ABSTRACT BOOK

Conference

EpiDiverse

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SCOPE

There is an ever-growing interest in the mechanisms underlying epigenetic phenomena, which may be particularly crucial for the survival of plants within dynamic environments as they cannot respond behaviourally or migrate immediately. Understanding the epigenetic contribution to plant phenotypic variation, stress responses and long-term adaptation will help to better understanding species responses to global environmental change, and can open new directions for sustainable agriculture and crop breeding. Moving forward this field requires multidisciplinary cross-talk between ecologists, molecular geneticists and bioinformaticians. This was the aim of this *EpiDiverse* Conference that received funding from the European Union's Horizon 2020 research and innovation programme under Marie Skłodowska-Curie grant agreement No 764965 for *EpiDiverse* Network.

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KEYNOTE SPEAKER

EPIGENETICS AND SUBINDIVIDUAL VARIATION IN PLANTS

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Recent research on ecological epigenetics of non-model plants has often focused on the relationships between epigenetic and phenotypic variation across populations or individuals of the same species. In plant populations, however, subindividual variance in phenotypic traits of reiterated, homologous organs that perform the same function (leaves, flowers, fruits, seeds) is often very high, sometimes contributing more to total population variance than variation among individuals. This subindividual component of phenotypic variance can arise from multiple proximate causes and ultimately influence the fitness of individuals via its ecological consequences. Epigenetic mosaicism, whereby different parts of the same genetic individual differ in the extent or pattern of DNA cytosine methylation, has recently emerged as one hitherto unrecognized mechanism potentially contributing to subindividual phenotypic variance in perennial plants. Observational studies reveal considerable variance in DNA methylation among modules of the same individual, exceeding the variance between individuals. Within-plant relationships exist across modules between DNA methylation and phenotypic traits at the module level (e.g., seed production, seed size), and experimental augmentation of within-plant heterogeneity in DNA methylation can increase phenotypic variance. Modular construction by continual organogenesis and reiterated production of homologous structures is a quintessential plant feature which motivates the consideration of plant individuals as non-unitary metapopulations of semi-autonomous parts. The emerging relationships between phenotypic and epigenetic variation within individual plants, along with recent findings showing that extant subindividual epigenetic mosaics result from steady epigenetic diversification over the lifetimes of genetic individuals, provide grounds for proposing an ‘epigenetic mosaicism hypothesis’ with many ecological and evolutionary implications

SESSION 1
POPULATION EPIGENOMICS IN NATURAL SYSTEMS

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OPENING LECTURE

POPULATION EPIGENOMICS IN NATURAL SYSTEMS

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One single genome can enable multiple evolutionary trajectories as a result of molecular variation beyond the DNA sequence. This epigenetic variation can manifest itself in the methylome (DNA methylation), the chromatin interactome (3D chromatin landscape) and the mobilome (transposable elements) as major components of the epigenome. Epigenetic variation in any of these epigenetic processes can allow individuals to cope with newly emerging stressors and changing environmental conditions, and therefore represents a core element of individual fitness. As a consequence, intra- and inter-population variability in the epigenomic signature is key to population evolutionary potential and persistence. This epigenomics perspective on evolution in natural systems propels the field of population epigenomics, and new insights into the spatial distribution and drivers of epigenetic variation keep emerging. Although the involvement of epigenetic variation in fitness and genotype × environment interactions is beyond doubt, the magnitude of epigenetic inheritance, and the mechanisms by which epigenetic variation can boost evolution (e.g. genetic assimilation), remain underexplored. Such open questions, however, provide opportunities for further research aiming to resolve the highly intertwined nature of genetic and epigenetic variation, and to bring the field to the forefront of evolutionary and conservation science. Here, I show how “population epigenomics” has moved in the past decade, I highlight major advances in the field, and I present research directions aiming to unravel when, and to what extent, epigenomic variation shapes evolutionary trajectories in natural systems

S1.1. Natural epigenetic variation in wild strawberry populations along a European climatic gradient

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Epigenetic modifications, such as DNA methylation, are thought to contribute to local adaptation of plant populations. Unlike genetic mutations, environmentally induced DNA methylation variants can arise rapidly and generate locally adapted phenotypes, potentially heritable across multiple generations. However, the extent of DNA methylation variation in wild populations is poorly understood. Here we evaluated patterns of DNA methylation variation in wild strawberry (*Fragaria vesca*) populations in the field and in clones of the same individuals grown for one year in garden conditions. Using a single-base resolution technique of cytosine DNA methylation, we assessed variation in epigenetic marks among 21 natural populations along climatic gradients in Italy, Czechia and Norway. Principal component analysis showed that overall epigenetic variation was closely related to the geographic origin of the populations, in both field and garden conditions. In addition, we found crucial differences in functional DNA regions and a potential variation in transposable elements regulation between populations with different climatic origin. Our findings suggest that the habitat of origin was associated with an epigenetic signature heritable across multiple clonal generations. They also suggest that environmentally induced DNA methylation variants play a key role on gene activity and transposable elements regulation, potentially contributing to natural phenotypic variation. We conclude that DNA methylation variation in the wild is common and relevant for plant adaptation.

Keywords: ecological epigenetics, natural variation, heritability

S.1.2. Does epigenetic diversity contribute to the invasion of Japanese knotweeds?

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Japanese knotweeds are an invasive species of plants in Europe and the USA which can cause serious damage to roads and buildings. The source of the European invasion of *R. japonica* is thought to be a single plant taken by Philipp von Siebold from Japan in the early 1840s, and distributed across Europe. After this, at least two introductions from both Europe and Asia were identified in the USA based on phylogenetic research. As the genetic diversity of these plants is expected to be extremely low, we will test the hypothesis that epigenetic variation has contributed to the invasion of new environments for this species.

As a first step to test this hypothesis, we have collected leaves from 5 individuals at each of 50 USA and European invasive populations and in 50 native populations from China. In the transects spanning 2000 km across each of the three continents, we collected a total of 750 samples. We will present the preliminary epiGBS and GBS results documenting genetic and epigenetic patterns in natural populations for all three continents. In addition, we will link these results to patterns of trait variation and habitat characteristics.

Keywords: epiGBS, Japanese knotweed, invasion, clonality

S.1.3. An emerging relation between epimutations and gene expression in a clonally propagated perennial tree

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In plants DNA methylation is often associated with chromatin repression, typically at silenced repetitive elements. The signature of such epigenetic control is the methylation of cytosines in all the three contexts (CG, CHG and CHH) that are amenable for such modification. Another pattern of DNA methylation, occurring only in the CG context, is instead associated with middle-highly expressed genes, but the biological role of this phenomenon, defined as Gene Body Methylation (GBM), is unclear. The origin of GBM has been ascribed to a byproduct of epigenetic silencing of gene-associated repetitive elements through a CHG methylation self-reinforcing loop. However, the link between CHG and CG methylation is still poorly understood.

