a species or a species complex. They can be described in terms of gene presence / absence variations (PAVs) but a more recent alternative aims to integrate full length genomes in a sequence graph (1). In these "Variation Graphs", nodes represent words of genome fragments and edges represent the contiguity of these words in at least one of the genomes. Each genome corresponds to a path in the graph. It has been showed that VGs can improve variant calling and genotyping processes (2). In particular, reference-based biases are reduced when large structural variations (> 50bp) are targeted.
Identifying new variants via a pangenome graph requires a compulsory preliminary step of sequence to graph mapping. Several approaches have been proposed (see (3) for a review), with algorithms dedicated to either long or short sequence reads. In practice, it remains unclear how this preliminary will impact further variants predictions.

The poster will present a benchmark produced by Hajar Bouamout during her Master internship to evaluate several graph read mapping tools. It will briefly describe the main ideas behind the algorithms proposed by 4 tools: GraphAligner (4), vg map (5), vg giraffe (5) and Minichain (6).
(1) Paten B et al. Genome Res. 2017. (2) Garrison E et al. Nat Biotechnol. 2018. (3) Shuo Wet al. Jour of Exp Botany 2023. (4) Rautiainen M et al. Genome Biol. 2020. (5) Hickey G et al. Genome Biol. 2020 (6) Ghanshyam C et al. bioRxiv 2022.08.29.505691.

Keywords: pangenome, variation graph, read mapping, genotyping

*Speaker

33 - Canine genomics: Deciphering lifelong traits, diseases, and longevity for integrated One Health advances.

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Through extensive artificial selection by humans to build modern dog breeds, canines serve as a unique mammalian model for unraveling the (epi-)genetic underpinnings diverse phenotypes (e.g., size, coat color, behavior), diseases, and longevity. Our team focuses on the genetic and epigenetic basis of lifelong traits using dogs as a natural model (https://igdr.univ-rennes.fr/equipe-genetique-du-chien). We created and manage the Cani-DNA BRC, housing over 30,000 dog DNAs and over 6,000 tissue samples, and which supports our ethical and participatory science approach, aligned with the "One Health" concept.
Our efforts have yielded the identification of genes and mutations in dogs, later extrapolated to orthologous genes in humans, with applications in rare cancers, dermatological and neurological diseases, fostering reciprocal benefits for human and veterinary medicine. We explored recently the canine model's potential in studying longevity which showed an inverse correlation between breed average lifespan and body weight (SCC-AGRIA).
Now supported by France Génomique in the context of the GOLDogs project (https://www.france-genomique.org/projet/goldogs/), we aim to create a comprehensive catalog of genetic variations in 25 dog breeds. This involves low-pass sequencing of 500 aged dogs, long-read DNA sequencing of 100 dogs (with our IGDRion plateform (https://igdr.univ-rennes.fr/igdrion)), and genome assemblies for a diverse panel representing the primary breeds. This extensive resource not only enhances our understanding of genomic diversity in dog populations but also introduces a novel strategy for mapping genetic variations to specific phenotypes, exemplified by the fascinating case of longevity in dogs.

Keywords: Dog model, Longevity, Structural Variants, LongRead sequencing

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34 - Genotyping two structural variants with the Graphtyper2 pan-genome approach in honey bee populations

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The current honey bee reference genome, HAv3.1, was produced from a commercial line sample, with a largely dominant Apis mellifera ligustica genetic background. The black bee Apis mellifera mellifera has a separate evolutionary history and is the original type in western and northern Europe. A specific genome assembly for this subspecies is essential for conservation purposes, for deciphering genome backgrounds in hybrid honey bees and initiating pan-genome approaches. PaciBio technology long reads were produced from a single haploid black bee drone and scaffolding contigs was done using a genetic map, to produce the black bee reference genome AMelMel_1.1. To demonstrate its utility, AMelMel_1.1 was used together with HAv3.1, for genotyping two nuclear mitochondrial DNA insertions (NUMTs), both of which present only in one or the other genome assembly, by a pan-genome graph approach with Graphtyper2. A total of 80 samples were genotyped and call rates were 89 and 76 % for the two variants respectively, which were consistent with an approach based on local sequencing depth. Our new assembly will therefore be an invaluable resource for future studies, such as including structural variants having different mutation rates than SNPs, in population genomics or GWAS.

Keywords: Honey bee, Genome, Structural variant, pan, genome

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Poster - Technological advances: producing and analyzing genomic data

35 - Combination of capture methyl seq technology and molecular tools to highlight imprinted loci in pig

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Genomic imprinting (GI) is an epigenetic phenomenon in which genes are mono allelically expressed depending on the parental origin. GI is regulated through epigenetic marks that also show parent-of-origin (PofO) methylation resulting in differentially methylated regions (DMRs) between both parental alleles. Identifying such specific patterns requires to integrate the most suitable molecular and computational tools which can be challenging especially in species such as livestock in which knowledge on GI is still sparse.
Here, we propose to characterize in pigs, the imprinting status of regions that are known to be imprinted in humans and mice, based on a method developed by our team. We identified 135 regions in blood showing molecular signatures of GI from capture methyl-seq trio data, combining DMR information and informative variants. The regions of interest include the GNAS locus which is associated with growth traits in pigs. We showed that the GNAS region carries PofO methylation within 5 DMRs including a potentially new one. Using independent methods of genotyping and methylation sequencing, these results were validated in blood and confirmed in brain and muscle.
Our results support the relevance of our novel technology to advance in pigs the characterization of GI mechanisms especially DMRs. For the GNAS locus, we are currently working on the specificity of the pig region showing the potential interest of enlarging the study of GI to a broad range of species to better understand the evolution of GI.

Keywords: Genomic imprinting, Pig, capture methyl seq technology, parent, of, origin, methylation, DMR

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36 - Comparative Analysis of the Circular Transcriptome in Sheep and Cow Blood Cells

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Circular RNAs (circRNAs) have been observed in a large number of species and tissues and are now recognized as a clear component of the transcriptome. Exonic circRNAs can be produced by a significant proportion of coding genes simultaneously with linear transcripts. Most circRNAs contain only exonic sequences, as they are generated by the circularization of exons through a back-splicing process where the end of an exon is joined to the beginning of an upstream exon. The ratio of circular to linear transcripts is dependent on the parental gene. Various physiological and environmental conditions have been shown to be associated with changes in circRNA levels. This study examines the relationship between parental circRNA genes and the position of the circular transcriptome in relation to the coding transcriptome. Total-RNA-Seq sequences from blood samples of 12 sheep and blood cells maintained ex-vivo from 6 cattle were analyzed. Using *CIRCexplorer2*, 3425 and 4123 exonic circRNAs were detected in sheep and in cattle respectively. A significant overlap was observed when parental genes of circRNAs were compared. We expanded this analysis to identify parental genes that produce varying proportions of circular and linear transcripts depending on the species. To do this, we quantified the expression of circRNAs using *CLEAR*, with the expression of the coding transcript (linear) produced by the same parental gene as the reference. This approach also enabled us to detect outliers among bovine samples (two replicates, 4h and 24h), much better than by monitoring circRNA expression alone.

Keywords: Circular RNA, Sheep, Cow, Transcripts ratio, Total RNA, Seq

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37 - Cross-species use of neural networks to improve pig genome annotation – a proof of concept

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A better knowledge of functional characterization of livestock species seems a lever linking genome to phenome. However, data describing gene regulation mechanisms and chromatin state in various experimental conditions are lacking. To overcome this bottleneck, predictive biology seems a good alternative. Human and mouse are organisms phylogenetically close to pig, we can assume that molecular mechanisms are similar. Furthermore, they offer much more data which is a condition to train powerful deep learning algorithms.
Here, we use artificial neural networks trained with human and murine data to predict gene regulation mechanisms from pig DNA sequences. We focused our analysis on a genomic region known to be associated with production traits in pigs. Because of the abundance of CTCF binding sites on genome, we used this protein as an indicator to estimate the accuracy of the predictions. For different tissues, at least half of observed peaks were predicted. Four reference chromatin marks also show correlations between observations and predictions from 0.5 to 0.8.
To conclude, the prediction results dedicated on a specific genomic region seem promising. An extended whole pig genome analysis will be performed and those predictions will enrich a database accessible to scientific community. A fine-tuned optimisation with data augmentation by orthology may improve predictions. Furthermore, this approach may also help us to predict variant impact and associate it with phenotypes of interest.