In this study, we leveraged whole-genome DNA methylation profiles of 201 genetically identical clones of the *Populus nigra italica* cultivar from 12 geographic locations in Europe to investigate variation in GBM and other patterns of DNA methylation. Analyzing a unique genotype propagated in different environments, allowed us to combine methylation data across all the individuals, making hidden patterns detectable. The cumulative signal of rare epimutations pinpointed across several individuals proved as useful as steady-state methylation measurements to identify specific methylation patterns.

In addition, it provided information on ongoing epigenetic processes, such as the enrichment of CG and CHG hypermethylation events in highly expressed genes and the correlation between GBM and CHG hypermethylation. Our population-level approach suggests a mechanistic link between expression and methylation accumulation in gene bodies and further supports the current model of GBM triggered by CHG methylation.

Keywords: whole-genome-DNA-methylation, epimutations, gene-body-methylation, gene-expression

S.1.4. No geographical pattern in methylome variation in the Lombardy poplar clone across Europe

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Environmental variability can induce phenotypic variation in plants through epigenetic mechanisms, but strong genetic influences make it difficult to isolate and study such epigenetic effects. For this reason, clonal tree species or varieties like the Lombardy poplar (*Populus nigra* cv. 'Italica' Duroi) offer a unique system to study natural epigenetic variation caused by the environment. We collected poplar ramets across Europe and planted them under common garden conditions in the Marburg Botanical Garden. We performed whole-genome bisulfite sequencing in 164 ramets growing in the common garden and a subset of 35 of the original ortets. Using historical bioclimatic data from the ortet localities, we tested the correlation between epigenetic variation and climatic gradients. We found that global methylation levels differ significantly between ramets from some of the geographic origins and that methylation levels correlate with biologically relevant climatic variables. Furthermore, we found that epigenetic marks that occur in symmetric genomic contexts (CG and CHG) can be transmitted to the next clonal generation, but a fraction of the methylome was dynamic and changed relatively fast when comparing the ramets to the ortets. Despite these differences, we saw that most of the methylation variation could not be linked to the ortet localities. Our results suggest that the poplar methylome is highly dynamic but that changes can be transmitted to clonal offspring and potentially affect how poplars adapt to new environmental conditions.

Keywords: *Populus nigra*, adaptation, memory

S.1.5. Genetic and environmental determinants of natural DNA methylation variation in *Thlaspi arvense*

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Intraspecific patterns of DNA methylation vary substantially in natural plant populations. However, the ecological and evolutionary significance of this epigenetic variation remains largely unknown. We do not fully understand its relationships with phenotypic and environmental variation, its degreed of stability and especially to which extent it is controlled by genetic variants. So far, such complex questions were studied only in the model species *A. thaliana*. Aiming to study a more complex genome, we sampled 36 *Thlaspi arvense* (Brassicaceae) populations along a large climatic gradient, from southern France to central Sweden. We grew the first generation under common conditions and screened more than 200 lines for genetic (Whole Genome Sequencing) and DNA methylation (Whole Genome Bisulfite Sequencing) variation, as well as for phenotypic variation. By means of Genome Wide Association Studies, we detected several genetic variants associated with context specific (CpG, CHG and CHH) DNA methylation levels, suggesting partial genetic control of methylation. In addition, upon population structure correction, methylation patterns correlate with climate of origin variables, suggesting an adaptive role. In particular, methylation is generally lower in accessions from cold and thermally fluctuating environments. Finally, by variance decomposition of individual Differentially Methylated Regions (DMRs), we detected an increasing proportion of "environment-related-DMRs" from CpG to CHG and CHH contexts respectively. Our study shows that, although natural DNA methylation variation is largely under multi-locus genetic control, it is also partially shaped by climate of origin and the contribution of the latter varies with sequence context.

Keywords: Population epigenetics, DNA methylation, GWAS,DMRs, WGBS

SESSION 2
EPIGENETIC REGULATION OF PLANT PHENOTYPES

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OPENING LECTURE

EPIGENETIC REPROGRAMMING IN PLANT GERMLINES

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The plant male germline undergoes DNA methylation reprogramming, which methylates genes de novo and thereby alters gene expression and facilitates meiosis. Why reprogramming is limited to the germline and how specific genes are chosen is unknown. Here, we demonstrate that genic methylation in the male germline, from meiocytes to sperm, is established by germline-specific siRNAs transcribed from transposons with imperfect sequence homology. These siRNAs are synthesized by meiocyte nurse cells (tapetum) via activity of the tapetum-specific chromatin remodeler CLASSY3. Remarkably, tapetal siRNAs govern germline methylation throughout the genome, including the inherited methylation patterns in sperm. Finally, we demonstrate that these nurse cell-derived siRNAs (niRNAs) silence germline transposons, thereby safeguarding genome integrity. Our results reveal that tapetal niRNAs are sufficient to reconstitute germline methylation patterns and drive extensive, functional methylation reprogramming analogous to piRNA-mediated reprogramming in animal germlines.

S.2.1. Epigenetic and transcriptional regulation of Volatile Organic Compounds: *Dianthus broteri* as a polyploid study system

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Volatile organic compounds (VOCs) are emitted by plants as a consequence of their interaction with biotic and abiotic factors, having a very important role in plant evolution. Floral VOCs are often involved in defense and pollinator attraction. These interactions often change rapidly over time, so a quick response to those changes is required. Epigenetic factors, such as DNA methylation and histone modification, which regulate both genes and transcription factors, might trigger adaptive responses to these evolutionary pressures as well as regulating the rhythmic emission of VOCs through circadian clock regulation. In addition, transgenerational epigenetic effects and whole genome polyploidy could modify the generation of VOCs' profiles of offspring, contributing to long-term evolutionary shifts.

We reviewed the available knowledge about the mechanisms that may act as epigenetic regulators of the main VOC biosynthetic pathways, and their importance in plant evolution, in order to study these mechanisms in *Dianthus broteri*, which represents the largest polyploid series known in the genus *Dianthus*, with four different cytotypes which present different scents profiles. We propose this polyploidy system to study the role of epigenetics and polyploidy on the evolutionary change of floral volatiles.

Keywords: VOCs, epigenetics, transcriptional-regulation, polyploidy

S.2.2. Role of histone demethylases in somatic embryogenesis in *Arabidopsis*

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Somatic embryogenesis (SE) is a process by which plants can regenerate bipolar embryo-like structures from somatic cells. Somatic embryogenesis is the most studied regeneration model in plants, yet the precise molecular mechanisms implicated remain poorly understood. Genetic studies have revealed that somatic embryogenesis is associated with changes in chromatin structure and the activation of transcription factors preferentially activated during embryo development. Specifically, the tri-methylation of lysine 27 of histone 3 (H3K27me3), a silent histone modification deposited by the catalytic activity of the Polycomb repressive complex 2 (PRC2), has been implicated with the repression of embryonic transcriptional programs in somatic cells. To dissect this complex regulatory network, we have targeted different H3K27me3 demethylases to the regulatory sequences of genes implicated in somatic embryogenesis. Our data suggest that the targeted removal of H3K27me3 results in the activation of distinct transcriptional programs, which ultimately contribute to an increase in the developmental plasticity of somatic cells. This work will provide valuable knowledge to dissect the complex molecular networks implicated in somatic embryogenesis in plants.