Keywords: pig genetic annotation, artificial intelligence, deeplearning

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38 - Multichromosomal and dynamic mitogenomes of Ludwigia sp.

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To better understand genetic processes involved in invasive species acclimatization, genomic resources are needed, which, for non-model species, represent a challenge. *Ludwigia grandiflora* subsp. *hexapetala* (*Lgh*; decaploid: 2n=10x=80) and *Ludwigia peploides* subsp. *montevidensis* (*Lpm*; diploid: 2n=2x=16) are two invasive aquatic plants, very common in France. In order to generate adequate genomic resources, we first decided to assemble organellar genomes, easier than nuclear genomes. Following the assembly and annotation of these two *Ludwigia* plastomes, we also managed to assemble their mitochondrial genomes using hybrid (Oxford Nanopore and Illumina Mi-seq) sequencing and combined assembly strategies. Our results show that the mitogenomes of both *Ludwigia* species consist of two circular molecules, named M1 and M2 of size 544,782 and 166,796 in *Lgh* and 555,518 and 167,000 in *Lpm*, respectively. The M2 molecules are collinear between the 2 species while numerous reorganizations of LCB (long colinear blocks) are observed for the M1 molecules. We also show that due to the presence of long repeats, M1 molecules recombine, generating alternative forms. We observe chloroplast insertions, mostly as fragments or pseudogenes, fragments of mobile elements gene (mitoviruses, LINE elements, non-LTR retrotransposons). Analysis of repeated sequences, SNPs as well as genomic comparisons are currently being carried out. These assemblies will serve for further transcriptome and methylome analysis in order to understand the capacity of *Ludwigia* to invade terrestrial habitat.

Keywords: Ludwigia, hybrid assembly, mitochondria, multichromosomal, mobile elements

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39 - Targeting the pig imprintome with EMseq

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Genomic imprinting (GI) represents an original model of epigenetic regulation resulting in the parent-of-origin (PofO)-specific expression of up to 2% of the mammalian gene content. This PofO-specific expression is primarily controlled by differentially methylated regions in a parental way as well. As it has a critical role in mammalian growth, metabolism and brain function, GI is involved in many traits in humans but also contributes to variability of major agronomic phenotypes. Imprinted domains are therefore highly attractive regions, representing only a subset of the genome while displaying a heterogeneity that makes their exhaustive characterization non-trivial. Faced with the lack of dedicated tools, we offer a turnkey strategy based on enzymatic methyl-seq (EMseq) to specifically target imprintomes (i.e., the set of DNA methylation marks regulating GI) and associated sequence variation, opening the way to scalable routine genome scans. Briefly, we *(i)* selected 165 regions in the pig genome based on human and mouse orthologies, *(ii)* exploited reciprocal crosses to identify PofO methylation, *(iii)* tested and optimised the novel Twist Bioscience Methylation Detection System and *(iv)* developed a novel pipeline to detect potential signatures of GI from methyl-seq trio data. Overall our results show that EMseq outperforms the current technological standard for methylation quantification. More specifically, our orthology-based capture offers an affordable and comprehensive picture of the impact of GI on a mammalian genome from a single experiment, which has implications for basic research, agrigenomics and clinical practice.

Keywords: Epigenomics, Genomic imprinting, Allele specific methylation, Bisulfite free sequencing

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40 - Whole genome sequencing and epigenotyping for multiple generations of selection using Oxford Nanopore Technology

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Livestock species offer a unique opportunity to trace genetic and epigenetic evolutionary trajectories. Genetic variation through time is well studied whereas in epigenetics it is insufficiently known. Yet epigenetic changes and their potential transmissibility across multiple generations is crucial, especially in the current context of adaptation to contemporary challenges for more animal resilience to climate change, reduced resource use and environmental impact. In this study we aim to better grasp the evolution of epigenetic patterns and their link to genetic variation. We had access to a dataset from two decades of selection, 15 continuous generations, for a sino-european pig breed. For each generation we sampled a pool of sperm from the most influential boars. Recent technological developments from Oxford Nanopore Technology provided us with high quality sequencing for both the genome and the epigenome. For each sample we obtained whole genome sequences with 30X coverage and the methylation status for about 30 million CpG sites, which is the full information on CpG for Sus scrofa genome (GCF_000003025.6). We identified regions showing changes in methylation status across generations and we clustered CpG sites having similar evolution with the objective to see variation along the genome and through time. Additionally we inferred the correlation between genetic and epigenetic changes. These results will contribute to the development of a statistical model to identify epigenetic selection signatures and to adjust selection models to include multi and transgenerational epigenetic changes, thus better accounting for the non genetic heritability in selection decisions.

Keywords: Pool sequencing, Oxford Nanopore Technology, long reads, sus scrofa, sperm, genera-

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tions, methylation

Poster - Genomics of plants and animals and their microbiota

41 - Climate, heat-stress, and genetics impacts the whole-blood gene expression levels in crossbred pigs

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The rising frequency of heat waves puts the livestock industry at risk, as animals are affected by the rise of temperatures. Pigs are also affected, causing both discomfort to the animal and an economical loss to farmers. Tropical pig breeds such as the Creole pigs are well adapted to high temperatures, meaning they might have the key to compensate for this increase in global temperature. A crossbreeding between Creole pigs and Large-White cosmopolitan pigs was setup to investigate the genetic determinism of heat adaptation in pigs.
We studied the whole blood transcriptome of the backcrossed offspring (n = 358). We processed a differential expression analysis between genetically related pigs living in tropical environment and in temperate environment, as well as between different time points along a 2 weeks experimental heat stress of the pigs living in the temperate environment.

Pigs living in a tropical environment overexpressed genes involved in the response to cellular stress, while pigs living in a temperate environment overexpressed genes that can be associated with their more sanitized living conditions. Gene differentially expressed in temperate environment after an experimental heat stress were different than the genes overexpressed in pigs raised under a tropical climate, highlighting different regulatory mechanisms for climate adaptation and meteorological variations.
A total of 5,917 quantitative trait loci affecting gene expression (eQTLs) were detected, with 3,427 located in cis and 1 ,750 located in trans. Some eQTL were correlated with the expression of several genes. Candidate genes for such eQTLs were MMP28 and ARSG. Furthermore, we detected 57 eQTLs associated with a Genotype by Environment (GxE) interactions.

Keywords: heat stress, transcriptome, GWAS, eQTL, pigs

*Speaker

42 - Diversity and functional potential of midgut symbionts in Culex pipiens from single and pooled mosquito individuals

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Mosquitoes cause about 700,000 global deaths annually by transmitting pathogens. Due to an increased resistance to insecticides and in the absence of effective vaccines against diseases caused by the pathogens they carry, new biocontrol methods are required. The endosymbiont *Wolbachia* infects most insects and is known to modulate host reproduction or confer protection against some pathogens. These abilities are currently used in biocontrol methods that aim at either reducing mosquito populations or offering protection to reduce the pathogen infection rate in mosquitoes. However, the microbiota and more particularly the midgut microbiota is also known to play a key role in survival, development and immune system of the mosquito host. Study of the mosquito microbiota is essential because a better understanding of its composition and functional potential could help develop new targeted biocontrol methods or improve existing ones. To this aim, we set up a laboratory protocol to reduce host contamination in genomic DNA extracted from individual and pooled dissected midguts of *Culex pipiens molestus*. From these bacterial enriched samples, we reconstructed multiple Metagenome-Assembled Genomes (MAGs) using shotgun metagenomics strategy in order to analyze their diversity, functions, and putative interactions. First, we characterized multiple high-quality MAGs corresponding to various (cultivable and non-cultivable) bacterial genera and we investigated their potential micro-diversity using fine-scale variability analyses. Then, we attempted drawing a "metabolic overview" from these newly reconstructed genomes that illustrates different levels of contribution from some taxa to specific or shared metabolic pathways.