Keywords: chromatin modifications, histone demethylase H3K27

S.2.3. Improvement of plant growth and immunity by impediment of MYST histone acetyltransferases

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Part of plant immune responses to pathogens is reprogramming of gene expression. Acetylation of the N-terminal tails of histones by histone acetyltransferases (HATs) and deacetylation by histone deacetylases (HDACs) can regulate transcriptional responses. Arabidopsis HAM1 together with its close homologue HAM2 belong to the MYST-HAT family. According to previous studies, AtHAM1 and AtHAM2 work redundantly to regulate gametophyte development and flowering time. Our results show that the two MYST-HATs interact with distinct set of transcription factors and have different functions. AtHAM1 regulates immunity against Verticillium dahliae while AtHAM2 regulates plant growth and immunity against Pseudomonas syringae pv. tomato. Interestingly, not all plant species have two homologues of MYST-HATs. Tomato (*Solanum lycopersicum*) has a single copy but Brassica (*Brassica oleracea*) has two copies of MYST-HATs, which raise the question if a single copy of MYST-HAT can perform both functions of MYST family. To address this question we have generated mutants of Brassica plants and performed an in silico screen to identify chemical inhibitors of tomato and Brassica MYST-HATs.

Keywords: histone acetylation, plant epigenetics, plant-microbe interactions

S.2.4. Molecular function of Meiosis-Associated Argonaute (MAGO) proteins in maize

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Abstract- Argonaute (AGOs) proteins are key players involved in the regulation of gene expression in most Eukaryotes. AGO proteins bind small RNAs and direct gene silencing by targeting complementary mRNAs for degradation or induce epigenetic changes that alter transcription. These silencing mechanisms are essential to preserve developmental transitions as well as to maintain genome integrity by repressing the activity of transposable elements.

Both plants and animals have a discrete class of AGOs that are important to maintain the integrity of the germline- from meiosis to gametogenesis. It has been recently found that these AGOs are critical to maintain male fertility in response to stress, likely by regulating the activity of transposable elements (TEs). However, the precise mechanisms implicated in the control of TEs remain unknown. The aim of the project is to define the role of meiosis associated AGO proteins (MAGO) and associated phasiRNAs in plant fertility. Using maize as a model system and performing molecular, biochemical and computational analyses I will investigate the role of MAGO proteins in the biogenesis of MAGO-associated sRNA to determine their role in TE silencing and ultimately in safeguarding male meiosis in plants.

S.2.5. TreeGeneClimate – Sustainable wood and biomass production: novel traits, resilience to climate change, pests and diseases

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Forest trees have important roles not only because their significant economic and ecological value in biodiversity conservation but also because of their mitigating effect on clmate change and pollution abatement, and their value as an alternative and sustainable source of raw material and bioenergy. Wood and associated products as timber or plywood contribute significantly to the revenue generation of many countries in the world. However, new climatic conditions together with new pests and diseases, may pose a threat to the supply of forest products. The main goal of the TreeGeneClimate project is the analysis of the genetic basis underlying wood quality traits in Norway spruce (*Picea abies* (L.) H.Karst), the economically most important tree species in Europe, through a combination of genetic and genome-wide analysis of genetic and epigenetic markers associated with variation in morphological, physiological and biochemical traits, response to environment, resistance to disease and decay, heartwood extracts, and traits that might be necessary to limit degradation of wood and timber. Genotyping and epigenotyping of 620 individuals (clones and unrelated trees) will be performed by means of exome-capture and targeted-bisulfite sequencing using target enrichment probes. The phenotypic, biochemical and genomic data will be then used for GWAS to identify SNPs associated to these traits. The results obtained will be useful for the development of Genomic Selection programmes based on genome- and epigenome-wide markers and regressive prediction models, reducing in turn the usually long generation times of traditionall breeding programmes in tree species.

Keywords: GWAS, wood, Picea, genomics, epigenomics

S.2.6. The epigenome of the Brassicaceae *Thlaspi arvense*

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Reference genomes are often generated with a comprehensive annotation of protein coding genes and the mRNAs they produce, but this offers only a partial view of genome functions, many of which involve epigenetic mechanisms. In this work, we investigate epigenetic components of Thlaspi arvense genome by providing a detailed annotation of transposable elements (TE) and small RNA loci (sRNA).

We identified and annotated 423,249 individual TEs, which together constitute 61% of the T. arvense genome of. Among these TEs, we found that retroelements of the GYPSY superfamily are the most abundant, with a single family responsible for 6% of the total genome size. In contrast, some scarcer CACTA and HELITRON families are the most active, as observed from transcriptomic data profiles of several plant tissues.

To understand how TE activity is regulated, we complemented our TE annotation with sRNA data. Applying a custom pipeline to data from leaf, root, inflorescence and pollen, we identified 19,288 distinct sRNA loci, of which 72 were microRNAs. Most of the sRNA loci were located at the transition point between gene-rich and TE-dense regions, with sRNA being highly expressed in gene-rich regions.

Using this annotation, we will survey a diverse set of wild T. arvense populations for transposable insertion polymorphisms to gain insight into their evolutionary history and correlate them with sRNA expression profiles in a population subset. Our results should provide an important resource for comparative genomics and transposable element evolution research in the Brassicaceae.

Keywords: Population-genomics, Transposable-elements, sRNA annotation

S.2.7. Effects of parental and immediate light and soil moisture conditions on transcriptional activity in *Polygonum persicaria* offspring

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As transgenerational effects become increasingly recognized as a source of individual variation, important questions remain regarding (a) the relative impact of parental and current environments; and (b) genetic variation for these environmental effects. We investigated trans- and within-generational effects on transcriptional activity of two key environmental variables (light and soil moisture) in field-sourced genotypes of the annual species Polygonum persicaria. We grew isogenic parent plants of four highly inbred lines in Shade (Moist soil), Control (Sun/Moist soil), and Dry soil (Sun) conditions, to produce genetically uniform progeny from three contrasting parental environments. We then grew seedling progeny in these same treatments and quantified their transcriptional activity using RNA-seq in order to make the following comparisons: (i) effects of parental Sun versus Shade on transcriptomes of progeny growing in Shade; (ii) effects of parental Dry versus Moist soil on progeny growing in Dry soil; (iii) immediate effects of Shade and Dry soil versus Control seedling conditions on progeny of Control parents. For light versus shade, more transcripts were differentially expressed as a result of offspring conditions than parental conditions, while the reverse was true for soil moisture (i.e., more transcripts were differentially expressed due to parental treatment than offspring treatment). Genotypes varied in the number and identity of differentially expressed transcripts for both parent and offspring effects in all environments. These data provide insight to the way transcription varies among genotypes in response to key environmental factors experienced during the parental and current generation.