Keywords: metagenomics, mosquito, microbiota, Culex, midgut

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43 - Highlighting key genetic factors of the Plant Growth-Promoting Bacteria Pseudomonas siliginis mediating the dialog with Arabidopsis thaliana

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Current global changes, such as climate change, habitat degradation and long-distance dispersal of pests, result in yield losses in crops, which in turn reduce food security on a worldwide scale and cause substantial economic losses. While strategies such as varietal mixtures, temporal crop diversification, plant breeding for alleviating abiotic and biotic stresses have been successfully developed and implemented to increase crop yield and its stability across diverse spatio-temporal scales, exploring and exploiting the plant microbiota represent an additional opportunity to participate in developing eco-friendly and sustainable agricultural practices.
Plant microbiota includes, among others, Plant Growth-Promoting Bacteria (PGPB) that have the potential to mobilize and provision nutrients to the plants, alleviate abiotic stresses and provide protection against several pathogens. While the combination of 'omics' tool and mutational studies has revealed key genes underlying the benefits conferred by a PGPB on a plant, studies reporting the genetic and molecular mechanisms associated with natural variation of a given PGPB remain scarce.
We aimed at characterizing the genetic architecture of the benefits conferred by a PGPB on a plant. To do so, a genome wide association study was set up in two contrasted growth conditions (*in-vitro* and field conditions), with the inoculation of nine accessions of *Arabidopsis thaliana* from the south-west of France with 74 whole-genome sequenced strains of the PGPB *Pseudomonas siliginis* isolated from the same geographic area. The benefits conferred by *P. siliginis* on *A. thaliana* was driven by a polygenic architecture that was highly dependent on both growth conditions and plant genotype.

Keywords: Natural genetic variation, Plant growth, promoting bacteria, Native interaction, GWA mapping

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44 - Insect viruses as biological control agents: contribution of viral metagenomics

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The European legislation to ban many pesticides has led to increased demand for alternatives, including to use of viruses as biocontrol agents, which first requires knowledge of their diversity. Viral metagenomics has revolutionised our view of the complexity of arthropod viral communities, and has the potential to be used as a tool to discover and characterise promising viruses. However, as research effort has focussed on blood-feeding arthropods, we have very limited knowledge of the diversity of insect pest viruses.
Our objective was to inventory the full viral communities of major insect pest species.

We focussed our sampling effort on the cotton bollworm (*Helicoverpa armigera*), the green stink bug (*Nezara vridula*), the tomato leafminer (*Tuta absoluta*), and their host-plant (tomato). We collected 928 samples from several sampling sites in France and Italy, and processed them using a without *a priori* viral metagenomic approach based on the enrichment of encapsidated nucleic acids coupled to high-throughput sequencing. We processed 1.3 billion of short reads. After eliminating low-quality genomes, we retained 236 virus species belonging to 54 families. We reconstructed the complete genomes of 58 novel species, including 17 insect viruses, 26 plant viruses and 12 bacteriophages.
Finally, we studied differences in viral community composition between the insect species at the individual level using 536 unpooled samples. We observed disparities in the prevalence and abundance of insect viruses between each insect pest species, potentially reflecting a host species-specific assemblage. The impact of these newly discovered viruses on insect pests have yet to be elucidated.

Keywords: Metagenomics, Virus communities, Microbiota, Insect pests, Viral ecology, Virus discovery, Agroecosystems

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45 - Microbial communities' flux along the process of kaak making, a Lebanese baked product involving chickpea fermentation.

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One way to increase the consumption of legumes could be their incorporation into new food products, or through the preparation of fermented foods based on legumes. Numerous traditional foods in Mediterranean region are produced by using soaked chickpeas in the preparation of bread, cakes, crackers...Kaak is a traditional Lebanese baked product produced by using chickpea (*Cicer arietinum L.*) soaked in water as a leavening agent. We analyzed the kaak making practices and the microbial communities' diversity along the kaak processes in 26 different bakeries in Lebanon. A total of 113 samples were collected along the process, including Chickpeas fermented water, Chickpea water sourdough (chickpea fermented water mixed with wheat flour) and kaak doughs. Yeast population density increased along the process from 2.11 ($\pm$1.99) log CFU/g in fermented chickpea's water to 3.51 ($\pm$1.52) log CFU/g in the final dough with a gradual increase in pH from 4.81 ($\pm$0.27) for the fermented chickpea's water to 5.57 ($\pm$0.57) for the final dough. The microbial community was studied by V3-V4 16S rDNA and ITS1 metabarcoding analysis, performed by MiSeq Illumina sequencing. The bacterial richness ranged from 8 to 86 ASVs while fungal richness ranged from 1 to 150 ASVs. *Clostridium perfringens* was the most frequent bacteria species all along the process (frequency $>$ 0.5 in chickpea water, sourdoughs and doughs). *Weissella spp.* occur at low frequency in the chickpea water and sourdough (frequency $<$ 0.6) and increase in frequency in the final dough (frequency=0.2). Fungal communities were not dominated by single species. The bakery yeast, *Saccharomyces cerevisiae* was present in all samples at a frequency below 0.16. The plant pathogen, *Alternaria tenuissima* and *Alternaria infectoria* were also commonly found. A cultural dependent method was also used in order to obtain yeast isolates from all bakers and analyzed their phenotypic diversity. A total of 22 yeast species were identified as *Saccharomyces cerevisiae, Candida parapsilosis, Meyerozyma sp., Trichosporon sp., Candida tropicalis, Clavispora sp., Diutina mesorugosa.* More research could be carried out for analyzing the functional diversity of isolated kaak's strains and for testing new bakery formulation based on chickpeas.

*Speaker

Keywords: fermentation, baked product, chickpea, metabarcoding, microbial diversity

46 - Structure and dynamic of the rhizosphere microbiome of a desert barley under salt conditions.

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Soil salinization is one of the major factors of soil degradation. It has reached about 20% of the irrigated land on the globe (F.A.O, 2000).
Inhibition of plant growth by salinity is the result of osmotic and ionic effects and plant species have developed different mechanisms to cope with these effects (Munnes, 2002). Recent research, however, has revealed that plant growth promoting bacteria including both endophyticendrhizospheric bacteria can improve salinity tolerance (YaishMWet *al.*,2015; Ali S et *al.*, 2014).

In the El Meniaa oasis in the Algerian desert (Sahara) Barley is used as crop. We make use of a field station in El Meniaa. This station has areas with a relatively high salt concentrations due to bad irrigation practices as well as areas with low. We determined the microbiomes of barley rhizosphere in a concentration gradient NaCl in a field experiment. This revealed that the composition of the microbiome of the rhizosphere compartment is markedly influenced by the salt (450mM). Barley was grown on soil that was pre-cultured with barley.
The barley rhizosphere microbiome was markedly different. Currently the function of the microbiome in conferring salt tolerance is studied under lab conditions using the barley soil from El Meniaa.