Keywords: transcriptome, transgenerational plasticity, plant plasticity

SESSION 6
EPIGENETIC REGULATION OF BIOTIC INTERACTIONS

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OPENING LECTURE

THE EPIGENETIC DRIVERS OF PLANT IMMUNE MEMORY

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Short-term immune reactions in plants often lead to long-lasting induced resistance (IR), which is based on priming of inducible defences. We study the epigenetic basis of this immune memory, which stems from our earlier finding that diseased Arabidopsis produce progeny that are primed to resist biotrophic pathogens. This transgenerational IR is reversed in the absence of stress and requires ROS1-dependent DNA demethylation of transposable elements (TEs). Monitoring changes in DNA methylome from parent to F1 embryos and F1 plants revealed loss of DNA methylation at TEs/repeat sequences at non-CG context. Using artificial epigenetic recombinant inbred lines (epiRILs) of Arabidopsis, we furthermore found that DNA hypomethylation of TE-rich pericentromeric regions primes global defence genes against biotrophic pathogens. Current research also focuses on long-term IR against herbivores within one generation. Treatment of seedlings with the defence hormone jasmonic acid (JA) induces long-term IR against caterpillars, which is maintained for several weeks. Transcriptome analysis demonstrated that this long-term JA-IR is associated with priming and/or up-regulation of MYC2/3/4-dependent defence genes. In contrast to short-term JA-IR, this long-term JA-IR requires the DNA demethylase ROS1 and the sRNA-binding protein AGO1. Although DNA methylome sequencing failed to identify consistent changes in DNA methylation near MYC2/3/4-controlled defence genes, plants from JA-treated seedlings were specifically enriched with hypo-methylated TEs from the AtREP2 family. Analysis of JA-inducible AGO1-associated sRNAs revealed enrichment with AtREP2-derived sRNAs, suggesting that hypo-methylated AtREP2 TEs prime MYC2/3/4-dependent defences via AGO1-associated sRNAs. Our research indicates that trans-regulatory responses to TE hypomethylation control long-term immune memory in plants.

S.6.1. Exogenous alteration of DNA methylation in soybean reduces the plant's ability to assemble beneficial soil microbial communities

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Microbes provide multiple benefits to their plant hosts, including better access to nutrients, enhanced growth, and improved tolerance to biotic/abiotic insult. Previous studies have shown that domestication has inadvertently altered the microbial communities of crops, suggesting that microbiota composition is a trait that can be bred. This makes the understanding of what makes a "good microbiota host" critical in the conceptualization of breeding programs aimed at improving productivity, quality, and sustainability via the enhancement of the holobiont. The generation of stochastically hypomethylated plant populations can be used to determine the putative function of non-characterized genes, and to identify genomic locations responsible for traits of interest. In this study, we used next generation sequencing to study the effect of DNA demethylation induced by the demethylating agent 5-Azacytidine on the ability of soybean to assemble soil microbial communities. Our results indicate that: the loss of DNA methylation negatively affects the plant's ability to alter the soil microbial communities; demethylated plants showed higher variability in their capacity to alter the bulk soil microbiomes, and a significantly lower ability than wild-type plants to prevent the growth of pathogenic bacterial species and promote the growth of plant growth-promoting taxa. Taken collectively, these results strongly support our hypothesis that the stochastic perturbation of DNA methylation uniquely alters the ability of plants to direct the assembly of its microbiota. These findings signify the importance of epimutant plant populations as a resource for the identification of plant genes regulating soil microbiota assembly.

Keywords: DNA methylation, epimutation, Rhizosphere, PGPB

S.6.2. Effects of short-term herbivory on plant response and its association with DNA methylation

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Genetic and chemical mechanisms involved in plant-herbivory stress are well-studied, but the impact of epigenetic factors as modulators of the responses is less understood.

Here, we studied the role of DNA cytosine methylation on phenotypic responses after short-term herbivory in the annual plant *Thlaspi arvense*. We investigate the effect of experimental demethylation and herbivory treatments following a 2x3 factorial design in two European populations. Seeds were submerged in demethylating agent 5-azacytidine and only in water as controls. The controls and demethylated plants were assigned to three herbivory categories (i) insect herbivory, (ii) artificial herbivory, and (iii) undamaged plants. The effects of treatments were assessed by quantifying genome-wide global DNA cytosine methylation, concentration of glucosinolates in leaves and fecundity. In Swedish plants, global methylation did not differ between control and demethylated plants but it was significantly reduced by herbivory. Conversely, in German plants, demethylation at seed-stage was still evident in reproductive individuals whereas herbivory did not affect their global methylation. For Swedish plants, artificial herbivory imposed a stronger reduction than insect herbivory in global methylation, higher seed mass and a higher aliphatic glucosinolate concentration. In German plants, insect herbivory plants had higher fruit number and both herbivory led to higher leaf glucosinolates concentrations. Finally, the effect of insect herbivory on stem biomass and reproductive parameters varied with the level of demethylation and the study population. The complexity of our results suggests that the genetic background of experimental plants can affect herbivory-induced phenotypic plasticity.

Keywords: Brassicaceae, Glucosinolates, herbivory, methylation, *Thlaspi*

S.6.3. What epigenetic control (DNA methylation) during the mutualistic symbiotic interaction between a tree (*Populus tremula* x *alba*) and a mycorrhizal fungus (Lacarria bicolor)?

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The current climate changes are causing forests decline, especially in connection with repeated episodes of drought. In trees, it is very common for water supply to be achieved through a mycorrhizal symbiosis with their roots. The mechanisms that allow the establishment of this mutualistic symbiotic interaction have yet to be clarified, particularly on epigenetic control in the two protagonists.

To study the responses of the tree, we are working in the ARCHE team on the poplar which is a model tree. My thesis work is part of the ANR EPITREE project, which aims to study the role of one of the epigenetic marks: DNA methylation in the phenotypic plasticity of poplar (Amaral et al., 2020; Vigneaud et Maury, 2020). This study uses RNAi poplar lines modified for DNA methylation: hyper or hypomethylated. Here, in collaboration with the UMR IAM of Nancy (C. Venault-Fourrey and A. Kohler), the RNAi lines were mycorrhizal then phenotyped and sampled for -OMIC type analyzes: RNAseq and WGBS on the poplar genome and on the fungus. The results will be presented and discussed to highlight the role of DNA methylation in this interaction.

Keywords: Drought, epigenetics, DNA-methylation, poplar, mycorrhizae

S.6.4. Stress memory in response to benzoxazinoid allelochemicals in Thlaspi arvense

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Plants grow in dynamic environments and experience many different types of biotic and abiotic stresses. To be able to respond to fluctuating conditions, plants have evolved sophisticated adaptive mechanisms. One strategy involves “memorizing” stress, enabling the plant to respond more efficiently when the stressor reoccurs (somatic memory) or even to prepare offspring for harsh conditions (transgenerational memory). Although plants generally display such responses, little is known about the specific mechanisms behind such memory formation. Here we address this issue in the context of one particularly common source of plant stress, allelopathy, a phenomenon in which plants release toxins, termed allelochemicals, which inhibit growth of surrounding flora. We explore adaptation to allelopathic environments in *Thlaspi arvense* with the aim of understanding the molecular basis underlying memory acquisition. To this end, we exposed *T. arvense* to the allelochemical 2-aminophenoxazin-3-one (APO) in vitro and assess the epigenetic stress response in young seedlings through Whole Genome Bisulfite Sequencing (WGBS) and calling of Differentially Methylated Regions (DMRs) between stress samples vs non-stress. Result showed that APO stress causes methylation changes in CHH context on regions near genes related to stress response, detoxification mechanisms and cell wall integrity.

Keywords: Stress response, Thlaspi, allelochemicals

S.6.5. Competition-induced transgenerational plasticity influences competitive interactions and leaf decomposition of offspring

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Phenotypic plasticity, within and across generations (transgenerational plasticity), allows organisms and their progeny to adapt to the environment without modification of the underlying DNA. Recent findings suggest that epigenetic modifications are important mediators of such plasticity. However, empirical studies have, so far, mainly focused on plasticity in response to abiotic factors, overlooking the response to biotic interactions such as competition. We tested for within-generation and transgenerational phenotypic plasticity triggered by plant–plant competition intensity, and tested whether it was mediated via DNA methylation, using the perennial, apomictic herb Taraxacum brevicorniculatum in four coordinated experiments. We then tested the consequences of
transgenerational plasticity affecting competitive interactions of the offspring and ecosystem processes such as decomposition. We found that, by promoting differences in DNA methylation, offspring of plants under stronger competition developed faster and presented more resource-conservative phenotypes. Further, these adjustments associated with less degradable leaves which have the potential to reduce nutrient turnover and might, in turn, favour plants with more conservative traits. Greater parental competition enhanced competitive abilities of the offspring by triggering adaptive phenotypic plasticity, and decreased offspring leaf decomposability. Our results suggest that competition-induced transgenerational effects could promote rapid adaptations and species coexistence, and feed back on biodiversity assembly and nutrient cycling.

Keywords: Adaptation, Functional_traits, Competition, nutrient_cycling

SESSION 4

EPIGENETIC MEMORY AND TRANSGENERATIONAL INHERITANCE

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OPENING LECTURE

PRIMING – EPIGENETIC MEMORY IN KELP AND SEAGRASS FOR ENHANCED RESTORATION SUCCESS AND FOOD SECURITY IN FUTURE OCEANS

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Marine macrophytes, including seagrasses and macroalgae, form the basis of diverse and productive coastal ecosystems that deliver important ecosystem services and are targets for a new sustainable blue bio-economy. However, seagrass meadows and macroalgal forests are threatened by a variety of anthropogenic stressors. Most notably, rising temperatures and marine heatwaves are already devastating these ecosystems around the globe, and threaten production security of farmed kelp. I will present recent studies that show that seagrass and macroalgae can become less susceptible to heat events once they have been primed thermally. Early evidence in clonal seagrass shows that heat-resistance, and the formation of a stress memory, partly relies on DNA methylation marks that switch state and permanently trigger stress-preventive genes after the first stress exposure. Thus, the priming-induced formation of epigenetic stress memories have considerable potential for both ecosystem restoration and macroalgae farming to immediately improve performance and stress resistance and, thus, to enhance restoration success and production security under environmental challenges. I conclude with specific research gaps that need to be filled before priming can be established as new bio-engineering technique in these primary producers.

S.4.1. Heritability of stress-induced DNA methylation patterns in the diploid woodland strawberry (*Fragaria vesca* sp.)

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Environmental stresses can induce a wide range of phenotypic changes in plants and impact their epigenome. DNA methylation is one of the few heritable epigenetic marks and one of its functions is to silence transposable elements (TEs). In this study we investigated heritable genetic and epigenetic changes induced by environmental stresses in strawberry (*Fragaria vesca*). This plant is an ideal model to study such processes given its dual modes of reproduction –sexually, through seeds, and asexually through runners. Using global DNA methylation and transcriptomic approaches our objectives in this study are to: (1) Test the impact of heat stress on the methylome and transcriptome of *F. vesca*. (2) Screening for multi-generational persistence of stress-induced changes in DNA methylation patterns and their association with gene and TE silencing and/or activity. (3) Investigation of transgenerational DNA methylation stability differences between somatic and sexual reproduction. In this study we show that the strawberry methylome as well as transcriptome respond with a high level of flexibility to ecologically relevant stresses. Moreover, we describe that the heritability of DNA methylation differences is affected by meiotic reproduction in comparison to mitotic reproduction. We identified differences in global methylation among three clonal generations and conserved genes that are down- and up- regulated over the first two clonal generations following stress exposure. Similarly, we detected transposable elements actively transcribed in the parental lines after the stress but not in the clones, suggesting that they are silenced under normal conditions to maintain genome stability in the offspring.

Keywords: methylome, transcriptome, clonal, strawberry, stress

S.4.2. Phenotypic plasticity in plant defense across life stages: inducibility, transgenerational induction and transgenerational priming in wild radish

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As they develop, many plants deploy shifts in anti-herbivore defense allocation due to changing costs and benefits of their defensive traits. Plant defenses are known to be primed or directly induced by herbivore damage within generations, and across generations by long-lasting epigenetic mechanisms.

However, little is known about the ontogenetic trajectories' differences between life stages of epigenetically inducible defensive traits across generations and their consequences. To help fill this knowledge gap, we conducted a multigenerational experiment to determine whether defense induction in wild radish plants was reflected in chromatin modifications (DNA methylation); we then examined ontogenetic trajectories (differences between seedlings to and reproductive plants) in of current and transgenerational plasticity in anti-herbivore chemical (glucosinolates) and physical (trichomes) defenses in this species. Herbivory triggered genome methylation both in targeted plants and their offspring. Within one generation, both defenses were highly inducible at the seedling stage but only marginally or non-inducible chemical defenses were inducible in reproductive plants. Across generations, herbivory experienced by mother plants caused strong direct induction of physical defenses in their progeny, with effects lasting from seedling to reproductive stages. For chemical defenses, however, this transgenerational induction was evident only in adults. Transgenerational priming was observed in physical defenses both for seedlings and adult plants. Our results show that transgenerational induction and priming in response to herbivore offense differ for physical and chemical defense and change across plant life stages.

Keywords: Ontogenetic trajectories, Transgenerational induction, Transgenerational priming

S.4.3. Stability of epigenetic inheritance in the highly clonal common Duckweed, *Lemna minor*

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With climate change increasing at an alarming rate, organisms must adapt to rapidly changing environments. Whether an organism is capable of adapting fast enough is a prominent question in ecology and evolution. There is increasing evidence, particularly from plants, that epigenetic mechanisms can contribute to adaptive responses. However, it is not clear what the evolutionary implications are, as environment-induced epigenetic modifications are often reset between generations and therefore will not cause a lasting response to selection.