Keywords: Salt stress, microbiome, rhizosphere, Sahara, barley, desert farming

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47 - Transgenerational response to endocrine disruptor : phenotypic, genotypic and epigenotypic analyses in quail

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While the typical evaluation of an offspring's phenotypic traits usually focuses on the inheritance of parental alleles, variations across generations can also result from the transmission of non-genetic factors. Epigenetic marks like DNA methylation offer a dynamic molecular signature of an individual's history, as they can carry a memory of the individual's environmental past, and also of its parents. A recent study in quail (Coturnix japonica) found that alterations in the embryonic environment can have an impact on subsequent generations, resulting in significant reduction of the offspring's body weight. To better disentangle the complex interplay between genetic and epigenetic effects, a specific quail breeding plan was designed. Twenty initial founder families were crossed to generate two epilines with similar genetic backgrounds. In the first generation, one epiline received a genistein supplementation whereas the other was only provided with regular feed. After this initial diet disruption, the groups were maintained under standard breeding conditions, and animals from the next two generations were mated following a mirrored design to ensure a balanced genetic structure in both groups. By recording multiple phenotypes (production, breeding, behaviour), genotypes and epigenotypes of both epilines, this approach aims to investigate the transgenerational inheritance of an environmental effect through changes in DNA methylation profiles.

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Keywords: Transgenerational inheritance, Epigenetics, DNA methylation, Coturnix japonica

Poster - Genomics of biological interactions: holobionts, pathogens, symbionts

48 - Reconstruction of near-complete endosymbiotic Wolbachia genomes from long and short-read sequencing data of individual Culex quinquefasciatus ovaries

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The endosymbiotic and maternally-inherited bacteria *Wolbachia*, which can selfishly manipulate the reproduction of their host and interfere with viral pathogens, are the focus of intense study for their potential application to vector control strategies. In this context, it is crucial to obtain high-quality reference genomes to further characterize the naturally occurring diversity of *Wolbachia* at a large scale. Next-generation sequencing technologies are key to achieve this goal for obligate intracellular symbionts that remain recalcitrant to cultivation. Nevertheless, the presence of repeated prophage regions in *Wolbachia* genomes leads to fragmented assemblies when relying on Illumina short reads only. Here, we present three near-complete genomes of *Wolbachia* from individual *Culex quinquefasciatus* from Martinique (French Caribbean Island), reconstructed from long- and short-read sequencing data of mosquito ovaries. The final refined assemblies contain between 4 and 15 linear contigs, with an additional circular contig corresponding to pWCP (plasmid of *Wolbachia* in *Culex pipiens*) recovered from each sample. The full *Wolbachia* genome sequences are estimated to be 94.8% to 100% complete and less than 1.57% redundant. We believe the acquisition of these fairly unfragmented genomes is a stepping stone for further investigation of *Wolbachia* genomic diversity and rearrangement in *Culex* mosquitoes collected from distinct geographic locations and settings around the globe.

Keywords: long read sequencing, symbiont, Wolbachia

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49 - The Interplay between Symbiotic Egg Microbiota and Fusarium Pathogens in a threatened Amazonian River turtle.

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Fusarium pathogens are among the most harmful mycotoxigenic fungi, threatening food safety, human health and biodiversity and have been listed as priority fungal pathogens by the WHO since 2022. In particular, species of the *Fusarium solani* species complex (FSSC) are considered highly pathogenic, due to their global distribution, ability to adapt to changing environments and their trans-kingdom pathogenicity. They cause infections in multiple hosts, from crops to humans and are threatening wildlife. For about a decade, fusariosis, caused by FSSC pathogens, in sea turtle eggs is considered a fungal emerging disease, leading to hatching failure and mass hatchling mortalities worldwide. In 2019, we observed and reported hatching failure caused by fusariosis in the eggs of the yellow-spotted Amazon River turtle (*Podocnemis unifilis*), a turtle species inhabiting a pristine environment in the Ecuadorian Amazon. In addition, we demonstrated that the egg microbiome is influenced by river sand and water environment, which shapes egg and hatchling microbial composition and impacts health. Finally, after exploring the internal egg microbiota, we observed differences in microbial diversity and composition of FSSC uninfected and infected eggs, suggesting that *Fusarium* pathogens interact with the internal egg microbiota. With this prior knowledge we show the critical role of the host-associated internal egg microbiota in hatching success, pathogen resistance, and turtle health.

Keywords: egg microbiota, fusariosis, fungal diseases, hatching success

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Author Index