Nevertheless, a general hypothesis is that plants that reproduce without a germline (asexual species) will undergo no or low epigenetic resetting, allowing for stronger transgenerational stability of spontaneous and environmentally induced DNA methylation variants. This talk will present evidence supporting this hypothesis using acquired transgenerational DNA methylation results from the highly clonal aquatic species, the common Duckweed *Lemna minor*. *L.minor* individuals, which have a clonal generation time of ~2days, were exposed to either control or high temperatures for 6 weeks. Using epiGBS reduced representation bisulfite sequencing, we show that high temperature induces many changes in DNA methylation in both CG and CHG context. Moreover, after removing the high-temperature trigger, a subset of induced changes in the CHG context (but not CG) persisted during a 3 weeks growth in control temperatures. Furthermore, structural annotation show that the majority of these transgenerationally inherited CHGs are found near or within gene bodies. In brief, these results demonstrate that environment-induced changes in DNA methylation patterns can be stably inherited across many clonal generations.

Keywords: DNAmethylation, Transgenerational, Duckweed, epiGBS

S.4.4. Developmental variation in transgenerational transcriptional plasticity in *Persicaria maculosa*

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Transgenerational plasticity, also described as epigenetic inheritance or parental effects, can influence the response to environmental cues and shape organismal phenotype and fitness. The magnitude of these effects may also change over the development of an indivdiual, creating a ongoing, dynamic feedback between generational cues. We survey the transcriptomes of 12 genotypes of Persicaria maculosa and find that in general, transcription is strongly influenced by ancestral light conditions early in life, but is more influenced by present conditions later in life. However, we also find individual genes and gene networks whose expression is stably altered by ancestral cues, regardless of present conditions. Finally, we find genetic variation for the ability to respond to both past and present cues at the level of gene expression. In sum, our work suggests that within- and transgenerational cues interact to alter the integration of environmental information into gene expression patterns across development. In turn, developmental integration might itself be a evolving trait in natural populations.

Keywords: Transgenerational plasticity, inheritance, gene regulation

S.4.5. Transgenerational non-genetic inheritance has fitness costs and benefits in the clonal duckweed *Spirodela polyrhiza*

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Although non-genetic inheritance is thought to play an important role in plant ecology and evolution, evidence for adaptive transgenerational plasticity is scarce. Here, we investigated the consequences of copper excess on offspring fitness under recurring stress in the duckweed *Spirodela polyrhiza* across multiple asexual generations. Growing large monoclonal populations for 30 generations under copper excess had negative fitness effects after short and no fitness effect after prolonged growth under recurring stress. These time-dependent growth rates were likely influenced by environment-induced transgenerational responses, as propagating plants as single descendants for 2 to 10 generations under copper excess had positive, negative or neutral effects on offspring fitness depending on the interval between initial and recurring stress (5 to 15 generations). Copper excess modified offspring fitness under recurring stress in a genotype-specific manner, and increasing the interval between initial and recurring stress reversed these genotype-specific fitness effects. Experiments to assess the underlying molecular mechanisms, particularly DNA methylation, are on the way. Taken together, these data demonstrate time- and genotype-dependent adaptive and non-adaptive transgenerational responses under recurring stress, which suggests that non-genetic inheritance alters the evolutionary trajectory of clonal plant lineages in fluctuating environments.

SESSION 5

EPIGENETIC REGULATION AS A RESPONSE TO CHANGES IN ENVIRONMENTAL CONDITIONS

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OPENING LECTURE

EVOLUTIONARY AND FUNCTIONAL IMPACT OF EPIGENETIC VARIATIONS IN FOREST TREES FACING CLIMATE CHANGE (EPITREE)

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Forest die-off is reported all around the world due to heat and drought stress episodes. As fixed and long living organisms subjected to repeated environmental stresses, trees have developed mechanisms enabling them to cope with fluctuating environmental conditions. We proposed epigenetics as a hub of integration in meristematic cells linking developmental and hormonal responses with changing environment. This was based on our studies in the shoot apical meristem of poplar under water deficit conditions (Lafon-Placette et al., 2018; Le Gac et al., 2018; Sow et al., 2018a; Le Gac et al., 2019; Maury et al., 2019; Vigneaud et Maury, 2020). Our objective is now to evaluate how DNA methylation can significantly participate to plasticity and adaptation in natural populations of trees facing climate change in order to test potential uses for trees breeders and forest managers (Sow et al., 2018b; Amaral et al., 2020; Kakoulidou et al., in submission).

Recently, we used a reverse genetic approach and found that poplar trees with altered methylation profiles are affected for their tolerance to drought. In addition, we have identified genes and transposable elements targeted during this process and highlight a trade-off situation between plasticity and genome integrity in meristematic cells (Sow/Le Gac et al., 2021 revision).

Finally, in the EPITREE project, we are evaluating the adaptive potential of DNA methylation variations in natural tree populations (Poplar and Oak). This study is also associated to the EPICATCH COST action aiming at defining the role of epigenetic mechanisms for crop adaptation to climate change.

S.5.1. DNA methylation aids adaptation and population persistence in a changing climate

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Epigenetic modifications are increasingly recognized as an important source of phenotypic variation. They are also postulated to play a role in adaptation to fast and erratic environmental changes but experimental evidence is scarce.
Here we present evidence of epigenetic based adaptation to environmental changes. At the Buxton Climate Change Impacts Laboratory (BCCIL), a semi-natural grassland has been exposed for more than 2 decades of simulated climate change including drought, additional rainfall, and winter warming treatments. The short-lived perennial *Scabiosa columbaria* proved remarkably resistant to these climatic manipulations.
We transplanted *Scabiosa columbaria* from BCCIL to a common garden and studied changes over a period of four years. DNA methylation was measured before and four years after transplantation using epiGBS2. Consistent and stable epigenetic differentiation in response to different climate change treatments was observed, in particular in CHH context. Additionally, offspring from plants originating from long-term summer drought and additional summer rain treatments at BCCIL were used in a drought experiment. F2-generation plants from grandmothers that received summer drought at BCCIL grew significantly larger in the drought treatment compared to offspring from control or additional summer rain treatments.
Our results show stable maintenance of environmentally-induced epigenetic variation that may have contributed to the stability and adaptability of *Scabiosa columbaria* through long-term climatic manipulations.

Keywords: DNA methylation, adaptation, climate change

S.5.2. Drought induces genome-wide hypermethylation of short interspersed nuclear elements (SINE) in *Populus nigra* var. *italica*

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In long-lived species, such as trees, environmental-induced epigenetic modifications have the potential to influence plant ability to respond to novel situations. DNA methylation is the most studied epigenetic mark in plants, and plays a key role in the transcriptional silencing of transposable elements (TEs) and gene expression regulation.

However, the involvement of DNA methylation in plant response to environmental cues remains poorly characterized. In this project, we aimed to identify specific and general methylation changes induced by several stresses in the clonal tree Populus nigra italica. Hardwood cuttings were collected from different European regions, grown in common greenhouse conditions for 4 months, and subsequently exposed to different stresses (heat, cold, drought, herbivory, rust infection, and salicylic acid). Then, DNA methylation was measured in leaf tissue by whole-genome bisulfite sequencing (WGBS) in 8 replicate individuals per treatment. Our work highlighted a strong genome-wide methylation stability in CG and CHG sequence context after any stress condition. Global CHH methylation levels significantly increased after drought, mainly over gene flanking regions and TEs, and preferentially targeting SINE retrotransposons. In addition, all stresses induced methylation changes around a specific family of auxin-responsive genes. Our study shows how trees are able to remodel its methylome, and a cytosine context-specific drought response of SINE methylation, which open new insights into the possible role of genome-wide DNA methylation in response to stress.

Keywords: WGBS, hypermethylation, drought, poplar, SINE

S.5.3. Changes in DNA methylation and gene expression in response to copper stress in bryophytes

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Bryophytes are one of the most resilient taxa on Earth due to their capacity to thrive in extreme environments. Yet, the molecular basis of this resilience remains largely unexplored. Here, we used the moss *Scopelophila cataractae* (Sc) as a model to study the role of epigenetic regulation to Cu tolerance in bryophytes. We also assessed the molecular mechanisms involved. We collected four populations of Sc from different contamination levels within a former Cu mine. After clonally propagating them under control and Cu treatments, we measured plant performance and Cu accumulation to understand the plant response to Cu, and used reduced representation bisulfite sequencing (epiGBS) and RNA sequencing to detect DNA methylation and functional changes associated with Cu exposure, respectively.

Two Sc isolates from the most polluted locations in the mine were more tolerant to Cu than the other two. Cu accumulation, however, did not differ among isolates. Exposure to Cu had a significant, isolate-dependent, effect on genome-wide DNA methylation: epigenetic distances between control and Cu-treated samples of the two most tolerant isolates were greater than between those of the other two (167-222 vs. 130-178). More than 90% of the differentially expressed genes within each isolate were downregulated in response to Cu suggesting an overall shut down of the plant metabolism, including pathways typically involved in heavy metal stress response (e.g. membrane transporters and redox regulating enzymes), and proteins potentially involved in epigenetic regulation (histone deacetylases and histone variants). We provide evidence that Cu induces an epigenetic response that differs in magnitude among isolates from the same species.

Keywords: copper, epigenetic regulation, RNA seq

S.5.4. Characterization of the genome wide distribution of H3K27me3 and H3K4me in the marine microalgae *Ostreococcus tauri* over diurnal and seasonal cycles

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The Earth's tilted rotation and translation around the Sun produce repetitive environmental signals giving rise to diurnal and seasonal cycles. This has promoted the evolution of cell-autonomous molecular systems consisting of oscillators known as circadian clocks that predict and anticipate these changes. Seasonal regulated circadian clocks are especially relevant in photosynthetic organisms since light is their main source of energy. Nevertheless, multiomics integrative analysis of seasonal and diurnal cycle effects over the physiology of microalgae remains largely unexplored despise their wide range of promising biotechnological applications. In order to fill this gap we have chosen the marine picoeukaryote *Ostreococcus tauri* as a model microalgae. We have analysed and integrated RNA-seq and ChIP-seq data generated under diurnal and seasonal cycles. Our analysis has unveiled that more than 80% of the Ostreococus transcriptome present rhythmic expression. In order to explore the mechanisms regulating rhythmic gene expression we have studied the genome wide distribution of the epigenetic marks H3K27me3 and H3K4me3 associated with gene repression and activation respectively. No change in the levels of these marks were detected over diurnal cycles instead they were involved in the control of specific processes such as the repression by H3K27me3 of viral infection response genes. This suggests that rhythmic gene expression is predominantly regulated by transcription factors. In this respect, we have determined using ChIP-seq data the gene targets of the Ostreococcus CCA1 ortholog at dawn identifying the cell cycle and starch biosynthesis as biological processes repressed by this transcription factor.

Keywords: Microalgae, Ostreococcus, H3k27me3, H3k4me3, circadian

S.5.5. Selection and cross resistance in monoclonal *Spirodela polyrhiza* populations under oxidative stress outdoors

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Plants may alter their phenotype and fitness across generations in the absence of genetic change. However, it remains unclear whether non-genetically inherited traits are subject to natural selection. We grew monoclonal populations of the Great Duckweed, *Spirodela polyrhiza*, for approximately eight generations in the presence and absence of copper excess in outdoor ponds, and manipulated the degree of intra-clonal selection in each pond by either subjecting large populations to recurrent bottlenecks (with intra-clonal selection), or growing plants as single descendant lineages (without intra-clonal selection), and assessed their fitness under different oxidative stresses. Populations grown in the absence and presence of copper excess did not differ in their fitness under recurring oxidative stresses. However, populations grown under copper but not control conditions tended to suffer lower growth reduction under salt stress compared to single descendant lineages, suggesting that non-genetically inherited traits are subject to intra-clonal selection and allow cross resistance. Using chemical inhibition, we are currently studying the contribution of DNA methylation and histone acetylation for the observed differences in resistance to salt stress. Taken together, this study provides the first evidence that intra-clonal selection improves plant resistance in the absence genetic change under natural conditions outdoors.

S.5.6. Dynamics of DNA methylation in *Posidonia oceanica* (L.) Delile from different environments

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The dynamics of DNA methylation play important roles in the regulation of gene expression and genome stability. These epigenetic modifications are regulated by environmental factors through the activation of different writers/erasers that can be at the basis of the appearance of different phenotypic responses to environmental stresses. In the context of climate change, understanding the impact of ocean warming in combination with local pressures is fundamental for exploring resilience capacity of marine organisms. Seagrasses, which are marine angiosperms particularly vulnerable to environmental changes, display high degree of phenotypic plasticity colonizing heterogeneous environments. Here we explore, for the first time, the dynamics of DNA-methylation in *Posidonia oceanica* shoots from meadows experiencing different nutrient conditions (oligotrophic, Ol; eutrophic, Eu).

The expression of key genes involved in the de *novo* DNA methylation, maintenance, demethylation and histone modifications were selected and analysed after one, two and five weeks of exposure to single (temperature and nutrients) and combined stressors (nutrients + temperature). Global DNA methylation levels were also measured in both Ol and Eu plants at the same time points. Our results revealed that the global DNA methylation and the expression dynamics of selected genes were influenced by both plants origin and the duration of the imposed stresses. Temperature was the main driver modulating gene expression during the experiment. These findings suggest that DNA methylation in marine plants is a dynamic process that could potentially regulate phenotypic responses to environmental changes.

Keywords: DNA methylation, gene expression, seagrasses, environmental stress

SESSION 3
BIOINFORMATIC ANALYSIS FOR PLANT EPIGENETICS

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OPENING LECTURE

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Third-generation sequencing techniques are currently revolutionizing the field of genome analysis by generating formerly unreachable read lengths with averages in the tens of thousands of nucleotides. Current established applications are de novo genome assemblies, structural variant and splicing detection. In addition, formerly almost unsequencable regions like telomeres and highly repetitive elements become now accessible. However, the potential of Nanopore sequencing is not yet fully exhausted: it does not require any amplification and thus enables direct readout of sequence including base modifications on a single DNA or RNA molecule level.
To date, methylation of CpGs is the best-established base modification read-out from mammalian DNA. The DNA methylome provides information of cell type identity, DNA accessibility and plays a critical role during embryonic development, particularly in repressing retrotransposons. The mammalian methylation landscape is dependent on the combined activities of the canonical maintenance enzyme Dnmt1 and the de novo Dnmts, 3a and 3b.
Using long-read Nanopore and whole-genome bisulfite sequencing in genetically engineered methylation depleted mouse embryonic stem cells, we demonstrate that Dnmt1 displays de novo methylation activity in vitro and in vivo with specific retrotransposon targeting. Utilizing additional knockout lines and molecular characterization, we show that Dnmt1's de novo methylation activity depends on Uhrf1 and its genomic recruitment overlaps with targets that enrich for Uhrf1, Trim28, and H3K9 trimethylation, especially at retrotransposons where this mechanism may provide additional stability for long-term repression and epigenetic propagation throughout development.

S.3.1. The EpiDiverse epigenome-wide association studies (EWAS) pipeline

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Epigenetics covers heritable changes via DNA, chromatin modification, and small RNA interactions that can alter the transcriptional activity without changing the DNA sequence. DNA methylation is the most frequently studied mechanism due to its easy accessibility and can be determined by a widely used technique called bisulfite treatment to associate genetics and epigenetics with phenotypic traits. This association has become possible with genome-wide association studies (GWAS) and epigenome-wide association studies (EWAS).
Very scarce use of EWAS with plants and general insufficiency of existing tools prompted us to develop the EpiDiverse EWAS pipeline. Luckily, an R package called GEM is compatible with all species and allows missing data imputation.
Therefore, the pipeline is based on the GEM R package including graphical outputs, novel missing data imputation, compatible with new input types, and was tested on *Quercus lobata* (valley oak) *Picea abies* (Norway spruce) data sets.
Q. lobata dataset showed that nearly all significant epigenetic markers were reproduced by the EWAS pipeline, even the statistical methods are different between the two studies. *P. abies* dataset showed that significant epigenetic markers in outputs with different input types vary and most of the gene ontology (GO) terms are shared.
In conclusion, we revealed the reliability of missing data estimation and showed beta distribution is an accurate choice for approximation as concluded from significant overlap. Moreover, we presented that a model and input choice depend on the user's research question, GO could help cluster suggestive biological functions that might be missed in single gene descriptions.

Keywords: EWAS, GWAS, DNA methylation, non-model species, pipeline

S.3.2. epiGBS2: an improved protocol for reduced representation bisulfite sequencing with or without reference genome

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Several reduced representation bisulfite sequencing methods have been developed in recent years to determine cytosine methylation de *novo* in non-model species. Here, we present epiGBS2, an improved version of the reduced representation bisulfite sequencing method epiGBS. epiGBS2 has a revised and user-friendly bioinformatics pipeline suitable for a wide range of species with or without reference genome. To evaluate the performance of this revised pipeline we compared the methylation levels and genetic structure of different *Arabidopsis thaliana* lines to known baselines. Here we provide recommendations, highlight strengths and indicate limitations for the use of epiGBS2. We illustrate this using examples from both model and non-model species in which we answer ecologically relevant questions.

Keywords: epiGBS pipeline

S.3.3. Ecc_finder: a robust and accurate tool for detecting extrachromosomal circular DNA (eccDNA) from eccDNA sequencing data

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Taking advantage of investigating extrachromosomal circular DNA (eccDNA) using Nanopore long reads will undoubtedly accelerate eccDNA detection and narrow down functional important regions producing eccDNA in any plant or animal genome. However, there are no tools currently available to specifically identify eccDNA from Nanopore reads. More importantly, the current tools based on Illumina short reads lack an efficient standard pipeline and cannot be applied to very large genomes. Here we introduce a comprehensive tool to solve both of these two problems (https://github.com/njaupan/ecc_finder). Applying ecc_finder to mobilome-seq and eccDNA-seq data from Arabidopsis, human , and wheat (with genome sizes ranging from 120Mb to 17Gb), we document the improvement of computational time, sensitivity , and accuracy and prove its wide applicability and functionality.

Keywords: eccDNA, long reads, mobilome-seq

S.3.4. A new role for histone demethylases in the maintenance of genome integrity in *Arabidopsis*

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Histone modifications deposited by the Polycomb repressive complex 2 (PRC2) play a critical role in the control of growth, development, and adaptation to environmental fluctuations of most multicellular eukaryotes. The deposition of H3K27me3 by PRC2 is counteracted by Jumonji-type (JMJ) histone demethylases specific for H3K27, and this shapes the genomic distribution of H3K27me3. In Arabidopsis, two JMJ histone demethylases, EARLY FLOWERING 6 (ELF6) and RELATIVE OF EARLY FLOWERING 6 (REF6), were thought to be redundant in the active removal of H3K27me3. Using ChIP-seq analysis on mutants for these enzymes we found that they play distinct roles in H3K27me3 homeostasis and that REF6 is responsible for the maintenance of H3K27me1 in lowly expressed genes —a histone modification previously associated with heterochromatin. We discovered that failure to reset these chromatin marks during sexual reproduction results in the transgenerational inheritance of histone marks to wild type progeny and expansion of H3K27me3 into pericentromeric regions, which cause a loss of DNA methylation at heterochromatic loci and transposon activation, accompanied by pleiotropic phenotypes. Thus, Jumonji-type histone demethylases play a dual role in plants by helping to maintain transcriptional states through development and safeguard genome integrity during sexual reproduction

Keywords: H3K27m

S.3.5. Manipulating base quality scores enables variant calling from bisulfite sequencing alignments using conventional Bayesian approaches

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Calling germline SNP variants from bisulfite-converted sequencing data poses a challenge for conventional tools, which have no inherent capability to dissociate true polymorphisms from artificial mutations induced by the chemical treatment. Nevertheless, SNP data is desirable both for genotyping and for resolving the interaction between genetic and epigenetic effects when elucidating the DNA methylome. Existing software for this purpose have reduced sensitivity, precision and computational performance relative to conventional variant callers, due in part to the obfuscating nature of bisulfite sequencing libraries, but also to assumptions made when elucidating between SNPs and methylated positions. Instead, we seek to leverage the underlying mechanisms of conventional variant callers, such as GATK and Freebayes, to resolve the confounding effect of bisulfite conversion by observing differences in allele counts on a per-strand basis. Herein, we present a computational pre-processing approach for adapting such data, thus enabling downstream analysis in this way using conventional variant calling software. The method is applicable for (i) alignment, (ii) haplotype and (iii) local assembly-based approaches for deriving SNPs. On plant and human benchmark datasets, the method consistently outpeforms existing software whilst providing variants in a format more familiar to those who work with conventional sequencing data.

Keywords: DNA methylation, variant calling, bisulfite sequencing

CLOSING LECTURE

BIOSKETCH

Koen Verhoeven 1

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Koen Verhoeven is an ecological geneticist at the Netherlands Institute of Ecology (NIOO-KNAW), Wageningen, The Netherlands. His research on plant adaptation uses genetic and genomic tools and applies them in ecologically relevant contexts in order to understand how plant populations respond to changing environments. One of his research interests is in ecological epigenetics: what role does epigenetic variation play in ecology and microevolution? In this research he is coordinator of the EPiDiverse Marie Curie European training network. He will present here some ideas learned during this network.